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Immunotherapy

Myths, Reality, Ideas, Future

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IMMUNOTHERAPY - MYTHS, REALITY, IDEAS, FUTURE

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Meet the editor

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He has published over 250 papers, IF 175.00, 9 books on medicine, and 2 books on poetry. He is the editor in chief of the *Journal of IMAB* and editorial board member of IJIP, EJI, CEJM, JAA, and JISAO. He has edited *Risk Infections and Possibilities for Biomedical Terrorism* (IOS Press, NATO), *Immunodeficiency* (InTech Press), and *Immunopathology and Immunomodulation (*InTech Press). Prof. Dr. Metodiev was an invited speaker and a chairperson of congresses and symposiums, in over 60 countries worldwide. He is a holder of prestigious awards in Bulgaria, Israel, the UK, Germany, Italy, Greece, the USA, Japan, Canada, Australia, Brazil, Serbia, Turkey, Romania, and Slovenia. He is a board member of ISC, FESCI, EUCAST, ESC, ISAO/IFAO, EANO, ICHS, etc. Prof. Dr. Metodiev is also an honorary consul of Israel to Bulgaria.

Contents

Preface

Bona diagnosis, bona curatio—good diagnosis, good cure

but important is:

Fiat secundum artem—Let it be made according to art

The present book is a combined achievement of a number of distinguished specialists in immunology, immunogenetics, oncology, infections, allergy, and experimental medicine.

The topic immunotherapy is a huge ocean of subtopics, theoretical and practical issues, successes and dissatisfactions, and every attempt to find out the best meaning and role of this discipline is a step forward to the victory over diseases.

Starting with the definition of immunotherapy, treatment, or prevention of disease (such as an autoimmune disorder, allergy, infection, or cancer) that involves the stimulation, enhancement, suppression, or desensitization of the immune system, we immediately deepen our current knowledge, tending to add new skills and apply them for the benefit of human health.

Activation immunotherapies induce or amplify an immune response and are used in vaccines and as cancer immunotherapies.

Suppression immunotherapies reduce or suppress an immune response and are used to prevent graft rejection and treat autoimmunity and allergy.

We are witnessing how for the past few decades breakthroughs in cell and molecular biology have allowed significant advances in science and medicine.

Up-to-date cell-based products are rather often genetically engineered: immune cells for immunotherapies or stem cells for regenerative medicine, including anticancer vaccines.

Nowadays, immunotherapy is one of the most exciting areas of new discoveries and treatments for a number of diseases, on the first line being the many different kinds of cancer.

Understanding how the immune system works is opening the doors to developing new treatments that are changing the way we think about and treat cancer.

The human immune system is a network of cells, tissues, and organs that work together to recognize and destroy foreign aggressors, such as bacteria and viruses or abnormal or unhealthy cells in your body—the most important function of the immune system is to know the difference between self and nonself.

Thus, the immunotherapy is designed to harness the ability of the body's immune system to combat infection or disease.

Another essential part of the general immunotherapy is the allergen immunotherapy, usually cited as allergy shots—this is a form of long-term treatment, tending to decrease the symptoms of many people with allergic rhinitis, allergic asthma, conjunctivitis (eye allergy), or stinging insect allergy.

These allergy shots minimize the sensitivity to allergens and often lead to lasting relief of allergy symptoms even after completion of the treatment procedure.

Immunotherapy or biological therapy includes preparations like monoclonal antibodies, interferon, interleukin-2 (IL-2), several types of colony-stimulating factors (CSF, GM-CSF, G-CSF), TNF, biological response modifiers (BRMs), and a number of topical pharmaceutical products to activate or suppress the immune reactivity.

A variety of diseases are reported to be successfully manipulated by applying one or a combination of two or several immunotherapeutic drugs: advanced malignant melanoma, hepatitis C, Crohn's disease, and rheumatoid arthritis.

However, despite the efforts of the specialists, not everything goes smoothly: different side effects of biological therapy are registered, depending on the type of treatment—flu-like symptoms such as chills, fever, muscle aches, weakness, loss of appetite, nausea, vomiting, and diarrhea. Some patients demonstrate a rash; others bleed or bruise easily. The interleukin therapy can cause swelling as well. The good news is that these side effects are usually short term and can gradually disappear after the end of the treatment.

To be exact and honest enough—immunotherapy in general and/or especially anticancer immunotherapy in particular: this lauded breakthrough is far more dangerous than advertised!

Therefore, not every announced "progress" in anticancer immunotherapy is a real victory over tumors, and the attention of the specialists, applying or prescribing immunotherapy drugs against cancer, is highly required.

In summary, this book throws certain light on the research, production, and application of a number of immunotherapy products in various fields of human pathology, thus serving as a very good tool for additional knowledge, experience, and educational and scientific approaches of medical and biological students, postdocs, specialists, and experts.

The authors of this book, as well as I, as the editor in chief, would wish to extend our warmest thanks to the Junior Commissioning Editor, Ms. Ana Simcic, from InTech—Open Science Open Minds, for her never-ending assistance and contributions to the success of our publications. She was with us from the very beginning of the project until the last minute of the entire procedure of the book processing.

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Reviews

Chapter 1

Hematopoietic Cell Transplantation for Autoimmune Diseases: A Review of History, Current State, and Future Issues

Igor B. Resnick, Krassimir Metodiev and Paula Lazarova

Additional information is available at the end of the chapter

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Abstract

Autoimmune diseases are characterized by recurrent attacks and remissions, but as a rule they progress and eventually cause a severe disability and death. The present chapter contains general characteristics of autoimmune disease pathogenesis, ways to cause immune tolerance by hematopoietic cell transplantation (HCT), clinical aspects of the treatment for established autoimmune diseases with a special attention to multiple sclerosis (MS) and systemic sclerosis (SSc). A profound analysis of authors' point of view and of the available literature has been performed. The promising results allows to consider HCT as a relevant treatment option for a certain autoimmune diseases.

Keywords: hematopoiesis, autoimmune diseases, immunomodulation, hematopoietic cells

1. Introduction

Autoimmune disorders are affecting from 5 to 10% of the population. Usually, they are characterized by recurrent attacks and remissions, but as a rule they could develop with further progression and eventually development of a severe disability and death. The usual treatment of the vast majority of autoimmune diseases is immunosuppression. Newly proposed pharmacological agents can cause pronounce effect for the disease course and bring to a long-term remission. Evidences that certain autoimmune disorders can develop into a prolong treatment-free remission after hematopoietic cell transplantation (HCT) have been recently discussed in terms of human and animal models, including cases with accompanying

malignancies. The hypothesis that a strong immunosuppressive or myeloablative therapy can eliminate auto-reactive clones and cause a prolonged treatment-free remission is still open for analysis. It is still not clear whether myeloablative conditioning regimen with autologous HCT is more beneficial compared to a modern reduced intensity immune ablation with hematopoietic cell rescue. Other types of cell therapy are under intensive investigation at present too. Our present review will contain general characteristics of autoimmune disease pathogenesis, ways to cause immune tolerance (immunosuppression versus repertoire replacement), clinical aspects of HCT for established autoimmune diseases with a special attention to multiple sclerosis (MS) and systemic sclerosis (SSc), treatment regimens of autoimmune diseases and approaches for future therapies.

2. Back to immune tolerance: immunosuppression versus repertoire replacement and restoration of immune regulation

The treatment of autoimmune diseases, as pivotal goal, is to cause immune tolerance and therefore interrupt disease progression. The basic treatment of autoimmune disease is the immunosuppressive (and anti-inflammatory) therapy. In addition to different groups of cytotoxic immunosuppressive drugs, an increase in different types of monoclonal antibodies is seen. All they are directed to damage the number or function of lymphocytes. Immunomodulatory approaches are also in the center of research evaluation and clinical trial, performed with some indications to show effectiveness of this concept. For example, intravenous IgG therapy has major effects on idiotypic network immune regulation and demonstrates clinical effectiveness in many autoimmune diseases.

Thus, due to decreasing lymphocyte infiltration, cytokine production and secondary inflammatory changes, a repair of misbalanced immune regulation and pathogenic and networked anti-idiotypic antibodies can lead to interruption of inflammatory attack, slowing disease progression, prolong life expectancy and improve the quality of life of patients with autoimmune diseases. However, most if not all patients should stay lifelong on their treatment, and ultimately in addition to the disease itself, an accumulation of side effects brings them to irreversible deterioration.

The first modern fashion hematopoietic cell transplantation (HCT) was performed in 1967 by Gatti et al. [1], but a few approaches for allogeneic transplantation before MHC/HLA discovery were performed several years prior to them [2, 3]. If the aims of allogeneic HCT are the use of immune graft-versus-tumor effect or substitute the inborn or acquired error in hematopoiesis, the immunogenesis or metabolism will result accordingly. Autologous transplantations are performed with a goal to reach the highest tolerable level of cytotoxic antitumor effect saving hematopoietic system maximally intact and minimally impaired. The latter approach became standard of care for multiple myeloma and lymphomas, and in addition, it finds its place in some other malignancies (neuroblastoma, breast cancer, etc.).

Myeloablative conditioning with subsequent hematopoietic cells rescue (autologous transplantation) can re-establish the life-saving three lineage hematopoiesis, usually fast enough;

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the white blood cells, the platelets, and the red blood cell engraftment occur in most of the cases within 2–3 weeks, registered in the vast majority of patients.

But while innate immunity usually restores in a few weeks, it takes much more time for recovery of adaptive immune system. In fact, despite a huge number of publications, there is no complex understanding concerning recovery of immunity after transplantation. What we exactly know is that this process is not simultaneous and that some segments of immune system can stay compromised for years. Those researchers, interested in specific details, can consider several resent reviews, but still majority of data are quantitative and fragmental [4].

Data concerning immune reconstitution after allogeneic HCT are dominating in medical literature. At the same time, there are less but still numerous publications and reviews concerning immune recovery after high-dose chemotherapy with hematopoietic cells rescue autologous transplantation. Again, there are many specific details that are reviewed elsewhere [5] but major conclusions could be briefly seen here.

Comparison of autologous and allogeneic HCT in terms of modification of immune system shows that auto-HCT, in general, causes less long and less deep disturbance of immune function. In case of allogeneic transplantation, the most important and significant factors are conditioning regimen (e.g., a myeloablative, MA or non-myeloablative stem cell transplantation, and NST), the use of serotherapy (e.g., antithymocyte globulin, ATG: more often late infections, more serious prevention of infections is necessary, etc.), graft manipulation (e.g., T repleted, T depleted and if depleted, then which way), prophylaxis, and treatment of developed graft-versus-host disease (GvHD) and procedures associated with the intensity and duration of immunosuppression.

Most of the mentioned factors are not existing in case of autologous transplantation by definition. Conditioning regimens in all standard cases of auto-HCT are myeloablative. They cause short-term deep aplasia and mucosa injury which eventually restore fast enough. More ancient phylogenetic defense mechanisms and cells, such as granulocytes, monocytes and NK cells, recover usually in 2–4 weeks. Lymphopenia normally stays longer and can be profound for a year after transplantation. But adaptive immune defense mechanisms does not expose to immunosuppressive medications, to circulating for several weeks antilymphocyte antibodies, GvHD (so-called autologous GvHD is more rare and much less severe and dangerous than GvHD after allogeneic transplantation), T cell depletion with need to rebuild acceptable quantity and repertoire of all lymphocyte subsets (except rare cases of graft purging by positive CD34+ selection), etc.

We can mention in advance that many of the rules of autologous transplantation are infringed in case of transplantation for autoimmune diseases; this will be discussed in details further in the text.

When immune reconstitution is discussed, the main attention goes to protection from infections and, in case of malignant diseases, to "graft-versus-tumor" effect. These factors shall govern and prevail treatment (transplant)-related mortality (TRM), overall survival (OS), and disease-free survival (DFS). Along with all that, there is one more aspect of immune

reconstitution and this is a reconstruction of self-tolerance [6]. In case of application of transplantation to autoimmune diseases, it becomes critical.

3. Concept

It is well established that the existing standard immunosuppressive/immunomodulating and anti-inflammatory treatment, even if prolonged, can lead to a remission, but the patient can never stay out of his or her medicamentous treatment and therefore cannot be defined as "cured." Moreover, lifelong therapy is clearly associated with side effects of prolonged use of immunosuppressors and/or anti-inflammatory drugs; a list of complication consists of a range of systems involvement, starting from gastrointestinal tract damage from nonsteroidal anti-inflammatory drugs and finishing with complex Cushing's syndrome due to steroids. Altogether, these complications tend to infections, cardiovascular problems, depression and social deprivation, and finally to a seriously compromised quality of life of these patients.

On the basis of this knowledge, the concept of total eradication of immune system, including auto-aggressive clones and auto-reactive immune memory with subsequent rebuilding of "normal" self-tolerant repertoire, looks extremely attractive. The concept of reconfiguration, "resetting" of immune system using HCT, has the aim "to cure," meaning to keep patient without disease progression and without any chronic disease-modifying antirheumatic drugs (DMARDs).

4. Background

Indeed and logically, there is enough background information to presume that HCT can be effective in autoimmune diseases according to our understanding of pathogenesis [7, 8].

Firstly, in the animal studies, a bulk of the experimental data is provided by models of syngeneic transplantations for adjuvant arthritis or collagen-induced arthritis (AA or CIA), for rheumatoid arthritis (RA) and experimental allergic encephalomyelitis (EAE) for multiple sclerosis (MS) on laboratory rodents [9]. It was clearly shown that the transplantation protects the cited conditions from relapse [10–12]. Similar effect was shown in autoimmune diseases with other target organs [13]. Re-induction with antigen after such transplantation did not provoke a relapse, and curative effect was shown in case of substitution of syngeneic transplantation with autologous one and with different conditioning regimens (some of them were shown as inadequate) [10, 14, 15]. This effect brought some investigators to the conclusion that at least some animal autoimmune diseases were stem cell diseases [16–18]. This still questionable opinion is in line with a few anecdotal observations of passive transfer of autoimmune diseases from the donor by allogeneic HCT; examples include insulin-dependent diabetes mellitus, former name for type 1 diabetes (T1D), mellitus and hypothyroidism [19], toxic diffuse goiter [20], myasthenia gravis [21], and multiple sclerosis [22, 23].

Our opinion, based on multiple published evidences that underestimation of specific local factors is incorrect, can be discussed. For example, transplantation performed in late stage of EAE leads to inferior outcomes [24]. Moreover, by monitoring of tracking of transplanted green fluorescent protein-transduced cells, the endogenous origin of microglia in advanced disease was shown. Host macrophages/microglial cells demonstrated robust activation and their number was higher in the stage of disease progression [25]. The same demonstration of different local trigger mechanisms can be made for systemic sclerosis (SSc), inflammatory bowel disease (IBD), rheumatoid arthritis (RA), and other autoimmune diseases.

Secondly, before 1995, there were several cases or small series reports of coexistence of autoimmune diseases with other malignant conditions, which were the primary indication for HCT. After transplantation, the autoimmune diseases developed stable long-term remission, improvement of their symptoms, or alternatively proposed cure procedure. These cases included improvement or complete remission of RA by HCT for gold-induced aplastic anemia [26–28], full remission of psoriasis and ulcerative colitis [29], and resolution of autoimmune hepatitis after HCT for leukemia [30]. In addition, Nelson et al. [31] have reported 13 patients with either preexisting autoimmune diseases (11 patients), or diseases that are possibly autoimmune in nature (two patients), who underwent allogeneic HCT for the treatment of another pathology. None of these patients was found to have the autoimmune disease recurrence after HCT. However, there are other reports for patients whose RA have progressed [32] or had only a short period of relief from joint pain [33] following HCT.

5. Progress in transplantation for autoimmune diseases

The first report of HCT for autoimmune disease treatment as a primary indication was published in 1996 [57]. In the late 1990s of 20th century, a Joint Committee of the European League Against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT), joined by several North American and Australian centers, referred to as the International Autoimmune Disease Stem Cell Project, initiated a phase I/II study to assess feasibility, mortality, and preliminary response for this treatment model, performed for isolated autoimmune disease [34–36].

For over 20 years of experience, a reasonably big pool of clinical data of over thousands patients has been accumulated; this issue will be discussed in detail in the second half of this chapter. From the initial case reports, via small series and bigger retrospective group analysis, the studies came to phase II/III clinical trials. The concept of transplantation in autoimmune diseases itself has undergone a significant transformation. It looks naïve now, in terms of earlier views, when seriously discussed questions of syngeneic transplantations (which is casuistic), allogeneic transplantation (at present considered as too dangerous procedure for autoimmune disease, as potentially having incapacitating consequences, like GvHD) or absolutely indicated autoimmune transplantations (considered like panacea), were discussed [37].

5.1. So, what was changed?

Initially, and this is still the dominant dogma, that transplantation is considered exclusively as prolonged immune suppression with rebuilding de novo immunopoiesis and therefore a tolerance. But comprehension is coming that this is only one side of the immune "resetting" and HCT is not a simple immunosuppression. Reinfusion of hematopoietic stem cells after severe immunoablative causes regeneration of a new naïve immune repertoire from the patient's thymus [38]. Moreover, autologous HCT probably causes restoration of immune regulation and abnormal FoxP3 function of CD4+CD25+ (Treg) cells, as one of the main pathogenic mechanism of many autoimmune diseases [24, 39].

Gradually but persistently, conditioning regimens tend to undergo certain changes. In the first report of EULAR/EBMT, Tyndall (1999) [36] listed four main conditioning regimens that were used: (i) BEAM polychemotherapy (BCNU, VP-16, Ara-C, and melphalan) ± antithymocyte globulin (ATG), (ii) CyATG, consisted of 200 mg/kg of cyclophosphamide (Cy) ± ATG, sometimes substituted with monoclonal antibodies, usually Campath (alemtuzumab, antibody targeted CD52-expressing cells: lymphocytes, monocytes and dendritic cells), (iii) busulfan and cyclophosphamide (BuCy), and (iv) Cy and total body irradiation, usually 8 Gy (CyTBI) ± ATG. BEAM-ATG till nowadays keeps its role as a central conditioning protocol despite the fact that it causes high rate of side effects, including life-threatening ones [40]. Non-myeloablative protocols based on maximal dose of cyclophosphamide demonstrate probably similar effectiveness with impression of lower toxicity [41, 42]. Other procedures of different reduced toxicity of immunoablative but not always myeloablative conditioning were published as well: mini BEAM-like, BCNU/CCNU with intermediate dose of melphalan, ±ATG or alemtuzumab, rituximab, or Cy ± thiotepa or fludarabine, etc. Some could lead to the possibility of performing this procedure in outpatient manner [43–45].

Accompanying additional therapy is also improving during the entire period of analysis.

In other words, all discussed progressively resulting models developed different philosophy of autologous transplantations for autoimmune diseases, compared to other types of autotransplants (possibility to use non-myeloablative but immunoablative regimens, wide use of antilymphocyte antibodies, potential for reduction of toxicity, etc.). The critical advance of this evolution brings to major reduction of TRM from 20 to 30% up to very close to 0%. This is especially important when referred to application of HCT with early stages of slow progressive diseases with life expectancy of dozens of years.

6. Potential effectiveness of HCT for specific diseases

HCT was reportedly applied to 2–3 thousands patients worldwide, and since 2010, the yearly number of HCT procedures registered has increased by 30%, reflecting a change in practice [46]. Despite major basic similarities between different nosology entities, neither data reliability nor even experience with hematopoietic cells transplantation was considered uniform. Therefore, quite a few specific details should be overviewed to make picture certain. Some diseases are

prevalent while some are rare; some can be long time controlled with minimal and available measures while some characterized with fast progression of severe disability and dramatically shortened life expectancy. Altogether, this heterogeneous group of conditions, affecting 8–10% of the population [47], but at present only for several forms of HCT, can be considered as an optional or investigational treatment. Phase I/II studies and then randomized trials have been designed for SSc, MS, IBD, RA, and chronic inflammatory demyelinating polyneuropathy (CIDP).

6.1. HCT for systemic sclerosis

SSc is a relatively rare connective tissue disease (prevalence ranged from 7 to 489 cases per million individuals [48]). It is characterized by early vasculopathy, autoantibody formation, low-grade inflammation, enhanced collagen synthesis, and fibrosis in skin and internal organs. Autoimmunity is triggered by antigens and has some genetic background, associated with loci at HLA-DPB1 and HLA-DPB2 [49], and several polymorphisms of other genes involved in immune regulation, including BANK1, C8orf13-BLK, IL-23R, IRF5, STAT4, TBX21, and TNFSF4 [50]. Both T and B lymphocytes are involved in the immune process, and it is accompanied by profibrotic cytokine production, such as transforming growth factor β (TGF-β) and connective tissue growth factor (CTGF), as well as fibroblast activation. It is interesting that many of the polymorphisms associated with SSc are shared with systemic lupus erythematosus (SLE) and other autoimmune diseases; they reflect on their pathophysiological importance, but not for SSc as a separate nosology. Polymorphisms that have failed to be confirmed in follow-up studies include TGF-β and CTGF [51]. In the Caucasian cohorts, the associations were significant for SSc patients with either antitopoisomerase-1 or anticentromere autoantibodies [50].

SSc is clinically characterized by extensive involvement of skin and visceral organs. For skin assessment, the modified Rodnan's skin score (mRSS) is used: a semi-quantitative skin thickness evaluation in 17 different body areas. Upon the degree of skin involvement, extended and limited SSc are differentiated. The extensive skin damage, associated with a degree of visceral organs involvement and the presence of heart, lung or renal disease, can increase the 5 year mortality rate up to 40–50% [52–56].

In terms of such serious prognosis, SSc was one of the first reported cases of autoimmune disease where HCT was applied [57]. Since then, most of numerous small case series and phase I/II studies and three randomized trials demonstrated encouraging data.

The two randomized studies are described in detail and discussed in the present study: ASSIST performed by USA Chicago group [58]: a phase II study, including 19 patients, aged younger than 60 years, with diffuse SSc, mRSS of 14 or more, and internal organ involvement or restricted skin involvement (mRSS < 14), but coexistent pulmonary involvement. The patients were randomly allocated in two equal groups to receive HCT (n = 10) or to receive 1 g/m^2 intravenous cyclophosphamide once per month for a period of 6 months (n = 9). The conditioning was non-myeloablative of intermediate toxicity, and consisted of 200 mg/kg intravenous cyclophosphamide and 6.5 mg/kg rabbit ATG, CyATG protocol. All 10 patients,

who received HCT, had no disease progression, and all 10 improved at or before 12 month follow-up, compared with none of nine treated with monthly cyclophosphamide (odds ratio 110, 95% CI 14.04–∞; p = 0.00001); eight of these nine controls had disease progression (p = 0.0001 versus HCT group), and 7 patients switched to HCT.

ASTIS [42] is an EBMT/EULAR phase III, multicenter, randomized, open-label, and parallel-group clinical trial, conducted at 29 centers of 10 European countries. It included 156 patients between 18 and 65 years of age with mRSS 15, with disease duration of 4 years and involvement of heart, lungs, or kidneys. In addition, inclusion of patients was allowed with disease duration of 2 years or less, and no major organ dysfunction as defined above provided they had an mRSS of ≥20 and an erythrocyte sedimentation rate greater than 25 mm/h and/or hemoglobin less than 11 g/DL, not explained by causes other than active scleroderma. Patients were randomly assigned to receive HCT (n = 79) or cyclophosphamide (n = 77). The CyATG conditioning regimen was very similar to Chicago study (total cyclophosphamide 200 mg/kg and intravenous rabbit ATG (Genzyme) in a total dose of 7.5 mg/kg). The dose of cyclophosphamide in the control group was 750 mg/m^2, repeated in 12 monthly pulses. During the first year, there were more irreversible events with organ failure or death in the HCT group, 13 (16.5%) versus 8 (10.4%) in the cyclophosphamide group. However, during the second year, the cumulative events were similar in both groups: 14 (17.7%) versus 14 (18.2%). And by the 4-th year, the cumulative events in HCT group 15 (19%) were less than cyclophosphamide group 20 (26%).

At present, one more phase III clinical trial scleroderma: cyclophosphamide or transplantation (SCOT) [59] is completed but the results are not published yet. The SCOT protocol employs a lymphoablative preparative regimen, including 800 cGy TBI, delivered in two 200 cGy fractions twice a day before CD34+ selected autologous hematopoietic stem cell transplantation [60]. The late results will be especially important to evaluate appearance of secondary malignancies in association with the radiotherapy.

Therefore, employment of HCT has resulted in rapid and sustained improvement of skin thickening and functional ability, stabilization of major organ function with some improvement of vital capacity in pilot studies, registry analyses, and the phase II–III trials. Some patients have achieved complete remission (CR) including unexpected and dramatic clinical and biopsy regression of dermal fibrosis as well as normalization of the microvasculature [61].

Despite an early treatment-related mortality rate of around 6–10%, potential long-term complications and an increase in serious adverse events, HCT conferred a long-term survival benefit.

6.2. HCT for multiple sclerosis

In MS, a chronic inflammation of the central nervous system (CNS) is caused by an autoimmune reactivity of T cells toward CNS myelin components and therefore has classical autoimmune nature [62].

Primary susceptibility to MS in the majority of various populations is associated with HLA-DrB1*15 [63, 64]. Recent genome-wide association studies (GWAS) identified multiple loci

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affecting the risk of developing disease. The reported screen implicates a majority of these genes as immune related and coding for cytokine pathway (CXCR5, IL2RA, IL7R, IL7, IL12RB1, IL22RA2, IL12A, IL12B, IRF8, TNFRSF1A, TNFRSF14, TNFSF14), co-stimulatory (CD37, CD40, CD58, CD80, CD86, CLECL1) and signal transduction (CBLB, GPR65, MALT1, RGS1, STAT3, TAGAP, TYK2) molecules. In addition, some other genes are related to previously reported environmental risk factors such as vitamin D—CYP27B1, CYP24A1 and therapies for multiple sclerosis including natalizumab—VCAM1 and daclizumab—IL2RA [65].

At present, four different clinical patterns of MS are considered: clinically isolated syndromes (CIS; the first attack of a disease compatible with MS), relapsing-remitting MS (RRMS; clearly defined relapses without or with minimal residual deficit upon recovery), secondary progressive MS (SPMS, as a result of conversion of RRMS with or without occasional relapses and with gradual worsening), and primary progressive MS (PPMS, accumulation of disability from the very beginning of the disease and worse prognosis compare to RRMS/SPMS). The term progressive-relapsing multiple sclerosis (PRMS) is now obsolete [66].

The most commonly used rating scale to grade neurological disability in patients with MS is the expanded disability status scale (EDSS) [67].

Magnetic resonance imaging (MRI) is sensitive to focal CNS lesions and has been included in the diagnostic workup of patients in whom MS is suspected. Conventional MRI measures of the disease burden are useful tool to monitor the disease course.

Over the years, therapeutic approaches to MS were aimed at suppressing the immune system, in order to control the inflammatory process which causes the demyelination and finally irreversible axonal damage [68, 69].

The long list of registered therapies for MS includes corticosteroids (used mainly in a high-dose fore acute attack), immunosuppressive and immunomodulatory drugs (such as gilenya, teriflunomide, dimethyl fumarate, etc.), cytokines (interferons, IFN-alpha and IFN-beta), and strong immunosuppressive modalities (alemtuzumab, natalizumab, mitoxantrone, and cyclophosphamide). In many cases, registered disease-modifying treatments do not provide satisfactory control of MS due to their inability to eradicate the self-specific T-cell clones and compartmentized inflammation *in situ*, which is less affected by the conventional modalities and seems to be the reason for lack of efficacy of any of the registered treatment models in the progressive phase of MS. That is why the best available conventional therapy has only partial beneficial effects [64, 70, 71].

According to the recent published databases from Europe, North America, and South America, multiple sclerosis (followed by SSc) is constantly the most common indication for HCT [48, 72, 73].

The pioneer publication of Fassas et al. [74] described a phase I/II study, involving 15 patients with progressive median EDSS of 6 (5–7.5). The patients were treated with BEAM protocol followed by autologous HCT and antithymocyte globulin (ATG). Results were encouraging: short time (6–18 months) neurologic improvements have been detected using EDSS in 7 of 15 patients, and what was more obvious, using Scripps Neurologic Rating Scale (SNRS), which is more sensitive but not based predominantly on walking ability. One patient worsened

after 3 months and two have relapsed. There were no toxic deaths and reasonable number of side effects, mainly due to ATG infusion and infections during neutropenia. Since that time, BEAM-ATG became a gold standard for future trials and in use until nowadays.

The BEAM-ATG demonstrates its effectiveness in several trials, including the most recent ones.

The autologous hematopoietic stem cell transplantation trial in MS (ASTIMS) is promoted by the EBMT multicenter, randomized trial. Initiated as phase III study, it was transformed to phase II with a primary laboratory endpoint [40]. The aim was to compare BEAM-ATG with mitoxantrone 20 mg monthly for 6 months. The including criteria were SPMS or RRMS, with an increase of the EDSS in the last year, despite conventional therapy, and the presence of one or more active gadolinium-enhancing areas on MRI. Twenty-one recruited patients were randomized in either HCT (n = 9) or mitoxantrone (n = 12) arm. All but two patients were followed up for 4 years. The relapse rate was reduced in patients treated with HCT, when compared with mitoxantrone. HCT significantly reduced by 79% of the number of new T2 lesions compared to mitoxantrone (median number 2.5 versus 8). In the AHCT group, no new gadolinium-enhancing lesions appeared on brain MRI, while 56% of patients treated with mitoxantrone presented at least one active lesion. Despite the fact that there was no treatment-related mortality, serious adverse events (SAEs) were seen only in HCT arm. They were defined as life threatening in 2 patients at least. There were no deaths or late SAE. Adverse events were resolved without sequelae.

Another significant report was published by Burt's group [75] and it includes a single institution (Chicago, USA), experienced with treatment of 145 patients with RRMS (n = 123), or treated on a compassionate basis SPMS (n = 28), and with a median follow-up of 2 years. The main group consisted of patients aged 18–55 years, and their including criteria were RRMS. The therapy was unsuccessful with ≥1 conventional drug, EDSS from 2.0 to 6.0, and during the preceding year, the patients had either ≥2 relapses or 1 relapse treated with a corticosteroid and additional gadolinium-enhanced lesions on MRI scan at a separate time.

The conditioning regimen consisted of 200 mg/kg of cyclophosphamide divided into four single daily doses, plus either 20 mg of alemtuzumab given 2 days before stem cell infusion (22 patients) or 6 mg/kg of ATG (thymoglobulin), divided into five daily doses (129 patients).

Prior to each antithymocyte globulin infusion, additionally 1 g of methylprednisolone was infused.

There was a significant improvement in disability defined as decrease in EDSS score of ≥1.0, with proportion of patients 50% (95% CI, 39–61%) in 41 of 82 for improvement at 2 years and 64% (95% CI, 46–79%) in 23 of 36 for improvement at 4 years. The authors found a significant decrease of T2 lesion volume. Several other evaluated scores demonstrated pronounced and statistically significant improvement including notable advance in total quality of life scores. Treatment-related mortality was 0% and overall survival was 99.3%; the only death that occurred 30 months after transplantation was due to cardiovascular disease.

Hamerschlak et al. [76] performed a direct comparison of BEAM-ATG and CyATG regimens in a prospective multicentric Brazilian MS trial. The authors found that the rate of compli-

cations during transplantation was higher in the BEAM-ATG group (71.4%), compared to the CyATG group (40%; $p < 0.04$). Three subjects (7.5%) died of cardiac toxicity, sepsis, and alveolar hemorrhage, all of them from the BEAM-ATG group. The important conclusion was that despite the lower toxicity of CyATG, this regimen seems to be associated with the same outcome, but with lower toxicity, compared to BEAM-ATG.

Summarizing the cited protocols, with domination of BEAM and Cy (200 mg/kg), both ±ATG/alemtuzumab make an impression that in case of MS, lower intensity protocols demonstrate lower, up to 0%, mortality rate, while OS and progression-free survival are similar; in resent studies, they range from 65 to 100%, far better than the results of conventional MS therapies [77–81]. Our limited experience demonstrates 71% of progression-free survival after non-myeloablative transplantations performed in Hadassah-HUJI Medical Center (14 patients, 1998–2016, 12 after autologous and 2 after allogeneic transplantation; S. Savin, R. Or, M. Shapira and I. Resnick; unpublished data).

The risk of treatment-related mortality in HCT conventionally perceived to be unacceptably high. In a similar approach, the vein statistical analysis demonstrates a decrease in TRM to 1.3%, according to an analysis of the EBMT registry [82], and 0% in the most recent published profound enough series. The major role resulting from the studies is the development and choosing of less toxic conditioning protocols and adequate patient selection.

It is clear that there is a need for a solid phase III trial of HCT, firstly, for aggressive forms of MS and effectiveness of save low toxicity immunoablative conditioning for less incapacitating patients. There are several ongoing trials. A prospective, randomized, controlled multicentre trial has been already outlined in a positioning article of Saccardi et al. [83].

6.3. HCT in rheumatoid arthritis and juvenile idiopathic arthritis

Rheumatoid arthritis (RA) is affecting approximately 1% of the population. It is characterized by autoantibody production with progressive joint destruction due to the formation of an inflammatory hypertrophied synovium, erosion of the synovial cartilage and the surrounding bone [84]. Break of tolerance causes accumulation of immune effector cells, including macrophages and osteoclasts, DCs, B and T cells, especially Th17 subsets. Reduced T-cell receptor (TCR) excision circles and shortened telomeres result in a contracted TCR repertoire in both naïve and memory cells [85, 86].

An adequate control and a possibility of remission are usually limited to early-stage disease.

Pilot studies of HCT in RA date back to middle 1990s of the 20th century. It was shown that sustained remission responses were shortly activated for up to 6–12 months, which was followed by reintroduction of DMARDs/anti-TNF therapy. Following HCT, there was a somewhat better response to DMARDs supporting the immunomodulating effect of HCT. There has been variable success of HCT in RA, but the results have not been encouraging as compared to diseases like SSc or MS [87–89].

Published data from the EBMT registry showed no transplant-related mortality of RA patients with OS 98%, while in JIA patients, TRM was detected in 7 of 65 patients [90].

At present, HCT for RA or JRA, in general, cannot be recommended, and can be considered very seriously only in context of RA/JRA oriented well-established clinical trials.

6.4. HCT in systemic lupus erythematosus

SLE is a prototype autoimmune disease with prevalence of 20–150 cases per 100,000 populations. It is characterized by wide abundance of self-reactive antibodies, including those against nuclear and cytoplasmic antigens, as well as autoimmune activity associated with complement activation [91]. A typical characteristic of SLE is an extremely variable clinical manifestation that can make the diagnosis difficult and late.

The plasma cells are key players in pathogenesis of SLE. The immunological hallmarks of the disease are short-lived (HLA-DRhigh) plasmablasts, which are easily detectable in the circulation during active disease [92]; the upregulation of IFN-regulated gene transcripts, therefore IFN-α and its response proteins IP-10 and Siglec-1, are established markers for monitoring disease activity in SLE [93, 94]; finally, circulating Foxp3+ Tregs, especially Helios+ subpopulation, are associated with disease activity [95].

Major visceral involvement and persistent disease activities are predictors of poor outcome [96].

Treatment response varies in population subsets owing to the genetic composition and racial differences, as well as hormonal influences in both the adult and pediatric patients [97].

Immunosuppressive therapy is often protracted for adequate disease control and to minimize organ damage in patients with very high disease activity, but prolonged uses of corticosteroids and repeated courses of higher doses of immunosuppressant have resulted in unfavorable long-term disease-free outcomes or drug-free intervals [98].

Results of autologous HCT are less consistent. In an American trial by Burt et al. [99], reduced intensity HCT (Cy-ATG) in refractory SLE showed significant advantages of HCT in terms of progression-free survival and attenuation of nephritic symptoms in patients with SLE. The study (n = 50) showed promising results with respect to the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score and such activity markers as ANA, anti-dsDNA and complement with increasing 5 year progression-free survival. There was either stabilization or reversal of organ dysfunction, including renal function. With a mean follow-up of 29 months, the 5 year probability of overall survival and disease-free survival (DFS) following HCT was 84 and 50%. TRM was 2% (1/50).

In EBMT too, positive trends in progression-free and overall survival were noted but the numbers are less encouraging [44, 90, 100]. The last analysis of 28 patients, transplanted between 2001 and 2008 in eight centers of six countries, using a spectrum of conditioning protocols and with median follow-up of 38 months after transplantation, demonstrated that the 5 year overall survival was 81 ± 8%, disease-free survival was 29 ± 9%, and non-relapse mortality (NRM) was 15 ± 7%. OS tended to be lower when using intermediate as compared to low-intensity conditioning ($p = 0.08$). OS was not significantly associated with the presence of renal, neurologic or hematologic involvement or of SLEDAI >20 before ASCT, anti-dsDNA antibodies at mobilization or ex vivo graft manipulation.

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The 3 year NRM was 0% in the low-intensity conditioning versus 23 ± 10% in the intermediate-intensity conditioning (p = 0.13). It is interesting that DFS and relapse incidence were not associated with any immediate pretransplant variables, including the use of low versus intermediate conditioning regimens.

A follow-up study using third-generation "rituximab sandwich" conditioning regimen (CyATG + rituximab, a B cell targeted anti-CD20 monoclonal antibody) is ongoing [101].

6.5. HCT for Crohn's disease

Crohn's disease is a relapsing inflammatory disease, mainly affecting the gastrointestinal tract, and frequently is presented with abdominal pain, fever and clinical signs of bowel obstruction or diarrhoea with passage of blood or mucus, or both. It represents one of two major forms of IBD [102].

It is thought that the disease develops due to abnormal mucosal immune responses to the gut flora. GWAS identified >100 susceptibility loci to Crohn's disease in Caucasians but their heritability is not fully explained [103, 104]. Recent studies revealed an altered local and circulating T-cell phenotype, in particular involvement of Th17 cells and IL-21/IL-22-producing CD4+ T cells [105, 106].

Initially, it was a clear impression that HCT is an effective approach. After a few cases or small series reports [107–111], the important publication of Chicago group appeared in Blood, 2010 [112] and demonstrated that in all 25 patients, who received CyATG with autologous stem cell, the risk to develop clinical remission with Crohn's disease activity index (CDAI) < 150 (inclusion criteria CDAI > 250) was open. Relapse-free survival was 91, 63, 57, 39 and 39% at 1st, 2nd, 3rd, 4th and 5th year, respectively. Five years after transplantation, 70% of patients were in remission, 80% were steroid free and 60% medication free. There was no treatment-related mortality. In line with other cell therapies, the non-myeloablative transplantation was considered as the best studied/investigated idea of Crohn's disease treatment [102].

The EBMT paper came out in 2015 [113], presenting a parallel-group randomized clinical trial conducted in 11 European transplant units (Autologous Stem Cell Transplantation International Crohn Disease—ASTIC—trial); the medial follow-up was 1 year. Comparison of immunoablation, with use of the similar CyATG protocol and HCT (n = 23) and control treatment (n = 22), demonstrated no statistically significant in-between group differences in proportions of patients achieving sustained disease remission, with CDAI less than 150 in the last 3 months, or free of active disease. There was a statistically significant difference among patients able to discontinue active treatment in the last 3 months. There were 76 serious adverse events in patients undergoing HCT versus 38 in controls; 1 patient from HCT group died 20 days after the start of conditioning with postmortem evidence of sinusoidal obstructive syndrome (SOS). Whether the SOS, seen in the patient who died, may be an agonal event in a septic patient with development of a fulminant liver failure, or it can be a result of endothelial injury induced by high dose cyclophosphamide, is still open for discussion.

Therefore, evaluation of presented experience of HCT for refractory Crohn's disease is not straightforward; it makes further study highly necessary and strongly recommended.

6.6. HCT in other autoimmune diseases

In accordance with the EBMT data for December 2016, from 2227 reported HCT for autoimmune diseases (exact numbers not yet published), the cumulative percent of afore-discussed five conditions (SSc, MS, SLE, RA with JIA, and Crohn's disease) is 83% (data not yet published). All cases of application of transplantation for other multiple autoimmune diseases are outnumbered. There are published reports giving some evidence that transplantation might be an effective treatment option in case of severe primary systemic vasculitis. For example, in 15 transplanted patients of different forms of vasculitis with an overall response rate of 93% (46% complete), partial responses were observed [114]. HCT has been promoted in polymyositis/dermatomyositis, Sjogren's syndrome, psoriatic arthritis and ankylosing arthritis, chronic inflammatory demyelinating polyneuropathy and autoimmune cytopenias, including hemolytic anemia, ITP, Evans syndrome and other rare combinations [115, 116]. Promising preliminary results were registered in small groups of patients with type 1 diabetes (T1D) from Brazil [117, 118], China [119, 120], and Poland [121]. Preliminary lessons from these small trials suggest that: (i) majority of patients can reach independence of exogenous insulin for a period of few months to years; (ii) according to our knowledge, there were no described transplant-related deaths; and (iii) diabetic ketoacidosis at onset, probably due to a severe depletion of islet cells, can be a poor predicting factor.

However, the experience with almost all autoimmune diseases, plus some others, recently included into clinical trials, is limited to allow any generally accepted conclusions.

7. Conclusion

HCT treatment has revolutionized the approach to autoimmune diseases treatment.

The results vary with different diseases, and there is certainly a special room for well designed clinical trials. Late results sometimes also provide surprises, tending to review the initial concept. Does that mean that the existing data are not enough to make a decision-concerning transplantation, either a more positive one (for instance MS) or a more negative one (e.g., RA)? Specific issues vary significantly depending on the country's social and economic climate, differences in medical system or medical insurance barriers, and legislation requiring third-party payers, and as a result, a large portion of patients cannot afford the best, or even equivalent "conventional" lifelong treatment. The majority of failures come in the form of TRM, as well as nonresponsiveness and high relapse rate [121]. The attempts to decrease the treatment toxicity, without sacrificing the efficiency, are directed to balancing the failure-to-benefit ratio. Different conditioning protocols may be more appropriate at various stages of the disease, such as RRMS with EDSS <6 versus SPMS with EDSS > 6. However, the questions are still widely open for a profound discussion, because in many cases, the efficiency itself remains unclear (e.g., Crohn's disease). Despite a sufficient number of open issues, the number of treated patients with very promising results, especially concerning SSc and MS published in major publications in peer-reviewed prestige journals, already allow us to consider HCT, a relevant clinical option, for a successful treatment of certain autoimmune diseases.

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References

[1] Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet. 1968 Dec 28;2(7583):1366–9.

[2] Thomas ED, Lochte HL, Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. N Engl J Med. 1957;257:491.

[3] Mathe G et al. Trial treatment of patients afflicted with acute leukemia in remission with total irradiation followed by homologous bone marrow transfusion. Rev Fr Etud Clin Biol. 1959 Sep;4:675–704 [Article in French].

[4] de Koning C, Plantinga M, Besseling P, Boelens JJ, Nierkens S. Immune reconstitution after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2016 Feb;22(2):195–206.

[5] Rueff J. et al. Lymphocyte subset recovery and outcome after autologous hematopoietic stem cell transplantation for plasma cell myeloma. Biol Blood Marrow Transplant. 2014;20:881–903.

[6] Hess A. Reconstitution of self-tolerance after hematopoietic stem cell transplantation. Immunol Res. 2010 July;47(1–3):143–52.

[7] Metodiev K. Immunopathology and immunomodulation. InTech, Open Science/Open Minds. ISBN: 978-953-51-2210-4. 2012, pp 302.

[8] Metodiev K. Immunodeficiency. InTech, Open Science/Open Minds. ISBN: 978-953-51-0791-0. 2012, pp 392.

[9] van Bekkum DW. Stem cell transplantation for autoimmune disorders. Preclinical experiments. Best Pract Res Clin Haematol. 2004 Jun;17(2):201–22.

[10] Karussis DM, Vourka-Karussis U, Lehmann D, Ovadia H, Mizrachi-Koll R, Ben-Nun A, Abramsky O, Slavin S. Prevention and reversal of adoptively transferred, chronic relapsing experimental autoimmune encephalomyelitis with a single high dose cytoreductive treatment followed by syngeneic bone marrow transplantation. J Clin Invest. 1993 Aug;92(2):765–72.

[11] Karussis DM, Slavin S, Ben-Nun A, Ovadia H, Vourka-Karussis U, Lehmann D, Mizrachi-Kol R, Abramsky O. Chronic-relapsing experimental autoimmune encephalomyelitis (CR-EAE): treatment and induction of tolerance, with high dose cyclophosphamide followed by syngeneic bone marrow transplantation. J Neuroimmunol. 1992 Aug;39(3):201–10.

[12] van Bekkum DW, Bohre EP, Houben PF, Knaan-Shanzer S. Regression of adjuvant-induced arthritis in rats following bone marrow transplantation. Proc Natl Acad Sci USA. 1989 Dec;86(24):10090–4.

[13] Alderuccio F, Murphy K, Biondo M, Field J, Toh BH. Reversing the autoimmune condition: experience with experimental autoimmune gastritis. Int Rev Immunol. 2005 Jan–Apr;24(1–2):135–55.

[14] van Bekkum DW. Conditioning regimens for the treatment of experimental arthritis with autologous bone marrow transplantation. Bone Marrow Transplant. 2000 Feb;25(4):357–64.

[15] van Bekkum DW. Stem cell transplantation in experimental models of autoimmune disease. J Clin Immunol. 2000 Jan;20(1):10–6.

[16] Ikehara S. Treatment of autoimmune diseases by hematopoietic stem cell transplantation. Exp Hematol. 2001;29(6):661–9.

[17] Ikehara S. Stem cell transplantation for autoimmune diseases: what can we learn from experimental models?. Autoimmunity. 2008; 41(8):563–9.

[18] Marmont du Haut Champ AM. Hematopoietic stem cell transplantation for systemic lupus erythematosus. Clin Dev Immunol. 2012;2012:380391.

[19] Vialettes B, Maranchini D, San Marco MP, et al. Autoimmune polyendocrine failure-type I (insulin-dependent) diabetes mellitus and hypothyroidism-after allogeneic bone marrow transplantation in a patient with lymphoblastic leukaemia. Diabetologia. 1993;36:541–6.

[20] Aldouri MA, Ruggier R, Epstein O, Prentice HG. Adoptive transfer of hyperthyroidism and autoimmune thyroiditis following allogeneic bone marrow transplantation for chronic myeloid leukaemia. Br J Haematol. 1990;74:118–9.

[21] Grau J M, Casademont J, Monforte R, et al. Myasthenia gravis after allogeneic bone marrow transplantation: report of a new case and pathogenetic considerations. Bone Marrow Transplant. 1990;5:435–7.

[22] McAllister LD, Beatty PG, Rose J. Allogeneic bone marrow transplant for chronic myelogenous leukemia in a patient with multiple sclerosis. Bone Marrow Transplant. 1997 Feb;19(4):395–7.

[23] Meloni G, Capria S, Salvetti M, Cordone I, Mancini M, Mandelli F. Autologous peripheral blood stem cell transplantation in a patient with multiple sclerosis and concomitant Ph^+ acute leukemia. Haematologica. 1999 Jul;84(7):665–7.

[24] Sullivan KM, Muraro P, Tyndall A. Hematopoietic cell transplantation for autoimmune disease: updates from Europe and the United States. Biol Blood Marrow Transplant. 2010 Jan;16(1 Suppl):S48–56.

[25] Cassiani-Ingoni R, Muraro PA, Magnus T, Reichert-Scrivner S, Schmidt J, Huh J, Quandt JA, Bratincsak A, Shahar T, Eusebi F, Sherman LS, Mattson MP, Martin R, Rao MS. Disease progression after bone marrow transplantation in a model of multiple sclerosis is associated with chronic microglial and glial progenitor response. J Neuropathol Exp Neurol. 2007 Jul;66(7):637–49.

[26] Mant MJ et al. Immunosuppression as initial treatment for gold induced aplastic anemia. J Rheumatol. 1987;14:1026–9.

[27] Lowenthal RM, Cohen ML, Atkinson K, Biggs JC. Apparent cure of rheumatoid arthritis by bone marrow transplantation. J Rheumatol. 1993;20:137–40.

[28] Baldwin JL, Storb R, Thomas ED, Mannik M. Bone marrow transplantation in patients with gold-induced marrow aplasia. Arthritis Rheum. 1977;20:1043–8.

[29] Yin JA, Jowitt SN. Resolution of immune-mediated diseases following allogeneic bone marrow transplantation for leukaemia. Bone Marrow Transplant. 1992;9:31–3.

[30] Vento S et al. Resolution of autoimmune hepatitis after bone-marrow transplantation (letter). Lancet. 1996;348:544–5.

[31] Nelson JL et al. Pre-existing autoimmune disease in patients with long- term survival after allogeneic bone marrow transplantation. J Rheumatol. 1997;48 (Suppl):23–9.

[32] McKendry RJ, Huebsch L, Leclair B. Progression of rheumatoid arthritis following bone marrow transplantation. Arthritis Rheum. 1996;39:1246–53.

[33] Jacobs P, Vincent MD, Martell RW. Prolonged remission of severe refractory rheumatoid arthritis following allogeneic bone marrow transplantation for drug-induced aplastic anemia. Bone Marrow Transplant. 1986;1:237–9.

[34] Marmont A, Tyndall A, Gratwohl A, Vischer T. Haematopoietic precursor cell transplants for autoimmune diseases. Lancet. 1995;345:978.

[35] Tyndall A, Gratwohl A. Blood and marrow stem cell trans- plants in autoimmune disease. A consensus report written on behalf of the European League Against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT). Br J Rheumatol. 1997;36:390–2.

[36] Tyndall A, Fassas A, Passweg J, Ruiz de Elvira C, Attal M, Brooks P, Black C, Durez P, Finke J, Forman S, Fouillard L, Furst D, Holmes J, Joske D, Jouet J, Kötter I, Locatelli F, Prentice H, Marmont AM, McSweeney P, Musso M, Peter HH, Snowden JA, Sullivan K, Gratwohl A, et al. Autologous haematopoietic stem cell transplants for autoimmune disease: feasibility and transplant-related mortality. Autoimmune Disease and Lymphoma Working Parties of the European Group for Blood and Marrow Transplantation, the European League Against Rheumatism and the International Stem Cell Project for Autoimmune Disease. Bone Marrow Transplant. 1999 Oct;24(7):729–34.

[37] Sherer Y, Shoenfeld Y. Stem cells transplantation-a cure for autoimmune diseases. Lupus. 1998;7(3):137–40. Review.

[38] Muraro PA, Douek DC, Packer A, et al. Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. J Exp Med. 2005; 201:805–16.

[39] Tao JH, Cheng M, Tang JP, Liu Q, Pan F, Li XP. Foxp3, regulatory T cell, and autoimmune diseases. Inflammation. 2016 Nov 24; 41: 328–39. [Epub ahead of print].

[40] Mancardi GL, Sormani MP, Gualandi F, Saiz A, Carreras E, Merelli E, Donelli A, Lugaresi A, Di Bartolomeo P, Rottoli MR, Rambaldi A, Amato MP, Massacesi L, Di Gioia M, Vuolo L, Currò D, Roccatagliata L, Filippi M, Aguglia U, Iacopino P, Farge D, Saccardi R; ASTIMS Haemato-Neurological Collaborative Group, On behalf of the Autoimmune Disease Working Party (ADWP) of the European Group for Blood and Marrow Transplantation (EBMT).; ASTIMS Haemato-Neurological Collaborative Group On behalf of the Autoimmune Disease Working Party ADWP of the European Group for Blood and Marrow Transplantation EBMT. Autologous hematopoietic stem cell transplantation in multiple sclerosis: a phase II trial. Neurology. 2015 Mar 10;84(10):981–8.

[41] Burt RK, Loh Y, Cohen B, Stefoski D, Balabanov R, Katsamakis G, Oyama Y, Russell EJ, Stern J, Muraro P, Rose J, Testori A, Bucha J, Jovanovic B, Milanetti F, Storek J, Voltarelli JC, Burns WH. Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study. Lancet Neurol. 2009 Mar;8(3):244–53.

[42] van Laar JM, Farge D, Sont JK, Naraghi K, Marjanovic Z, Larghero J, Schuerwegh AJ, Marijt EW, Vonk MC, Schattenberg AV, Matucci-Cerinic M, Voskuyl AE, van de Loosdrecht AA, Daikeler T, Kötter I, Schmalzing M, Martin T, Lioure B, Weiner SM, Kreuter A, Deligny C, Durand JM, Emery P, Machold KP, Sarrot-Reynauld F, Warnatz K, Adoue DF, Constans J, Tony HP, Del Papa N, Fassas A, Himsel A, Launay D, Lo Monaco A, Philippe P, Quéré I, Rich É, Westhovens R, Griffiths B, Saccardi R, van den Hoogen FH, Fibbe WE, Socié G, Gratwohl A, Tyndall A; EBMT/EULAR Scleroderma Study Group. Autologous hematopoietic stem cell transplantation versus intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis: a randomized clinical trial. JAMA. 2014 Jun 25;311(24):2490–8.

[43] Shevchenko JL, Kuznetsov AN, Ionova TI, Melnichenko VY, Fedorenko DA, Kurbatova KA, Gorodokin GI, Novik AA. Long-term outcomes of autologous hematopoietic stem cell transplantation with reduced-intensity conditioning in multiple sclerosis: physician's and patient's perspectives. Ann Hematol. 2015 Jul;94(7):1149–57.

[44] Alchi B, Jayne D, Labopin M, Demin A, Sergeevicheva V, Alexander T, Gualandi F, Gruhn B, Ouyang J, Rzepecki P, Held G, Sampol A, Voswinkel J, Ljungman P, Fassas A, Badoglio M, Saccardi R, Farge D; EBMT Autoimmune Disease Working Party members. Autologous haematopoietic stem cell transplantation for systemic lupus erythematosus: data from the European Group for Blood and Marrow Transplantation registry. Lupus. 2013 Mar;22(3):245–53.

[45] Ruiz-Arguelles GJ et al. A Feasibility Study of the Full Outpatient Conduction of Hematopoietic Transplants in Persons with Multiple Sclerosis Employing Autologous Non- Cryopreserved Peripheral Blood Stem Cells. ASH, 58th Annual Meeting, San-Diego, CA. December 3–6, 2016. Abst 2262, https://ash.confex.com/ash/2016/webprogram/Paper91244.html.

[46] EBMT Annual Report 2015, p 15. Available from: https://www.ebmt.org/Contents/Resources/Library/Annualreport/Documents/EBMT_AnnualRep_2015.pdf.

[47] Alexander T, Bondanza A, Muraro PA, Greco R, Saccardi R, Daikeler T, Kazmi M, Hawkey C, Simoes BP, Leblanc K, Fibbe WE, Moore J, Snarski E, Martin T, Hiepe F, Velardi A, Toubert A, Snowden JA, Farge D. SCT for severe autoimmune diseases: consensus guidelines of the European Society for Blood and Marrow Transplantation for immune monitoring and biobanking. Bone Marrow Transplant. 2015 Feb;50(2):173–80.

[48] Chifflot H, Fautrel B, Sordet C, Chatelus E, Sibilia J. Incidence and prevalence of systemic sclerosis: a systematic literature review. Semin Arthritis Rheum. 2008;37(4):223.

[49] Zhou X, Lee JE, Arnett FC, Xiong M, Park MY, et al. 2009. HLA-DPB1 and DPB2 are genetic loci for systemic sclerosis: a genome-wide association study in Koreans with replication in North Americans. Arthritis Rheum. 2009;60:3807–14.

[50] Katsumoto TR, Whitfield ML, Connolly MK. The pathogenesis of systemic sclerosis. Annu Rev Pathol. 2011;6:509–37.

[51] Gourh P, Mayes MD, Arnett FC. 2008. CTGF polymorphism associated with systemic sclerosis. N Engl J Med. 358:308–9.

[52] Altman RD, Medsger TA, Bloch DA, Michel BA. Predictors of survival in systemic sclerosis (scleroderma). Arthritis Rheum. 1991;34:403–13.

[53] Jacobsen S, Halberg P, Ullman S. Mortality and causes of death of 344 Danish patients with systemic sclerosis (scleroderma). Br J Rheumatol. 1998;37:750–5.

[54] Bryan C, Howard Y, Brennan P, Black C, Silman A. Survival following the onset of scleroderma: results from a retrospective inception cohort study of the UK patient population. Br J Rheumatol. 1996;35:1122–6.

[55] Abu-Shakra M, Lee P. Mortality in systemic sclerosis: a comparison with the general population. J Rheumatol. 1995;22:2100–2.

[56] Dumoitier N, Lofek S, Mouthon L. Pathophysiology of systemic sclerosis: state of the art in 2014. Presse Med. 2014;43:e267–78.

[57] Tamm M, Gratwohl A, Tichelli A, Perruchoud AP, Tyndall A. Autologous haemopoietic stem cell transplantation in a patient with severe pulmonary hypertension complicating connective tissue disease. Ann Rheum Dis. 1996 Oct;55(10):779–80.

[58] Burt RK, Shah SJ, Dill K, Grant T, Gheorghiade M, Schroeder J, Craig R, Hirano I, Marshall K, Ruderman E, Jovanovic B, Milanetti F, Jain S, Boyce K, Morgan A, Carr J, Barr W. Autologous non-myeloablative haemopoietic stem-cell transplantation compared with pulse cyclophosphamide once per month for systemic sclerosis (ASSIST): an open-label, randomised phase 2 trial. Lancet. 2011 Aug 6;378(9790):498–506.

[59] Naraghi K, van Laar JM. Update on stem cell transplantation for systemic sclerosis: recent trial results. Curr Rheumatol Rep. 2013 May;15(5):326.

[60] Craciunescu OI, Steffey BA, Kelsey CR, Larrier NA, Paarz-Largay CJ, Prosnitz RG, Chao N, Chute J, Gasparetto C, Horwitz M, Long G, Rizzieri D, Sullivan KM. Renal shielding and dosimetry for patients with severe systemic sclerosis receiving immunoablation with total body irradiation in the scleroderma: cyclophosphamide or transplantation trial. Int J Radiat Oncol Biol Phys. 2011 Mar 15;79(4):1248–55.

[61] Fleming JN, Nash RA, McLeod DO, Fiorentino DF, Shulman HM, Connolly MK, Molitor JA, Henstorf G, Lafyatis R, Pritchard DK, Adams LD, Furst DE, Schwartz SM. Capillary regeneration in scleroderma: stem cell therapy reverses phenotype?. PLoS One. 2008 Jan 16;3(1):e1452.

[62] Hafler DA. Multiple sclerosis. J Clin Invest. 2004;113:788–94.

[63] Lincoln MR, Montpetit A, Cader MZ, Saarela J, Dyment DA, Tiislar M et al. A predominant role for the HLA class II region in the association of the MHC region with multiple sclerosis. Nat Genet 2005;37:1108–12.

[64] Caillier SJ, Briggs F, Cree BA, Baranzini SE, Fernandez-Vina M, Ramsay PP et al. Uncoupling the roles of HLA-DRB1 and HLA-DRB5 genes in multiple sclerosis. J Immunol. 2008;181:5473–80.

[65] Sawcer S, Hellenthal G, Pirinen M, Spencer CC, Patsopoulos NA, Moutsianas L et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 2011;476:214–9.

[66] Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sørensen PS, Thompson AJ, Wolinsky JS, Balcer LJ, Banwell B, Barkhof F, Bebo B Jr, Calabresi PA, Clanet M, Comi G, Fox RJ, Freedman MS, Goodman AD, Inglese M, Kappos L, Kieseier BC, Lincoln JA, Lubetzki C, Miller AE, Montalban X, O'Connor PW, Petkau J, Pozzilli C, Rudick RA, Sormani MP,

Stüve O, Waubant E, Polman CH. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology. 2014;83(3):278.

[67] Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33:1444–52.

[68] Steinman L. Multiple sclerosis: a two-stage disease. Nat Immunol. 2001 Sep;2(9):762–4.

[69] Einstein O, Grigoriadis N, Mizrachi-Kol R, Reinhartz E, Polyzoidou E, Lavon I, et al. Transplanted neural precursor cells reduce brain inflammation to attenuate chronic experimental autoimmune encephalomyelitis. Exp Neurol. 2006 Apr;198(2):275–84.

[70] Levin MC, Jackson WC. Developing a therapeutic plan for treating MS: evidence for new treatments. J Clin Psychiatry. 2014 Dec;75(12):e34. doi:10.4088/JCP.12100nr8c.

[71] Kappos L. Therapy. In: Kesselring J, McDonald WI, eds. Multiple Sclerosis. Boston: Cambridge University Press; 1997. pp 148–67.

[72] Snowden JA et al. On behalf of the EBMT Autoimmune Disease Working Party (ADWP) and Paediatric Diseases Working Party (PDWP). Haematopoietic SCT in severe autoimmune diseases: updated guidelines of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant. 2012;47:770–90.

[73] Pasquini MC, Voltarelli J, Atkins HL, Hamerschlak N, Zhong X, Ahn KW, Sullivan KM, Carrum G, Andrey J, Bredeson CN, Cairo M, Gale RP, Hahn T, Storek J, Horowitz MM, McSweeney PA, Griffith LM, Muraro PA, Pavletic SZ, Nash RA. Transplantation for autoimmune diseases in North and South America: a report of the Center for International Blood and Marrow Transplant Research. Biol Blood Marrow Transplant. 2012 Oct;18(10):1471–8.

[74] Fassas A, Anagnostopoulos A, Kazis A, Kapinas K, Sakellari I, Kimiskidis V, Tsompanakou A. Peripheral blood stem cell transplantation in the treatment of progressive multiple sclerosis: first results of a pilot study. Bone Marrow Transplant. 1997 Oct;20(8):631–8.

[75] Burt RK, Balabanov R, Han X, Sharrack B, Morgan A, Quigley K, Yaung K, Helenowski IB, Jovanovic B, Spahovic D, Arnautovic I, Lee DC, Benefield BC, Futterer S, Oliveira MC, Burman J. Association of nonmyeloablative hematopoietic stem cell transplantation with neurological disability in patients with relapsing-remitting multiple sclerosis. JAMA. 2015 Jan 20;313(3):275–84.

[76] Hamerschlak N, Rodrigues M, Moraes DA, Oliveira MC, Stracieri AB, Pieroni F, Barros GM, Madeira MI, Simões BP, Barreira AA, Brum DG, Ribeiro AA, Kutner JM, Tylberi CP, Porto PP, Santana CL, Neto JZ, Barros JC, Paes AT, Burt RK, Oliveira EA, Mastropietro AP, Santos AC, Voltarelli JC. Brazilian experience with two conditioning regimens in patients with multiple sclerosis: BEAM/horse ATG and CY/rabbit ATG. Bone Marrow Transplant. 2010 Feb;45(2):239–48.

[77] Nash RA, Hutton GJ, Racke MK, et al. High-dose immunosuppressive therapy and autologous hematopoietic cell transplantation for relapsing-remitting multiple sclerosis (HALT-MS): a 3 year interim report. JAMA Neurol. 2015 Feb 1;72(2):159–69.

[78] Krasulova E, Trneny M, Kozak T, et al. High-dose immunoablation with autologous haematopoietic stem cell transplantation in aggressive multiple sclerosis: a single centre 10 year experience. Mult Scler. 2010;16:685–93.

[79] Burt R, Loh Y, Cohen B, et al. Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing–remitting multiple sclerosis: a phase I/II study. Lancet Neurol. 2009;8:244–53.

[80] Shevchenko JL, Kuznetsova AN, Ionova TI et al. Autologous hematopoietic stem cell transplantation with reduced-intensity conditioning in multiple sclerosis. Exp Hematol. 2012;40:892–98.

[81] Currò D, Mancardi G. Autologous hematopoietic stem cell transplantation in multiple sclerosis: 20 years of experience. Neurol Sci. 2016 Jun;37(6):857–65.

[82] Mancardi G, Saccardi R. Autologous haematopoietic stem-cell transplantation in multiple sclerosis. Lancet Neurol 2008;7:626–36.

[83] Saccardi R, Freedman MS, Sormani MP, Atkins H, Farge D, Griffith LM, Kraft G, Mancardi GL, Nash R, Pasquini M, Martin R, Muraro PA; European Blood and Marrow Transplantation Group.; Center for International Blood and Marrow Research.; HCT in MS International Study Group. A prospective, randomized, controlled trial of autologous haematopoietic stem cell transplantation for aggressive multiple sclerosis: a position paper. Mult Scler. 2012 Jun;18(6):825–34.

[84] McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365:2205–19.

[85] Koetz K, Bryl E, Spickschen K, O'Fallon WM, Goronzy JJ, Weyand CM. T cell homeostasis in patients with rheumatoid arthritis. Proc Natl Acad Sci USA. 2000;97:9203–08.

[86] Nistala K, Wedderburn LR. Th17 and regulatory T cells: rebalancing pro- and anti-inflammatory forces in autoimmune arthritis. Rheumatology. (Oxford) 2009;48:602–06.

[87] Moore J, Brooks P, Milliken S, Biggs J, Ma D, Handel M, Cannell P, Will R, Rule S, Joske D. A pilot randomized trial comparing CD34-selected versus unmanipulated hemopoietic stem cell transplantation for severe, refractory rheumatoid arthritis. Arthritis Rheum. 2002;46:2301–9.

[88] Burt RK, Georganas C, Schroeder J, Traynor A, Stefka J, Schuening F, Graziano F, Mineishi S, Brush M, Fishman M. Autologous hematopoietic stem cell transplantation in refractory rheumatoid arthritis: sustained response in two of four patients. Arthritis Rheum. 1999;42:2281–5.

[89] Verburg RJ, Kruize AA, van den Hoogen FH, Fibbe WE, Petersen EJ, Preijers F, Sont JK, Barge RM, Bijlsma JW, van de Putte LB. High-dose chemotherapy and autologous hematopoietic stem cell transplantation in patients with rheumatoid arthritis: results of an open study to assess feasibility, safety, and efficacy. Arthritis Rheum. 2001;44:754–60.

[90] Gratwohl A, Passweg J, Bocelli-Tyndall C, Fassas A, van Laar JM, Farge D, Andolina M, Arnold R, Carreras E, Finke J. Autologous hematopoietic stem cell transplantation for autoimmune diseases. Bone Marrow Transplant. 2005;35:869–79.

[91] Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011;365:2110–21.

[92] Jacobi AM, Mei H, Hoyer BF, Mumtaz IM, Thiele K, Radbruch A et al. HLA-DRhigh/CD27high plasmablasts indicate active disease in patients with systemic lupus erythematosus. Ann Rheum Dis. 2010;69:305–8.

[93] Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J et al. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. J Exp Med. 2003;197:711–23.

[94] Rose T, Grutzkau A, Hirseland H, Huscher D, Dahnrich C, Dzionek A et al. IFNalpha and its response proteins, IP-10 and SIGLEC-1, are biomarkers of disease activity in systemic lupus erythematosus. Ann Rheum Dis. 2013;72:1639–45.

[95] Alexander T, Sattler A, Templin L, Kohler S, Gross C, Meisel A et al. Foxp3+ Helios+ regulatory T cells are expanded in active systemic lupus erythematosus. Ann Rheum Dis. 2013;72:1549–58.

[96] Ippolito A, Petri M. An update on mortality in systemic lupus erythematosus. Clin Exp Rheumatol. 2008;26:S72–9.

[97] Mirabelli G, Cannarile F, Bruni C, Vagelli R, De Luca R, Carli L. One year in review 2015: systemic lupus erythematosus. Clin Exp Rheumatol. 2015;33:414–25.

[98] Illei GG, Austin HA, Crane M, Collins L, Gourley MF, Yarboro CH, Vaughan EM, Kuroiwa T, Danning CL, Steinberg AD. Combination therapy with pulse cyclophosphamide plus pulse methylprednisolone improves long-term renal outcome without adding toxicity in patients with lupus nephritis. Ann Intern Med. 2001;135:248–57.

[99] Burt RK, Traynor A, Statkute L, Barr WG, Rosa R, Schroeder J, Verda L, Krosnjar N, Quigley K, Yaung K. Nonmyeloablative hematopoietic stem cell transplantation for systemic lupus erythematosus. JAMA. 2006;295:527–35.

[100] Farge D, Labopin M, Tyndall A, Fassas A, Mancardi GL, Van Laar J, Ouyang J, Kozak T, Moore J, Kötter I. Autologous hematopoietic stem cell transplantation for autoimmune diseases: an observational study on 12 years' experience from the European Group for Blood and Marrow Transplantation Working Party on Autoimmune Diseases. Haematologica. 2010;95:284–92.

[101] Cyclophosphamide and rATG/Rituximab in patients with systemic lupus erythematosus. Clinicaltrials.gov, NCT00278538. Available from: https://clinicaltrials.gov/ct2/show/NCT00278538.

[102] Baumgart DC, Sandborn WJ. Crohn's disease. Lancet. 2012 Nov 3;380(9853):1590–605. doi:10.1016/S0140-6736(12)60026-9.

[103] Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011;474:307–17.

[104] Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 2012;491:119–24.

[105] Dige A, Stoy S, Rasmussen TK, Kelsen J, Hvas CL, Sandahl TD et al. Increased levels of circulating Th17 cells in quiescent versus active Crohn's disease. J Crohns Colitis. 2013;7:248–55.

[106] Hedin CR, McCarthy NE, Louis P, Farquharson FM, McCartney S, Taylor K et al. Altered intestinal microbiota and blood T cell phenotype are shared by patients with Crohn's disease and their unaffected siblings. Gut. 2014;63:1578–86.

[107] Kashyap A, Forman SJ. Autologous bone marrow transplantation for non-Hodgkin's lymphoma resulting in long-term remission of coincidental Crohn's disease. Br J Haematol. 1998;103(3):651–2.

[108] Kreisel W, Potthoff K, Bertz H, et al. Complete remission of Crohn's disease after high-dose cylophosphamide and autologous stem cell transplantation. Bone Marrow Transplant. 2003;32(3):337–40.

[109] Hawkey CJ. Stem cell transplantation for Crohn's disease. Best Pract Res Clin Haematol. 2004;17(2):317–25.

[110] Oyama Y, Craig RM, Traynor AE, et al. Autologous hematopoietic stem cell transplantation in patients with refractory Crohn's disease. Gastroenterology. 2005;128(3):552–63.

[111] Lopez-Cubero SO, Sullivan KM, McDonald GB. Course of Crohn's disease after allogeneic marrow transplantation. Gastroenterology. 1998;114(3):433–40.

[112] Burt RK, Craig RM, Milanetti F, Quigley K, Gozdziak P, Bucha J, Testori A, Halverson A, Verda L, de Villiers WJ, Jovanovic B, Oyama Y. Autologous nonmyeloablative hematopoietic stem cell transplantation in patients with severe anti-TNF refractory Crohn disease: long-term follow-up. Blood. 2010;116:6123–32.

[113] Hawkey CJ, Allez M, Clark MM, Labopin M, Lindsay JO, Ricart E, Rogler G, Rovira M, Satsangi J, Danese S, Russell N, Gribben J, Johnson P, Larghero J, Thieblemont C, Ardizzone S, Dierickx D, Ibatici A, Littlewood T, Onida F, Schanz U, Vermeire S, Colombel JF, Jouet JP, Clark E, Saccardi R, Tyndall A, Travis S, Farge D. Autologous hematopoetic stem cell transplantation for refractory Crohn disease: a randomized clinical trial. JAMA. 2015 Dec 15;314(23):2524–34.

[114] Daikeler T, Kötter I, Bocelli Tyndall C, Apperley J, Attarbaschi A, Guardiola P, Gratwohl A, Jantunen E, Marmont A, Porretto F. Haematopoietic stem cell transplantation for vasculitis including Behcet's disease and polychondritis: a retrospective analysis of patients recorded in the European Bone Marrow Transplantation and European League Against Rheumatism databases and a review of the literature. Ann Rheum Dis. 2007;66:202–7.

[115] Jantunen E, Myllykangas-Luosujärvi R, Kaipiainen-Seppänen O, Nousiainen T. Autologous stem cell transplantation in a lymphoma patient with a long history of ankylosing spondylitis. Rheumatology (Oxford). 2000;39:563–4.

[116] Khorshid O, Hosing C, Bibawi S, Ueno N, Reveille J, Mayes MD, Champlin RE. Nonmyeloablative stem cell transplant in a patient with advanced systemic sclerosis and systemic lupus erythematosus. J Rheumatol. 2004;31:2513–6.

[117] Voharelli JC, Couri EB, Boris N et al. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type l diabetes mellitus. JAMA. 2007;297:1568–77.

[118] Couri CE, Oliveira MC, Stracieri AB et al. C-Peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA. 2009;301:1573–9.

[119] Li L, Shen S, Ouyang J et al. Autologous hematopoietic stem cell transplantation modulates immunocompetent cells and improves beta cell function in Chinese patients with new onset of type 1 diabetes. J Clin Endocrinol Metab. 2012;97:1729–36.

[120] Gu W, Hu J, Wang W et al. Diabetic ketoacidosis at diagnosis influences complete remission after treatment with hematopoietic stem cell transplantation in adolescents with type 1 diabetes. Diabetes Care. 2012;35:1413–9.

[121] Snarski E, Milczarczyk A, Torosian T et al. Independence of exogenous insulin following immunoabla- tion and stem cell reconstitution in newly diagnosed diabetes type I. Bone Marrow Transplant. 2011;46:562–6.

Chapter 2

Management and Supportive Care of Patients Undergoing Immunotherapy

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Additional information is available at the end of the chapter

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Abstract

In many tumor types, where the prognosis was shown to be extremely dismal before, immunotherapy is now a new beacon of hope to many patients. Immunotherapy has been approved for use in a many different cancers including metastatic melanoma, advanced non-small cell lung cancer, metastatic renal cell carcinoma, refractory Hodgkin's lymphoma, metastatic bladder cancer advanced head and neck cancer, and the list keeps growing each day. It seems to be generally better tolerated in most patients and less toxic compared to what we have seen in different anticancer treatments from before. However, the toxicities here are termed immune-related adverse events. There is almost no prospective data on these toxicities, and guidelines or recommendations are mostly based on symptomatic management from the ongoing clinical trials. Treating oncologists need to be aware of the subtleties in presentation and the huge difference in the way we mange these side effects. Although most adverse events are low-grade and manageable, they have the potential to be life-threatening if not treated promptly. In this chapter, we address the different immune-related adverse events relating to the organ system they can involve, presentation and symptomatology, general recommendations of management, and individual toxicities. Keywords: immunotherapy, PD-1, CTLA-4.

Keywords: immunotherapy, PD-1, CTLA-4, immune-related adverse events, iRAE, supportive care

1. Introduction

Immunotherapy has emerged as the utmost oncological advance of 2016 [1]. It encompasses the enhancement, suppression, or induction of the body's own immune system to battle

cancer [1]. There has been a paradigm shift toward immunooncology therapy, and its side effects are often referred to as immune-related adverse events (irAEs). These side effects are in some cases unique and very different than those associated with chemotherapy or targeted drugs. The spectrum of irAEs is typically low-grade and manageable; however, the reporting of irAEs is generally suboptimal [2]. Therefore, oncologists should be aware that there is a broad range of additional toxicities and side effects that can be both unpredictable and even severe in nature. Early recognition of irAEs and aggressive management is crucial to reduce morbidity and mortality. Toxicities associated with PD-1 inhibitors are generally less severe than those associated with CTLA-4 inhibitors; however, grade 3–4 toxicities occur in about 21% of immunotherapy cases [3, 4].

Monoclonal antibodies that are currently registered include the following: anti-PD-1 (nivolumab and pembrolizumab), anti-PD-L1 (atezolizumab), and anti-CTLA-4 antibodies (ipilimumab) [5, 6].

2. Pathogenesis

The pathogenesis of irAEs is primarily based on and can be understood by the immune pathophysiology that leads to hyperactivation of T-cells. PD-1 and CTLA-4 are immune checkpoints that are expressed on the surface of antigen-presenting cells in the initiator and effector phase of T-cell activation, respectively. They are responsible for "switching off" the T-cell. Inhibition of these checkpoints allows for overexpression of the immune system, which is a powerful mechanism to defeat tumor cells.

Two signals are required by T cells to become fully activated [7]. The first signal originates from the interaction between T-cell receptors (TCR) and the antigen-peptide major-histocompatibility complex (MHC), which contributes to the specificity of the immune response. Additionally, T cells require a costimulatory antigen-dependent signal that occurs through the interaction between CD28 on T cells and B7-1 and B7-2 on the antigen-presenting cells (APC), to become entirely activated. On the other hand, expression of CTLA-4 by T cells constitutes a mechanism to prevent overstimulation of the immune system. CTLA-4 has a 100-fold higher affinity with the B7 complex than CD28, and this interaction is associated with an inhibitory function on the cell [8]. CTLA-4 inhibitors such as monoclonal antibodies ipilimumab and tremelimumab have been developed to block and release these breaks. Ipilimumab is currently approved for the treatment of metastatic malignant melanoma and is under investigation in the treatment of patients with non-small cell cancer (NSCLC).

Another well-established mechanism of immune-response evasion is regulated by expression of PD-L1 in the malignant cells. PD-L1 binds to PD-1 on the T cells and thus initiates a dual mechanism of inhibition by promoting apoptosis in antigen-specific T cells in lymph nodes and simultaneously reducing apoptosis in regulatory T cells referred to as T regs [9].

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The mechanism of defeating tumor cells can be understood by the three phases of immunoediting [1]. The first phase, elimination, consists of the eradication of tumor cells by working with the innate and adaptive immune system. It activates several effector cells by inflammatory cytokines released by the tumor cells. The second phase, named equilibrium, is the development of resistance to the elimination phase by the tumour cells. Finally, the escape phase is where further resistance develops toward immune detection. The overactivation of the immune system, and blocking of suppressor checkpoints, also affects normal body tissues, which is the possible mechanism by which toxicities arise, although this remains largely unknown [1]. Checkpoint inhibitors CTLA-4, PD-1, and PD-L1 blockers are approved for use in metastatic melanoma, nonsmall cell lung cancer (NSCLC), renal cell carcinoma, head and neck cancer, Hodgkin's disease, and bladder cancer. They show improvement in overall survival in these tumor types.

3. irAEs' general concepts

The incidence of grade 3 or 4 adverse events is higher with CTLA-4 blockers, and PD-1 inhibitors appear to have better tolerability [2, 3, 10]. The grade of irAEs varies according to the dose of drug administered to patients, where smaller doses of drug are used, side effects are similar but are less frequent [11]. The incidence of irAEs can vary with tumor type and between different classes of drugs. The combination of PD-1 inhibitor with a CTLA-4 inhibitor was recently approved for the treatment of metastatic malignant melanoma; however, more adverse reactions were seen when the two drugs were used together. In combination, there are especially more grade 3 or 4 events (55%). It is important to point out that although greater overall response rates were seen, it was also noted that the combination led to a higher incidence of severe irAEs and treatment discontinuations due to severe toxicity [12–14].

Generally, the most frequent irAEs are seen in the gastrointestinal (35%) and dermatological (44%) systems [11]. The incidence of hepatic and endocrine system involvement follows with about 5–6%. Other systems less frequently affected are neurological, ophthalmological, pulmonary, renal, hematological, cardiovascular, respiratory, and musculoskeletal [3, 11, 13]. IrAEs typically develop within 6–12 weeks of initial dosing and resolution occurs within 12 weeks of onset. irAEs may develop after the first dose administered [15, 16]. It has been also hypothesized that the severity of the adverse correlates positively with a response to treatment [4, 14, 17]. However, the correlation of response to treatment and toxicity remains controversial. When managed correctly and promptly and with close monitoring, most are irAEs are reversible [11, 12, 14]. In general, the optimal management of irAEs includes early recognition (by far being the most important), proper assessment of severity so that the choice of therapy, either supportive or immunosuppressive, can be quickly and correctly implemented. Usually, mild adverse events can be observed or treated symptomatically with supportive care. As a guide, with the exception of irAE endocrine moderate events, what is usually required is stopping the offending agent, implementing oral corticosteroid therapy, and restarting therapy again once symptoms have resolved. Severe irAEs warrant permanent discontinuation of the drug, patient hospitalization, and high-dose intravenous corticosteroids, with slow

weaning. In very severe cases, other immunosuppressive agents such as infliximab or mycophenolate mofetil may be necessary [18].

In the following chapter sections, the different systems will be discussed.

3.1. Dermatological

A diffused, erythematous maculopapular rash and pruritus can occur in up to 50% of patients treated with anti-CTLA4 or up to 37% of patients treated with anti-PD-1 [4, 13, 15, 17]. The rash can occur after the initial dose of treatment and can be ongoing (**Figure 1A–C**). However, symptoms on an average start 3–4 weeks after treatment. Vitiligo has also been reported [19, 20] (**Figure 2**). In severe cases, toxic epidermal necrolysis and Stevens-Johnson syndrome can occur, but in less than 1% of patients [15, 19]. Most of the dermatological eruptions and pruritus associated with these agents are managed symptomatically and usually do not require treatment delays or discontinuation. A recent meta-analysis of a total of 1208 patients demonstrated that the overall incidence of all-grade rash associated with ipilimumab was 24.3% (95% confidence interval [CI]: 21.4–27.6%), with a relative risk of 4.00 (95% CI: 2.63–6.08, $P < 0.001$). The overall incidence of high-grade rash was 2.4% (95% CI: 1.1–5.1%), with a relative risk of 3.31 (95% CI: 0.70–15.76, $P = 0.13$) [21]. A second meta-analysis from a total of nine clinical trials in patients receiving ipilimumab, nivolumab, tremelimumab, pidlizumab, and pembrolizumab was included. The relative risk of all-grade rash was 4.06 (95% CI: 3.35–4.91; $P < 0.0001$), vitiligo 16.3 (95% CI: 3.21–82.8; $P = 0.0008$), and pruritus was 3.4 (95% CI: 2.24–5.16; $P < 0.00001$) [22].

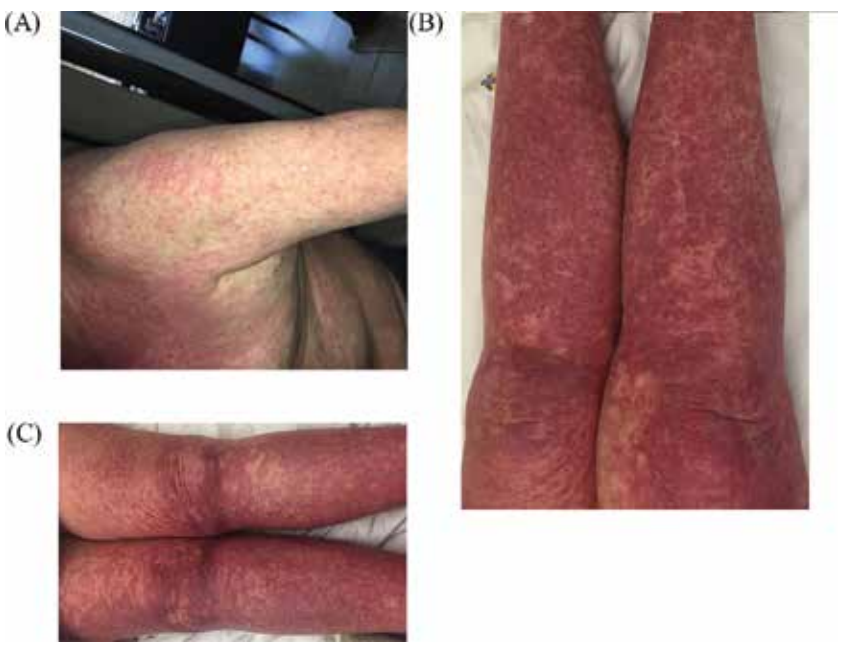

Figure 1. Severe generalized maculo-papular rash associated with a combination of ipilimumab and nivolumab.

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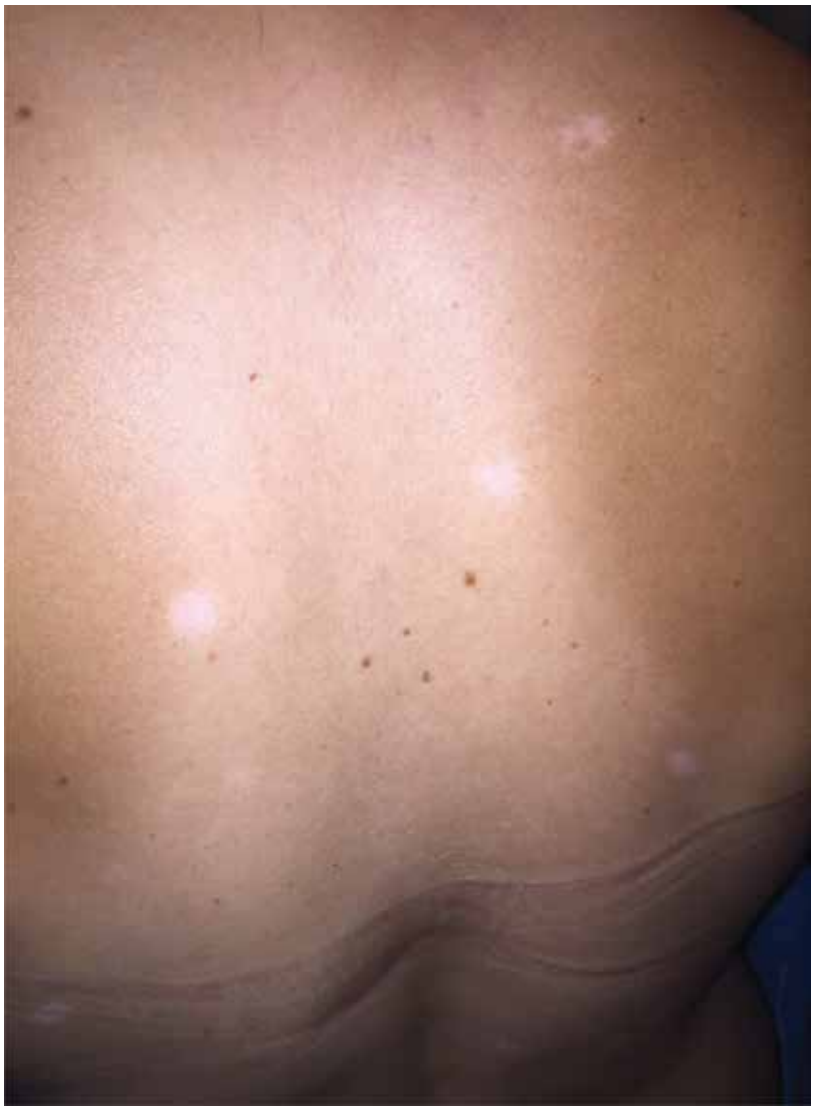

Figure 2. Vitiligo associated with Ipilimumab.

Management

Topical glucocorticosteroids (e.g., betamethasone cream) or urea-containing creams in combination with oral antipruritics (e.g., diphen-hydramine HCl or hydroxyzine HCl) are recommended. The recommendation patients with a moderate rash, nonlocalized, and covers more than 50% of the skin surface area are to omit the offending agent. For grade 3 dermatological irAEs, hold treatment and administer a 3–4-week course of oral corticosteroids in the form of prednisone at a dose of 1 mg/kg or dexamethasone at a dose of 4 mg four times a day given orally daily. Treatment should be permanently discontinued for severe, life-threatening skin

toxicity and prednisone at a dose of 1–2 mg/kg orally or equivalent formulations given at least for 30 days [23]. When a high-dose corticosteroid therapy is used, once symptoms are controlled, tapering of the steroids should occur over a one-month period at least [18]. Vitiligo may be associated with clinical benefit. Although it occurs in a small percentage of patients undergoing immunotherapy, there is a clear survival benefit in patients who do develop vitiligo during treatment [19, 20]. In some patients, vitiligo is associated with long-term survival [19, 20].

3.2. Gastrointestinal

Side effects can occur anywhere along the gastrointestinal tract, ranging from mucositis, aphthous ulcers, gastritis, and abdominal pain. More commonly, diarrhea related to colitis can be observed. This will be elaborated on in the next section [4, 13, 15].

3.2.1. Diarrhea and colitis

Diarrhea and colitis are very common side effects of checkpoint inhibitors. It is more frequently seen when using CTLA-4 inhibitors than when using PDL-1 inhibitors. It is reported in about 30% of patients receiving CTLA-4 therapy, whereas it is as little as only 1–2% of patients receiving PDL-1 therapy [2, 4, 10, 24]. There is a higher incidence and a greater severity in grade when bigger doses are used as seen in the initial trials of ipilimumab when comparing 10 mg vs. 3 mg [4, 11, 24]. It is also more frequently seen and with a higher incidence in grade 3 and grade 4 events when the two checkpoint inhibitors are used in combination [2, 3, 12, 14]. This irAE is most likely to manifest within the first 6 weeks after checkpoint inhibitor therapy has been initiated, slightly later than dermatological irAEs, although this is not absolute, as it can also occur anywhere in the treatment course [15, 16, 24]. Diarrhea, which is an increase in the frequency of stool is related to, but a different clinical entity from colitis. The CTCAE states that symptoms related to colitis are associated with abdominal pain and include patients who have blood or mucus in their stool. If there is evidence of inflammation on endoscopic investigation or radiographically, it is also then defined as colitis. It is important to exclude other infectious causes of diarrhea, for instance, *Clostridium difficile* infection in all cases [4, 13, 15]. In very selected cases, where patients have accompanying symptoms of high fevers, leukocytosis, and those who have been on immunosuppressive therapy for long periods of time rendering them more susceptible to infections, prophylactic antibiotics can be considered [15]. A colonoscopy can be considered in patients with severe or persistent symptoms or if the cause is unclear [13, 15, 24] (**Figure 3**).

In severe conditions, perforation can occur and lead to death and must be excluded in patients with symptoms of peritonitis. These patients may require surgery and possible colostomy [3, 15].

Mild symptoms can be treated symptomatically with rehydration, replacing electrolyte losses, and loperamide [3, 4, 18, 24]. Grade 2 irAEs require the offending immunotherapy agent to be omitted. If symptoms are ongoing for more than one week, there should be an immediate commencement of oral corticosteroid therapy at a dose of 1 mg/kg/day. When symptoms are resolved, the immunotherapy drug can be recommenced [4, 6, 13, 15, 24].

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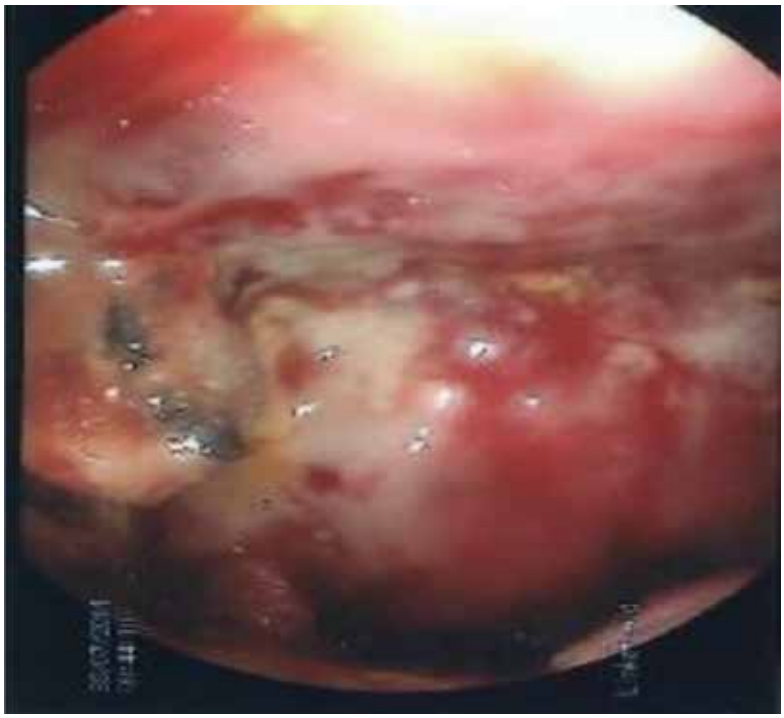

Figure 3. Severe colitis associated with ipilimumab.

Severe or life-threatening colitis and symptoms consistent with perforation, ileus, or fever is a serious complication. High-dose intravenous corticosteroids commencing at a starting dose of 2 mg/kg/day must be initiated promptly [15, 18].

If symptoms persist, a single dose of immunosuppressive infliximab therapy at 5 mg/kg must be considered unless there is a contraindication [15, 18, 24]. The dose of infliximab be repeated after 2 weeks if symptoms persist [13, 15, 24]. Mycophenolate mofetil can also be considered in severe and refractory cases [15]. The most important part of management of a patient with colitis is recognition and early initiation of aggressive treatment. Diarrhea treatment guidelines have been shown to reduce bowel perforation and colectomy rates and serious irAEs by up to 50% when this is done. There is anecdotal evidence that shows that high-dose therapy initiated for irAEs does not affect efficacy of treatment [2, 12]. Furthermore, it is postulated that the severity of the adverse event correlates with a better response to treatment [11, 14, 17].

3.3. Hepatic

Hepatotoxicity can be observed following treatment with anti-CTLA4 or anti-PD-1/anti-PDL1 therapy usually at about 6 weeks after initiation. It frequently manifests as an asymptomatic increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and/or total bilirubin. Hepatotoxicity has been observed in 3–9% of patients receiving ipilimumab [25, 26]. A meta-analysis of a total of nine randomized controlled trials in patients with solid tumors

demonstrated that the use of PD-1 inhibitors, when compared to the control group of chemotherapy or everolimus, significantly increased the risk of developing all, but high-grade hepatic AEs. Additionally, the risk of all grades of hepatic AEs was considerably higher when a nivolumab and ipilimumab combination was used compared to ipilimumab monotherapy. No significant differences in the risk of all-grade and high-grade hepatic irAEs were found between PD-1 inhibitors monotherapy and ipilimumab monotherapy [27].

3.3.1. Management

The differential diagnosis of immune-related hepatotoxity includes progressive metastatic liver disease, viral hepatitis, or another drug-specific toxic reaction. Diagnostic workup includes viral hepatitis studies, liver imaging, and excluding other drug-related causes for abnormal liver functions. A liver biopsy is indicated when the etiology is unclear [15]. It is important to point out that hepatic toxicity can occur in the absence of symptoms. Baseline liver functions should be obtained before commencement of therapy [15, 18]. When derangements are documented, other infectious causes, concurrent medications used by patients and disease progression must be excluded by appropriate investigations [15, 18].

Severe hepatotoxicity requires permanent discontinuation of the drug. Additionally, high-dose intravenous glucocorticosteroids for 24–48 hours followed by an oral steroid taper with dexamethasone at a dose of 4 mg every 4 hours or prednisone at 1–2 mg/kg tapered over not less than 30 days. If the levels of serum transaminase do not decrease 48 hours after commencement of systemic steroids, oral mycophenolate mofetil 500 mg every 12 hours should be considered [28]. Infliximab is associated with hepatotoxicity and should be avoided in this clinical setting.

3.4. Endocrine

Endocrine irAEs are in general inconstantly described in recent published data. Assessment and reporting of endocrine irAEs in clinical trials should be done using standardized diagnostic criteria and terminology. Unfortunately, as a consequence of the lack of standardization, the true incidence of endocrine adverse events on patients undergoing anti-CTLA-4 and antiPD-1/PD-L1 pathway blockades is unknown. Thyroid dysfunction is the most common irAE reported. Hypophysitis has merged as a distinctive side effect of CTLA-4-blocking antibodies [2, 13, 29]. The spectrum of endocrine disease in patients treated with ipilimumab includes hypophysitis, and occasionally primary adrenal insufficiency. This complication, if not promptly diagnosed, can be life-threatening (due to secondary hypoadrenalism). Hypopituitarism caused by CTLA-4-blocking antibodies is rarely reversible, and prolonged or lifelong hormonal replacement treatment is often required. The mechanism of injury and pathogenesis to the endocrine system triggered by ipilimumab needs to be clarified.

Presenting symptoms of hypothyroidism, such as fatigue, weakness, depression, memory loss, cold intolerance, and cardiovascular abnormalities, may be incorrectly attributed to the primary malignant disease. The onset of hypothyroidism is variable and can occur within the first 5 months and up to 2 years following immune-therapy. Some patients may develop autoimmune thyroiditis [30]. The prevalence of abnormal thyroid tests in one series was 15%

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[31]. A recent meta-analysis of ten clinical trials showed that relative risk of all grades hypothyroidism 8.26 (95% CI: 4.67–14.62; $P < 0.00001$), hyperthyroidism 5.48 (95% CI: 1.33–22.53; $P = 0.02$), hypophyisitis 22.03 (95% CI: 8.52–56.94; $P < 0.00001$), and adrenal insufficiency 3.87 (95% CI: 1.12–13.41; $P = 0.03$) [32].

Baseline thyroid function tests are also recommended. Pituitary hormones, in the presence of symptoms, are indicated if thyroid functions are normal. Primary adrenal and primary pituitary insufficiency can be differentiated with an early morning cortisol [4, 13, 15]. MRI can be obtained to visualize the pituitary gland to confirm the diagnosis of hypophysitis [4, 15]. MRI findings can be nonspecific, but can show a general enlargement of the pituitary gland [33, 34]. In a review, about 85% of patients had pituitary gland abnormality on MRI [5]. Treatment of hypothyroidism usually requires replacement of thyroid hormone, and in mild cases of adrenal insufficiency, oral corticosteroid therapy can be used [4, 8]. Adrenal insufficiency or crisis is a medical emergency. This warrants hospitalization, high-dose intravenous corticosteroids with mineralocorticoid activity. Infection or sepsis should be excluded in these cases. A consultation with an endocrinologist is needed to ascertain if long-term hormone replacement is necessary [13, 15, 18].

3.5. Pulmonary

Immune-related pneumonitis is a serious IrAE associated with immunotherapy. This is more common with PD-1 blockers, although the incidence is <1% and presents far later into treatment phase [13]. Patients undergoing immunotherapy, experiencing new symptoms of cough or dyspnea, should arouse suspicion for the development of pneumonitis (**Figure 4**). In a nivolumab monotherapy, early dose-finding study (CA209-003) that evaluated various tumor types, three treatment-related deaths (1%) due to pneumonitis were reported in two patients with NSCLC and one patient with colorectal cancer [36]. A recent meta-analysis of 11 clinical trials showed that the odds ratio was 3.96 (95% confidence interval [CI]: 2.02–7.79; $P < 0.0001$) for all-grade pneumonitis and 2.87 (95% CI: 0.90–9.20; $P = 0.08$) for high-grade pneumonitis. Additionally, the odds ratio of all grades of pneumonitis with a nivolumab and ipilimumab combination vs. ipilimumab monotherapy was 3.68 (95% CI: 1.59–8.50; $P = 0.002$), and for high-grade pneumonitis, it was 1.86 (95% CI: 0.36–9.53; $P = 0.46$). Subgroup analysis did not demonstrate a significant difference between lung cancer patients and other types of cancer in the risk of pneumonitis. This is an irAE that can occur both with anti-CTLA-4 or anti-PD-1 agents. It has been reported in approximately 1% of patients treated with anti-PD-1 agents and occurs more frequently than with anti-CTLA-4 agent ipilimumab. Deaths related to immune-onset pneumonitis have been reported in NSCLC patients. Pneumonitis management involves prompt initiation of high-dose corticosteroids, close symptoms monitoring, and oxygen requirements. Immunosuppressive interventions may be required in a minority of patients [37]. Radiological findings should be monitored closely.

A second meta-analysis comprised 20 PD-1 inhibitor trials in 4496 patients with malignant melanoma (12 trials), NSCLC (5 trials), and RCC (3 trials). The overall incidence of pneumonitis during PD-1 inhibitor monotherapy was 2.7% (95% CI, 1.9–3.6%) for all-grade and 0.8% (95% CI, 0.4–1.2%) for grade 3 or higher pneumonitis. The incidence was higher in NSCLC for all-grade

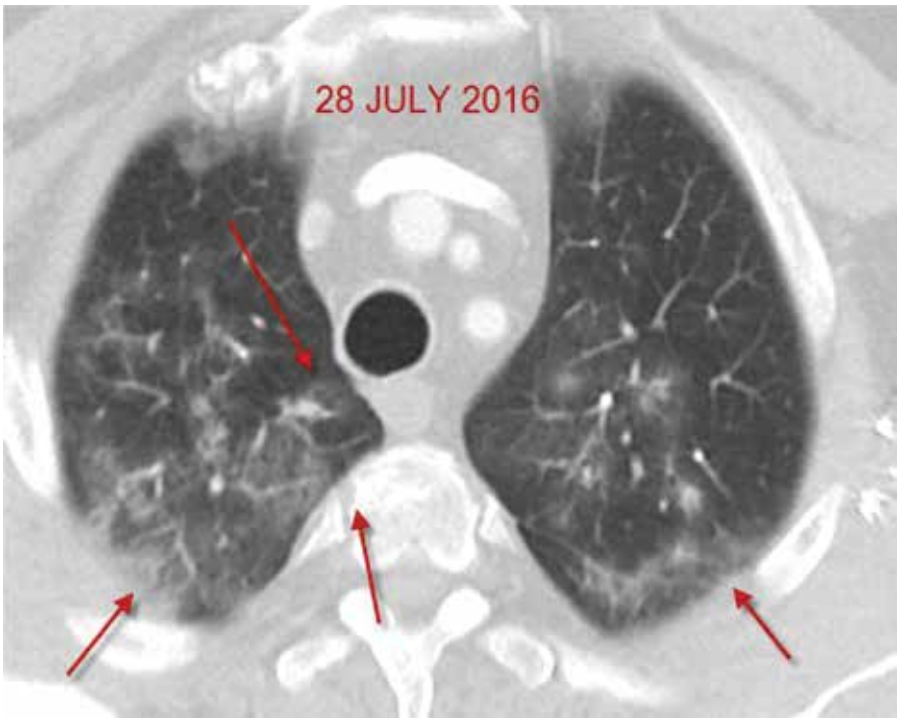

Figure 4. Pneumonitis associated with Nivolumab.

(4.1 vs. 1.6%; $P = 0.002$) and grade 3 or higher pneumonitis (1.8 vs. 0.2%; $P < 0.001$) compared with melanoma. The incidence in RCC was higher than in melanoma for all grades of pneumonitis (4.1 vs. 1.6%; $P < 0.001$) but not for grade 3 or higher. Four pneumonitis-related deaths were documented in patients with NSCLC in the monotherapy group. Pneumonitis was more frequent during combination immunotherapy than monotherapy for all grades (6.6 vs. 1.6%; $P < 0.001$) and for grade 3 or higher (1.5 vs. 0.2%; $P = 0.001$) in melanoma, with one pneumonitis-related death during combination therapy. Multivariable analyses demonstrated higher odds of pneumonitis in NSCLC for all-grade (odds ratio [OR], 1.43; 95% CI, 1.08–1.89; $P = 0.005$) and grade 3 or higher pneumonitis (OR, 2.85; 95% CI, 1.60–5.08; $P < 0.001$) and in RCC for all-grade pneumonitis (OR, 1.59; 95% CI, 1.32–1.92; $P < 0.001$) compared with melanoma. The combination therapy had significantly higher odds than monotherapy for all-grade (OR, 2.04; 95% CI, 1.69–2.50; $P < 0.001$) and grade 3 or higher pneumonitis (OR, 2.86; 95% CI, 1.79–4.35; $P < 0.001$). The authors concluded that the incidence of PD-1 inhibitor-related pneumonitis was higher in NSCLC and RCC and during combination therapy [38].

Several pulmonary inflammatory conditions have also been seen in patients treated with ipilimumab, including sarcoidosis [39, 40] and organizing inflammatory pneumonia [41].

In any patient undergoing anti-CTLA-4 or anti-PD-1/PD-L1 immunotherapy, presenting with pulmonary symptoms, such as an upper respiratory infection, new cough, or shortness of breath, pneumonitis should be considered and evaluated with imaging. Because the onset and

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symptoms of pneumonitis are often vague and diagnosis is often delayed, clinicians should be aware of this and consider diagnostic radiology (X-rays, CT scans) early. Bronchoscopy and lung biopsy should be considered to rule out other causes such as infectious etiologies before starting treatment, especially in moderate-to-severe cases [13, 15]. Differential diagnosis includes disease progression of cancer, lymphangitis carcinomatosis, opportunistic infections, severe pneumonitis, early cardiac failure, alveolar hemorrhage, or congestive cardiac failure. In severe cases, treatment should comprise high doses of corticosteroids such as intravenous methylprednisone at a dose of 2 mg/kg. Additional immunosuppression with infliximab, mycophenolate mofetil, or cyclophosphamide may be required and is a reasonable approach in nonresponding patients [13, 15].

3.6. Ophthalmological

Ophthalmological immune-related adverse events are extremely rare and occur in less than 1% of patients treated with anti-CTLA-4 therapy. The incidence with anti-PD-1 antibodies is unknown [42, 43]. Besides, from the direct toxicity of immunotherapy agents, the eye can also indirectly be affected via other immune-related adverse endocrinopathies such as hyperthyroidism form autoimmune thyroiditis [30, 43]. There have been case reports of Grave's opthalmopathy with symptoms and signs of proptosis associated with swelling of extraocular muscles and xeropthalmia [30, 42, 44]. Ophthalmological side effects include episcleritis, conjunctivitis, and uveitis [3]. A rare case of bilateral iridocyclitis and of bilateral choroidal neovascularization was reported [4, 42, 45]. Most cases can be managed with topical corticosteroids [34]. Systemic corticosteroids can be implemented in patients who do not respond to topical management or in grade 3 or in grade 4 cases. It is always recommended to consult an opthalomologist [43].

3.7. Neurological

Neurological symptoms can vary widely and present as a range of different conditions. It is postulated that neurological toxicity can occur in about 1–3% of patients from literature reviews [46]. Most information collected about neurological toxicity from immunotherapy is from case reports. Posterior reversible encephalopathy syndrome, Guillain-Barre, aseptic meningitis, enteric neuropathy, and transverse myelitis cases have been reported [4, 13]. There have also been isolated reports of chronic inflammatory demyelinating polyneuropathy and a Myasthenia-Gravis type syndrome [47]. Most times, if the adverse event is low-grade, stopping the offending agent until symptoms dissipate suffices or commencing low-dose oral corticosteroids [18, 47]. In grade 3 or grade 4 events, high-dose intravenous corticosteroids are warranted, and at times, plasmapheresis and intravenous immunoglobulin are warranted [4, 13]. It is worthwhile to involve neurologists to assist with diagnosis and what treatment is necessary for each individual case according to severity [4, 13].

3.8. Hematological

The evidence regarding hematological side effects is all anecdotal and based on case reports as well. Severe anemia requiring transfusions and febrile neutropenia requiring support

with granulocyte colony stimulating factor (GCSF) may occur [4, 48]. One case reported a patient with neutropenia receiving a CTLA-4 inhibitor that was refractory to GCSF therapy and required immunoglobulin therapy [49]. Red cell aplasia, acquired hemophilia A, and thrombocytopenia have all been described as well [4, 13]. Recently, cases of hemolytic-uremic syndrome occurring in a patient receiving ipilimumab have been reported [50]. Generally, hematological immune-related adverse events respond to steroid therapy, but in severe cases, may need more intense therapy.

3.9. Renal

Renal toxicity due to checkpoint inhibitors is extremely rare. A case series of thirteen patients provides information of different clinical presentations of patients with immune-related nephritis and different histological diagnoses [51]. It showed that the median time to develop kidney injury from immune checkpoint inhibitors was around 91 days though it ranged widely. It is estimated that about 1–2% of patients can have acute kidney injury from checkpoint inhibitors, with less than 1% of those patients having a serious grade 3 or 4 events [15, 51]. Histology in these patients showed a dominance of tubule-interstitial nephritis, and in one patient, showed a thrombotic microangiopathy [51, 52]. Initiating corticosteroid early therapy and stopping drug is the recommended treatment for acute kidney injury/interstitial nephritis from checkpoint inhibitor therapy. Most patients respond to steroid therapy [15]. Other causes of kidney injury such as infection or other medications should be excluded, and when etiology is in doubt, a renal biopsy should always be performed if not contraindicated. Close monitoring of patient's serum creatinine should be followed during treatment, especially if there is even a slight increase in creatinine. Grade 1 toxicity according to management guidelines is defined as an increase in creatinine up to 1.5 times above baseline, grade 2 or grade 3, defined as a creatinine above 1.5 times above baseline to 6 times above normal. Grade 4 events are life-threatening [15]. Mycophenolate Mophetil in refractory cases can be considered and potentially anti-TNF agents [51]. Data regarding management in these patients is very limited, and general supportive measure should be carried out as well such as fluid therapy and correcting electrolytes. Early involvement with a nephrologist is recommended as there were dialyses-requiring patients in the series as well [15, 51].

3.10. Pancreatitis

There have been reports of elevated amylase and lipase levels in clinical trials with unknown clinical significance. It is not recommended in general guidelines to monitor pancreatic enzymes unless there is a clinical suspicion of active or acute pancreatitis. There have been very few case reports of patients who developed fulminant pancreatitis. General guidelines for immune-related adverse events should be followed with close monitoring in these patients [15, 43, 53].

3.11. Cardiac

This is also extremely rare. There are case reports of varying cardiac conditions in patients with toxicity form checkpoint inhibitors. In a series, eight cases of immune-related cardiac toxicity were reviewed. Patients were asymptomatic of any cardiac-related issues before

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initiating treatment with checkpoint inhibitors. Cases ranged from myocarditis and cardiomyopathy that responded well to corticosteroid therapy as well as cases that were fatal and refractory to treatment. Myocardial fibrosis was found in one patient's autopsy findings, in combination with multiorgan failure. The patients in this series were both very young and very old with no cardiac history and included patients with predisposing cardiac dysfunction. A patient also suffered a cardiac arrest. A total of 63% of patients had other organ systems involved in combination with the cardiac toxicity [54]. The review can allude to many hypotheses about cardiac related toxicity. There is a possibility of higher risk to develop cardiac toxicity if there are predisposing conditions and a higher incidence if there are other systems involved. As with other rare irAEs, more prospective data are needed. More case reports are emerging and include fulminant myocarditis and pericardial effusions with tamponade [55, 56]. It is clear that treating physicians need to be aware of the possibility of this irAEs and to start treatment with supportive and corticosteroid therapy promptly to avoid serious complications and death. There is currently no recommendations regarding monitoring of cardiac enzymes during therapy [54].

4. Conclusion

When managing a patient with suspected irAEs, the patients should be treated as individuals, and a thorough workup of each side effect should be done to ascertain whether or not there is truly an irAE and not other treatable causes. Most importantly, a high index of suspicion must always be kept in mind even though most are self-limiting and low-grade in severe cases if treatment is not given promptly and correctly, it can be life-threatening and result in death. Early recognition and aggressive treatment with immunosuppression is vital to prevent morbidity and mortality.

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References

[1] K. Ryungsa, E. Manabu and T. Kazuak. Cancer immunoediting from immune surveillance to immune escape. Immunology. Vol: 121, No: 1, pp. 1-14, 2007.

[2] T. Chen, A. Razak, P. Bedard et al. A systematic review of immune-related adverse event reporting in clinical trials of immune checkpoint inhibitors. Ann Oncol. Vol: 26, No: 9, pp. 1824-1829, 2015.

[3] A. Bertrand, M. Kostine, T. Barnetche. Immune-related adverse events associated with anti-CTLA-4 antibodies: systematic review and meta analysis. BMC Med. Vol: 13, p. 211, 2015.

[4] A. Tarhini. Immune-mediated adverse events associated with Ipilimumab CTLA-4 blockade therapy: the underlying mechanisms and clinical management. Scientifica (Cairo). Vol: 2013, p. 857519, 2013.

[5] J. M. Redman, G. T. Gibney, M. B. Atkins. Advances in immunotherapy for melanoma. BMC Med. Vol: 6, pp. 14-20, 2016.

[6] M. Tsiatas, P. Grivas. Immunobiology and immunotherapy in genitourinary malignancies. Ann Transl Med. Vol: 4, No: 14, p. 270, 2016.

[7] K. J. Lafferty, H. S. Warren, J. A. Woolnough. Immunological induction of T lymphocytes: role of antigen and the lymphocyte costimulator. Blood Cells. Vol: 4, No: 3, pp. 395-406, 1978.

[8] D. M. Pardoll. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. Vol: 12, No: 4, pp. 252-264, 2012.

[9] C. Blank, T. F. Gajewski, A. Mackensen. Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: implications for tumor immunotherapy. Cancer Immunol Immunother. Vol: 54, No: 4, pp. 307-314, 2005.

[10] J. Weber, K. Kahler, A. Hauschild. Management of immune-related adverse events and kinetics of response with ipilimumab. J Clin Oncol. Vol: 30, No: 21, pp. 12691-12697, 2015.

[11] J. Weber, R. Dummer, V. de Pril et al. Patterns of onset and resolution of immune-related adverse events of special interest with ipilimumab: detailed safety analysis from a phase 3 trial in patients with advanced melanoma. Cancer. Vol: 119, No: 9, pp. 1675-1682, 2013.

[12] J. Larkin, V. Chiarion-Sileni, R. Gonzalez. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. Vol: 373, No: 1, pp. 23-34, 2015.

[13] M. Postow. Managing immune checkpoint-blocking antibody side effects. Am Soc Clin Oncol Educ Book. pp. 76-83, 2015.

[14] J. Liu, S. Blake, M. Smyth et al. Improved mouse models to assess tumour immunity and irAEs after combination cancer immunotherapies. Clin Transl Immunol. Vol: 3, No: 8, e22, 2014.

[15] J. Villadolid, A. Amin. Immune checkpoint inhibitors in clinical practice: update on management of immune-related toxicities. Transl Lung Cancer Res. Vol: 4, No: 5, pp. 560-575, 2015.

[16] Y. Bronstein, C. Ng, P. Hwu et al. Radiologic manifestations of immune-related adverse events in patients with metastatic melanoma undergoing anti–CTLA-4 antibody therapy. AJR Am J Roentgenol. Vol: 197, No: 6, pp. W992-W1000, 2011.

[17] B. Teply, E. Lipson. Identification and management of toxicities from immune checkpoint-blocking drugs. Oncology (Williston Park). Vol: 28 Suppl 3, pp. 30-38, 2014.

http://dx.doi.org/10.5772/67372

[18] Yervoy (ipilimumab). Princeton, NJ: Bristol-Myers Squibb, 2013 (package insert).

[19] C. Hua, L. Boussemart, C. Mateus et al. Association of vitiligo with tumor response in patients with metastatic melanoma treated with pembrolizumab. JAMA Dermatol. Vol: 152, No: 1, pp. 45-51, 2016.

[20] H. Teulings, J. Limpens, S. Jansen et al. Vitiligo-Like depigmentation in patients with stage III–IV melanoma receiving immunotherapy and its association with survival: a systematic review and meta-analysis. J Clin Oncol. Vol: 33, No: 7, pp. 773-781, 2015.

[21] K. Minkis, B. C. Garden, S. Wu et al. The risk of rash associated with ipilimumab in patients with cancer: a systematic review of the literature and meta-analysis. J Am Acad Dermatol. Vol: 69, No: 3, pp. e121-e128, 2013.

[22] O. Abdel-Rahman, H. ElHalawani, M. Fouad. Risk of cutaneous toxicities in patients with solid tumors treated with immune checkpoint inhibitors: a meta-analysis. Future Oncol. Vol: 11, No: 17, pp. 2471-2484, 2015.

[23] J. S. Weber, S. P. D'Angelo, D. Minor et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol. Vol: 16 No: 4, pp. 375-384, 2015.

[24] C. Friedman and M. Postow. Managing immunotherapy-related side effects. Oncol Hematol Rev. Vol: 11, No: 2, pp. 143-144, 2015.

[25] F. S. Hodi, S. J. O'Day, D. F. McDermott, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. Vol: 363, No: 8, pp. 711-723, 2010.

[26] C. Robert, L. Thomas, I. Bondarenko et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. Vol: 364, No: 26, pp. 2517-2526, 2011.

[27] X. Zhang, Y. Ran, K. Wang, et al. Incidence and risk of hepatic toxicities with PD-1 inhibitors in cancer patients: a meta-analysis. Drug Des Devel Ther. Vol: 10, pp. 3153-3161, 2016.

[28] R. Cheng, A. Cooper, J. Kench et al. Ipilimumab-induced toxicities and the gastroenterologist. J Gastroenterol Hepatol. Vol: 30, No: 4, pp. 657-666, 2015.

[29] S. M. Corsello, A. Barnabei, P. Marchetti et al. Endocrine side effects induced by immune checkpoint inhibitors. J Clin Endocrinol Metab. Vol: 98, No: 4, pp. 1361-1375, 2013.

[30] L. Min, A. Vaidya, C. Becker. Thyroid autoimmunity and ophthalmopathy related to melanoma biological therapy. Eur J Endocrinol. Vol: 164, No: 2, pp. 303-307, 2011.

[31] M. Ryder, M. Callahan, MA Postow et al. Endocrine-related adverse events following ipilimumab in patients with advanced melanoma: a comprehensive retrospective review from a single institution. Endocr Relat Cancer. Vol: 21 No: 2, pp. 371-381, 2014.

[32] O. Abdel-Rahman, H. ElHalawani, M. Fouad. Risk of endocrine complications in cancer patients treated with immune checkpoint inhibitors: a meta-analysis. Future Oncol. Vol: 12, No: 3, pp. 413-425, 2016.

[33] K. Carpenter, R. Murtagh, H. Lilienfeld et al. Ipilimumab-induced hypophysitis: MR imaging findings. AJNR Am J Neuroradiol. Vol: 30, No: 9, pp. 1751-1753, 2009.

[34] T. Tokudome. Anti-CTLA-4 antibodies immunotherapy of cancer. Chapter 18, pp. 263-282, Kurashiki, Japan. Springer.

[35] Faje A. Immunotherapy and hypophysitis: clinical presentation, treatment, and biologic insights. Pituitary. Vol:19, pp 82-92, 2016.

[36] S. L. Topalian, F. S. Hodi, J. R. Brahmer et al. Safety, activity, and immune correlates of anti-PD-1 anti-body in cancer. N Engl J Med. Vol: 366: No: 26, pp. 2443-2454, 2012.

[37] O. Abdel-Rahman, M. Fouad. Risk of pneumonitis in cancer patients treated with immune checkpoint inhibitors: a meta-analysis. Ther Adv Respir Dis. Vol: 10, No: 3, pp. 183-193, 2016.

[38] M. Nishino, A. Giobbie-Hurder, H. Hatabu et al. Incidence of programmed cell death 1 inhibitor-related pneumonitis in patients with advanced cancer: a systematic review and meta-analysis. JAMA Oncol. Vol: 2, Published online August 18, 2016. doi:10.1001/jamaoncol.2016.2453.

[39] C. Kim, J. Gao, VR Shannon et al. Systemic sarcoidosis first manifesting in a tattoo in the setting of immune checkpoint inhibition. BMJ Case Rep. Vol: 18, 2016 Oct 26;2016. pii: bcr2016216217.

[40] K. C. Suozzi, M. Stahl, C. J. Ko, et al. Immune-related sarcoidosis observed in combination ipilimumab and nivolumab therapy. JAAD Case Rep. Vol: 2, No: 3, pp. 264-268, 2016.

[41] P. Fragkou, M. Souli, M. Theochari et al. A case of organizing pneumonia (OP) associated with pembrolizumab. Drug Target Insights. Vol: 10, pp. 9-12, 2016.

[42] M. R. Robinson, C. C. Chan, J. C. Yang et al. Cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma: a new cause of uveitis. J Immunother. Vol: 27, No: 6, pp. 478-479, 2004.

[43] M. Postow, J. Wolchok. Toxicities associated with checkpoint inhibitor immunotherapy. UPTODATE http://www.uptodate.com/contents/toxicities-associated-with-checkpoint-inhibitor-immunotherapy.

[44] T. Hager and B. Seitz. Ocular side effects of biological agents in oncology: what should the clinician be aware of?. Onco Targets Ther. Vol: 7, pp. 69-77, 2014.

[45] B. S. Modjtahedi, H. Maibach, S. Park. Multifocal bilateral choroidal neovascularization in a patient on ipilimumab for metastatic melanoma. Cutan Ocul Toxicol. Vol: 32, No: 4, pp. 341-343, 2013.

[46] L. Spain, G. Walls, M. Julve et al. Neurotoxicity from immune-checkpoint inhibition in the treatment of melanoma: a single centre experience and review of the literature. Ann Oncol. Published online October 25, 2016.

http://dx.doi.org/10.5772/67372

[47] B. Liao, S. Shroff, C. Kamiya-Matsuoka et al. Atypical neurological complications of ipilimumab therapy in patients with metastatic melanoma. Neuro Oncol. Vol: 16, No: 4, pp. 589-593, 2014.

[48] E. Simeone, A. M. Grimaldi, A. Esposito et al. Serious haematological toxicity during and after ipilimumab treatment: a case series. J Med Case Rep. Vol: 8, p. 240, 2014.

[49] M. Akhtari, E. K. Waller, D. L. Jaye et al. Neutropenia in a patient treated with ipilimumab (anti-CTLA-4 Antibody). J Immunother. Vol: 32, No: 3, pp. 322-324, 2009.

[50] R. Nair, S. Gheith, S. Nair et al. Immunotherapy-associated hemolytic anemia with pure red-cell aplasia. N Engl J Med. Vol: 374, No: 11, pp. 1096-1097, 2016.

[51] F. B. Cortazar, K. A. Marrone, M. L. Troxell et al. Clinicopathological features of acute kidney injury associated with immune checkpoint inhibitors. Kidney Int. Vol: 90, No: 3, pp. 638-647, 2016.

[52] N. Murakami, T. J. Borges, M. Yamashita et al. Severe acute interstitial nephritis after combination immune-checkpoint inhibitor therapy for metastatic melanoma. Clin Kidney J. Vol: 9, No: 3, pp. 411-417, 2016.

[53] A. M. Di Giacomo, R. Danielli, M. Guidoboni et al. Therapeutic efficacy of ipilimumab, an anti-CTLA-4 monoclonal antibody, in patients with metastatic melanoma unresponsive to prior systemic treatments: clinical and immunological evidence from three patient cases. Cancer Immunol Immunother. Vol: 58, No: 8, pp. 1297-1306, 2009.

[54] D. B. Johnson, J. M. Balko, M. L. Compton et al. Fulminant myocarditis with combination immune checkpoint blockade. N Engl J Med. Vol: 375, No: 18, pp. 1749-1755, 2016.

[55] I. Kushnir, I. Wolf. Nivolumab-induced pericardial tamponade: a case report and discussion. Cardiology. Vol: 136, No: 1, pp. 49-51, 2016.

[56] L. Heinzerling, P. A. Ott, F. S. Hodi et al. Cardiotoxicity associated with CTLA4 and PD1 blocking immunotherapy. J Immunother Cancer. Vol: 4, p. 50, 2016.

Chapter 3

Immune Checkpoint Blockade and Adaptive Immune Resistance in Cancer

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Additional information is available at the end of the chapter

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Abstract

The clinical success of immune checkpoint blockers is a pivotal advancement for treating an increasing number of cancer types. However, immune checkpoint blockers still rarely induce complete remission and show little to no therapeutic efficacy in a significant percentage of cancer patients. Efforts are now underway to identify biomarkers that accurately predict which patients benefit from immune checkpoint blockers. Moreover, adaptive immune resistance can develop in tumors during treatment with immune checkpoint blockers. These adaptive resistance mechanisms in tumors might be disrupted by combining adjunctive immunotherapies, which could potentially improve the therapeutic efficacy of immune checkpoint blockers. This chapter discusses the mechanism of action of cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) immune checkpoint blockers and biomarkers that might predict clinical responses to these drugs. Lastly, ongoing research on mechanisms of tumor adaptive resistance could facilitate rationale design of adjunctive immunotherapies that can be synergistically combined with immune checkpoint blockers to more effectively treat cancer.

Keywords: immunotherapy, T lymphocytes, immune checkpoints, CTLA-4, PD-1, PD-L1

1. Introduction

Immune checkpoints are inhibitory pathways that are critical for maintaining self-tolerance. Immune checkpoints also control the magnitude and duration of physiological immune responses in peripheral tissues in order to minimize collateral damage. Immune checkpoint receptors and their cognate ligands are naturally expressed on a variety of cell types, including antigen-presenting cells, T cells, B cells, tumor cells, tumor stroma, and also normal tissue.

A number of immune checkpoint pathways have been identified, including cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed death-1 (PD-1), programmed death ligand-1 (PD-L1), T cell immunoglobulin and mucin domain 1 (TIM-1), T cell immunoglobulin and mucin domain 3 (TIM-3), lymphocyte-activation gene 3 (LAG-3), T cell immunoreceptor with Ig and ITIM domains (TIGIT), V-domain Ig suppressor of T cell activation (VISTA), carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), leukocyte-associated immunoglobulin-like receptor 1 (LAIR-1), herpesvirus entry mediator (HVEM), B- and T-lymphocyte attenuator (BTLA), CD160, CD200, CD200 receptor, and adenosine 2A receptor (A2Ar). For brevity, this chapter will focus on CTLA-4 and PD-1/PD-L1, as clinical drugs targeting these pathways have been successfully developed to treat an increasing variety of human cancer types.

2. Main body

2.1. CTLA-4

CTLA-4 is the first immune checkpoint receptor to be clinically targeted. CTLA-4 is expressed mainly on the surface of activated T cells. While certain subsets of T regulatory cells constitutively express CTLA-4, it is virtually undetectable on naïve, inactivated T cells. Upon activation, both $CD4^+$ and $CD8^+$ T cells upregulate CTLA-4 on the surface, reaching maximum level within 2–3 days. $CD4^+$ T cells are reported to express more CTLA-4 mRNA and protein compared to $CD8^+$ T cells, suggesting that CTLA-4 has a more significant regulatory effect on $CD4^+$ T cells [1].

CTLA-4 downregulates T cell activation by sequestering CD80 and CD86 costimulatory molecules on antigen-presenting cells. This prevents CD80 and CD86 from delivering costimulatory activation signals to T cells through the CD28 receptor. CTLA-4 binds to CD80 and CD86 with ~10 times higher affinity than CD28 [2]. CTLA-4 expressed on T cells can also remove CD80 and CD86 molecules from neighboring antigen-presenting cells through a process called trans-endocytosis [3]. CTLA-4 also prevents CD28 recruitment to the immunological synapse, further impairing T cell activation [4].

CTLA-4 knockout mice die within 2–3 weeks of age due to massive lymphoproliferation, resulting in destruction of vital organs [5]. This lethal phenotype is associated primarily with hyperactivated $CD4^+$ T cells, which are skewed toward a T helper type-2 phenotype and have increased resistance to apoptosis. These hyperactivated $CD4^+$ T cells abnormally infiltrate into peripheral tissues, resulting in organ failure. These observations led cancer immunology researchers to hypothesize that blockade of CTLA-4 signaling could potentially induce effective T cell-mediated immune responses against tumor tissue.

A pivotal laboratory study reported in 1996 by James Allison's group showed that treatment of tumor-bearing mice with a CTLA-4-blocking antibody could effectively induce tumor regression [6]. Despite much subsequent investigation, the in vivo mechanism of action of CTLA-4 blockade immunotherapy has remained elusive. The prevailing hypothesis is that CTLA-4 blockade not only enhances T cell infiltration into tumors but also reduces the

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relative presence of immunosuppressive T regulatory cells in tumor tissue [7]. This alteration in the ratio of effector T cells versus T regulatory cells in tumors tilts the immunological balance in favor of T cell-mediated destruction of tumor cells.

These studies led to pharmaceutical development of the first immune checkpoint blocker, ipilimumab (Yervoy®). Ipilimumab is a fully human monoclonal antibody that blocks the CTLA-4 receptor, thereby preventing its ability to sequester CD80 and CD86 costimulatory molecules. It was initially tested in melanoma, and demonstrated extended overall survival in patients versus a comparator melanoma peptide-based immunotherapy vaccine called gp100. In a randomized phase III clinical trial, melanoma patients receiving ipilimumab had a median overall survival of 10.4 months versus 6.4 months in those receiving only the gp100 peptide vaccine (Hodi 2010). Objective response rates (measureable tumor regression) were 10.9% in the ipilimumab group versus 1.5% in the gp100 vaccine group. The responses to ipilimumab were durable, with the 1-year and 2-year survival rate being 46 and 24%, respectively. By comparison, the 1-year and 2-year survival rate in patients receiving only the gp100 peptide vaccine was only 25 and 14%, respectively [8]. These trial results led to US FDA approval of ipilimumab for melanoma in 2011.

2.2. PD-1

PD-1 is another major immune checkpoint receptor that regulates T cell activity against tumor tissue. PD-1 is a cell surface receptor originally identified in a murine T cell hybridoma undergoing programmed cell death [9]. PD-1 is absent on naïve inactivated immune cells but is significantly upregulated on activated T cells, B cells, natural killer cells and myeloid-derived cells [10]. In T cells, PD-1 expression is induced by T cell receptor signaling [11] and also by certain pro-inflammatory cytokines including interleukin-2, interleukin-7, interleukin-15, and interleukin-21 [12].

PD-1 signaling downregulates T cell activity primarily via interaction with its two natural ligands: Programmed Death Ligand-1 (PD-L1) and Programmed Death Ligand-2 (PD-L2). PD-L1 is expressed on a wide variety of cell types including hematopoietic cells, T cells, B cells, myeloid cells, and dendritic cells [10]. It is also expressed on a wide variety of peripheral tissues such as skeletal muscle, lung, heart, and placenta [10]. Notably, PD-L1 is also expressed on a wide variety of cancer cells and generally is associated with poorer patient prognosis [13]. PD-L2 expression is generally more restricted, being found primarily on dendritic cells, macrophages, and occasionally cancer cells [14]. PD-L2 binds to PD-1 with two- to sixfold higher relative affinity than PD-L1 [15]. However, PD-L2 is generally expressed at lower relative levels [16]. Thus, it is believed that PD-L1 is the predominant ligand for PD-1.

Signaling through the PD-1 receptor on T cells results in downstream inhibition of PI3K/AKT activation [17]. The net effect is downregulation of a number of effector functions including cytokine secretion and cytolytic activity. PD-1 knockout mice have various autoimmune pathologies, including autoantibody-induced cardiomyopathy [18], arthritis and lupus-like disease [19], and diabetes [20]. In peripheral tissues, the immunosuppressive activity of PD-1 is mediated primarily by interaction with PD-L1 [21]. PD-L1 expressed in tumor tissue also impairs host antitumor immune responses [22]. PD-L1 and/or PD-L2 in tumor tissue facilitates

evasion from host immune responses via multiple mechanisms including induction of T cell anergy and exhaustion [23], promoting T cell apoptosis [24], and also by enhancing the expansion and activity of immunosuppressive T regulatory cells [25]. Moreover, PD-1 can transmit an antiapoptotic signal to PD-L1-expressing tumor cells, which renders them resistant to lysis by cytotoxic T lymphocytes [26].

This fundamental understanding of the PD-1/PD-L1 axis in suppressing host antitumor immune responses led to development of the first clinical PD-1 blockers, nivolumab (Opdivo®) and pembrolizumab (Keytruda®). Both nivolumab and pembrolizumab are fully human monoclonal antibodies that block the PD-1 receptor, thereby preventing its ability to bind its natural ligands PD-L1 and PD-L2. In large phase I clinical trials, nivolumab and pembrolizumab each demonstrated durable clinical response rates with acceptable safety profiles in patients with advanced melanoma, non-small cell lung cancer, renal cell carcinoma or Hodgkin's lymphoma [27–30]. Nivolumab and pembrolizumab are now both FDA approved for treating melanoma and non-small cell lung cancer. Nivolumab is additionally approved for treating renal cell carcinoma, Hodgkin's lymphoma, and also for use in combination with the CTLA-4 blocker, ipilimumab, for treating melanoma. Remarkably, in two separate melanoma clinical trials, the combination of nivolumab and ipilimumab induced objective responses in ~60% of patients, with complete responses seen in ~11.5–22% of patients [31–32].

Pembrolizumab and nivolumab (and a third investigational PD-1 blocker, pidilizumab) are now collectively continuing in 500+ clinical trials. Virtually all cancer types are now being targeted with PD-1/PD-L1 blockers in some capacity. Notably, there is a significant effort to test nivolumab or pembrolizumab with other adjunctive therapies to determine synergistic combinatorial regimens. Conventional treatments like chemotherapy and radiation have shown in animal tumor models to potentially synergize with PD-1/PD-L1 blockers [33–35]. In addition, PD-1 blockers are now also being tested in combination with small molecule drugs (investigational and Food and Drug Administration (FDA) approved) and also experimental immunotherapies such as vaccines and chimeric antigen receptor T cells.

All clinical PD-1 blockers have the same mechanism of action. Slight variances in the protein structure among different PD-1 blockers could potentially confer differences in binding affinity for the PD-1 receptor and also differences in half-life (i.e. persistence in the body). The physiological significance and clinical effectiveness of such variances remain undetermined.

2.3. PD-L1

Expression of PD-L1 is found on diverse cell types, including normal and malignant tissue, antigen presenting cells, myeloid cells, B cells, and T cells. PD-L1 downregulates T cells via multiple mechanisms. PD-L1 expressed on various cells primarily interacts with PD-1 expressed on T cells, delivering an inhibitory signal that downregulates T cell activity. PD-L1 also binds to CD80 expressed on both antigen-presenting cells and activated T cells [36]. Interaction of PD-L1 with CD80 on antigen-presenting cells prevents CD80 from delivering costimulatory activating signals to T cells. When PD-L1 binds to CD80 expressed on activated T cells, an inhibitory signal is delivered to T cells. Currently, it is unknown exactly what intracellular

signaling pathways are altered when PD-L1 binds to CD80 on T cells. Nonetheless, it is now generally understood that blocking PD-L1 results in enhanced T cell activation.

Atezolizumab (Tecentriq®) was the first PD-L1 blocker to enter clinical trials. Atezolizumab is a fully human monoclonal antibody that prevents PD-L1 from binding to PD-1 and CD80. It was initially tested in patients with PD-L1-positive metastatic bladder cancer [37]. Bladder cancer patients with PD-L1-negative tumors were subsequently included for treatment. Clinical response rates were ~15% of PD-L1-negative patients and ~25% of PD-L1-positive patients [37]. Because of the higher clinical activity of atezolizumab in PD-L1-positive bladder cancer, a companion diagnostic called the Ventana PD-L1 (SP142) assay is offered to provide tumor PD-L1 expression status of patients considering atezolizumab treatment. In 2016, atezolizumab was FDA approved for urothelial carcinoma, the most common form of bladder cancer. Like nivolumab and pembrolizumab PD-1 blockers, atezolizumab is now continuing in clinical trials for a wide variety cancer types and also being tested in combination with conventional cancer treatments, small molecule drugs and other investigational immunotherapies. Alternative PD-L1 blockers, such as avelumab and durvalumab, are also now in clinical trials.

2.4. Predictive biomarkers for CTLA-4 and PD-1/PD-L1 blockers

CTLA-4 and PD-1/PD-L1 immune checkpoint blockers have proven to be pivotal advancements in cancer treatment. However, a significant proportion of cancer patients still experience little to no clinical benefit from treatment. Even among responding patients, only a small minority achieve complete remission. Studies using clinical tumor specimens from patients treated with immune checkpoint blockers have revealed some potentially important differences between responders versus nonresponders.

During early clinical development of PD-1 blockers, it was hypothesized that differential expression levels of PD-L1 in tumor tissue would correlate with clinical responses. It was anticipated that PD-L1 expression in tumor tissue could therefore be a predictive biomarker to accurately identify patients likely to respond to PD-1 or PD-L1 blockers. However, a definitive correlation has thus far not been established. Both PD-L1-positive and PD-L1-negative tumors can respond to PD-1 or PD-L1 blockers. Further confounding factors include variability of PD-L1 expression in different anatomical areas of tumor tissue. In addition, PD-L1 expression in tumor tissue may be transient—appearing and disappearing due to treatments or other poorly understood influences. Lastly, assays measuring PD-L1 in tumors have yet to establish a clear threshold of expression that defines what is considered "PD-L1-positive." For instance, the FDA-approved Ventana PD-L1 assay defines ≥5% PD-L1-positive cells in bladder cancer tissue to be associated with higher clinical response rates to atezolizumab [38]. However, alternative PD-L1 assays used in various other clinical trials of nivolumab or pembrolizumab have wide variability in PD-L1 expression analysis methodologies. Overall, it is generally agreed upon that low or absent PD-L1 expression in tumors is not sufficient to preclude a patient from treatment with PD-1/PD-L1 blockers [39].

Alternative predictive biomarkers for clinical response to PD-1/PD-L1 blockers are currently being explored. $CD8^+$ T cell infiltration into tumors might be predictive of clinical response to

PD-1 blockers. Specifically, the density of pretreatment $CD8^+$ T cells at both the tumor invasive margin and tumor center may be correlated with clinical response to pembrolizumab. In serially biopsied tumors from melanoma patients undergoing pembrolizumab treatment, it was shown that responding patients generally had higher densities of $CD8^+/PD\text{-}1^+$ cells in close proximity to PD-L1-expressing tumor cells [40]. Furthermore, serial analysis of tumor biopsies showed that intratumoral $CD8^+/PD\text{-}1^+$ T cells actively proliferate during pembrolizumab treatment [40]. These data offer insights on a potential mechanism of PD-1 blockade efficacy, whereby presence of pretreatment $CD8^+$ T cells in tumors is a prerequisite for clinical response. However, like tumor PD-L1 expression assays, establishing a standard cut-off threshold value for $CD8^+$ T cell levels in tumors that accurately predicts clinical response to PD-1/PD-L1 blockade will be challenging. Tumors of various tissue origins often contain infiltrating T cells that can vary greatly in absolute number, density, and also anatomical location within the intratumoral space. Nonetheless, establishing a "scoring system" based on pretreatment $CD8^+$ T cell infiltration warrants further investigation as a potential predictive biomarker.

Another intriguing biomarker with predictive potential may be intratumoral expression of indoleamine-2,3-dioxygenase (IDO). IDO is a tryptophan catabolizing enzyme that is occasionally expressed in various tumor types. Depletion of tryptophan within tumors by IDO may be a rate-limiting step for effective antitumor T cell activity. Studies in melanoma patients treated with ipilimumab suggest a correlation between pretreatment IDO expression and clinical response. In one study, intratumoral IDO was detected in 37.5% of responding melanoma patients and only 11.1% in nonresponders [41]. It remains to be seen if similar patterns are seen in other cancer types and also patients treated with PD-1/PD-L1 blockers.

Genetic signatures of tumors are yet another parameter with potential for yielding predictive biomarkers for clinical response to immune checkpoint blockers. Certain tumors, such as colorectal cancer, are highly refractory to treatment with PD-1 blockers. In early clinical trials of nivolumab, it was found that only 1 in 33 colorectal cancer patients responded to treatment [27–28]. Subsequently, it was hypothesized that the single responding colorectal cancer patient harbored a defect in DNA mismatch repair in tumor tissue, resulting in a significantly high load of somatic mutations [42]. Defects in tumor tissue mismatch repair can result in thousands of somatic mutations, providing a larger pool of neo-antigens for immune recognition. Immune checkpoint blockade therapy could therefore amplify the natural adaptive immune response to mutated neo-antigens. Hence, mutational load in pretreatment tumor tissue might be predictive of clinical response to immune checkpoint blockers. To test this hypothesis, a small clinical trial focusing primarily on colorectal cancer showed that patients with defects in tumor tissue mismatch repair harbored significantly higher loads of somatic mutations versus those with mismatch repair-proficient tumors. Upon treatment with pembrolizumab, higher response rates and longer survival times were seen in patients with mismatch repair defects versus those with proficient mismatch repair [42]. This pivotal study has catalyzed further investigation of tumor mutational profiles to determine if a correlation with clinical responses can be established in large studies of diverse cancer types.

2.5. Adaptive immune resistance

Mechanisms of inherent and acquired resistance to immune checkpoint blockade are poorly understood. Clinical responses to CTLA-4 and PD-1/PD-L1 blockers are often durable, sometimes lasting years. However, complete regressions are still relatively rare and eventual disease relapse among responding patients is frequent. Recent studies have offered insights that immunological parameters of tumor tissue adapt in response to T cell-mediated attack induced by immune checkpoint blockers. Enhanced T cell activity within tumors involves local production of inflammatory mediators, such as interferon (IFN)-γ, which is known to upregulate PD-L1 on peripheral tissues [43]. Upregulation of PD-L1 on various cell types within tumor tissue might result in heightened CD80-mediated inhibition of proximal effector T cells.

Furthermore, augmentation of effector T cell activity in tumor tissue via PD-1 blockade may subsequently induce compensatory upregulation of alternative immune checkpoint receptors, TIM-3. TIM-3 is a receptor expressed primarily on IFN-γ-secreting $CD4^+$ and $CD8^+$ T cells [44]. TIM-3 is bound by multiple ligands, including galectin-9, CEACAM-1, and high-mobility group box 1 (HMGB-1). Signaling through TIM-3 in activated T cells triggers the release of human leukocyte antigen B-associated transcript 3 (BAT3) from the TIM-3 cytoplasmic domain. This results in defective production of IL-2, IFN-γ, and likely other pro-inflammatory cytokines [44]. Although the TIM-3 signaling pathway has yet to be fully elucidated, it seems clear that TIM-3 affects T cell receptor downstream signaling via a mechanism distinct from PD-1 and CTLA-4.

TIM-3 appears to be co-expressed with PD-1 in tumor-infiltrating lymphocytes of cancer patients and is upregulated on T cells upon therapeutic PD-1 blockade [45]. This may provide a mechanism of immunological escape and a possible reason for incomplete clinical responses upon PD-1 blockade immunotherapy. It might also be a contributing factor toward acquired resistance to PD-1 blockade clinically, whereby patients initially respond to treatment but eventually relapse despite continuous therapy. Preclinical studies in animal tumor models show that PD-1 blockade immunotherapy results in upregulation of TIM-3 on T cells. Co-blockade of both TIM3 and PD-1 can prevent resistance to PD-1 blockade immunotherapy [45]. As such, TIM-3 blocking antibodies are now in early phase clinical trials to evaluate their safety, tolerability, and dosing ranges. **Figure 1** illustrates how PD-1/PD-L1 blockade may result in compensatory upregulation of TIM-3 and/or PD-L1 on T cells and tumor cells.

Downregulation of major histocompatibility (MHC) receptor expression in tumors might also contribute to acquired resistance to PD-1 blockers. Loss-of-function mutations in the MHC beta-2 microglobulin antigen-presenting protein have been noted in selected melanoma patients who initially responded to pembrolizumab therapy but subsequently relapsed [46]. Further studies in larger patient populations are necessary to confirm the association of MHC-related mutations and acquired resistance to PD-1 blockers.

2.6. Strategies to counteract adaptive resistance to immune checkpoint blockade

The mechanism of inherent and acquired/adaptive resistance to CTLA-4 and PD-1/PD-L1 immune checkpoint blockers is not fully understood and could possibly vary between

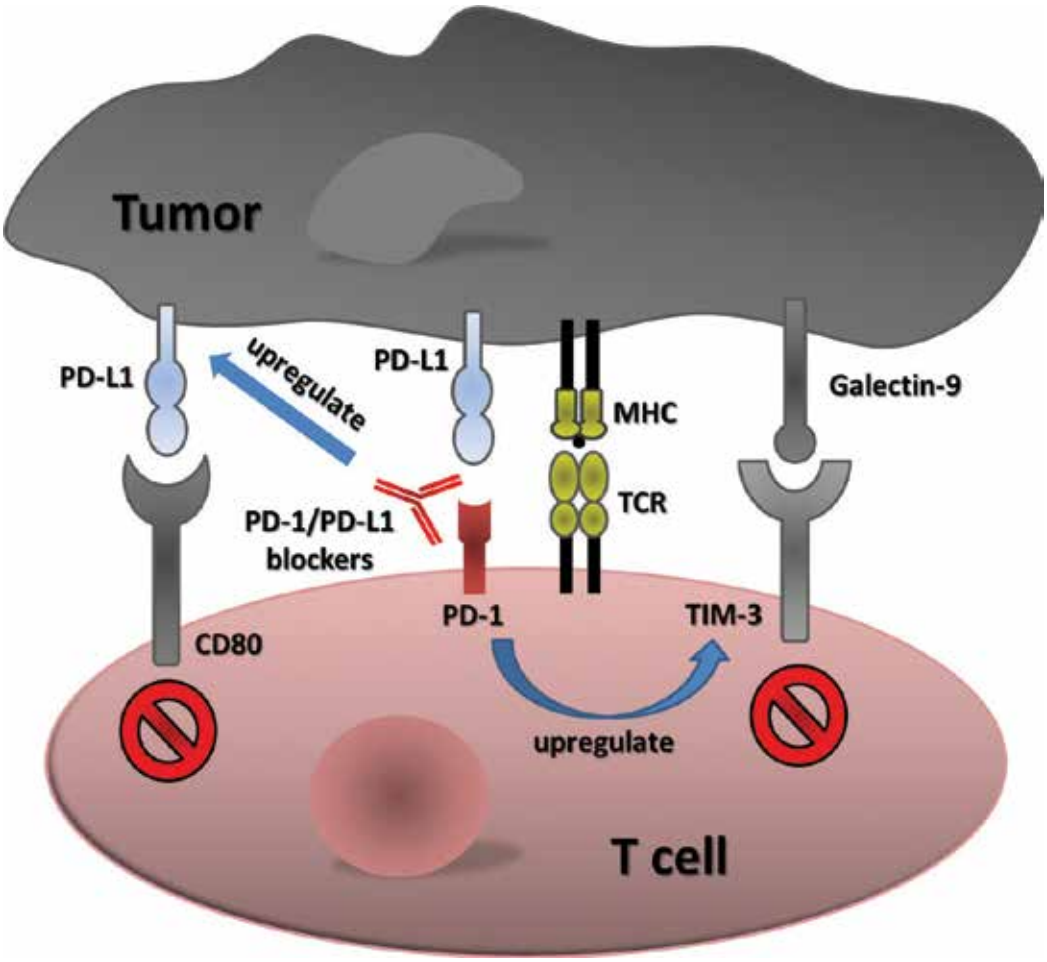

Figure 1. PD-1/PD-L1 blockade promotes T cell-mediated inflammation in tumors. In turn, this can trigger upregulation of PD-L1 on various cells within tumor tissue. This can also trigger compensatory upregulation of TIM-3 on effector T cells. Upregulation of PD-L1 and TIM-3, even during continuous treatment with PD-1 blockers, can impair T cell activity and result in clinical resistance.

individual patients and different tumor types. However, research on predictive biomarkers and mechanisms of adaptive resistance to PD-1 blockers have yielded insight that might be extrapolated to rationally design combination immunotherapies that synergistically enhance the efficacy of immune checkpoint blockers. For instance, it is now generally understood that PD-1 blockers augment T cell-mediated inflammation in tumor tissue. In turn, this can promote upregulation of PD-L1 on various cells in tumors, likely due to IFN-γ signaling [43]. Upregulation of PD-L1 expression in tumor tissue can promote enhanced CD80 signaling in T cells, which impairs T cell activity [36]. PD-1 blockade may also induce compensatory upregulation of alternative immune checkpoint receptors, such as TIM-3, on T cells within tumor tissue [45]. TIM-3 signaling results in downregulation of T cell activity. Next-generation immunotherapeutic regimens might combine PD-1 blockers such as

nivolumab/pembrolizumab with PD-L1 blockers like atezolizumab, to counteract PD-L1 upregulation induced by T cell-mediated inflammation in tumor tissue. Other rational combinations might include PD-1/PD-L1 blockers combined with investigational TIM-3 blockers, to counteract the effects of TIM-3 upregulation on activated T cells.

Another strategy to enhance the efficacy of immune checkpoint blockers might involve improving T cell trafficking to tumor tissue. The extent of T cell infiltration into tumor tissue may be a predictive biomarker and a prerequisite for efficacy of both CTLA-4 and PD-1/PD-L1 blockers. As such, therapies that promote T cell trafficking to tumors could potentially improve tumor sensitivity to immune checkpoint blockers. Studies of human melanoma tumors have identified a set of chemokines that are associated with enhanced recruitment of T cells toward tumor tissue. These chemokines, including CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL10, might have utility as clinical therapies to improve T cell trafficking to tumors [47]. However, such chemokines or other T cell recruitment factors must be targeted specifically to tumor tissue in order to effectively recruit T cells. T cell recruitment factors might be coupled to antibodies that bind to tumor cell receptors, thus providing a vehicle for tumor targeting. In animal tumor studies, a T cell recruitment factor called LIGHT (also called tumor necrosis factor superfamily member 14) was fused to an anti-epidermal growth factor receptor (EGFR) antibody. This LIGHT-anti-EGFR fusion molecule was able to promote more extensive T cell infiltration into EGFR-expressing tumors. In turn, this prevented resistance to PD-L1 blockade immunotherapy [48]. Similar strategies that target other T cell recruitment factors toward tumors might be feasible.

Our group at the Pacific Heart, Lung & Blood Institute (Los Angeles, CA) is conducting research on gene-modified human mesenchymal stem cells (MSCs) as a strategy to alter the tumor microenvironment and prevent resistance to immune checkpoint blockers. MSCs can be isolated and expanded from various adult tissues including bone marrow, fat, umbilical cord blood, and term placentas. MSCs are known to preferentially migrate to tumor tissue, making them potentially useful drug delivery vectors to alter the immunological microenvironment of tumors [49]. In animal tumor models, MSCs have been genetically modified in diverse ways to effectively treat tumors. These include modification to produce immunostimulatory cytokines (e.g. IFN-α, IFN-β, IL-12) and T cell trafficking molecules such as LIGHT [50–53].

Both autologous and allogeneic MSCs have been used extensively in clinical trials for treating severe inflammatory disorders and certain degenerative conditions, and generally have an acceptable safety profile [54]. Autologous gene-modified MSCs have recently entered clinical trials for cancer [55]. It remains to be seen if MSCs and other tumor-targeting systems can effectively deliver pro-inflammatory agents to tumor tissue and improve sensitivity to clinical immune checkpoint blockers.

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References

[1] Chan DV, Gibson HM, Aufiero BM, Wilson AJ, Hafner MS, Mi QS, Wong HK. Differential CTLA-4 expression in human CD4+ versus CD8+ T cells is associated with increased NFAT1 and inhibition of CD4+ proliferation. *Genes Immun*. 15(1):25–32; 2014.

[2] van der Merwe PA, Bodian DL, Daenke S, Linsley P, Davis SJ. CD80 (B7-1) binds both CD28 and CTLA-4 with a low affinity and very fast kinetics. *J Exp Med*. 185(3):393–403; 1997.

[3] Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, Baker J, Jeffery LE, Kaur S, Briggs Z, Hou TZ, Futter CE, Anderson G, Walker LS, Sansom DM. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science*. 332(6029):600–3; 2011.

[4] Greene JL, Leytze GM, Emswiler J, Peach R, Bajorath J, Cosand W, Linsley PS. Covalent dimerization of CD28/CTLA-4 and oligomerization of CD80/CD86 regulate T cell costimulatory interactions. *J Biol Chem*. 271(43):26762–71; 1996.

[5] Khattri R, Auger JA, Griffin MD, Sharpe AH, Bluestone JA. Lymphoproliferative disorder in CTLA-4 knockout mice is characterized by CD28-regulated activation of Th2 responses. *J Immunol*. 162(10):5784–91; 1999.

[6] Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science*. 271(5256):1734–6; 1996.

[7] Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell*. 161(2):205–14; 2015.

[8] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 363(8):711–23; 2010.

[9] Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J*. 11(11):3887–95; 1992.

[10] Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*. 26:677–704; 2008.

[11] Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubata T, Yagita H, Honjo T. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int Immunol*. 8(5):765–72; 1996.

[12] Kinter AL, Godbout EJ, McNally JP, Sereti I, Roby GA, O'Shea MA, Fauci AS. The common gamma-chain cytokines IL-2, IL-7, IL-15, and IL-21 induce the expression of programmed death-1 and its ligands. *J Immunol*. 181(10):6738–46; 2008.

[13] Ohaegbulam KC, Assal A, Lazar-Molnar E, Yao Y, Zang X. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. *Trends Mol Med*. 21(1):24–33; 2015.

[14] Rozali EN, Hato SV, Robinson BW, Lake RA, Lesterhuis WJ. Programmed death ligand 2 in cancer-induced immune suppression. *Clin Dev Immunol*. 2012:656340; 2012.

[15] Youngnak P, Kozono Y, Kozono H, Iwai H, Otsuki N, Jin H, Omura K, Yagita H, Pardoll DM, Chen L, Azuma M. Differential binding properties of B7-H1 and B7-DC to programmed death-1. *Biochem Biophys Res Commun*. 307(3):672–7; 2003.

[16] Liang SC, Latchman YE, Buhlmann JE, Tomczak MF, Horwitz BH, Freeman GJ, Sharpe AH. Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. *Eur J Immunol*. 33(10):2706–16; 2003.

[17] Riley JL. PD-1 signaling in primary T cells. *Immunol Rev*. 229(1):114–25; 2009.

[18] Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, Sasayama S, Mizoguchi A, Hiai H, Minato N, Honjo T. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science*. 291(5502):319–22; 2001.

[19] Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity*. 11(2):141–51; 1999.

[20] Wang J, Yoshida T, Nakaki F, Hiai H, Okazaki T, Honjo T. Establishment of NOD-Pdcd1-/- mice as an efficient animal model of type I diabetes. *Proc Natl Acad Sci U S A*. 102(33):11823–8; 2005.

[21] Tsushima F, Yao S, Shin T, Flies A, Flies S, Xu H, Tamada K, Pardoll DM, Chen L. Interaction between B7-H1 and PD-1 determines initiation and reversal of T-cell anergy. *Blood*. 110(1):180–5; 2007.

[22] Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. *Nat Rev Immunol*. 8(6):467–77; 2008.

[23] Crespo J, Sun H, Welling TH, Tian Z, Zou W. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. *Curr Opin Immunol*. 25(2):214–21; 2013.

[24] Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu G, Tamada K, Lennon VA, Celis E, Chen L. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med*. 8(8):793–800; 2002.

[25] Amarnath S, Mangus CW, Wang JC, Wei F, He A, Kapoor V, Foley JE, Massey PR, Felizardo TC, Riley JL, Levine BL, June CH, Medin JA, Fowler DH. The PDL1-PD1 axis converts human TH1 cells into regulatory T cells. *Sci Transl Med*. 3(111):111ra120; 2011.

[26] Azuma T, Yao S, Zhu G, Flies AS, Flies SJ, Chen L. B7-H1 is a ubiquitous antiapoptotic receptor on cancer cells. *Blood*. 111(7):3635–43; 2008.

[27] Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, Stankevich E, Pons A, Salay TM, McMiller TL, Gilson MM, Wang C, Selby M, Taube JM, Anders R, Chen L, Korman AJ, Pardoll DM, Lowy I, Topalian SL. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol*. 28(19):3167–75; 2010.

[28] Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 366(26):2443–54; 2012.

[29] Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, Schuster SJ, Millenson MM, Cattry D, Freeman GJ, Rodig SJ, Chapuy B, Ligon AH, Zhu L, Grosso JF, Kim SY, Timmerman JM, Shipp MA, Armand P. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med*. 372(4):311–9; 2015.

[30] Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, Wolchok JD, Hersey P, Joseph RW, Weber JS, Dronca R, Gangadhar TC, Patnaik A, Zarour H, Joshua AM, Gergich K, Elassaiss-Schaap J, Algazi A, Mateus C, Boasberg P, Tumeh PC, Chmielowski B, Ebbinghaus SW, Li XN, Kang SP, Ribas A. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med*. 369(2):134–44; 2013.

[31] Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, Linette GP, Meyer N, Giguere JK, Agarwala SS, Shaheen M, Ernstoff MS, Minor D, Salama AK, Taylor M, Ott PA, Rollin LM, Horak C, Gagnier P, Wolchok JD, Hodi FS. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med*. 372(21):2006–17; 2015.

[32] Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, Burke MM, Caldwell A, Kronenberg SA, Agunwamba BU, Zhang X, Lowy I, Inzunza HD, Feely W, Horak CE, Hong Q, Korman AJ, Wigginton

JM, Gupta A, Sznol M. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med*. 369(2):122–33; 2013.

[33] Rios-Doria J, Durham N, Wetzel L, Rothstein R, Chesebrough J, Holoweckyj N, Zhao W, Leow CC, Hollingsworth R. Doxil synergizes with cancer immunotherapies to enhance antitumor responses in syngeneic mouse models. *Neoplasia*. 17(8):661–70; 2015.

[34] Deng L, Liang H, Burnette B, Beckett M, Darga T, Weichselbaum RR, Fu YX. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J Clin Invest*. 124(2):687–95; 2014.

[35] Zeng J, See AP, Phallen J, Jackson CM, Belcaid Z, Ruzevick J, Durham N, Meyer C, Harris TJ, Albesiano E, Pradilla G, Ford E, Wong J, Hammers HJ, Mathios D, Tyler B, Brem H, Tran PT, Pardoll D, Drake CG, Lim M. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. *Int J Radiat Oncol Biol Phys*. 86(2):343–9; 2013.

[36] Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. *Immunity*. 27(1):111–22; 2007.

[37] Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, Bellmunt J, Burris HA, Petrylak DP, Teng SL, Shen X, Boyd Z, Hegde PS, Chen DS, Vogelzang NJ. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature*. 515(7528):558–62; 2014.

[38] McDermott DF, Sosman JA, Sznol M, Massard C, Gordon MS, Hamid O, Powderly JD, Infante JR, Fassò M, Wang YV, Zou W, Hegde PS, Fine GD, Powles T. Atezolizumab, an anti-programmed death-ligand 1 antibody, in metastatic renal cell carcinoma: long-term safety, clinical activity, and immune correlates from a phase Ia study. *J Clin Oncol*. 34(8):833–42; 2016.

[39] Grigg C, Rizvi NA. PD-L1 biomarker testing for non-small cell lung cancer: truth or fiction? *J Immunother Cancer*. 4:48; 2016.

[40] Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature*. 515(7528):568–71; 2014.

[41] Hamid O, Schmidt H, Nissan A, Ridolfi L, Aamdal S, Hansson J, Guida M, Hyams DM, Gómez H, Bastholt L, Chasalow SD, Berman D. A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. *J Transl Med*. 9:204; 2011.

[42] Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T,

Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA Jr. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 372(26):2509–20; 2015.

[43] Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMiller TL, Chen S, Klein AP, Pardoll DM, Topalian SL, Chen L. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. *Sci Transl Med*. 4(127):127ra37; 2012.

[44] Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. *Immunity*. 44(5):989–1004; 2016.

[45] Koyama S, Akbay EA, Li YY, Herter-Sprie GS, Buczkowski KA, Richards WG, Gandhi L, Redig AJ, Rodig SJ, Asahina H, Jones RE, Kulkarni MM, Kuraguchi M, Palakurthi S, Fecci PE, Johnson BE, Janne PA, Engelman JA, Gangadharan SP, Costa DB, Freeman GJ, Bueno R, Hodi FS, Dranoff G, Wong KK, Hammerman PS. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. *Nat Commun*. 7:10501; 2016.

[46] Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, Torrejon DY, Abril-Rodriguez G, Sandoval S, Barthly L, Saco J, Homet Moreno B, Mezzadra R, Chmielowski B, Ruchalski K, Shintaku IP, Sanchez PJ, Puig-Saus C, Cherry G, Seja E, Kong X, Pang J, Berent-Maoz B, Comin-Anduix B, Graeber TG, Tumeh PC, Schumacher TN, Lo RS, Ribas A. Mutations associated with acquired resistance to PD-1 blockade in melanoma. *N Engl J Med*. 375(9):819–29; 2016.

[47] Harlin H, Meng Y, Peterson AC, Zha Y, Tretiakova M, Slingluff C, McKee M, Gajewski TF. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. *Cancer Res*. 69(7):3077–85; 2009.

[48] Tang H, Wang Y, Chlewicki LK, Zhang Y, Guo J, Liang W, Wang J, Wang X, Fu YX. Facilitating T cell infiltration in tumor microenvironment overcomes resistance to PD-L1 blockade. *Cancer Cell*. 29(3):285–96; 2016.

[49] Porada CD, Almeida-Porada G. Mesenchymal stem cells as therapeutics and vehicles for gene and drug delivery. *Adv Drug Deliv Rev*. 62(12):1156–66; 2010.

[50] Studeny M, Marini FC, Dembinski JL, Zompetta C, Cabreira-Hansen M, Bekele BN, Champlin RE, Andreeff M. Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents. *J Natl Cancer Inst*. 96(21):1593–603; 2004.

[51] Sartoris S, Mazzocco M, Tinelli M, Martini M, Mosna F, Lisi V, Indraccolo S, Moserle L, Cestari T, Riviera AP, Bifari F, Tridente G, Pizzolo G, Krampera M. Efficacy assessment of interferon-alpha-engineered mesenchymal stromal cells in a mouse plasmacytoma model. *Stem Cells Dev*. 20(4):709–19; 2011.

[52] Jeong KY, Lee EJ, Kim SJ, Yang SH, Sung YC, Seong J. Irradiation-induced localization of IL-12-expressing mesenchymal stem cells to enhance the curative effect in murine metastatic hepatoma. *Int J Cancer*. 137(3):721–30; 2015.

[53] Zou W, Zheng H, He TC, Chang J, Fu YX, Fan W. LIGHT delivery to tumors by mesenchymal stem cells mobilizes an effective antitumor immune response. *Cancer Res*. 72(12):2980–9; 2012.

[54] Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, Granton J, Stewart DJ; Canadian Critical Care Trials Group. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS One*. 7(10):e47559; 2012.

[55] Niess H, von Einem JC, Thomas MN, Michl M, Angele MK, Huss R, Günther C, Nelson PJ, Bruns CJ, Heinemann V. Treatment of advanced gastrointestinal tumors with genetically modified autologous mesenchymal stromal cells (TREAT-ME1): study protocol of a phase I/II clinical trial. *BMC Cancer*. 15:237; 2015.

Chapter 4

Present and Future of Subcutaneous Aero-Allergen Immunotherapy

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Additional information is available at the end of the chapter

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Abstract

The present review summarizes the literature-acquired knowledge as well as author's own experience in conducting aero-allergen immunotherapy, particularly in subcutaneous route of administration (SCIT) of all modalities of respiratory allergy disease from allergic rhinosinusitis, bronchial asthma to united airway disease. Because of the better adherence resulting in appropriate efficacy in connection with satisfactory safety, the author favours conventional schedules of subcutaneous route of therapeutic intervention. Given the lack of specific biomarker in monitoring treatment course, the main control mechanism of efficacy is the evaluation of quality of life using simple evaluation scale as visual analogue scale or standardized respiratory allergy questionnaires. The future of allergen immunotherapy should be focused on new routes of allergen administration (e.g. oral, epicutaneous, intradermal, intralymphatic) and on the searching potential biomarkers which could be objectively measured and easily accessible from body fluids (blood, nasal secretion, sputum). The combination of estimated biomarkers obtained from biological samples in conjunction with evaluation of quality of life could lead to the generation of the overall satisfactory monitoring protocol.

Keywords: allergen immunotherapy, routes of administration, adjuvants, compliance, future treatment

1. Introduction

Allergic diseases are considered as major global public health issue. Among them, respiratory allergies represent 1 of 10 most common diseases of affluence. At present, IgE sensitization to foreign proteins in the environment is rising up to 40% of the worldwide population, especially in highly industrialized countries [1]. Only in the United States, prevalence of

asthma reported in 2014 was 8.6% (6.3 million) and 7.4% (17.7 million) among children and adults, respectively. Mortality on asthma in 2013 reaches 1.1 deaths per 100,000 of the United States population [2, 3]. At least similar percentage rate could be reported in European countries also. Approximately, one-fifth of the world population suffers from upper respiratory allergies (hay fever, allergic rhinosinusitis).

Prevalence of asthma is still rising in many high as well as low income countries, likewise impact of allergic diseases continue to grow. According to the World Health Organization (WHO), the number of patients having asthma is 300 million, and with the rising trends, it is expected to increase to 400 million, by 2025. Even though in majority of cases respiratory allergies are not life-threatening diseases, it is necessary to say that patients with asthma and/or other respiratory allergies have reduced quality of life [4] which is comparable to moderate chronic coronary ischemia.

Asthma and allergic rhinosinusitis are linked by epidemiological, physiological, and pathological characteristics. The genetic predisposition to develop IgE-mediated sensitivity to common aero-allergens is the strongest predicting factor for the development of both diseases [5]. Facts are supported by the concept of unifying the management of these disorders. The united airway disease (UAD) hypothesis proposes that upper and lower airway disease, both are manifestations of a single inflammatory process within the respiratory tract.

First-line treatment includes avoidance and minimization of exposure when possible. Medication, including antihistamines, bronchodilators, leukotriene inhibitors, and steroids, may be used to reverse some of the symptoms of allergic reactions. Pharmacotherapy alone has no effect on the progression of the disease and treatment has to be administered repeatedly as long as symptoms prevail, which often means life-long [6]. It can be postulated that allergen avoidance and pharmacotherapy alone cannot control the disease. Only allergen immunotherapy has the disease-modifying potential and should be included in the algorithm of optimal therapeutic strategy.

Allergen immunotherapy is a form of parenteral (subcutaneous) or oral (sublingual) medication, designed to prevent or lessen an allergic reaction. Its mechanism of action is based upon the body's production of different antibodies to an antigen depending on how the antigen is introduced into the body. Allergy immunotherapy induces immunological tolerance and changes the course of disease. It is typically used in individuals after a trial of conservative treatment, when avoidance and medications has been found to be inadequate.

In 2000, American College of Allergy, Asthma and Immunology (ACAAI) organized an international conference "Immunotherapy in Allergic Asthma" where key board of the meeting summarized that allergen immunotherapy is an effective treatment for allergic asthma and also it prevents the early onset of asthma in children with allergic rhinitis. These conclusions were subsequently confirmed by The Preventive Allergy Treatment (PAT) study published in February 2002 [7]. The study concluded that pollen immunotherapy significantly reduces the development of asthma in children with seasonal allergic rhinoconjunctivitis, and also methacholine-induced bronchial hyper responsiveness was improved. Allergen immunotherapy can also prevent the development of sensitization to new allergens [8]. Active

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therapy resulted in a statistically significant reduction in rhinitis, conjunctivitis, and bronchial reactivity, showed a reduction in the need for medication, a reduction in bronchial hyperresponsiveness, and improvement in forced expiratory volume in 1 s (FEV1) [9].

Allergen immunotherapy does not cure allergies; immunotherapy aims to make a person less sensitive to allergens. In some cases, allergic symptoms may be controlled to the point of disappearance, allowing a person to avoid allergen reactions. Both forms of allergen immunotherapy (subcutaneous, sublingual) are used for the management of allergic rhinitis, allergic conjunctivitis, and allergic asthma, however, subcutaneous administration route is used for hymenoptera sensitivity only [10].

2. History of allergen immunotherapy

The first known historical remark about perception of immunity is dated to 430 B.C. when Thucydides recorded "recovery from plague-endowed protection from repeated attacks" [11]. Other pre-Christian reference by Plinius described the "principle" of allergen-specific immunotherapy when King Mithridates VI from Pontos (132–163 B.C.) tried to protect himself against poisoning. He had used increased doses of snake venom to make himself immune against the toxin. Plinius did not report the result of such procedure [12].

The real development in immunological treatment started approximately at the end of nineteenth and at the beginning of the twentieth century, but research was mostly orientated on how to protect humans against infective diseases. Parallel with these trends, scientists as Besredka, Pirquet, Dunbar, Holbrock-Curtis experimented with induction of "tolerance" by administration of various sera (hyper immune animal sera, mixtures of various pollens) in animal experiments as well as on treatment of human beings. However, due to significant side-effects of treatment (including one report of death), procedures were discontinued [12].

In the year 2011, worldwide allergy-immunology community celebrated 100 years of allergen immunotherapy, since the first successful use of this form of treatment by Leonard Noon (1878–1913) at St. Mary's Hospital in London in 1911. It is interesting to say that in 1928 in the same hospital one floor above Alexander Fleming discovered penicillin, the first antibiotic which has broad consequences for mankind.

Noon and Cantab published, in 10 June 1911 in Lancet [13], successful desensitization with pollen extract (*Phleum pratense*). In this first use of parental immunotherapy, they administered very low, increasing doses of the pollen extract by intradermal injections at intervals of 3–4 days. Following this therapy, the researchers demonstrated an improvement in hay fever symptoms. They monitored the reactivity of their hay fever patient with conjunctival provocation tests and observed that a single drop of highly diluted grass pollen extract prepared according to Dunbar's method was still sufficient to trigger a conjunctival reaction in sensitive patients. Noon left his work for following hay fever seasons in hands of his colleague and close friend John Freeman, while he knew his advanced tuberculosis would keep him from finishing his work. In February 1913, only 2 years after the discovery, Leonard Noon died from florid pulmonary tuberculosis at the age of only 35 [14].

Noon's immune-pathologic interpretation of possible mechanisms which was strongly influenced by Dunbar's thought was incorrect in claiming that the disease is caused by the exposure to a toxin, present in pollen, which could even induce antitoxin when injected into rabbits or horses. Administering little quantities of pollen extract to patients would actively immunize them [15].

John Freeman (1877–1962) continued in pending work and he had published early results in the same year 1911 [16]. After 3 years of treatment in 1914 (1 year after Noon's death), he summarized their results in the paper "Vaccination against hay fever: report of results during the last three years" in the same medical journal [17].

On the opposite coast of the Atlantic Ocean, the pioneer publication concerning the allergen immunotherapy appeared in 1913. George Cloves reported on the treatment of autumnal hay fever by vaccination with aqueous extract of the pollen of ragweed. He concluded all eight treated cases experienced improvement of general symptoms [18]. In 1915, Robert Cooke, the founding director of one of the first allergy clinics in the United States: "The Institute of Allergy Roosevelt Hospital, New York" published his own experience in Laryngoscope "The treatment of hay fever by active immunization" [19].

In years 1918–1922, Robert Cooke introduced a suggested mechanism of action for allergen injections as a "desensitization or hyposensitization," analogous to tolerance achieved in experimental anaphylaxis induced in animals. This concept suggested that the injections of an increasing amount of allergen or antigen slowly neutralized those antibodies responsible for the allergic reaction [11]. Cooke together with Mary Loveless have introduced the concept of specific blocking antibody: "the development under treatment of a peculiar blocking or inhibiting type of immune body that prevents the action of allergen on the sensitizing antibody" [20]. Twenty years later, Cooke confirmed his assumption that "serum factor" for inhibition was most likely gamma globulin (IgG) in electrophoretic mobility studies in ragweed-treated patients [21].

Next 30 years were strongly influenced by notable socio-economic disturbances as WWI, Wall Street Crash, Great Depression, and WWII. One remarkable publication from that period (1937), which has to be mentioned, was the report about depot allergenic vaccines for delayed absorption: alum adsorption [22]. Aluminum adjuvants function as delivery systems by generating depots that trap antigens/allergen at the injection site, providing slow release in order to continue the stimulation of the immune system (see Chapter 4).

Negative historical conditions slowed down medical and scientific world, so the next important event in the field of allergy was the first DBPC trial of grass pollen subcutaneous immunotherapy published by Alfred Frankland in 1954 in Lancet [23], which proved beyond doubt that subcutaneous immunotherapy was effective. The adequate number of patients (200), the exact description of randomization (four randomization groups), blinding, and of dropouts makes this study even today being rated as of moderately high scientific quality [15]. In 1957 Douglas Johnstone published early results and in 1968 late results of the study which was realized on the same group of paediatric patients. The research was focused on a preventive

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and dose–response effect of immunotherapy in terms of bronchial hypersensitivity and development of asthma [24, 25].

At the 1987 meeting of the EAACI on Mallorca, 40 specialists formed a subcommittee on immunotherapy and decided to create some guidelines for indications of allergen immunotherapy, monitoring of effect and side effect, practical information, and requirements for allergen extracts. The new common guidelines would serve for all specialists not only in the European countries, but also on a worldwide basis. So the first position paper was published in 1988 as Supplement of Journal Allergy [26]. New insights into the pathogenesis of allergic diseases and new publications on immunotherapy have called for its revision. Immunotherapy position paper was introduced to public in 1993 [27].

In 1996 in the United States, AAAAI together with ACAAI published practice parameters for allergen immunotherapy [28]. After the great discussion at the level of World Health Organization (WHO) and various allergy, asthma, and immunology societies throughout the world specialists took the decision to prepare common guidelines and in 1998 WHO position paper "Allergen immunotherapy: therapeutic vaccines for allergic diseases" was published [29]. Practice parameters for allergen immunotherapy in the United States were updated in 2007 and 2011 under the principal editor Linda Cox. In the preparation of these updates, the comprehensive search of the literature, information from articles known to the authors were considered. Published clinical studies were rated by category of evidence and used to establish the strength of a clinical recommendation. Published updates represent an evidence-based, broadly accepted consensus opinion [30, 31]. All these clinical guidelines are designed to assist clinicians by providing a framework for the evaluation and treatment of patients and are not intended to replace a clinician's judgment or establish a protocol for all patients [31].

3. Mechanisms of subcutaneous allergen immunotherapy

WHO position paper defines allergen immunotherapy as the administration of gradually increasing quantities of an allergen vaccine to an allergic subject, reaching a dose which is effective in ameliorating the symptoms associated with subsequent exposure to the causative allergen [29]. The ultimate goal of the therapy is to induce immune tolerance, a change in the immune response to specific antigens so that discontinuation of the therapy results in sustained long-lasting therapeutic benefits.

Allergen immunotherapy modifies the response to allergen exposure by inducing tolerance, but the mechanisms by which immunotherapy mediates its anti-inflammatory effects remain incompletely defined because of the use of heterogeneous medicaments, treatment protocols, routes of administration, and outcome measures in different studies. However, several common features emerge from the multiple studies show that allergen immunotherapy modifies the responses of antigen-presenting cells, T-cells and B-cells, as well as both the number and the function of effector cells. So allergen immunotherapy regulate regulate the local and systemic allergic inflammation [32].

Successful immunotherapy in respiratory allergy is associated with the immunodeviation of Th2 response to a more protective allergen-specific Th1 cells and with the induction of IL-10-/TGF-β-producing T regulatory cells in blood and also locally in inflamed airways. In subcutaneous route of administration (SCIT), allergen-specific T-cell proliferation has been reduced because of peripheral tolerance mechanisms. Immunoregulatory activity of T regulatory cells has been claimed to be the main mechanism for clinical efficacy of SCIT [33]. Production of IL-10 and TGF-b from an expression of cytotoxic T-lymphocyte-associated protein-4 by T regulatory cells have importance in immune regulation in SCIT.

3.1. Immunological processes

Four groups of immunological processes can be classified during the course of allergen immunotherapy [34]:

3.1.1. Group 1

An initial event is desensitization of FcεRI-bearing mast cells and basophils by allergen. The mechanism of this desensitization is not fully elucidated, although its major role is assigned to a rapid upregulation of the histamine 2 receptor, which is a major suppressor of basophil activation. At the beginning of treatment, decreases in mast cell and basophil activity, degranulation and tendency for systemic anaphylaxis degranulation take place within the first hours. Histamine 2 receptor strongly suppressed FcεRI-induced activation and mediator release of basophils, including histamine and leukotriene sulphides, as well as cytokine production *in vitro* [35].

3.1.2. Group 2

Second group represents generation of allergen-specific T and B regulatory cells and suppression of allergen-specific Th1 and Th2 cells. T regulatory cells are a diverse group of T cells that are active in the regulation of immune responses, and allergen-specific T regulatory cells (CD4+CD25+) have been demonstrated after allergen immunotherapy [36]. T regulatory cells have distinct cytokine profiles other than Th1 and Th2 cells, are characterized by IL-10 and TGF-β secretion capacity, and express suppressor molecules, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) [37].

IL-10 is the leading cytokine, which in T regulatory cell/B cell interaction suppresses specific IgE production. In addition, IL-10 induces specific IgG4 production. IgG4 and probably IgG1 compete with IgE on the surface of mast cells and basophils for allergen binding [37]. They produce interleukin IL-10 and transforming growth factor TGF-β, and have the potential to suppress local Th2 cell responses and redirect antibody class switching in favour of IgG4 (IL10 isotype switch factor), and IgA (TGF-β isotype switch factor) [5].

Allergen-specific IgG4 antibodies interrupt allergen presentation to Th2 cells and, in addition, block allergen-induced activation of mast cells and basophils, thereby significantly weakening the allergic reaction [5]. Although multiple factors contribute, it could be supposed that the tolerant state of specific cells essentially results from increased IL-10 secretion [38]. IL-10

particularly originates from activated and antigen-specific T regulatory and B regulatory cell populations and increases during allergen immunotherapy as well as in natural allergen exposure [39].

3.1.3. Group 3

These processes include regulation of antibody isotypes demonstrating an early increase in specific IgE levels, which later decrease, and an early and continuous increase in specific IgG4 levels. Natural exposure to a relevant allergen is often associated with an increase in IgE synthesis. Similarly, allergen immunotherapy often induces a transient increase IgE levels in serum, followed by a gradual decrease over months or years of continued treatment [40].

Allergen immunotherapy decreases allergen-specific IgE production and promotes allergen-specific IgG4 production, which competes with IgE by blocking the binding of allergens to FcεRI on the surface of mast cells and basophils [41]. IL-10 reduces allergen-specific IgE production through IL-4-induced IgE switching and enhances allergen-specific IgG4 production by inducing IL-4-induced gamma 4 transcript expression [42]. Grass pollen SCIT has reduced seasonal increases in serum allergen-specific IgE, whereas 60- to 80-fold increases in allergen-specific IgG and 100-fold increases in allergen-specific, IgG4 have been observed [43]. Thus, measuring IgG4 levels could be a good indicator of clinical efficacy of AIT during follow-up [44].

Mechanisms of innate immunity are also stimulated during the course of allergen immunotherapy. Human blood dendritic cells from allergic subjects have impaired IFN-α production following toll-like receptor-9 (TLR9) dependent innate immune stimulation. Tversky et al. [45] found out subcutaneous allergen immunotherapy resulting in a fivefold increase in IFN-α production and thus increases dendritic cell TLR9-mediated innate immune function, which is impaired in allergic subjects.

3.1.4. Group 4

The fourth group of events takes place after several months from the beginning of the treatment and these processes are characterized with decreases in tissue mast cells and eosinophils and release of their mediators. The phase is referred as the late-phase response and is localized in the peripheral tissues such as mucous membranes of respiratory organs (nose, bronchi) or in the skin. When comparing immediate reactions mediated by mast cells, last-phase response involves activation of T cells and the recruitment, activation and persistence of eosinophils at sites of allergen exposure. Chronic exposition to inhalant allergens causes immunopathologic changes seen during the late-phase. Mucosal changes are associated with positivity of nasal and bronchial provocation tests and suggest the pathologic conditions of chronic allergic inflammation. Van Bever and Stevens [46] postulated that allergen immunotherapy may resolve and/or reduce the severity of the late-phase reaction in treated children. Rak et al. reported reduction in plasma levels of eosinophil cationic protein, a marker of eosinophil activation, and chemotactic factors for eosinophils and neutrophils correlate with decreased bronchial hyperreactivity and clinical improvement [47]. After grass

pollen allergen immunotherapy decreased eosinophil and mast cell infiltration in nasal and bronchial mucosa correlates with an anti-inflammatory effect [48, 49].

3.2. Involved cells

When describing above mentioned immunological processes, the expected role of many immunological cells can be deduced (**Figure 1**). All these cells are involved in regulatory processes and might contribute to the control of allergen-induced immune responses.

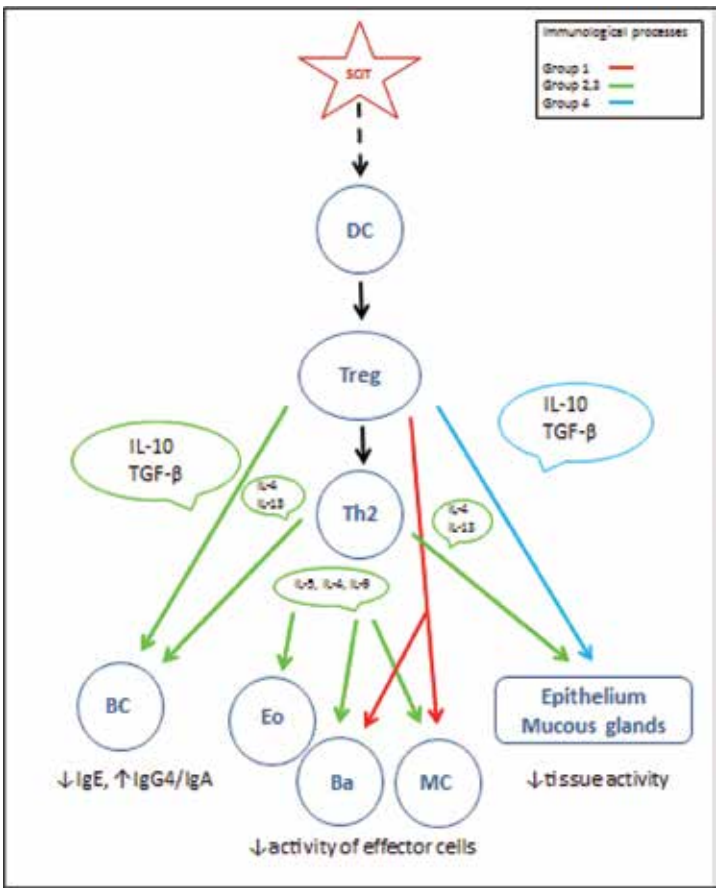

Figure 1. Cells and processes during allergen immunotherapy. DC, dendritic cell; Treg, T regulatory cell; Th2, Th2 cell; BC, B-cell; Eo, eosinophil; Ba, basophil; MC, mast cell; SCIT, subcutaneous allergen immunotherapy.

3.2.1. Antigen presenting cells (APCs)

APCs, particularly DCs, control both peripheral tolerance and immunity through the interpretation of environmental signals that are associated with antigen encounter (such as pathogen-associated molecular patterns). DCs in the airways control the pulmonary immune

response and determine tolerance and immunity to newly encountered antigens. Several studies support a role for DCs in the induction of T cells with a regulatory phenotype and function, particularly IL-10-secreting T regulatory cells. These T regulatory cells are involved in the inhibition of subsequent inflammatory response as well in protection against sensitization to allergen and development of asthma in a mouse model, so T regulatory cells might be important mediators of the beneficial action of allergen immunotherapy [32].

3.2.2. T cells

Allergen immunotherapy has been shown to modify T-cell responses to allergen in several ways. The main role is in switching Th1/Th2 ratio by increasing the allergen-induced Th1 cytokines to Th2 cytokines [50]. In other way, allergen immunotherapy can induce epitope-specific T-cell anergy, generate allergen-specific T regulatory cells that can suppress the responses of effector T cells following delivery of either whole allergen extracts or synthetic peptides that contain or consist of a T-cell epitope and increase the production of cytokines with regulatory activity [51]. Regulatory T cells also play an important role in controlling allergic inflammation. The transcription factor Foxp3 regulates the development and function of natural and adaptive CD4(+)CD25(+) T regulatory cells. Radulovic et al. detected the presence of local Foxp3(+)CD25(+)CD3(+) cells in the nasal mucosa, their increased numbers after immunotherapy, their association with clinical efficacy and suppression of seasonal allergic inflammation. In conclusion, they supported a putative role for T regulatory cells in the induction of allergen-specific tolerance in human subjects [52].

3.2.3. B cells

It is now generally accepted that peripheral tolerance is essential for a normal immune response and successful immunotherapy of allergic disorders. As seen above, the tolerant state essentially results from increased IL-10 secretion by T regulatory cells. Similar to Th cells, B cells can be classified into subsets according to the cytokines they produce. One functional B-cell subset, B regulatory cells, has recently been shown to contribute to the maintenance of the fine equilibrium required for tolerance. B regulatory cells control excessive inflammatory responses through IL-10, which inhibits proinflammatory cytokines and supports T regulatory cell differentiation [53]. IL-10 not only generates tolerance in T cells, but it also regulates specific isotype formation and skews B cells specific response from an IgE to an IgG4-dominated phenotype. In addition to IgE/IgG4 switching, recent studies have also provided evidence for increases in the amount of TGF-β driven allergen-specific IgA following allergen immunotherapy, indicating that other B cell production (antibody classes) might contribute to clinical efficacy [54].

3.2.4. Effector cells (mast cells, basophils, eosinophils) and indirect influences

Late-phase reaction involves the recruitment, activation, and persistence of eosinophils, mast cells, and activation of T cells at sites of allergen exposure. It is usually associated with increased bronchial and nasal hyper responsiveness and suggests the pathologic conditions present in chronic allergic inflammation. It has been postulated that the effect of allergen

immunotherapy on the late-phase reaction is relevant to its clinical efficacy [46]. After a few months, a decrease in tissue mast cell and eosinophil numbers and release of their mediators is observed, as well as a decrease in the late-phase response. These effects are partially demonstrated in SLIT and are rather weak compared with those seen in SCIT [55].

In addition, allergen immunotherapy exhibits indirect inhibition of Th2 cell-associated phenomena (such as mucus production, and endothelial cell activation and cellular influx) and Th1 cell-associated phenomena (such as epithelial cell activation and apoptosis).

In conclusion, when comparing clinical significance of SCIT and SLIT, due to well-established safety profile, SLIT is considered an alternative to SCIT [55]. However, immunologic mechanisms of SLIT are less well-elucidated than those for SCIT. All potential mechanisms seem to be similar in both forms of allergen immunotherapy—in induction of T-cell tolerance, generation of T regulatory cells, in the role of IL-10 and TGF-β as well as in the late-phase response (decrease the presence of mast cells, eosinophils and release of their mediators). Furthermore, subcutaneous administration in contrast to sublingual immunotherapy modifies the immune response also in very early phase of desensitization, generates B regulatory cells and shows clearly decrease in IgE and increase in blocking IgG4 [34].

4. Aluminium—basic adjuvant in subcutaneous allergen immunotherapy

Mineral adjuvant molecules such as calcium phosphate or aluminium hydroxide are broadly used in human immunization as adjuvants in parenteral route of administration. While aluminium salts are commonly included in vaccines against infectious pathogens with the aim to elicit proinflammatory responses following activation of the inflammasome, in subcutaneous allergen immunotherapy, allergen extracts are adsorbed on aluminium hydroxide or calcium phosphate as adjuvants in Europe, whereas in North America only soluble allergens are used.

Like any vaccines, adjuvants to be associated with allergens are expected to allow simplifying immunization regimens, and reaching efficacy faster and for a longer duration. Mechanisms involved include both a depot effect (slow release of the allergen, formulation of the allergen as particles to target antigen presenting cells) as well as interaction with the innate immune system (activation of the inflammasome) [56].

Although allergy vaccines are usually well-tolerated, an additional expected benefit of adjuvants in this field is to help lowering the allergen dose, thus improving the safety profile with less local reactions to the site of administration. On the other side, none of commercially available noninvasive sublingual products, which are considered as a safe and efficacious alternative, contain any adjuvant. These vaccines are based on high-dose aqueous allergen extracts presented either as drops or more recently as fast dissolving tablets or lyocs [57].

History shows that aluminum salts are being used as adjuvants in allergen immunotherapy for many years. Aluminum is validated as safe adjuvant with few established side effects. Biological potential of aluminum lays on its reactivity not only at injection site, but also

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elsewhere in the body. Aluminium hydroxide modifies the immune response to a range of allergens and is generally used in multiple injections over extended time periods. Incidence of adverse events increases more likely in the subsets of individuals predisposed to such reactivity. Susceptibility to adverse events grows with the high body burden of aluminum, in which allergen immunotherapy is the most probable source of the adjuvant molecule. But neither the safety nor the toxicity of aluminum as adjuvant in subcutaneous allergen immunotherapy preparations have been confirmed [58].

Threshold values for foodstuffs established by authorities are regularly exceeded and aluminum compounds are routinely used as adjuvants in vaccinations. A big challenge for pharma industry is to conduct clinical trials which confirm the benefit–risk relationship of long-term use of aluminum as adjuvant in SCIT according to good pharmacovigilance practice. Long-life time of accumulation of aluminum in every individual human body has undoubtedly the potential to exert chronic toxic side effect, such as neurotoxicity. In the literature, one serious disease, a neuromuscular disorder called *macrophagic myofasciitis*, is attributed to the persistence of aluminium salts at injections sites in muscle [59].

However, there is still a lack of studies examining the possible relationship among the development of such diseases, which may have a latency period of many years after the application of SCIT. Predisposing an individual to an unnecessary high body burden of aluminium should be avoided and could reasonably be considered [60]. Adverse events associated with aluminium adjuvants in allergen immunotherapy could be also connected with other more common conditions such as chronic fatigue syndrome or and autoimmune diseases [61]. More common but less critical are local reactions, such as discolouration of skin, urticaria, foreign body granulomas, subcutaneous sarcoidosis, progressive circumscribed sclerosis, subcutaneous nodules, and pseudo-lymphoma. When indicating subcutaneous route of administration, we have to consider aluminium as strong potent adjuvant in stimulating or modifying immunity. However, on the other side, the toxicity, antigenicity, and in a long-term possible body burden of aluminium have to be considered.

The other potent adjuvant used in subcutaneous allergen immunotherapy is calcium phosphate. Many studies have compared the effects and adverse effects of immunologic adjuvants, and in most studies, it was reported that allergen immunotherapy that contained calcium phosphate causes fewer reactions [62]. Nacaroglu et al. reported no association between adjuvant content and the incidence of adverse effects. They also concluded that the frequencies of local and wide local reactions during SCIT were lower than expected, and although systemic reactions were frequently seen, no fatal reaction was observed in the published study. House dust mite SCIT and multiple allergen use increased the risk of reaction [63].

5. Treatment protocols in subcutaneous allergen immunotherapy

Although allergen specific immunotherapy represents the only immune-modifying and curative option available for patients with respiratory allergy, the optimal schedule for specific subcutaneous immunotherapy is still unknown. All injections are given in the doctor's surgery,

because there is a small risk of inducing allergic reactions, which can become severe or even life-threatening if not treated promptly and appropriately. Two major groups of parenteral treatment courses are used in clinical praxis: intermittent (pre-seasonal) or continual (year-long) course.

Intermittent treatment course is considered as pre-seasonal treatment with pollen allergens (trees or grasses). The allergens are prepared by conversion into allergoids by treatment with glutaraldehyde and are adsorbed onto L-tyrosine. The course should be completed before the onset of the tree/grass pollen season. The three graduated doses constitute a complete dose for 1 year and can be followed by the pre-seasonal extension injections with three highest-dose vials for continued clinical improvement. It is recommended that the treatment course should be given in each of 3 successive years [64, 65].

Continuous all-year courses are used in the treatment of allergy to pollens, dust mites, moulds, animal epithelia as well as in the treatment of insect venom allergy (bee/wasp). Duration of such course lasts from 3 to 5 years and the course is divided into a build-up and a maintenance phase. In the initial (build-up) phase, four administration schedules of immunotherapy have been reported: conventional and three accelerated (cluster, rush, and ultra-rush) schedules (**Table 1**). Conventional subcutaneous immunotherapy for allergy treatment needs one injection per week. The duration of the conventional build-up phase varies but typically ranges from 3 to 8 months to reach the maintenance dose. Maintenance treatment continues at constant dosing and in the case of airway allergy, the duration of all treatment should be at least 3 years [66, 67].

	Conventional	**Cluster**	**Rush**	**Ultra-rush**
Time consumption	1 injection/week	2–3 injection/ week	2–3 full days	1 full day (+night)
Build-up phase	14–25 weeks	6 weeks	2–3 days	1 day
Arrangements	Check after 20 min	Check after 30 min	Premedication/2–3 consecutive day stay	Premedication/day stay (or + overnight)

Table 1. Administration schedules of subcutaneous allergen immunotherapy.

Accelerated immunotherapy build-up schedules allow the patient to achieve the benefits of immunotherapy more rapidly, as the maintenance dose is reached sooner. Shorter up-dosing schedules are desired, provide earlier clinical improvement and improved convenience, though they introduce increased risk of adverse reactions. However, many cluster schedules have similar adverse reaction rates to conventional schedules, and premedication significantly decreases side effects. Additionally, there may be cost savings by reduced patient visits and medication requirements [68]. To assess the safety of cluster SCIT, meta-analysis showed that no differences existed in the incidence of either local adverse reaction or systemic adverse reaction between the cluster group and control group. Based on the current limited evidence,

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this meta-analysis could not conclude affirmatively that cluster subcutaneous immunotherapy was a safe and efficacious option for the treatment of patients with allergic rhinitis [69].

It is important to conclude that accelerated build-up schedules have advantages over conventional schedules. They bring better compliance, cost effectiveness, and a reduction in dosage errors since most patients can reach the maintenance dose in shorter time. The introduction of premedication provides a safety profile similar to that of conventional schedules [70–72]. But main decision in favouring the treatment course lies on the clinician who is the only responsible person also in considering possible side effect of preferring the route of administration and the chosen protocol.

At present, it is also unclear whether subcutaneous or sublingual allergen immunotherapy has better outcomes. Subcutaneous protocols seem to be more effective in reducing symptoms for dust mites and grass allergy, but no one could declare any conclusive evidence of superiority of SLIT or SCIT because of a lack of true head-to-head studies. However, trend has favoured SCIT as more effective therapy [73].

6. Future of subcutaneous allergen immunotherapy

Recent research of the cellular and molecular basis of allergic reactions has advanced contemporary understanding of the mechanisms involved in allergic diseases. Newly discovered mechanisms have also helped the development of innovative approaches that are likely to further improve the control of allergic responses in the future. Only allergen immunotherapy induces immunological tolerance and changes the course of disease. Novel vaccines should meet increasing needs for reduction in adverse effects, costs, and duration of treatment [74]. The vaccines have to induce long-term tolerance to allergens.

The efficacy of allergen immunotherapy for the treatment of respiratory allergy (allergic rhinoconjunctivitis with or without bronchial asthma) has been clearly demonstrated in numerous well-designed, placebo-controlled trials. One of the most important studies was the PAT study. PAT study was conducted on children with allergic rhinoconjunctivitis and followed for 10 years with asthma development as the primary outcome. It showed that three years of continuous subcutaneous allergen immunotherapy reduced the risk of developing asthma in comparison to the control group. The difference was maintained at follow-up after 10 years [75].

All preparations that are currently available (standardized extract, allergoids, and recombinant allergen) may trigger side effects. A higher risk is detected in subjects with accelerated dosing schedules, and in subjects with asthma [76]. Contemporary research which is focusing on different administration modalities includes epicutaneous and intralymphatic route of administration of allergen extracts. Both novel strategies showed similar efficacy in the treatment of grass pollen allergy. Results gathered from recent studies have shown less demand on numbers of shots as well as on less total dose of allergen [77, 78].

Other way for enhancing desirable immune response of allergens is the biological modification of allergen preparations. Modification can be achieved using recombinant technology resulting in modified proteins and peptides [74]. Such peptide-based allergen preparations which do not bind IgE, induce increase in Il-10 and so consequently reduce the activity of mast cells as well as the modulation of synthesis Th1 and Th2 cytokines [79].

Novel adjuvants, i.e. nucleotide immunostimulatory sequences derived from bacteria CpG or monophosphoryl lipid A could be an alternative strategy in potentiating Th1 response of subcutaneous allergen immunotherapy [79]. The addition of TLR agonists as adjuvants, their use by themselves (TLR4, 8, 9), allergens coupled to virus-like particles or to hepatitis B PreS-fusion peptide also have shown some benefits in the novel treatment strategies [73, 75].

Additive effect to the allergen immunotherapy in the treatment of allergic rhinitis and asthma could be achieved by administration of anti-IgE recombinant humanized monoclonal antibody—omalizumab. Omalizumab which blocks the effects of IgE, improves efficacy, potentiates immuno-modifying effect, and decreases adverse effects when administered along with allergen immunotherapy. Although the cost of the combination of immunotherapy with anti-IgE treatment is high, this should be considered in view of the enhanced benefit/risk ratio and the known long-term benefits of allergen immunotherapy [79].

7. Author's remarks

Subcutaneous allergen immunotherapy seems to be more effective, but still there is lack of true head-to-head studies, favouring it as more effective therapy over sublingual treatment. Accelerated build-up schedules have advantages over conventional schedules due to better compliance, cost effectiveness, and a reduction in dosage errors since most patients can reach the maintenance dose in shorter time. Even though final decision in favouring the treatment course lies on the clinician who is the only responsible person also in considering possible side effect of preferring the route of administration and the chosen protocol.

The author favours conventional schedules of subcutaneous route of therapeutic interventions. Possibly, such view on the treatment process looks very conservative and from the perspective of contemporary knowledge described above, could be "scientifically" unpopular. When starting the treatment process in our office physician provides the patient with an example that "Gaining lean body weight or training for muscle gain is a slow process that takes months and years rather than days and weeks" and so the most efficient treatment with minimum risk lies on application in subcutaneous form and under the conventional schedules.

Other argument using SCIT opposite to SLIT is the personal experience that almost no one (mostly out of season) takes drops/tablets regularly, so the maximal dose-related effect could not be expected. The idea of such non-compliance in common patient community in contrary to patient community underwent the trial treatment with regular follows-up are indirectly confirmed by information obtained from IMS reports (personal communication). Data from IMS reports show the decrease in selling SLIT drugs in the period out of season.

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Given the lack of specific biomarker in monitoring treatment course, the main control mechanism of efficacy is the evaluation of quality of life using simple evaluation scale as visual analogue scale (VAS) or standardized respiratory allergy questionnaires. In evaluation of quality of life-treated patients modified VAS-like questionnaire is used since 2015. Multiple "umbrella" shaped visual analogue questionnaire is not one-parametric as visual analogue scale, but it is not as complicated as many allergy-specific questionnaires either [80].

Allergen upload for 1-year treatment is determined in 10–12 injected doses (one injection in every 4–6 weeks recommended in SPCs). When patient forgets to keep advised dosing due to personal non-compliance, he is kindly asked to hold the regular visits on the ground of reaching highest efficacy after three to five year lasting treatment. It shows us good results of indicated treatment as seen in evaluation of QoL questionnaires after finishing the treatment course (unpublished data).

8. Conclusion

The presented review summarizes the literary-acquired knowledge as well as author's own experience in conducting aero-allergen immunotherapy, particularly in subcutaneous route of administration for all modalities of respiratory allergy disease from allergic rhinosinusitis, bronchial asthma to united airway disease. Because of appropriate efficacy in connection with satisfactory safety the author favours conventional schedules of subcutaneous route of therapeutic intervention.

The future of aero-allergen immunotherapy should be focused on new hypoallergenic molecules/adjuvants, on new routes of allergen administration and on searching potential biomarkers which could be objectively measured and easily accessible from body fluids (blood, nasal secretion, sputum). The combination of estimated biomarkers obtained from biological samples in conjunction with evaluation of quality of life could lead to the generation of the overall satisfactory monitoring protocol. Due to enormous overload seen in the scientific literature, it seems that ongoing research in the field of allergen immunotherapy will bring even brighter future.

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References

[1] Pawankar R, et al. World Allergy Organization. White Book on Allergy. Update 2013. Executive Summary. Milwaukee:WAO;2013. 13p.

[2] Summary Health Statistics for U.S. Children: National Health Interview Survey, Table 2. [Internet]. 2012. Available from: http://www.cdc.gov/nchs/fastats/asthma.htm [Accessed: 2016-09-09]

[3] Summary Health Statistics for U.S. Adults: National Health Interview Survey, Table 3, 4. [Internet]. 2012. Available from: http://www.cdc.gov/nchs/fastats/asthma.htm [Accessed: 2016-09-09]

[4] Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action. World Allergy Organ J. 2014;7(1):12. doi:10.1186/1939-4551-7-12

[5] Larsen JN, Broge L, Jacobi H. Allergy immunotherapy: the future of allergy treatment. Drug Discov Today. 2016;21(1):26–37. doi:10.1016/j.drudis.2015.07.010

[6] Pawankar R, et al. World Allergy Organization. White Book on Allergy. Milwaukee:WAO; 2011. 228p.

[7] Moller C, Dreborg S, Ferdousi HA, et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT study). J Allergy Clin Immunol. 2002;109(2):251–256. doi:10.1067/mai.2002.121317

[8] Pajno GB, Barberio G, De Luca F, et al. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. Clin Exp Allergy. 2001;31(9):1392–1397. doi:10.1046/j.1365-2222.2001.01161.x

[9] Abramson MJ, Puy RM, Weiner JM. Allergen immunotherapy for asthma. Cochrane Database Syst Rev. 2003;(4):CD001186. doi:10.1002/14651858.CD001186

[10] Allergy Immunotherapy (Subcutaneous). Guideline: CG-MED-52. [Internet]. 2016. Available from: https://www.anthem.com/medicalpolicies/guidelines/gl_pw_c183207.htm [Accessed: 2016-09-09]

[11] Fitzhugh D, Cohen SG, Evans III R. Allergen Immunotherapy in a Historical Perspective. In Lockey RF, Ledford DK (Eds): Allergens and Allergen Immunotherapy Subcutaneous, Sublingual and Oral. Fifth edition. Boca Raton: CRC Press;2014. 3–24pp.

[12] Ring J, Gutermuth J. 100 years of hyposensitization: history of allergen-specific immunotherapy (ASIT). Allergy. 2011;66(6):713–724. doi:10.1111/j.1398-9995.2010.02541.x

[13] Noon L, Cantab BC. Prophylactic inoculation against hay fever. Lancet. 1911;177(4580):1572–1573. (Originally published as Volume 1, Issue 4580).

[14] Immunotherapy: From the Beginning. [Internet]. Available from: http://www.allergy-therapeutics.com/immunotherapy/immunotherapy-from-the-beginning/ [Accessed: 2016-09-09]

[15] Larenas Linnemann DE. One hundred years of immunotherapy: review of the first landmark studies. Allergy Asthma Proc. 2012;33(2):122–128. doi:10.2500/aap.2012.33.3515

[16] Freeman J, Oxon MD. Further observation on the treatment of hay-fever by hypodermic inoculation of pollen vaccine. Lancet. 1911;178(4594):814–817. (Originally published as Volume 2, Issue 4594).

[17] Freeman J, Oxon MD. Vaccination against hay fever: report of results during the last three years. Lancet. 1914;183(4730):1178–1180. (Originally published as Volume 1, Issue 4730).

[18] Clowes GHA. A preliminary communication on the treatment of autumnal hay fever by vaccination with aqueous extract of the pollen of ragweed. Proc Soc Exp Biol Med. 1913;10:70–72.

[19] Cooke RA. The treatment of hay fever by active immunization. Laryngoscope. 1915;25(2):108–112.

[20] Cooke RA, Loveless M, Stull A. Studies on immunity in a type of human allergy (hay fever): serologic response of non-sensitive individuals to pollen injections. J Exp Med. 1937;66(6):689–696.

[21] Cooke RA, Menzel AE, Kessler WR, Myers PA. The antibody mechanisms of ragweed allergy; electrophoretic and chemical studies. I. The blocking antibody. J Exp Med. 1995;101(2):177–196.

[22] Zoss AR, Koch CA, Hirose RS. Alum-ragweed precipitate: preparation and clinical investigation. J Allergy.1937;8(4):329–335.

[23] Frankland AW, Augustin R. Prophylaxis of summer hay-fever and asthma: a controlled trial comparing crude grass-pollen extracts with the isolated main protein component. Lancet. 1954;266(6821):1055–1057.

[24] Johnstone DE. Study of the role of antigen dosage in the treatment of pollenosis and pollen asthma. AMA J Dis Child. 1957 Jul;94(1):1–5. doi:10.1001/archpedi.1957.04030020003001

[25] Johnstone DE, Dutton A. The value of hyposensitization therapy for bronchial asthma in children—a 14-year study. Pediatrics. 1968 Nov;42(5):793–802.

[26] Malling HJ (ed). EAACI Immunotherapy position paper. Allergy 1988;43(Suppl. 6):9–33. doi:10.1111/j.1398-9995.1988.tb04767.x

[27] Malling HJ, Weeke B. Position paper: immunotherapy. Allergy. 1993;48(Suppl. 14):9–35. doi:10.1111/j.1398-9995.1993.tb04754.x

[28] Nicklas RA, et al. Practice parameters for allergen immunotherapy. J Allergy Clin Immunol. 1996;98(6 Pt 1):1001–1011. doi:10.1016/S0091-6749(96)80183-7

[29] Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. J Allergy Clin Immunol. 1998;102(4 Pt 1):558–562. doi:10.1016/S0091-6749(98)70271-4

[30] Cox L, et al. Allergen immunotherapy: a practice parameter second update. J Allergy Clin Immunol. 2007;120(Suppl. 3):S25–85. doi:10.1016/j.jaci.2007.06.019

[31] Cox L, et al. Allergen immunotherapy: a practice parameter third update. J Allergy Clin Immunol. 2011;127(Suppl. 1):S1–55. doi:10.1016/j.jaci.2010.09.034

[32] Larche M, et al. Immunological mechanisms of allergen-specific immunotherapy. Nat Rev Immunol. 2006;6(10):761–771. doi:10.1038/nri1934

[33] Maggi E, Vultaggio A, Matucci A. T-cell responses during allergen-specific immunotherapy. Curr Opin Allergy Clin Immunol. 2012;12(1):1–6. doi:10.1097/ACI.0b013e32834ecc9a

[34] Akdis C, et al. Mechanisms of allergen-specific immunotherapy. J Allergy Clin Immunol. 2014;133(3):621–631. doi:10.1016/j.jaci.2013.12.1088

[35] Novak N, Mete N, Bussmann C, Maintz L, Bieber T, Akdis M, et al. Early suppression of basophil activation during allergen-specific immunotherapy by histamine receptor 2. J Allergy Clin Immunol. 2012;130(5):1153–1158.e2. doi:10.1016/j.jaci.2012.04.039

[36] Robinson DS, et al. Tregs and allergic disease. J Clin Invest. 2004;114(10):1389–1397. doi:10.1172/JCI23595

[37] Santos AF, James LK, Bahnson HT, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. J Allergy Clin Immunol. 2015;135(5):1249–1256. doi:10.1016/j.jaci.2015.01.012

[38] Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. J Clin Invest. 1998;102(1):98–106. doi:10.1172/JCI2250

[39] Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R, et al. Immune responses in healthy and allergic Individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. J Exp Med. 2004;199(11):1567–1575. doi:10.1084/jem.20032058

[40] Gleich GJ, Zimmermann EM, Henderson LL, Yunginger JW. Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. J Allergy Clin Immunol. 1982;70:261–271. doi:10.1016/0091-6749(82)90062-8

[41] Fujita H, Soyka MB, Akdis M, et al. Mechanisms of allergen-specific immuno-therapy. Clin Transl Allergy. 2012;2(1):2. doi:10.1186/2045-7022-2-2

[42] Meiler F, Klunker S, Zimmermann M, et al. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. Allergy. 2008;63(11):1455–1463. doi:10.1111/j.1398-9995.2008.01774.x

[43] Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol. 2005;116(3):608–613. doi:10.1016/j.jaci.2005.06.004

[44] Ozdemir C, Kucuksezer UC, Akdis M, Akdis CA. Mechanisms of aeroallergen immunotherapy: subcutaneous immunotherapy and sublingual immunotherapy. Immunol Allergy Clin North Am. 2016;36(1):71–86. doi:10.1016/j.iac.2015.08.003

[45] Tversky JR, Bieneman AP, Chichester KL, Hamilton RG, Schroeder JT. Subcutaneous allergen immunotherapy restores human dendritic cell innate immune function. Clin Exp Allergy. 2010;40(1):94–102. doi:10.1111/j.1365-2222.2009.03388.x

[46] Van Bever HP, Stevens WJ. Suppression of the late asthmatic reaction by hyposensitization in asthmatic children allergic to house dust mite (Dermatophagoides pteronyssinus). Clin Exp Allergy. 1989;19:399–404. doi:10.1111/j.1365-2222.1989.tb02405.x

[47] Rak S, Rowhagen O, Venge P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen allergic patients. J Allergy Clin Immunol. 1988;82(3 Pt 1):470–480. doi:10.1016/0091-6749(88)90021-8

[48] Durham SR, Kay AB, Hamid Q. Changes in allergic inflammation associated with successful immunotherapy. Int Arch Allergy Immunol. 1995;107(1–3):282–284. doi:10.1159/000237003

[49] Arvidsson MB, Löwhagen O, Rak S. Allergen specific immunotherapy attenuates early and late phase reactions in lower airways of birch pollen asthmatic patients: a double blind placebo-controlled study. Allergy. 2004;59(1):74–80. doi:10.1046/j.1398-9995.2003.00334.x

[50] Ebner C, et al. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH 1in T-cell clones specific for Phl p 1, a major grass pollen allergen. Clin Exp Allergy. 1997;27(9):1007–1015. doi:10.1111/j.1365-2222.1997.tb01252.x

[51] Verhoef A, Alexander C, Kay AB, Larché M. T cell epitope immunotherapy induces a CD4+ T cell population with regulatory activity. PLoS Med. 2005;2(3):e78. doi:10.1371/journal.pmed.0020078

[52] Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa. J Allergy Clin Immunol. 2008;121(6):1467–1472, 1472.e1. doi:10.1016/j.jaci.2008.03.013

[53] Mauri C, Bosma A. Immune regulatory function of B cells. Annu Rev Immunol. 2012;30:221–241. doi:10.1146/annurev-immunol-020711-074934

[54] Jutel M, et al. IL-10 and TGF-β cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol. 2003;33(5): 1205–1214. doi:10.1002/eji.200322919

[55] Burks AW, et al. Update on allergy immunotherapy: American Academy of Allergy, Asthma & Immunology/European Academy of Allergy and Clinical Immunology/ PRACTALL consensus report. J Allergy Clin Immunol. 2013;131(5):1288–1296.e3. doi: 10.1016/j.jaci.2013.01.049

[56] Moingeon P. Adjuvants for allergy vaccines. Hum Vaccin Immunother. 2012;8(10):1492–1498. doi:10.4161/hv.21688

[57] Canonica GW, Bousquet J, Casale T, Lockey RF, Baena-Cagnani CE, Pawankar R, et al. Sub-lingual immunotherapy: world allergy organization position paper. Allergy. 2009;64(Suppl. 91):1–59. doi:10.1111/j.1398-9995.2009.02309.x

[58] Exley C. Aluminium adjuvants and adverse events in sub-cutaneous allergy immunotherapy. Allergy Asthma Clin Immunol. 2014;10(1):4. doi:10.1186/1710-1492-10-4

[59] Gheradi RK, Coquet M, Cherin P, Belec L, Moretto P, Dreyfuss PA, Pellissier JF, Chariot P, Authier FJ. Macrophagic myofasciitis lesions assess long-term persistence of vaccine-derived aluminium hydroxide in muscle. Brain. 2001;124:1821–1831. doi:10.1093/brain/ 124.9.1821

[60] Kramer MF, Heath MD. Aluminium in allergen-specific subcutaneous immunotherapy–a German perspective. Vaccine. 2014;32(33):4140–4148. doi:10.1016/j.vaccine. 2014.05.063

[61] Tomljenovic L, Shaw CA. Aluminum vaccine adjuvants: are they safe? Curr Med Chem. 2011;18:2630–2637. doi:10.2174/092986711795933740

[62] Leynadier F, Banoun L, Dollois B, et al. Immunotherapy with a calcium phosphate adsorbed five-grass-pollen extract in seasonal rhinoconjunctivitis: a double-blind, placebo-controlled study. Clin Exp Allergy. 2001;31(7):988–996. doi:10.1046/j. 1365-2222.2001.01145.x

[63] Nacaroglu HT, Erdem SB, Sumer O, Karaman S, UnsalKarkıner CS, Asilsoy S, Gunay I, Can D. Local and systemic reactions to subcutaneous allergen immunotherapy: ten years' experience in a pediatric clinic. Ann Allergy Asthma Immunol. 2016;116(4):349–353. doi:10.1016/j.anai.2016.01.015

[64] SPC [Internet]. Available from: https://www.medicines.org.uk/emc/medicine/31601. [Accessed: 2016-09-09]

[65] SPC [Internet]. Available from: https://www.medicines.org.uk/emc/medicine/31610. [Accessed: 2016-09-09]

[66] Jutel M, et al. International consensus on allergy immunotherapy. J Allergy Clin Immunol. 2015;136(3):556–568. doi:10.1016/j.jaci.2015.04.047

[67] Pfaar O, et al. Guideline on allergen-specific immunotherapy in IgE-mediated allergic diseases: S2k Guideline of the German Society for Allergology and Clinical Immunology (DGAKI), the Society for Pediatric Allergy and Environmental Medicine (GPA), the Medical Association of German Allergologists (AeDA), the Austrian Society for Allergy and Immunology (ÖGAI), the Swiss Society for Allergy and Immunology (SGAI), the German Society of Dermatology (DDG), the German Society of Oto-Rhino-Laryngology, Head and Neck Surgery (DGHNO-KHC), the German Society of Pediatrics and Adolescent Medicine (DGKJ), the Society for PediatricPneumology (GPP), the German Respiratory Society (DGP), the German Association of ENT Surgeons (BV-HNO), the Professional Federation of Paediatricians and Youth Doctors (BVKJ), the Federal Association of Pulmonologists (BDP) and the German Dermatologists Association (BVDD). Allergo J Int. 2014;23(8):282–319. doi:10.1007/s40629-014-0032-2

[68] Calabria CW. Accelerated immunotherapy schedules. Curr Allergy Asthma Rep. 2013; 13(4):389–398. doi:10.1007/s11882-013-0356-x

[69] Feng S, Xu Y, Ma R, Sun Y, Luo X, Li H. Cluster subcutaneous allergen specific immunotherapy for the treatment of allergic rhinitis: a systematic review and meta-analysis. PLoS One. 2014;9(1):e86529. doi:10.1371/journal.pone.0086529

[70] Tabar AI, Echechipía S, García BE, Olaguibel JM, Lizaso MT, Gómez B, Aldunate MT, Martin S, Marcotegui F. Double-blind comparative study of cluster and conventional immunotherapy schedules with Dermatophagoides pteronyssinus. J Allergy Clin Immunol. 2005;116(1):109–118. doi:10.1016/j.jaci.2005.05.005

[71] Sharkey P, Portnoy J. Rush immunotherapy: experience with a one-day schedule. Ann Allergy Asthma Immunol. 1996;76(2):175–180. doi:10.1016/S1081-1206(10)63419-9

[72] Casanovas M, Martín R, Jiménez C, Caballero R, Fernández-Caldas E. Safety of an ultra-rush immunotherapy build-up schedule with therapeutic vaccines containing depigmented and polymerized allergen extracts. Int Arch Allergy Immunol. 2006;139(2):153–158. doi:10.1159/000090392

[73] Casale TB, Stokes JR. Immunotherapy: what lies beyond. J Allergy Clin Immunol. 2014; 133(3):612–619. quiz 620. doi:10.1016/j.jaci.2014.01.007

[74] Jutel M, Kosowska A, Smolinska S. Allergen immunotherapy: past, present, and future. Allergy Asthma Immunol Res. 2016;8(3):191–197. doi:10.4168/aair.2016.8.3.191

[75] Jacobsen L, et al. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. Allergy. 2007;62:943–948. doi:10.1111/j.1398-9995.2007.01451.x

[76] Cappella A, Durham SR. Allergen immunotherapy for allergic respiratory diseases. Hum Vaccin Immunother. 2012;8(10):1499–1512. doi:10.4161/hv.21629

[77] Senti G, von Moos S, Tay F, Graf N, Sonderegger T, Johansen P, et al. Epicutaneous allergen-specific immunotherapy ameliorates grass pollen-induced rhinoconjunctivitis:

a double-blind, placebo-controlled dose escalation study. J Allergy Clin Immunol. 2012;129(1):128–135. doi:10.1016/j.jaci.2011.08.036

[78] Hylander T, Latif L, Petersson-Westin U, Cardell LO. Intralymphatic allergen-specific immunotherapy: an effective and safe alternative treatment route for pollen-induced allergic rhinitis. J Allergy Clin Immunol. 2013;131(2):412–420. doi:10.1016/j.jaci.2012.10.056

[79] Nouri-Aria KT. Recent progress in allergen immunotherapy. Iran J Immunol. 2008;5(1):1–24. doi:IJIv5i1A1

[80] Lukan N, Chmelarova A, Petrovicova J. Applied multiple umbrella visual analogue scale for respiratory allergy-preliminary study. J Allergy Clin Immunol. 2015;135(Suppl. 2):AB48. doi:10.1016/j.jaci.2014.12.1087

Chapter 5

Vitamin B12: Could It Be a Promising Immunotherapy?

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Additional information is available at the end of the chapter

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Abstract

Vitamin B12 is a water-soluble vitamin and an important micronutrient with critical role in DNA, protein, and lipid synthesis. It is responsible for one-carbon metabolism and cell division of nervous and hematopoietic cells. Among its various functions, the role as immunomodulator in cellular immunity, especially in elevating the number of CD8+ cells and NK cells, attracts scientific interest. Many alternative anticancer and anti-inflammatory treatments involve the use of B12 together with other vitamins and nutrients, but still the scientific information is too obscure and insufficient. Controversial data link tumorigenesis with either increased or decreased B12 blood levels in different types of cancer. Dietary intake and additional supplement with the vitamin do not protect against cancer risk, but the dominant opinion is to integrate B12 as part of rational and healthy nutrition to ensure proper function of the immune system. This chapter will review in brief the most important facts for vitamin B12 functions and properties. We will try also to present in concise way the human immune system and the exact role of B12 in immune activity with emphasis on the questionable participation of vitamin B12 in the process of carcinogenesis and its significance as anticancer immunotherapy.

Keywords: vitamin B12, immunonutrition, immunomodulation, immunotherapy, tumorigenesis, cancer, inflammation

1. Introduction

Cancer is the final outcome of uncontrolled overgrowth of normal cells. Cancer cells remain insensitive to antiproliferative signals and apoptosis. As a result, they replicate, proliferate, and invade infinitely and aggressively. Although the genetic events are thought to be the most important in the process of carcinogenesis, other factors can facilitate abnormal cell development. For many years, inflammation and anti-inflammatory response were widely associated with malignancy [1, 2] and recognized as major elements that trigger carcinogenesis.

Extended inflammation, especially in chronic infections, predisposes to cancer, but still the mechanism(s) involved is (are) not definitely known. Usually all inflammatory processes are followed rapidly by anti-inflammatory defense response—excessive production of pro-inflammatory signals (mediators) and reactive oxygen and nitrogen species. The pro-inflammatory mediators (cytokines, chemokines, and eicosanoids) may stimulate proliferation of both untransformed and tumor cells [2]. The reactive oxygen and nitrogen species lead to oxidative stress and damage of macromolecules, especially DNA to increase the risk of genetic mutations and tumorigenesis [3].

A continuously increasing number of microelements, vitamins, and mineral salts are reported to modulate the immune response and counter the inflammation and cancer, when taken as part of the healthy diet or as nutrient supplements. This concept is becoming more and more popular and nowadays is widely accepted and known as immunonutrition—an important part of each healthful dietary regime and immunotherapy approach. Immunonutrition means "modulation of the activities of the immune system, and the consequences on the patient of immune activation, by nutrients or specific food items fed in amounts above those normally encountered in the diet" [4]. Many formulations and routes of administration have been tested but with inconsistent results. Arginine, glutamine, omega-3 fatty acids, nucleotides, copper, selenium, zinc, vitamins of group B, C, and E are the most popular immunonutrients used alone or in different formulas. Among them, vitamin B12 attracts a great proportion of both scientific and wide public interest, because of its complex biological function. Unfortunately, as for the other promising immunonutrients, the real scientifically based information is too obscure or even missing. In the current work, we will try to summarize the available data and to elucidate the true implication of vitamin B12 in the proper function of the immune system and in the inflammatory response.

2. Vitamin B12: a miraculous molecule or a modern falsification

2.1. Chemical structure of vitamin B12 (cobalamin)

Vitamin B12 (cobalamin) is a water-soluble vitamin. It is the largest (molecular weight > 1000 g) and the most complex vitamin [5]. The chemical structure of B12 consists of a cobalt atom and four pyrroles in the center of corrin ring bound to a nucleotide part (ribose)—5,6-dimethylbenzimidazole (**Figure 1**) [6].

Cobalamin (Cbl) has the ability to bind to various functional groups. When the group is cyanide, it is called cyanocobalamin—represents the most stable active form of vitamin B12 and the most popular synthetic form. Other active forms in the human body are 5-deoxyadenosylcobalamin—with 5'-deoxyadenosine; methylcobalamin—with methyl group; and hydroxocobalamin—with hydroxyl group [6, 7].

2.2. Food sources of vitamin B12

The main dietary sources of vitamin B12 are animal foods—meat, liver, fish, and dairy products. It is also found in cobalamin-synthesizing bacteria and oysters. Plant foods do not contain

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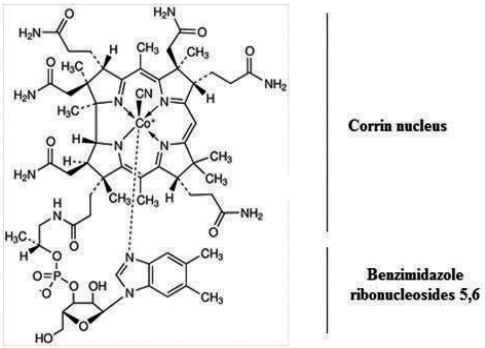

Figure 1. Chemical structure of cyanocobalamin.

vitamin B12 [8]. Some authors disagree with this fact [9, 10], but recent studies have shown that plant cell has the ability to produce only similar to B12 compounds (called pseudo-B12), which compete with B12 for the same cellular receptors, i.e., prevent normal physiological absorption of B12 [11].

2.3. Metabolism of vitamin B12

In the human body, cyanocobalamin is easily hydrolyzed to hydroxocobalamin. After the hydrolysis, it is converted to one of the two active forms—methylcobalamin or adenosylcobalamin (also known as coenzyme B12) [12]. Both forms act as enzyme cofactors.

Vitamin B12 is involved in two main enzymatic reactions. The first reaction, involving methylcobalamin, is remethylation of amino acid homocysteine to methionine and is catalyzed by methionine synthase (**Figure 2**) [9, 11, 13]. In this reaction, 5-methyltetrahydrofolic acid participates as a methyl group donor, while cobalamin is only an intermediate acceptor of the group.

In cobalamin deficiency, the synthesis of methionine is impaired and toxic amino acid homocysteine accumulates [11]. Vitamins B12, B6, and B9 are working together to control the levels of homocysteine in the blood (**Figure 2**). Homocysteine acts as a neurotoxin and as a toxin for the blood vessels increasing the risk of cardiovascular disease. In the laboratory diagnostics, high level of homocysteine is one of the signs of B12 deficiency [14].

The synthesis of methionine also produces tetrahydrofolic acid, which is essential for a number of folate-dependent reactions [11, 15], such as DNA synthesis [16]. The loss of this function is demonstrated in individuals with vitamin B12 deficiency, which explains why cobalamin deficiency often mimics folic acid deficiency.

The second enzymatic reaction requires adenosylcobalamin, which is located in the mitochondria and acts as coenzyme for methylmalonyl-CoA mutase [11, 17]. Methylmalonyl-CoA mutase catalyzes conversion of methylmalonyl-CoA to succinyl-CoA (**Figure 2**), an important metabolite in the Krebs cycle [16] and essential factor for the degradation of odd-chain fatty acids. In individuals with B12 deficiency, the activity of methylmalonyl-CoA mutase is dam-

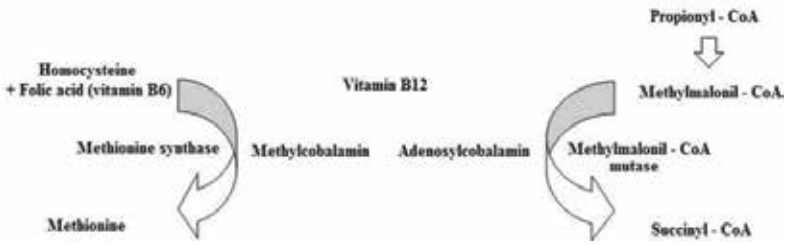

Figure 2. Metabolism of cobalamin (vitamin B12) in humans.

aged, and as a result, the levels of methylmalonic acid (MMA) in the body increase [11, 18]. The demonstration of elevated levels of MMA in urine or blood is a diagnostic sign of B12 deficiency too [14].

2.4. Digestion and absorption of vitamin B12

Simultaneously, with the digestion of animal products, vitamin B12 reaches the stomach. Under the action of the low pH (HCl) and pepsin, cobalamin is separated from the proteins to which it is connected in the food [19]. Then, it is associated with R-binders (or haptocorrins, transcobalamin I, R-factors)—glycoproteins secreted from the stomach cells and salivary glands. Their role is to protect vitamin B12 from chemical denaturation in the stomach (**Figure 3**) [19].

The intrinsic factors (IFs) have the main role in the vitamin B12 transport. IFs represent stomach-specific glycoproteins secreted by the stomach parietal cells and are essential for the absorption of B12 from the intestinal lumen into the bloodstream [17, 21]. Some genetic

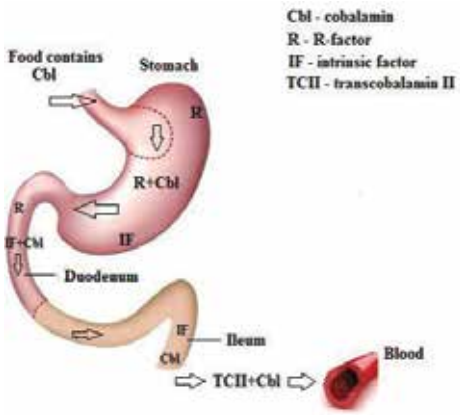

Figure 3. Absorption mechanism of vitamin B12 (based on Ref. [20]). Cobalamin (Cbl) is released from the food in the stomach, where it competes with the intrinsic factor (IF) to bind the R-factors. In duodenum R-factors are proteolytically degradated by pancreatic enzymes and released Cbl from the complex. IF and Cbl form a complex (IF-Cbl) which moves to the ileum and binds ileum receptors. The cells of the ileum absorb Cbl. Finally, Cbl binds to transcobalamin II (TC II), which delivers the complex to cells outside the intestinal tract.

defects or pathological changes in gastric and intestinal mucosa may lead to a deficiency of IF, in which case the transport of vitamin B12 is impeded and deficiency is present [17].

In circulation, B12 binds to other protein—transcobalamin II (TC II) [22], which ensures the transport to the liver, where the vitamin is stored for short time and finally is released and transported by the transcobalamin I to other tissues in the body. The excess vitamin B12 is stored for several hours in the liver without forming deposits (in contrast to fat-soluble vitamins) and is excreted in the urine.

2.5. Functions of vitamin B12 in the human body

The important reactions involved in the metabolism of vitamin B12 define its crucial role in a series of body processes. The main functions of vitamin B12 are summarized as follows [23]:

1. It carries and delivers methyl group to other molecules (including DNA and neurotransmitters). In this way, it has a significant role in cell division.
2. Vitamin B12 has activity as a coenzyme in many enzymatic reactions.
3. It participates in the synthesis of porphyrins, which are an important component of hemoglobin.
4. Together with folic acid, it is involved in the synthesis of red and white blood cells.
5. Without vitamin B12 folic acid cannot be absorbed and remains "trapped" in the intestinal wall (this is the reason why vitamin B12 deficiency causes the same symptoms as folate deficiency).
6. It supports the iron activity in the body and is involved in the synthesis of choline.
7. Vitamin B12 is necessary for reproduction and stability of DNA and RNA.
8. It helps the metabolism of vitamin A and more particularly absorption of carotene.
9. Vitamin B12, together with vitamin B6, facilitates the conversion of amino acids into hormones and neurotransmitters.
10. It supports the myelin sheath around nerve structures, working together with folic acid.

2.6. Causes of B12 deficiency in humans

Vitamin B12 deficiency is a common diagnosis, especially in older people [24]. Often the deficiency is due to mutations in the genes encoding important proteins in cobalamin metabolism, diet (vegetarian, vegan diet), and reduced production of stomach acids that are needed for the absorption of vitamin B12 [11, 23]. Other common causes are pernicious anemia (malabsorption of vitamin B12); atrophic gastritis; gastrectomy; Zollinger-Ellison syndrome; intestinal diseases, especially of the ileum (celiac disease, Crohn's disease, ileitis); pancreatic insufficiency; parasitism; bacterial overgrowth; medicament use (antiepileptic agents, proton pump inhibitors, histamine receptor antagonists, metformin, antibiotics); diabetes mellitus; renal insufficiency; smoking; and alcohol abuse.

Traditionally, the vitamin B12 deficiency is considered to be accompanied by low levels of B12 in the serum (or plasma) of the patient. This fact is disputed by some authors who believe that a significant proportion of people with normal or high levels of vitamin B12 actually have a deficiency [14, 19, 25].

The lower levels of vitamin B12 in serum are often used to assess the state of the vitamin, but this approach generates a high rate of false-negative results. A number of studies have shown that holotranscobalamin (the complex between transcobalamin II and vitamin B12) may be more reliable indicator of the status of vitamin B12 [14]. The holotranscobalamin binds only 20–30% of vitamin B12 circulating in the blood but is responsible for delivery to the cells and is considered to be the functionally important fraction. Therefore, testing for it can identify low vitamin B12 status before the total serum vitamin B12 levels drop.

The increase in homocysteine and methylmalonic acid (MMA) in the plasma is also sensitive indicators of the status of vitamin B12. The levels of plasma homocysteine may also elevate in folic acid or vitamin B6 deficiency, but the increase in MMA always indicates a poor status of vitamin B12 (the only other reason that explains the increased levels of MMA is renal insufficiency). MMA is considered the most representative marker for vitamin B12 deficiency, but the low accessibility of the assay in laboratory practice reduces its clinical utility [14].

2.7. Vitamin B12 excess

Whereas vitamin B12 deficiency has been studied intensively, the reverse situation—abnormal high levels—is rarely discussed in the literature. High plasma levels (when not associated with exterior supply) refer in all cases to some alteration in the metabolism of vitamin B12—either increased synthesis or decreased clearance of B12-binding proteins. In a routine blood tests, elevated levels of B12 are found in approximately 8–15% of patients referred for the measurement [26, 27]. The significance of this fact still needs to be clarified and linked to clinically important outcomes.

3. The immune system: how it works?

The immune system is a complex of cells, tissues, and organs that are specialized in defending against foreign agents. It is a remarkable defense mechanism and makes rapid, specific, and protective response against the innumerable potentially pathogenic microorganisms. The immune system also has a role in rejection of tumors.

3.1. Immune cells and organs

Cells of immune system are formed from pluripotent hematopoietic stem cells, capable of self-renewal and differentiation into lymphoid and myeloid progenitor cells. Lymphoid progenitors differentiate into T cells, B cells, and natural killer (NK) cells. Myeloid progenitors differentiate into monocytes and macrophage, granulocytes (eosinophils, basophils, and neutrophils), and other cell types.

In the primary lymphoid tissues (thymus and bone morrow), lymphocytes develop and mature to a stage at which they are able to respond to a pathogen. T and B lymphocytes both originate from lymphoid precursors in the bone morrow, but whereas B cells complete their maturation in the bone marrow, before entering the circulation, T cells leave the bone marrow at an immature stage and migrate to the thymus where they mature [28].

In the secondary lymphoid tissue (lymph glands, lymphatic vessels, spleen, and mucosa-associated lymphoid tissue), mature lymphocytes become stimulated to respond to invading pathogens [28]. The main function of lymph nodes is to trap antigens that flow into them via afferent lymphatic and to provide place for clonal expansion of lymphocytes. The spleen is the lymphoid organ that serves as filter for the blood to remove damaged or senescent red cells and protect against blood-borne pathogens. Splenic macrophages and dendritic cells grab the microorganisms and microbial products in the blood and then stimulate the T and B cells that arrive in the spleen from the blood. The thymus is an organ that lies behind the breast bone and where the T lymphocytes mature.

3.2. Innate and adaptive immunity

In regard to its function, the immune system is divided into two major components: innate immunity and adaptive immunity.

The innate immune response occurs rapidly and can generate effective mechanisms that start rapidly after the infection. The innate immune system consists of physical barriers such as the skin, chemical and microbiological barriers in the mucous membranes, phagocytic cells, and soluble factors [29]. The cells of the innate immunity are phagocytic cells (neutrophils, monocytes, macrophage), cells that release inflammatory mediators (mast cells, basophils, and eosinophils) and NK cells [30].

Neutrophils are the first line of defense in the body. They are recruited at the site of infection immediately after the invasion of a foreign antigen.

Monocytes are leucocytes that circulate in the blood and travel to tissues where they mature into macrophages able to engulf dead cells or invading pathogens [28]. After exposition to inflammatory stimuli, macrophages secrete cytokines such as tumor necrosis factor (TNF), interleukins (IL), leukotrienes, and prostaglandins. All these molecules increase vascular permeability and recruit inflammatory cells [31].

Eosinophils can kill large pathogens which cannot be phagocytized, while basophils and mast cells are implicated in the regulation of the immune response to parasites [28]. These cells play substantial roles in the induction of allergic inflammatory responses too. Mast cells and basophils can produce cytokines such as ILs, granulocyte-macrophage colony-stimulating factor, and TNF which are important for late consequences in allergic inflammatory responses [32].

Natural killer (NK) cells have the morphology of lymphocytes, but they do not bear specific antigen receptors [29]. NK cells are important in the defense against viral infection by killing infected cells and secreting of cytokines that hamper viral replication [28].

Soluble factors include the complement, acute-phase proteins, and cytokines [30]. Complement represents a key component of the innate immunity. It is composed of more than 40 proteins and produced mainly by the liver. It is a cascade of soluble proteins and membrane-expressed receptors and regulators, which operates in plasma, on cell membranes, and within cells [33]. The main roles of the complement activation are (1) opsonization of microbes and promoting phagocytosis, (2) triggering of inflammation process after diffusion of complement components away from the site of activation, (3) elimination of large immune complexes from the blood, and (4) membrane rupture of foreign cells.

Acute-phase proteins are a class of plasma proteins that include C-reactive protein, serum amyloid A protein, proteinase inhibitors, and coagulation proteins. They enhance the resistance to infection and support the repair of damaged tissue [30].

Cytokines are chemical messengers secreted by one cell to modify its own behavior or the activity of other cells. Cytokines that are produced by leucocytes and affect other white cells are named interleukins. Chemokines have chemoattractant activity and colony-stimulating factors cause differentiation and proliferation of stem cells. Interferons are a major class of cytokines which have antiviral activity [29].

The soluble factors are important in engaging monocytes, macrophages, and neutrophils in the phagocytosis [34], during which the foreign agents are destroyed by lysosomal enzymes, acidic pH, and radical attacks.

The second line of defense against a pathogen is the adaptive immunity, which takes several days to fully develop. Adaptive immune responses involve the proliferation of T and B lymphocytes after expressing on their surface of antigen-specific receptors.

B cells secrete immunoglobulins—the antigen-specific antibodies responsible for eliminating extracellular bacteria.

T cells help B cells to make antibody and can eradicate intracellular pathogens by activating macrophages and killing infected cells. Mature T cells display different surface markers and have different roles in adaptive immunity: cytotoxic T lymphocytes (also named CD8+ T cells) directly attack and kill infected or tumor cells; helper T lymphocytes (also named CD4+ T cells) send signals (cytokines) to other types of immune cells (CD8+ T cells); and regulatory T cells, called suppressor T cells, suppress the immune response [28].

Innate immunity and adaptive immunity interact and work together to eliminate pathogens and to protect the body from infection and disease.

4. Vitamin B12 as immunomodulator

4.1. Specific role of vitamin B12 in immune system functioning

Vitamin B12 plays a crucial role in the proper functioning of immune system. Methionine synthase, which uses methylcobalamin as a cofactor, is essential for the synthesis of purines

and pyrimidines in all cells, including fast-dividing immune cells. Several studies (both in man and on animal models) have reported the exact function of vitamin B12 in the immune response.

B12 deficiency leads to a low number of lymphocytes and impairs the activity of NK cells (the most important for destroying cancer cells) [35]. More specifically, CD8+ cells are decreased in patients with B12 deficiency anemia when compared to control population. Although the total number of CD4+ lymphocytes remains the same, the proportion of CD4+ is significantly elevated in such patients, and hence an abnormally high CD4/CD8 ratio is detected. A considerably suppressed NK cell activity was also noted in humans with B12 deficiency [35], as well as a decrease in the spleen NK activity was observed in rats on B12-deficient diet, although this effect was not statistically significant in the thymus or axillary nodes [36].

Intramuscular injections with B12 (under the form of methylcobalamin) in newly diagnosed B12-deficient patients completely restore the production of CD8+ T lymphocytes, the abnormally increased CD4/CD8 ratio, the CD3−CD16+ and CD16+CD57+ count (which possess strong NK cell activity), and hence the NK cells activity [35]. In contrast, serum levels of immunoglobulins are not affected by vitamin B12 deficiency or supplementation [35]. Intramuscular administration of cyanocobalamin in patients with pernicious anemia and low serum levels of vitamin B12 (three to ten times lower than reference level) increases the number of CD8+ and decreases CD4/CD8 ratio back to normal [37].

In addition, a significantly lower lymphoblastic response to *Mycobacterium paratuberculosis* and higher susceptibility toward gastrointestinal nematodes were reported in lambs put on B12-deficient diet, but no differences were found in white blood cell counts and antibody production against bovine herpesvirus type 1 and *M. paratuberculosis* [38].

An enhancing effect of methylcobalamin on the proliferative response to concanavalin A (a selective T cell mitogen) and autologous B cells was also observed in human T lymphocyte cultures in vitro [39].

Vitamin B12 could minimize the effects of protein malnutrition in the hematological or immune system—30-day addition of vitamin B12 to a low-protein diet restores white blood cell number in rats fed to protein-deficient diet [40]. All lymphocyte subpopulations are completely restored back to control levels except neutrophils and eosinophils. Rats fed a protein-deficient diet supplemented with vitamin B12 present also a normal CD4/CD8 ratio [40]. This finding is extremely important as protein malnutrition often happens in cancer patients in result of the higher demands of the tumor.

4.2. Vitamin B12 in cancer development

Most of the evidence does not absolutely clarify the role of vitamin B12 in the process of carcinogenesis and anticancer defense. This is due mainly on the dual modulatory effects that are constantly reported for vitamin B12. Another important question is the nature of

administrated vitamin B12—in some studies [41], a difference in the effect was noted between the food-administrated cobalamin and multivitamin-supplemented cobalamin.

One-carbon metabolism requires B vitamins, and hence the efficient dietary supply may protect against cancer by reducing DNA instability and by affecting DNA methylation [42], but vitamin B12, methylcobalamin, and coenzyme B12 were found to enhance DNA methylation in the presence of S-adenosylmethionine for concentrations up to 1 μM, but at higher concentrations, these compounds were found to inhibit DNA methylation [43].

The main immunological anticancer defenses in the organism include lymphocytes CD8+ and NK cells, which are strongly affected by B12 deficiency, as stated above. It is, therefore, intuitively logical that cobalamin will have positive effect on anticancer defense and will enhance anticancer treatment. One can also expect that vitamin B12 deficiency (mainly diagnosed as decreased plasma levels) will strongly correlate with the cancer risk. Interestingly, a considerable number of patients with different types of cancer or other chronic inflammatory diseases—acute and chronic liver diseases, malignant hemopathies (myelodysplasia, myeloproliferative diseases, and multiple myeloma) [44]; myeloproliferative disorders, such as chronic myeloid leukemia, polycythemia vera, and hypereosinophilic syndrome [26]; hepatocellular carcinoma [45]; and prostate cancer [46, 47]—have elevated levels of B12 in their blood. However, we should keep in mind that vitamin B12 deficiency can be present with either low or high serum levels of the vitamin, as the later ones can arise from impaired tissue uptake and activity at cellular level and it is irrelevant to establish direct causation.

In a population-based cohort study in Northern Denmark, which includes individuals without prevalent cancer and with plasma vitamin B12 levels ≥200 pmol/L (normal), the overall cancer risk was found to increase with high B12 levels [48]. This observation is especially significant in smoking-related, alcohol-related, and hematological cancers, thus provoking the authors to conclude that elevated B12 blood level can be successfully used as cancer markers. Furthermore, as patients on B12 therapy were excluded from the study and intestinal absorption capacity for B12 is limited, they hypothesized that elevated levels are directly related to malignization.

Together with vitamin B12, the haptocorrin levels (B12-binding protein) are also higher in cancer patients and may serve as additional cancer-provoking or cancer-resulting factor [48].

Additionally, patients with autoimmune lymphoproliferative syndrome also show high B12 levels [49], again without clear explanation of the nature of this finding—is it due to low absorption in gastrointestinal tract or inability to enter the cells and to serve its physiological role.

In contrast, circulating levels of vitamin B12 are not associated with pancreatic cancer risk, but this observation is limited to individuals using regular multivitamin supplements. Among individuals who do not use multivitamin supplements, the inverse relation (although modest) between circulating B12 and pancreatic cancer risk [41] was proven.

Inverse association of cobalamin to gastric cancer occurrence also exists, while MMA (which is elevated under vitamin B12 deficiency) is positively associated with gastric cancer [50]. This result could be due to worsen vitamin B12 status in atrophic gastritis that often precedes gastric cancer.

Similarly, plasma vitamin B12 levels are inversely associated with breast cancer risk, but again with one strong limitation—the finding is significant among premenopausal women but not

among postmenopausal women [51]. A more recent nested case-control study, in contrast, did not find any association between breast cancer risk and levels of vitamin B12 in the blood of tested patients [52].

In other case-control studies among multiethnic female population in Hawaii, vitamin B12 supplements showed inverse, dose-responsive associations with high-grade squamous intraepithelial lesions of the cervix [53], suggesting a protective role in cervical carcinogenesis.

Finally, vitamin B12 deficiency accelerates the development of AIDS in HIV-infected patients, whereas normalization of levels retards the development of immune dysfunction [54]. Decreased serum vitamin B12 levels occur in up to 20% of patients with AIDS and may adversely contribute to the hematologic and neurologic dysfunction [55].

4.3. Vitamin B12 as part of cancer immunotherapy

All these findings raise the question if there is a well-founded need to supplement our food with vitamin B12 in order to prevent future cancer development. Again no unanimous response exists. In a case-control study in Australia, vitamin B12 intake was not found to associate with childhood brain tumor risk [56]. Similarly, increased intake of it does not correlate with decreased risk of colorectal cancer [57], and also there is no significant effect when combine with folic acid and vitamin B6 on colorectal adenoma [58] and on total invasive cancer or breast cancer risk [59] among women at high risk for cardiovascular disease. Dietary and multivitamin supplement intake of cobalamin does not correlate with ovarian cancer diagnosis [60], nor with breast cancer [61]. In contrast, patients with high dietary intake of vitamin B12 have decreased tumor suppressor methylation of genes related to head and neck cancers [62]. Offspring of rats fed on vitamin B12-rich (together with methionine, choline, and folate) diet during pregnancy has significantly decreased breast cancer incidence, tumor multiplicity, and tumor volume [63].

The second question to answer is where it is relevant to include vitamin B12 in the nutrition scheme of cancer patients. To date, vitamin B12 is officially included as supplement to pemetrexed treatment (a chemotherapeutic used in pleural mesothelioma and non-small cell lung cancer because of its folate similarity and inhibition of purine and pyrimidine synthesis). In such patients, cobalamin efficiently reduces the toxicity of the main treatment [64].

Besides its direct effect in reducing the toxicity of anticancer drugs, as vitamin B12 is essential for red blood cell synthesis and neural functions, it should be included as part of the nutrition of cancer patients to avoid additional adverse effects (anemia, immune weakness, and cognitive problems). An eligible example is the use of cobalamin supplementation to decrease the severity of chemotherapy-induced peripheral neuropathy, which concerns approximately one third of all patients undergoing chemotherapy [65].

5. Conclusion

The current knowledge is insufficient to fully describe the link between tumorigenesis and vitamin B12 metabolism. Intuitively most of the specialists accept possible implication of B12 deficiency in the impairment of the immune system and hence a putative causation to cancer

development. Unfortunately, most of the studies do not support that elevated dietary intake and additional supplement with the vitamin could protect against cancer risk. However, the dominant opinion is to integrate B12 as part of rational and healthy nutrition to ensure proper function of the immune system.

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References

[1] Kuper H, Adami HO, Trichopoulos D. Infections as a major preventable cause of human cancer. J Intern Med. 2000 Sep;248(3):171–83.

[2] Rakoff-Nahoum S. Why cancer and inflammation? Yale J Biol Med. 2006 Dec;79(3–4): 123–30.

[3] Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer. 2003 Apr;3(4):276–85.

[4] Grimble RF. Nutritional modulation of immune function. Proc Nutr Soc. 2001 Aug; 60(3):389–97.

[5] Estevinho BN, Carlan I, Blaga A, Rocha F. Soluble vitamins (vitamin B12 and vitamin C) microencapsulated with different biopolymers by a spray drying process. Powder Technol. 2016;289:71–8.

[6] Castellanos-Sinco HB, Ramos-Peñafiel CO, Santoyo-Sánchez A, Collazo-Jaloma J, Martínez-Murillo C, Montaño-Figueroa E, et al. Megaloblastic anaemia: Folic acid and vitamin B12 metabolism. Rev Médica Del Hosp Gen México. 2015;78(3):135–43.

[7] Kumar SS, Chouhan RS, Thakur MS. Trends in analysis of vitamin B12. Anal Biochem. 2010 Mar;398(2):139–49.

[8] Allen RH, Stabler SP, Savage DG, Lindenbaum J. Metabolic abnormalities in cobalamin (vitamin B12) and folate deficiency. FASEB J. 1993 Nov;7(14):1344–53.

[9] Combs GF. Vitamin B12. In: The Vitamins: fundamental aspects in nutrition and health. 4th ed. Waltham: Elsevier Academic Press; 2012. p. 377–94.

[10] Watanabe F, Yabuta Y, Bito T, Teng F. Vitamin B12-containing plant food sources for vegetarians. Nutrients. 2014 May;6(5):1861–73.

[11] Wolters M, Ströhle A, Hahn A. Cobalamin: A critical vitamin in the elderly. Prev Med (Baltim). 2004 Dec;39(6):1256–66.

[12] Cheng Z, Yamamoto H, Bauer CE. Cobalamin's (vitamin B12) surprising function as a photoreceptor. Trends Biochem Sci. 2016 Aug;41(8):647–50.

[13] Marsh EN. Coenzyme B12 (cobalamin)-dependent enzymes. Essays Biochem. 1999;34:139–54.

[14] Sobczyńska-Malefora A, Gorska R, Pelisser M, Ruwona P, Witchlow B, Harrington DJ. An audit of holotranscobalamin (active B12) and methylmalonic acid assays for the assessment of vitamin B12 status: Application in a mixed patient population. Clin Biochem. 2014 Jan;47(1–2):82–6.

[15] Selhub J. Folate, vitamin B12 and vitamin B6 and one carbon metabolism. J Nutr Health Aging. 2002;6(1):39–42.

[16] Guéant J-L, Caillerez-Fofou M, Battaglia-Hsu S, Alberto J-M, Freund J-N, Dulluc I, et al. Molecular and cellular effects of vitamin B12 in brain, myocardium and liver through its role as co-factor of methionine synthase. Biochimie. 2013 May;95(5):1033–40.

[17] Roman-Garcia P, Quiros-Gonzalez I, Mottram L, Lieben L, Sharan K, Wangwiwatsin A, et al. Vitamin B12-dependent taurine synthesis regulates growth and bone mass. J Clin Invest. 2014 Jul;124(7):2988–3002.

[18] Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med. 1998 Apr;338(15):1042–50.

[19] Food and Agriculture Organization of the United Nations and World Health Organization. Vitamin B12. In: Human Vitamin and Mineral Requirements. Report of a joint FAO/WHO expert consultation Bangkok, Thailand 2001. Available from: http://www.fao.org/3/a-y2809e.pdf [Accessed 2016-08-13]

[20] Schick P. Pernicious Anemia: Background, Pathophysiology, Etiology [Internet]. 2015 [cited 2016 Aug 15]. Available from: http://emedicine.medscape.com/article/204930-overview

[21] Nielsen MJ, Rasmussen MR, Andersen CBF, Nexø E, Moestrup SK. Vitamin B12 transport from food to the body's cells-a sophisticated, multistep pathway. Nat Rev Gastroenterol Hepatol. 2012 Jun;9(6):345–54.

[22] Seetharam B, Yammani RR. Cobalamin transport proteins and their cell-surface receptors. Expert Rev Mol Med. 2003 Jun;5(18):1–18.

[23] Esperanca M. The Wonders of Vitamin B12: Keep Sane and Young. 1st ed. Bloomington: Xlibris Corporation; 2011.

[24] Orton DJ, Naugler C, Sadrzadeh SMH. Fasting time and vitamin B12 levels in a community-based population. Clin Chim Acta. 2016 Jul;458:129–32.

[25] Lindenbaum J, Savage DG, Stabler SP, Allen RH. Diagnosis of cobalamin deficiency: II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. Am J Hematol. 1990 Jun;34(2):99–107.

[26] Arendt JFB, Nexo E. Cobalamin related parameters and disease patterns in patients with increased serum cobalamin levels. PLoS One. 2012;7(9):e45979.

[27] Arendt JFB, Nexo E. Unexpected high plasma cobalamin : Proposal for a diagnostic strategy. Clin Chem Lab Med. 2013 Mar;51(3):489–96.

[28] Parham P. Elements of the immune system and their roles in defense. In: The Immune System. 4th ed. New York: Garland Science; 2014. p. 1–27.

[29] Parkin J, Cohen B. An overview of the immune system. Lancet. 2001;357(9270):1777–89.

[30] Delves P, Roitt I. The immune system (first of two parts). Adv Immunol. 2000;343(1):37–50.

[31] Duque GA, Descoteaux A. Macrophage cytokines: Involvement in immunity and infectious diseases. Front Immunol. 2014;5(Oct):1–12.

[32] Paul W. editor. Fundamental Immunology. 7th ed. Philadelphia: Lippincott Williams and Wilking; 2013.

[33] Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, Roumenina LT. Complement system part II: Role in immunity. Front Immunol. 2015;6(May):1–26.

[34] Chaplin D. Overview of the immune response. Allergy Clin Immunol. 2010;125(2 Suppl 2): S3–23.

[35] Tamura J, Kubota K, Murakami H, Sawamura M, Matsushima T, Tamura T, et al. Immunomodulation by vitamin B12: Augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. Clin Exp Immunol. 1999 Apr;116(1):28–32.

[36] Partearroyo T, Úbeda N, Montero A, Achón M, Varela-Moreiras G. Vitamin B(12) and folic acid imbalance modifies NK cytotoxicity, lymphocytes B and lymphoproliferation in aged rats. Nutrients. 2013 Dec;5(12):4836–48.

[37] Erkurt MA, Aydogdu I, Dikilitaş M, Kuku I, Kaya E, Bayraktar N, et al. Effects of cyanocobalamin on immunity in patients with pernicious anemia. Med Princ Pract. 2008;17(2):131–5.

[38] Vellema P, Rutten VP, Hoek A, Moll L, Wentink GH. The effect of cobalt supplementation on the immune response in vitamin B12 deficient Texel lambs. Vet Immunol Immunopathol. 1996 Dec;55(1–3):151–61.

[39] Sakane T, Takada S, Kotani H, Tsunematsu T. Effects of methyl-B12 on the in vitro immune functions of human T lymphocytes. J Clin Immunol. 1982 Apr;2(2):101–9.

[40] Lewicki S, Lewicka A, Kalicki B, Kłos A, Bertrandt J, Zdanowski R. The influence of vitamin B12 supplementation on the level of white blood cells and lymphocytes phenotype in rats fed a low-protein diet. Cent J Immunol. 2014;39(4):419–25.

[41] Schernhammer E, Wolpin B, Rifai N, Cochrane B, Manson JA, Ma J, et al. Plasma folate, vitamin B6, vitamin B12, and homocysteine and pancreatic cancer risk in four large cohorts. Cancer Res. 2007 Jun;67(11):5553–60.

[42] Kim Y-I. Folate and colorectal cancer: An evidence-based critical review. Mol Nutr Food Res. 2007 Mar;51(3):267–92.

[43] Pfohl-Leszkowicz A, Keith G, Dirheimer G. Effect of cobalamin derivatives on in vitro enzymatic DNA methylation: Methylcobalamin can act as a methyl donor. Biochemistry. 1991 Aug;30(32):8045–51.

[44] Chiche L, Jean R, Romain F, Roux F, Thomas G, Canavese S, et al. Clinical implications of high cobalamin blood levels for internal medicine. Rev Med Interne. 2008;29(3):187–94.

[45] Nörredam K, Chainuvati T, Gimsing P, Hippe E, Viranuvatti V. Plasma cobalamin and transcobalamin in patients with primary carcinoma of the liver. A study from Thailand. Scand J Gastroenterol. 1983 Mar;18(2):229–32.

[46] Collin SM, Metcalfe C, Refsum H, Lewis SJ, Zuccolo L, Smith GD, et al. Circulating folate, vitamin B12, homocysteine, vitamin B12 transport proteins, and risk of prostate cancer: A case-control study, systematic review, and meta-analysis. Cancer Epidemiol Biomarkers Prev. 2010 Jun;19(6):1632–42.

[47] Price AJ, Travis RC, Appleby PN, Albanes D, Barricarte Gurrea A, Bjørge T, et al. Circulating folate and vitamin B12 and risk of prostate cancer: A collaborative analysis of individual participant data from six cohorts including 6875 cases and 8104 controls. Eur Urol. 2016;70(6): 941–951.

[48] Arendt JFB, Pedersen L, Nexo E, Sørensen HT. Elevated plasma vitamin B12 levels as a marker for cancer: A population-based cohort study. J Natl Cancer Inst. 2013 Dec;105(23):1799–805.

[49] Oliveira JB, Bleesing JJ, Dianzani U, Fleisher TA, Jaffe ES, Lenardo MJ, et al. Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): Report from the 2009 NIH International Workshop. Blood. 2010 Oct;116(14):e35–40.

[50] Vollset SE, Igland J, Jenab M, Fredriksen A, Meyer K, Eussen S, et al. The association of gastric cancer risk with plasma folate, cobalamin, and methylenetetrahydrofolate reductase polymorphisms in the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol Biomarkers Prev. 2007 Nov;16(11):2416–24.

[51] Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD, et al. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. J Natl Cancer Inst. 2003 Mar;95(5):373–80.

[52] Agnoli C, Grioni S, Krogh V, Pala V, Allione A, Matullo G, et al. Plasma Riboflavin and Vitamin B-6, but not homocysteine, folate, or vitamin B-12, are inversely associated with breast cancer risk in the European Prospective Investigation into Cancer and Nutrition-Varese cohort. J Nutr. 2016 Jun;146(6):1227–34.

[53] Hernandez BY, McDuffie K, Wilkens LR, Kamemoto L, Goodman MT. Diet and premalignant lesions of the cervix: Evidence of a protective role for folate, riboflavin, thiamin, and vitamin B12. Cancer Causes Control. 2003 Nov;14(9):859–70.

[54] Liang B, Chung S, Araghiniknam M, Lane L, Watson R. Vitamins and immunomodulation in AIDS. Nutrition. 1996;12(1):1–7.

[55] Herzlich BC, Schiano TD. Reversal of apparent AIDS dementia complex following treatment with vitamin B12. J Intern Med. 1993 Jun;233(6):495–7.

[56] Greenop KR, Miller M, Bailey HD, de Klerk NH, Attia J, Kellie SJ, et al. Childhood folate, B6, B12, and food group intake and the risk of childhood brain tumors: Results from an Australian case-control study. Cancer Causes Control. 2015 Jun;26(6):871–9.

[57] Razzak AA, Oxentenko AS, Vierkant RA, Tillmans LS, Wang AH, Weisenberger DJ, et al. Associations between intake of folate and related micronutrients with molecularly defined colorectal cancer risks in the Iowa Women's Health Study. Nutr Cancer. 2012;64(7):899–910.

[58] Song Y, Manson JE, Lee I-M, Cook NR, Paul L, Selhub J, et al. Effect of combined folic acid, vitamin B(6), and vitamin B(12) on colorectal adenoma. J Natl Cancer Inst. 2012 Oct;104(20):1562–75.

[59] Zhang SM, Cook NR, Albert CM, Gaziano JM, Buring JE, Manson JE. Effect of combined folic acid, vitamin B6, and vitamin B12 on cancer risk in women: A randomized trial. JAMA. 2008 Nov;300(17):2012–21.

[60] Harris HR, Cramer DW, Vitonis AF, DePari M, Terry KL. Folate, vitamin B(6), vitamin B(12), methionine and alcohol intake in relation to ovarian cancer risk. Int J Cancer. 2012 Aug;131(4):E518–29.

[61] Zhang C-X, Ho SC, Chen Y-M, Lin F-Y, Fu J-H, Cheng S-Z. Dietary folate, vitamin B6, vitamin B12 and methionine intake and the risk of breast cancer by oestrogen and progesterone receptor status. Br J Nutr. 2011 Sep;106(6):936–43.

[62] Colacino JA, Arthur AE, Dolinoy DC, Sartor MA, Duffy SA, Chepeha DB, et al. Pretreatment dietary intake is associated with tumor suppressor DNA methylation in head and neck squamous cell carcinomas. Epigenetics. 2012 Aug;7(8):883–91.

[63] Cho K, Mabasa L, Bae S, Walters MW, Park CS. Maternal high-methyl diet suppresses mammary carcinogenesis in female rat offspring. Carcinogenesis. 2012 May;33(5): 1106–12.

[64] Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol. 2003 Jul;21(14):2636–44.

[65] Schloss JM, Colosimo M, Airey C, Vitetta L. Chemotherapy-induced peripheral neuropathy (CIPN) and vitamin B12 deficiency. Support Care Cancer. 2015 Jul;23(7):1843–50.

Section 2

Cancer Immunotherapy

Chapter 6

Immunotherapy in Gynecologic Cancers

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Additional information is available at the end of the chapter

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Abstract

During the last years, significant progress in the understanding of signaling pathways of immune cells has revive the field of immune therapy for cancer. In this chapter, we explain the recent immunotherapy-based strategies for the treatment of gynecological cancers including cervical cancer, endometrial cancer, ovarian cancer, and vulvar cancer. This work will mainly focus on emerging clinical data on immune checkpoint inhibitors. But also data on adoptive T cell therapies and vaccines will be presented. It is anticipated that in future biomarker-guided randomized trials will provide better approaches in terms of response and resistance to immune therapy. The use of combination therapy for gynecological cancer might be one possible approach to overcome resistance.

Keywords: gynecologic cancers, ovarian cancer, endometrial cancer, cervical cancer, immune therapy, immune checkpoint inhibitors

1. Introduction

Gynecologic cancers include vulva, vaginal, cervical, endometrial, and ovarian/tubal/peritoneal cancers, the latter of which are still classified as one disease. As these organ-classified cancers have different characteristics, biology, therapies, and outcomes, during the past decade, approaches have been undertaken to subclassify them as to their heterogeneity and based on next-generation profiling. The main cornerstone of treatment for gynecologic cancer comprises in most cancers of surgical resection with different possibilities of adjuvant further therapy like chemo-, radio-, targeted, and, increasingly, immunotherapy.

In the United States, almost 90,000 women were diagnosed with gynecologic cancers in 2015 and over 29,000 will die from their disease [1]. Many women are cured with combined modalities, however, in ovarian cancer, for example, over 70% of cancers are diagnosed in advanced

International Federation of Gynecology and Obstetrics (FIGO) stage III or IV, thus their five-year overall survival is only 30% [2].

Outcome in ovarian cancer in all stages is the worst of all gynecological cancers with a 10-year overall survival of 30%, followed by vaginal cancer with a 10-year overall survival of 35%. Cervical and vulvar cancers have a 10-year overall survival rate of 65%. Endometrial cancer has the best prognosis, with a 10-year survival rate of 80% [1].

Immunotherapy represents a new alternative and rational approach for the treatment of cancer, including gynecologic cancers [3, 4]. More than a decade ago, it was demonstrated for ovarian cancer that tumor-infiltrating lymphocytes (TILs) play an important role in tumor rejection and prognosis [5]. This was one of the first evidence that immune therapy might be beneficial in ovarian cancer patients. A meta-analysis confirmed the prognostic role of TILs for ovarian cancer patients [6]. Later it was also demonstrated that the ratio of different T cell subtypes plays an important role [7].

A major function of the immune system is to continually seek out and eliminate cancer cells as they arise in a process defined as cancer immunosurveillance [8]. This involves both innate and adaptive immune mechanisms that function complimentarily to promote tumor immunity. Most importantly is that antitumor immune responses can be induced by immunological agents. Various forms of immunotherapies are central components of treatment regimens for a number of malignancies [9]. To eliminate cancer cells by T cells is only one-step in a complex immunity cycle [10].

In general, there are three strategies to treat cancer with immunotherapeutic approaches:

(1) Increase tumor antigen presentation.

(2) Increase T-cell activity.

(3) Targeting the tumor environment (immune inhibitory mechanisms).

Strategies to increase tumor antigen presentation includes vaccinations, use of innate immune activators, oncolytic viruses, type I interferon, and toll-like receptor (TLR) agonists.

Especially for epithelial ovarian cancer (EOC), several vaccination approaches have been applied, e.g., cellular vaccines, dendritic cell (DC) vaccines, and virus-loaded vaccines. Several studies have used overexpressed proteins in EOC as a target, e.g., p53, surviving, and MUC1. Several studies have demonstrated immune response but clinical benefit rate was minimal in all of these studies. The vaccination approach is not used in clinical practice nowadays [11].

To increase T-cell activity, there are several approaches tested including cytokine therapies with IL-2 and IL-12, the use of checkpoint inhibitors, and adoptive T cell therapies [12, 13]. Rosenberg et al. demonstrated in 2015 an elegant new therapeutic approach by generating tumor-associated antigen-specific T cells via expression of T-cell receptor or chimeric antigen receptor (CAR) [14]. With this approach, CD19 targeting CAR therapy for acute lymphatic leukemia of the B cell lineage was applied with a very high remission rate of 90% [15]. In ovarian cancer, adoptive T-cell therapy might be also effective. For example, NY-ESO-1 is

specifically expressed in cancer, 42% expression has been seen in ovarian cancer. This might be an important target for adoptive T-cell therapy [16].

To target the tumor environment, there are also several therapeutic approaches. It has been demonstrated that several immune inhibitory mechanisms are associated with poor prognosis in gynecological cancer and in particular, in ovarian cancer, e.g., tumor infiltrating regulatory T cells, tumor-associated macrophages, expression of indoleamine 2, 3-dioxygenase (IDO) by tumor stromal cells [17, 18]. To target the responsible pathways might be effective, especially in combination with newer programmed cell death ligand-1 (PDL-1) or its receptor programmed cell death protein-1 (PD-1) checkpoint inhibition [19, 20].

For gynecological cancer, in particular for ovarian cancer, there is still an unmet challenge in cell therapy for cancer. The selection of the right target antigen, which is tumor cell-specific and has a robust expression, seems to be very important.

2. Cervical cancer

Cervical cancer is the fourth most common female cancer worldwide, with estimated 528,000 new cases and 266,000 deaths in 2014 [21]. Infection with high risk types of human papillomavirus (HPV) is the most crucial risk factor [22]. Human papillomavirus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 72, and 82 are associated with high risk of cervical cancer, whereas HPV types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, and 81 are considered to have low carcinogenic risk [23, 24].

Most common cases are diagnosed in less developed countries, where cervical cancer comprises nearly 15% of cancers in women. In Switzerland, with a small population of only eight million, the incidence is much lower, with about 240 cases diagnosed each year [25].

Better screening methods and vaccination against HPV in the past decades have led to an improvement of cervical cancer prognosis in developed countries, particularly where a broad prevention plan has been put in place [26]. To date, we have an efficacious vaccination available against the nine most important HPV types (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58) [27]; however, despite better prevention strategies, cervical cancer is still not sufficiently manageable worldwide with a stagnating mortality rate. Most cancers in the developed world present in early FIGO stage IA1–IIA, while primary metastatic disease is uncommon. Surgery including radical hysterectomy including pelvic lymph node resection for staging is the gold standard. Patients with high-risk features including insufficient margins, large tumors, and lymph vascular space invasion receive adjuvant radiochemotherapy (RCTX) with platinum [28]. From stage IIB onwards, patients are treated with combined radiochemotherapy with platinum. This was established in 1999 when five randomized controlled trials demonstrated a 30–50% survival benefit for patients treated with combined radiochemotherapy compared with radiation alone.

A large meta-analysis of chemoradiation trials demonstrated an absolute overall survival (OS) benefit of 12% [29]. Since these trials, no practice changing studies were published until 2014, when the findings of a phase III study with bevacizumab resulted in its approval for late-stage cervical cancer by the Food and Drug Administration (FDA) and European Medical

Agency (EMA). In a large randomized phase III trial, two chemotherapy regimens with cisplatin and paclitaxel or topotecan and paclitaxel plus or minus bevacizumab were examined [30]. Bevacizumab was applied during chemotherapy and as maintenance therapy until disease progression, achieving an increased OS benefit (17.0 months versus 13.3 months; hazard ratio (HR) for death, 0.71; 98% confidence interval (CI), 0.54–0.95; P = 0.004) and a higher response rate (48 versus 36%, P = 0.008). An additional quality of life (QoL) analysis confirmed the low toxicity profile and good tolerability without any deterioration of quality of life [31].

2.1. Immune system and cervical cancer

Most cervical cancers are associated with HPV infection. The cervical epithelium is the ideal area for HPV because of the absence of an inflammatory milieu, which provides a protective niche where the HPV is capable of evading the host immune response for many months. Research has provided some insight into the means of evasion by the virus in cervical cancer [32]. The presence of CD4+ lymphocytes in precursor lesions and CD8+ lymphocytes in malignant tumors in the absence of an effective immune response suggests that T cell cytotoxic responses are impaired [33, 34]. Indeed, the zeta chain of the T-cell receptor is downregulated in CD8+ lymphocytes in cervical cancer, suggesting defective T cell signaling [35]. Furthermore, NKG2D-expressing natural killer and cytotoxic T cells, which have a key role in the elimination of virus-infected and tumor cells, are present at reduced levels in both patients with cervical cancer and cervical intraepithelial neoplasia [36]. Increased T regulatory cell activity has also been reported [37]. The immunoregulatory enzyme, IDO, appears to facilitate the induction of immune escape together with T regulatory cells [38]. Understanding the different mechanisms of immune evasion in cervical cancer is key to establishing new treatments.

2.2. Checkpoint-inhibitors in cervical cancers

Despite this new regime, the prognosis for metastatic and locally advanced cervical cancer is still poor, with an OS of 12–17 months [30, 39]. To improve prognosis, new treatment options are urgently needed. One important strategy is to enable the immune system to reject the tumors facilitating checkpoint-inhibitors. An important strategy to improve T cell-dependent tumor attack is by inhibiting immune T cell checkpoints. A checkpoint-inhibitor is a drug that inhibits certain surface proteins made by specific immune cells, such as T cells and cancer cells. These specific proteins control the immune responses and prevent T cells from killing cancer cells. Inhibiting these proteins will remove the natural surveillance of the immune system and T cells will be activated to eliminate cancer cells.

Blocking inhibiting checkpoints like cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) or PD-1/PD-L1 results in activation of T cell proliferation and cytokine production. CTLA-4 begins to be expressed on the naïve T cell 48 hours after activation in lymph nodes and is closely associated with attenuation of these activating T cells [40]. PD-1 is expressed on effector T cells in peripheral tissues and binds with PD-L1 (B7-H1) and PD-L2 (B7-DC) expressed on DCs or tumor cells for attenuation of activated effector T cells [41, 42]. Under normal circumstances, interferon (IFN)-γ upregulates the expression of PD-L1, protecting DCs from

T cell-mediated cytotoxicity [43]. However, in head and neck squamous cell carcinomas associated with HPV, the number of CD8+ T cells expressing PD-1 has been reported to be higher in tumors than peripheral blood. This suggests that the expression of PD-1 by CD8+ T cells starts after entering into the tumor microenvironment.

Currently, there are several studies in the U.S. and EU examining different checkpoint-inhibitors and combinations, e.g., chemotherapy, PARP-inhibitors, or antiangiogenetic agents, in cervical cancer and other solid tumors (**Table 1**). Presently, most studied checkpoint-inhibitors are pembrolizumab, nivolumab, ipilimuab, and durvalumab.

Pembrolizumab, a PD-1 antibody, is approved for patients with advanced melanomas [44]. In cervical cancer, it is currently being investigated in a phase II trial (NCT02628067) based on the phase IB data presented at ASCO 2016 [45]. Patients with stage IVB or nonresectable cervical cancer received 10 mg/kg pembrolizumab every 2 weeks until disease progression or toxicity for a total treatment of 24 months. The overall response rate (ORR) was 17% (95% CI 5–36). While no grade 4 toxicity occurred, two treatment-related discontinuations were observed (grade 3 colitis and grade 3 Guillian-Barré syndrome). The median progression-free survival (PFS) and OS were 2 and 9 months, respectively. The 12 months PFS and OS were 8 and 33%, respectively. Some patients had very long remission rates that are promising and will lead to further evaluation in cervical cancer.

Nivolumab has been approved for metastatic and unresectable lung cancer [46], where it showed a survival benefit compared to conventional chemotherapy treatment. This PD-1 antibody has also been tested in a phase I/II study for patients with HPV-associated tumors, including cervical, vaginal, and vulvar cancer (www.clinicaltrials.gov:NCT02488759).

Ipilimumab is an anti-CTLA-4 antibody and was the first checkpoint inhibitor approved for metastatic melanoma and has significantly improved the OS of this disease [19]. It is at present tested in several other tumors including gynecologic cancers. In cervical cancer, it has been tested in a phase I study following standard radiochemotherapy in patients with

ClinicalTrials.gov Identifier:	Phase	Drug	Situation
NCT02471846	I	GDC-0919 *(small molecule investigational immunotherapy designed to inhibit IDO (Indoleamine 2,3-dioxygenase), a protein often overproduced by many cancer cells)* *plus atezolizumab*	Metastatic tumors including CC
NCT02812875	IB	CA-170 *Oral small molecule inhibiting PD-L-1/2*	Metastatic solid tumors including CC
NCT02635360	II	Pembrolizumab	Combing with RCTX for advanced CC
NCT02834013	II	Nivolumab plus ipilimumab	Metastatic rare tumors including CC

Notes: Ongoing checkpoint-inhibitor studies in cervical cancers. RCTX = radiochemotherapy; CC = cervical cancer.

Table 1. Data on immune therapy agents in particular checkpoint-inhibitors in cervical cancer.

locally advanced cervical cancer (www.clinicaltrials.gov: NCT01711515). The trial is currently recruiting patients.

Another phase 2 trial from Princess Margaret Hospital examines the role of ipilimumab in patients with metastatic or recurrent human papillomavirus-related cervical cancer (www.clinicaltrials.gov: NCT01693783).

Durvalumab (MEDI4736), an anti-PD-L1 antibody, is being tested in combination with tremelimumab, an anti-CTLA-4 antibody [47, 48] in a phase I trial for patients with six different types of cancer, including cervical cancer (www.clinicaltrials.gov: NCT01975831). Durvalumab inhibits PD-L1 interaction with PD-1 (IC_{50} 0.1 nM) and CD80/B7.1 (IC_{50} 0.04 nM), allowing T-cells to recognize and kill tumor cells.

Early single agent phase I evaluation in several tumor types, including triple negative breast cancer, showed a disease control rate of 33% and an overall response rate of 10% [49]. There were early (5 weeks) and also durable responses (56+ weeks). PD-L1 expression appears to enrich the response to durvalumab monotherapy. Drug-related events were observed in 46% of patients with 7% of patients reporting a grade ≥3 AE that led in 1% to discontinuation of the treatment. The most common drug-related AEs were fatigue, rash/pruritus, diarrhea, and vomiting.

3. Ovarian cancer

Epithelial ovarian cancer (EOC) is the fifth most common cancer in women and one of the main causes of death in relation to gynecologic cancer worldwide [50]. Ovarian cancer has a poor prognosis, probably as three in four cancers are diagnosed in advanced FIGO stages [51]. The 5-year survival rate is poor, estimated at 20–30% for stage I–IV disease. Not only the tumor stage, but also the histopathological subtype is prognosis defining, with poor differentiated serous cancers having the poorest outcomes [2]. Best prognosis is seen in mucinous subtype. These subtype is mostly localized FIGO stage I disease [52].

Surgery with optimal debulking still has a major influence on the outcome in advanced EOC. Best outcome has been reported for patients achieving maximal cytoreductive surgery without macroscopic residual disease [53].

The most promising novel agents for ovarian cancer are antiangiogenesis-based therapies, e.g., bevacizumab, pazopanib, cediranib, or trebananib and PARP-inhibitors, e.g., olaparib or niraparib [54–60]. Bevacizumab and olaparib are approved in the United States and Europe and demonstrated a PFS benefit of 3–4 months when used during or after platinum-based chemotherapy. In BRCA positive patients, there was a PFS benefit of more than 9 months for patients diagnosed with relapsed serous high grade EOC [61].

Recent data suggest that also patients without a BRCA mutation might benefit from a treatment with the PARP-inhibitor niraparib. In this recent study published by Mirza et al., niraparib was also beneficial in non-BRCA mutated patients [62]. They used homologous recombination deficiency (HRD) score to predict response on niraparib. In this study, non-BRCA mutated patients had also significant difference 9.3 versus 3.9 months in PFS.

Non-BRCA mutated and HRD-positive 12.9 versus 3.8 months (HR 0.38).

HRD positive and BRCA wildtype: 9.3 versus 3.7 months, somatic BRCA mutated 20.9 versus 11.0 months, HRD negative 6.9 versus 3.8 (HR 0.58).

3.1. Vaccination strategies for ovarian cancer

A number of methods have been used to enhance immune response in ovarian cancer to improve prognosis, yet, none of these methods have been approved. Several types of vaccination strategies have been tested, e.g.:

(1) Anti-idiotype AB-based vaccination, for example, Abagovomab.

(2) Peptide-/protein-based vaccination, for example, NY-ESO peptides.

(3) Lymphocytes-based vaccination, for example, Autologous LAK plus IL2.

(4) Carbohydrate-based vaccination, for example, MUC1-Sialyl-TN.

(5) DNA plasmid-based vaccination, for example, Poxviral Vector PANVAC-V.

(6) Combination-based vaccination, for example, with sunitinib.

(7) Vaccination-based on dendritic cells, for example, autologous DC pulsed with MUC1-derived peptides or HER-2/neu.

The following paragraph will focus on anti-idiotype AB-based vaccination only as this type is best developed and also phase III data are available.

3.2. Vaccination with idiopathic antibodies: abagovomab and oregovomab

In his theory of clonal selection published in 1974, Neils Jerne described how antibodies (Ab1) generated in response to a particular antigen may themselves be immunogenic [63, 64]. The immunogenic determinants of Ab1 antibodies are termed 'idiotopes'. Ab1 idiotopes can act as antigens, leading to the development of anti-idiotypic antibodies (Ab2) [64]. As idiotopes are largely located in the highly variable region of the antibody that serves as the antigen-binding site, in some cases Ab2 anti-idiotypic antibodies can mimic antigen structure. Indeed, research has shown that exposure to Ab2 anti-idiotypic antibodies can sometimes induce a more pronounced response than exposure to the antigen itself. Exposure to Ab2 anti-idiotypic antibodies may subsequently stimulate the generation of Ab3 antibodies, some of which target Ab2 idiotopes, and are also capable of binding to the antigen.

Abagovomab (ACA-126) is a murine IgG1k monoclonal antibody (Ab2) with an idiotope that imitates CA125. It is under investigation as an anti-idiotypic vaccine for ovarian cancer. CA125 is a mucin-like transmembrane glycoprotein that is upregulated in ovarian cancer and currently represents the most widely used ovarian cancer biomarker [65, 66]. The biological function of CA125 remains poorly understood, with putative roles in cell adhesion, migration, invasion, and possible immunosuppressive properties suggested [67, 68].

In phase I studies in patients with chemotherapy-resistant ovarian cancer, abagovomab was associated with induction of Ab3 and HAMA responses, increased serum levels of interferon (IFN)-γ, and increases in CA125-specific CD8+ T cells postvaccination [69], suggesting the induction of Th1 immune responses [70]. The induction of Ab3 response was confirmed in a phase Ib/II clinical trial with abagovomab in 119 patients with CA125-positive ovarian, tubal, and peritoneal cancer [71]. Ab3 response occurred in 68.1% of patients and was associated with prolonged overall survival (OS) compared with nonresponders (23.4 versus 4 months; $P < 0.0001$), regardless of FIGO stage, first-line chemotherapy, or previous treatment. Antibody-dependent cellular cytotoxicity (ADCC), observed in 26.9% of patients, was also associated with significantly prolonged survival (25 versus 10 months; $P = 0.0126$), suggesting a role for ADCC in the antitumor effect of abagovomab. Nevertheless, abagovomab was not associated with prolonged recurrence-free survival (RFS) or OS compared with placebo when administered as a maintenance therapy to patients ($n = 888$) with first remission of ovarian cancer (FIGO stage III/IV) during the phase III 'Monoclonal antibody Immunotherapy for Malignancies of Ovary by Subcutaneous Abagovomab' (MIMOSA) trial, despite the induction of measurable immune response [72].

Oregovomab (B43.13, OvRex) also targets CA125, binding with high affinity ($K_D = 1.2 \times 10^{10}\ M^{-1}$). This murine IgG1 monoclonal antibody was investigated for the treatment of ovarian cancer after a survival advantage was noted during its initial use as a technetium 99c-labeled agent for the immunoscintigraphic detection of recurrent ovarian cancer [73].

Infusion of the antibody results in the formation of immune complexes with circulating antigen that trigger generation of anti-CA125 antibodies [74]. Indeed, oregovomab appears to induce broad humoral and cellular anti-CA125 responses.

During a phase I trial, multiple infusions of oregovomab were associated with a greater than threefold increase in anti-CA125 antibody levels in nearly half (43%) of patients ($n = 184$) with ovarian cancer (FIGO stages I–IV) [74]. Anti-CA125 antibody response was associated with prolonged survival compared with nonresponse (22.9 versus 13.5 months; $P = 0.0089$), and an increase in T-cell proliferation was noted, which was also associated with prolonged survival. In a phase II study ($n = 20$), T-cell responses to CA125 and/or autologous tumors were also shown to correlate with prolonged survival in oregovomab-treated patients with platinum-resistant recurrent ovarian cancer (FIGO stages I–IV) compared with nonresponders (median not reached versus 51.9 weeks) [75].

While oregovomab elicits tumor-specific T-cell responses, it does not appear to be able to directly inhibit tumor growth. Anti-CA125 antibodies isolated from oregovomab-treated patients with ovarian cancer (FIGO stages I–IV) in one study were able to mediate ADCC in the presence of peripheral blood mononuclear cells, but not CDC [76].

In addition, there are conflicting data on the association between immune response to oregovomab and clinical benefit. A retrospective analysis of 44 patients with recurrent ovarian cancer (majority FIGO stages III and IV) who received technetium 99c-labeled oregovomab reported a significant relationship between immune response and survival [73]. More than 67% of patients had HAMA and Ab2 responses, with 28% of patients experiencing a more than threefold increase in anti-CA125 antibody levels. These immune responses were associated with prolonged survival compared with nonresponders: HAMA (22.6 versus 7.2 months;

$P = 0.0016$), Ab2 (18.3 versus 9.3 months; $P = 0.075$), and anti-CA125 (18.2 versus 13.1 months; $P = 0.0896$). By contrast, no reduction in tumor burden was detected in 13 oregovomab-treated patients with ovarian cancer during a pilot phase II study, despite measurable T- and B-cell responses in the majority of patients [77]. Furthermore, oregovomab monoimmunotherapy was associated with similar clinical outcomes to placebo during a phase III trial in 375 patients with advanced ovarian cancer (FIGO stage III/IV), despite measurable bioactivity [78].

The potential for combining oregovomab with front-line chemotherapy has been investigated during a phase II clinical trial in 40 patients with advanced ovarian cancer (FIGO stages III/IV) [79]. Patients were randomized to receive oregovomab via two dosing schedules: either on the same day as or 1 week after standard carboplatin-paclitaxel chemotherapy. Primary and secondary endpoints compared antibody and cellular response between the two dosing schedules, but the authors also noted that the immune responses triggered were stronger than those observed in previous studies using oregovomab monoimmunotherapy.

3.3. Immune checkpoint-inhibitors in ovarian cancer

Apart from the vaccination, immune checkpoint-inhibitors such as the programmed cell death 1 protein (anti-PD1)/PD-Ligand1 and CTL-A4 were also under research for ovarian cancer [42] (see also **Table 2**). Tumors with high mutational loads are ideal candidates for therapies with immune checkpoint-inhibitors. In melanoma and lung cancer, these new drugs are already approved [40, 46]. The role of immune checkpoint-inhibitors in ovarian cancer is not so clear so far, although PD1/PD-L1 pathway seems to play an important role in ovarian cancer. In ovarian cancers, PD-1 is expressed on TILs [80]. Expression of PD-L1 on tumors has been shown to be bad prognostic factor [81]. In a preclinical model inhibition of PD1 and PD-L1 demonstrated tumor rejection and reprogramming of tumor microenvironment [82]. In one of first phase I study for an anti-PD-L1 antibody, there were also responses seen for ovarian cancers [83].

First, data from phase a phase II studies demonstrated low response rates but a higher disease control rated and long-term remissions. The patients treated in these trials had poor prognostic disease and were platinum resistant [84]. The assessment of PD-L1 as a prognostic marker for ovarian cancer is less clear. The data from Hamannishi et al. demonstrated that PD-1 was not ideal as prognostic marker [81].

More importantly, the mutational landscape might be important to select the right treatment for the suitable tumor. In general, the mutational burden is lower in ovarian cancer than in other cancers. But ovarian tumors with germline or somatic BRCA1/2 mutations were found to have a higher frequency of exome mutations (67.5 on average) than tumors with wild-type BRCA (49.5 on average) [86].

3.3.1. Ipilimumab

Ipilimumab (MDX-CTLA-4, Yervoy) is a full human IgG1 monoclonal antibody to CTLA-4 approved by the FDA for the treatment of advanced melanoma on the basis of phase III observations of prolonged OS (median 4 months versus tumor vaccine) in patients with unresectable pretreated stages III and IV melanoma [40]. Immune response appears to underlie the antitumor

Drug	Target	Patients	N	PD-L1 Status	ORR (%)	DCR (%)	CR	PR	SD	Literature
Nivolumab	PD-1	Rezidiv platin-resistant	18	All	17	44	2	1	5	[84]
Pembrolizumab	PD-1	Fortgeschrittenes EOC	26	PD-L1+	11,5	34,6	1	2	6	[85]
Avelumab	PD-L1	Platin/Chemo-resistentes EOC	75	All	10,7	54,7	0	8	33	[92]
BMS-936559	PD-L1	Fortgeschrittenes EOC	17	All	1	23.5	0	1	3	[83]

Notes: The clinical data for checkpoint-inhibitors mainly anti-PD-1 in EOC. ORR = overall response rate; DCR = disease control rate; PR = partial remission; CR = complete remission; SD = stable disease; EOC = epithelial ovarian cancer.

Table 2. Data available on immune checkpoint-inhibitors used in ovarian cancer.

effect of ipilimumab. Studies with ipilimumab in melanoma have shown increased absolute lymphocyte counts [87], upregulation of inducible costimulator (ICOS) on CD4+ T cells [88], and enhanced antibody and T-cell responses to cancer-testis antigen NY-ESO-1 that largely correlate with clinical benefit and prolonged survival [88, 89].

Ipilimumab has also been investigated in a small number of patients with ovarian cancer. Findings from two studies in a total of 11 patients with previously vaccinated ovarian cancer (FIGO stage IV) suggest that ipilimumab is generally well tolerated and can trigger a decrease/stabilization of CA125 [90, 91]. Significant antitumor effects were observed in some patients. One patient experienced a marked reduction in serum CA125 levels during ipilimumab treatment with a substantial regression of a large cystic hepatic metastasis, complete resolution of mesenteric lymphadenopathy, and gastrocolic ligament thickening [91]. Increased antibody responses to NY-ESO-1, which is expressed in many ovarian carcinomas, were also detectable and correlated with therapeutic activity. Four additional patients achieved stable disease.

3.3.2. Avelumab

Avelumab (MSB0010718C; anti-PD-L1) is a full human anti-PD-L1 IgG1 antibody currently under clinical investigation for several cancers. It was tested in a phase IB study for chemotherapy refractory EOC. Safety and clinical activity data were reported at ASCO 2016 [92]. Patients with advanced EOC unselected for PD-L1 expression received avelumab 10 mg/kg IV every 2 weeks until progression, unacceptable toxicity, or withdrawal. Tumors were assessed every 6 weeks according to RECIST 1.1. Unconfirmed ORR, PFS, and OS were evaluated and AE graded by NCI-CTCAE v4.0. During a median of 12 weeks (range: 2–54 weeks), 124 patients were treated with AE only occurring in 82 patients (66.1%); most common ones (≥10%) were fatigue (13.7%), infusion-related reaction (12.1%), and diarrhea (11.3%). The ORR was 9.7%. The rate of stable disease was 44.4%. The disease control rate was 54.0%. PD-L1 expression was evaluable in 74 patients with PD-L1+ tumors expressing an ORR of 12.3% and in PD-L1 tumors of 5.9%.

In first line and maintenance setting, avelumab is currently evaluated in a phase III, open-label, international, multicenter study as additional maintenance therapy after debulking surgery (www.clinicaltrials.gov: NCT02718417). The study has three arms. Arm A includes chemotherapy with carboplatin plus paclitaxel (standard of care), arm B includes chemotherapy followed by avelumab in maintenance, and arm C includes chemotherapy in combination with avelumab followed by avelumab in maintenance. The primary endpoint of this study is PFS.

In this platinum-resistant and refractory setting, avelumab is combined with PEGylated doxorubicin versus single-agent PEGylated doxorubicin or avelumab alone (www.clinicaltrials.gov: NCT02580058). The primary endpoint of this study is OS.

4. Vulvar cancers

With approximately 4% of the tumors of the female genital tract, vulvar carcinoma is rare. In 75% of cases, it occurs as type 2 carcinoma in the elderly patient. The median age is 70 years.

As type 1 carcinoma, which is usually associated with HPV, it occurs in younger patients in combination with cervical carcinoma or anal carcinoma. The most important treatment is surgical resection. In approximately 30% of the cases, a complete R0 resection is not possible, and then combined procedures are used before or after surgery with chemotherapy and radiotherapy. For systemic approaches, in general, platinum-based chemotherapy regimens are used, frequently in combination with 5FU, mitomycine-c, taxanes, and ifosfamide [93].

Prognosis for vulvar cancer is still poor and there is a high recurrence rate. The 10-year survival rate is still modest with 65% [1]. Therefore, new treatment options are urgently needed. Standard of care for metastatic situations remains the standard platinum-based chemotherapy [94]. Newer targeted therapy failed to demonstrate a major survival benefit [95]. Immunotherapy especially with checkpoint-inhibitors might therefore be beneficial for squamous cell cancers of the female genital tract.

In anal cancer early data from the first 37 patients having received nivolumab every 2 weeks have recently been presented [96]. Here, two patients (5%) showed a complete response, seven (19%) had a partial response, and 17 (46%) had stable disease. The disease control rate was high with 79% and a median PFS of 3.9 months with 6 patients still remaining on the study at present. However, side effects included fatigue, anemia, rash, and one incident of pneumonitis. For vulvar cancers, currently three trials incorporate checkpoint inhibitors worldwide (www.clinicaltrials.gov: NCT 02858310, NCT02628067, NCT02834013).

5. Endometrial cancers

In the group of gynecologic cancers, endometrial cancers have the best outcome with a five-year overall survival rate of 80% [97]. In general, the disease can be cured with surgery including staging procedures and radiotherapy [98, 99]. For type II cancer and advanced stage disease, chemotherapy with carboplatin and paclitaxel is included in standard of care treatment [98, 99]. For relapsed disease and stage IV disease, surgery can be applied, but in general, standard of care is systemic therapy, including endocrine therapy such as medroxy-progestine, tamoxifen, or aromatase inhibitors [100]. Furthermore, combination chemotherapy with carboplatin and paclitaxel is used [101]. For further progression, agents like doxorubicin or topotecan have minimal activity [102, 103].

For advanced and relapsed endometrial cancer, new treatment options are urgently needed. Beside targeted therapy including combinations with endocrine therapy and CDK4/6 inhibitors or VEGF-(R) inhibitors or FGFR-inhibitors, application of immunotherapy might have strong impact in that stage of disease [104, 105].

The cancer genome atlas has recently classified endometrial cancers in four distinct subgroups [106, 107]:

(1) POLE-ultramutated.

(2) Microsatellite instability (MSI) hypermutated.

(3) Copy number low.

(4) Copy number high.

In endometrial cancers, there are only few data available for a specific immunotherapeutic approach, despite the knowledge that high mutational load tumors are expected to respond well. There are some data about dendritic cell vaccination [108–110] and about checkpoint inhibition, particularly in view of mismatch repair-deficient cancers [111]. In this phase II study, the authors examined the efficacy of pembrolizumab in several tumor types (n = 41), including two endometrial cancers. For mismatch repair-deficient colorectal cancers, the immune-related objective response rate was 40 versus 0% for mismatch-repair-proficient colorectal cancers. Patients with other mismatch repair-deficient tumors had comparable response rates. Whole-exome sequencing showed a much higher rate of somatic mutations for mismatch repair-deficient tumors (mean number of somatic mutations: 1782 versus 73 in mismatch repair-proficient tumors (P = 0.007)). The noncolorectal cohort included nine mismatch repair-deficient cancers including gastric cancer, ampullar or cholangiocarcinoma, small bowel cancer, and endometrial cancer. The objective response rate was 71% (95% CI 29—96) with a median time to response of 12 weeks (95% CI 10–13 months). The treatment was well tolerated; most common side effects (all grades) were rash, pruritus, diarrhoea, allergic rhinitis, and pain. The authors conclude that the treatment was well tolerated and the evaluation of mismatch repair deficiency might be a useful marker, independent of underlying tumor type.

6. Conclusion

There has been a tremendous success for immunotherapy in certain tumor types (e.g., melanoma, lung cancer etc.), in particular, for immune checkpoint-inhibitors. In gynecological cancers, the situation is less clear although there are some promising data, especially for treatment with anti-PD1 or anti-PD-L1 antibodies. Early clinical trials showed encouraging disease control rates in heavily pretreated patients. A combination of checkpoint-inhibitors, e.g., anti-PD-1 with CTLA-4 or anti-PD-1 with LAG3 or combinations with chemotherapy might overcome resistance in this type of disease. Current trials aim to examine the combination between immune checkpoint-inhibitors and VEGF inhibitors like bevacizumab or PARP-inhibitors like olaparib and niraparib. An important role of these combination trials is to improve quality of life for patients. Another important goal is the incorporation of appropriate biomarkers to identify new immunotherapeutic approaches. The situation about immunotherapy in other than ovarian cancer has to be called scarce, and no conclusion can be drawn from the data in these cancers up to date.

Abbreviations

AE Adverse event

AB Antibody

CAR	T-cell receptor or chimeric antigen receptor
CC	Cervical cancer
CD	Cluster of differentiation
CR	Complete remission
DC	Dendritic cells
EMA	European Medical Agency
EOC	Epithelial ovarian cancer
FDA	US Food and Drug Administration
FIGO	International Federation of Gynecology and Obstetrics
HPV	Human papillomavirus
IC_{50}	Half maximal inhibitory concentration
IFN-α	Interferon-alpha
IFN-γ	Interferon-gamma
nM	Nanomol
ORR	Overall response rate
OS	Overall survival
PD-1	Programmed death cell ligand 1
PFS	Progression free survival
PR	Partial remission
QoL	Quality of life
RCTX	Radiochemotherapy
RR	Response rate
TLR	Toll-like receptor

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References

[1] American Cancer Society. *Cancer Facts & Figures 2015*. Atlanta: American Cancer Society; 2015. https://old.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf

[2] Winter WE, Maxwell GL, Tian C, Carlson JW, Ozols RF, Rose PG, et al. Prognostic factors for stage III epithelial ovarian cancer: a Gynecologic Oncology Group Study. J Clin Oncol [Internet]. 2007;25(24):3621–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17704411

[3] Bourla AB, Zamarin D. Immunotherapy: new strategies for the treatment of gynecologic malignancies. Oncology (Williston Park) [Internet]. 2016 Jan;30(1):59–66, 69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26791846

[4] Pfisterer J, Harter P, Simonelli C, Peters M, Berek J, Sabbatini P, et al. Abagovomab for ovarian cancer. Expert Opin Biol Ther. 2011;11:395–403.

[5] Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348:203–13.

[6] Hwang W-T, Adams SF, Tahirovic E, Hagemann IS, Coukos G. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. Gynecol Oncol [Internet]. 2012 Feb;124(2):192–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22040834

[7] Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A. 2005;102:18538–43.

[8] Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv Immunol [Internet]. 2006;90:1–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16730260

[9] Herold M, Dölken G, Fiedler F, Franke A, Freund M, Helbig W, et al. Randomized phase III study for the treatment of advanced indolent non-Hodgkin's lymphomas (NHL) and mantle cell lymphoma: chemotherapy versus chemotherapy plus rituximab. Ann Hematol [Internet]. 2003 Feb;82(2):77–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12601483

[10] Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity [Internet]. 2013 Jul 25;39(1):1–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23890059

[11] Leffers N, Daemen T, Helfrich W, Boezen HM, Cohlen BJ, Melief CJM, et al. Antigen-specific active immunotherapy for ovarian cancer. Cochrane Database Syst Rev

[Internet]. 2014 Sep 17;(9):CD007287. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25229990

[12] Edwards RP, Gooding W, Lembersky BC, Colonello K, Hammond R, Paradise C, et al. Comparison of toxicity and survival following intraperitoneal recombinant interleukin-2 for persistent ovarian cancer after platinum: twenty-four-hour versus 7-day infusion. J Clin Oncol [Internet]. 1997 Nov;15(11):3399–407. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9363872

[13] Alvarez RD, Sill MW, Davidson SA, Muller CY, Bender DP, DeBernardo RL, et al. A phase II trial of intraperitoneal EGEN-001, an IL-12 plasmid formulated with PEG-PEI-cholesterol lipopolymer in the treatment of persistent or recurrent epithelial ovarian, fallopian tube or primary peritoneal cancer: a gynecologic oncology group study. Gynecol Oncol [Internet]. 2014 Jun;133(3):433–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24708919

[14] Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science [Internet]. 2015 Apr 3;348(6230):62–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25838374

[15] Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet (London, England) [Internet]. 2015 Feb 7;385(9967):517–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25319501

[16] Robbins PF, Kassim SH, Tran TLN, Crystal JS, Morgan RA, Feldman SA, et al. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. Clin Cancer Res [Internet]. 2015 Mar 1;21(5):1019–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25538264

[17] Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med [Internet]. 2004 Sep;10(9):942–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15322536

[18] Reinartz S, Schumann T, Finkernagel F, Wortmann A, Jansen JM, Meissner W, et al. Mixed-polarization phenotype of ascites-associated macrophages in human ovarian carcinoma: correlation of CD163 expression, cytokine levels and early relapse. Int J cancer [Internet]. 2014 Jan 1;134(1):32–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23784932

[19] Chevolet I, Speeckaert R, Schreuer M, Neyns B, Krysko O, Bachert C, et al. Characterization of the in vivo immune network of IDO, tryptophan metabolism, PD-L1, and CTLA-4 in circulating immune cells in melanoma. Oncoimmunology [Internet]. 2015 Mar;4(3):e982382. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25949897

[20] Wainwright DA, Chang AL, Dey M, Balyasnikova I V, Kim CK, Tobias A, et al. Durable therapeutic efficacy utilizing combinatorial blockade against IDO, CTLA-4, and PD-L1

in mice with brain tumors. Clin Cancer Res [Internet]. 2014 Oct 15;20(20):5290–301. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24691018

[21] Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64(1):9–29.

[22] Schiffman M, Solomon D. Clinical practice. Cervical-cancer screening with human papillomavirus and cytologic cotesting. N Engl J Med [Internet]. 2013 Dec 12;369(24):2324–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24328466

[23] Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med [Internet]. 2003 Feb 6;348(6):518–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12571259

[24] Choi YJ, Park JS. Clinical significance of human papillomavirus genotyping. J Gynecol Oncol [Internet]. 2016 Mar;27(2):e21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26768784

[25] Krebsliga Schweiz, NICER Report. Krebs in der Schweiz: wichtige Zahlen. 2012;2008: 2005–9. http://www.nicer.org/en/publications/scientific-publications/#content1957

[26] Kahn JA. HPV vaccination for the prevention of cervical intraepithelial neoplasia. N Engl J Med [Internet]. 2009 Jul 16;361(3):271–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19605832

[27] Vesikari T, Brodszki N, van Damme P, Diez-Domingo J, Icardi G, Petersen LK, et al. A randomized, double-blind, phase III study of the immunogenicity and safety of a 9-valent human papillomavirus L1 virus-like particle vaccine (V503) versus Gardasil® in 9-15-year-old girls. Pediatr Infect Dis J [Internet]. 2015 Sep;34(9):992–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26090572

[28] Koh W-J, Greer BE, Abu-Rustum NR, Apte SM, Campos SM, Cho KR, et al. Cervical cancer, Version 2.2015. J Natl Compr Canc Netw [Internet]. 2015 Apr;13(4):395–404; quiz 404. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25870376

[29] Green JA, Kirwan JM, Tierney JF, Symonds P, Fresco L, Collingwood M, et al. Survival and recurrence after concomitant chemotherapy and radiotherapy for cancer of the uterine cervix: a systematic review and meta-analysis. Lancet (London, England) [Internet]. 2001 Sep 8;358(9284):781–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11564482

[30] Tewari KS, Sill MW, Long HJ, Penson RT, Huang H, Ramondetta LM, et al. Improved survival with bevacizumab in advanced cervical cancer. N Engl J Med [Internet]. 2014 Feb 20;370(8):734–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24552320

[31] Penson RT, Huang HQ, Wenzel LB, Monk BJ, Stockman S, Long HJ, et al. Bevacizumab for advanced cervical cancer: patient-reported outcomes of a randomised, phase 3 trial (NRG Oncology-Gynecologic Oncology Group protocol 240). Lancet Oncol [Internet]. 2015 Mar;16(3):301–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25638326

[32] Patel S, Chiplunkar S. Host immune responses to cervical cancer. Curr Opin Obstet Gynecol [Internet]. 2009 Feb;21(1):54–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19125004

[33] Monnier-Benoit S, Mauny F, Riethmuller D, Guerrini J-S, Căpîlna M, Félix S, et al. Immunohistochemical analysis of CD4+ and CD8+ T-cell subsets in high risk human papillomavirus-associated pre-malignant and malignant lesions of the uterine cervix. Gynecol Oncol [Internet]. 2006 Jul;102(1):22–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16427684

[34] de Jong A, van Poelgeest MIE, van der Hulst JM, Drijfhout JW, Fleuren GJ, Melief CJM, et al. Human papillomavirus type 16-positive cervical cancer is associated with impaired CD4+ T-cell immunity against early antigens E2 and E6. Cancer Res [Internet]. 2004 Aug 1;64(15):5449–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15289354

[35] Santin AD, Bellone S, Palmieri M, Bossini B, Roman JJ, Cannon MJ, et al. Induction of tumor-specific cytotoxicity in tumor infiltrating lymphocytes by HPV16 and HPV18 E7-pulsed autologous dendritic cells in patients with cancer of the uterine cervix. Gynecol Oncol [Internet]. 2003 May;89(2):271–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12713991

[36] Arreygue-Garcia NA, Daneri-Navarro A, del Toro-Arreola A, Cid-Arregui A, Gonzalez-Ramella O, Jave-Suarez LF, et al. Augmented serum level of major histocompatibility complex class I-related chain A (MICA) protein and reduced NKG2D expression on NK and T cells in patients with cervical cancer and precursor lesions. BMC Cancer [Internet]. 2008 Jan 21;8:16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18208618

[37] Visser J, Nijman HW, Hoogenboom B-N, Jager P, van Baarle D, Schuuring E, et al. Frequencies and role of regulatory T cells in patients with (pre)malignant cervical neoplasia. Clin Exp Immunol [Internet]. 2007 Nov;150(2):199–209. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17937675

[38] Nakamura T, Shima T, Saeki A, Hidaka T, Nakashima A, Takikawa O, et al. Expression of indoleamine 2, 3-dioxygenase and the recruitment of Foxp3-expressing regulatory T cells in the development and progression of uterine cervical cancer. Cancer Sci [Internet]. 2007 Jun;98(6):874–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17433037

[39] Monk BJ, Sill MW, McMeekin DS, Cohn DE, Ramondetta LM, Boardman CH, et al. Phase III trial of four cisplatin-containing doublet combinations in stage IVB, recurrent, or persistent cervical carcinoma: a Gynecologic Oncology Group study. J Clin Oncol [Internet]. 2009 Oct 1;27(28):4649–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19720909

[40] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med [Internet]. 2010 Aug 19;363(8):711–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20525992

[41] Ribas A. Tumor immunotherapy directed at PD-1. N Engl J Med [Internet]. 2012 Jun 28;366(26):2517–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22658126

[42] Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med [Internet]. 2012 Jun 28;366(26):2443–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22658127

[43] Lyford-Pike S, Peng S, Young GD, Taube JM, Westra WH, Akpeng B, et al. Evidence for a role of the PD-1:PD-L1 pathway in immune resistance of HPV-associated head and neck squamous cell carcinoma. Cancer Res [Internet]. 2013 Mar 15;73(6):1733–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23288508

[44] Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol [Internet]. 2015 Aug;16(8):908–18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26115796

[45] Frenel J-S, Le Tourneau C, O'Neil BH, Ott PA, Piha-Paul SA, Gomez-Roca CA, Van Brummelen E, Rugo HS, Thomas S, Saraf S, Chen M, Varga A. Institut de Cancerologie de l' F. Pembrolizumab in patients with advanced cervical squamous cell cancer: preliminary results from the phase Ib KEYNOTE-028 study. J Clin Oncol. 2016;34(suppl; abstr 5515).

[46] Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med [Internet]. 2015 Oct 22;373(17):1627–39. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26412456

[47] Antonia S, Goldberg SB, Balmanoukian A, Chaft JE, Sanborn RE, Gupta A, et al. Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. Lancet Oncol [Internet]. 2016 Mar;17(3):299–308. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26858122

[48] Planchard D, Yokoi T, McCleod MJ, Fischer JR, Kim Y-C, Ballas M, et al. A phase III study of durvalumab (MEDI4736) with or without tremelimumab for previously treated patients with advanced NSCLC: rationale and protocol design of the ARCTIC Study. Clin Lung Cancer [Internet]. 2016 May;17(3):232–236.e1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27265743

[49] Segal NH, Hamid O, Hwu W, Massard C, Butler M, Antonia SJ, Blake-Haskins A, Robbins PB, Li X, Vasselli JSK. A phase I multi-arm dose-expansion study of the anti-programmed cell death-ligand-1 (PD-L1) antibody MEDI4736: preliminary data. Ann Oncol 25 iv361–72 101093/annonc/mdu342.

[50] Siegel R, Miller K, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65(1):29.

[51] Vergote I, Trope CG, Amant F, Kristensen GB, Ehlen T, Johnson N, et al. Neoadjuvant chemotherapy or primary surgery in stage IIIC or IV ovarian cancer. N Engl J Med. United States; 2010 Sep;363(10):943–53.

[52] Heinzelmann-Schwarz VA, Gardiner-Garden M, Henshall SM, Scurry JP, Scolyer RA, Smith AN, et al. A distinct molecular profile associated with mucinous epithelial ovarian cancer. Br J Cancer [Internet]. 2006 Mar 27;94(6):904–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16508639

[53] du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I, Pfisterer J. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzin. Cancer. United States; 2009 Mar;115(6):1234–44.

[54] du Bois A, Floquet A, Kim J-W, Rau J, del Campo JM, Friedlander M, et al. Incorporation of pazopanib in maintenance therapy of ovarian cancer. J Clin Oncol [Internet]. 2014;32(30):3374–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25225436

[55] Eichbaum M, Mayer C, Eickhoff R, Bischofs E, Gebauer G, Fehm T, et al. The PACOVAR-trial: a phase I/II study of pazopanib (GW786034) and cyclophosphamide in patients with platinum-resistant recurrent, pre-treated ovarian cancer. BMC Cancer [Internet]. 2011;11:453. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22014006

[56] Pignata S, Lorusso D, Scambia G, Sambataro D, Tamberi S, Cinieri S, et al. Pazopanib plus weekly paclitaxel versus weekly paclitaxel alone for platinum-resistant or platinum-refractory advanced ovarian cancer (MITO 11): a randomised, open-label, phase 2 trial. Lancet Oncol [Internet]. 2015;16(5):561–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25882986

[57] Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. N Engl J Med [Internet]. 2011;365(26):2473–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22204724

[58] Pujade-Lauraine E, Hilpert F, Weber B, Reuss A, Poveda A, Kristensen G, et al. Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: The AURELIA open-label randomized phase III trial. J Clin Oncol [Internet]. 2014;32(13):1302–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24637997

[59] Monk BJ, Poveda A, Vergote I, Raspagliesi F, Fujiwara K, Bae D-S, et al. Final results of a phase 3 study of trebananib plus weekly paclitaxel in recurrent ovarian cancer (TRINOVA-1): Long-term survival, impact of ascites, and progression-free survival-2. Gynecol Oncol [Internet]. 2016 Oct;143(1):27–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27546885

[60] Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med. 2012;366(15):1382–92.

[61] Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol [Internet]. 2014;15(8):852–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24882434

[62] Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. N Engl J Med [Internet]. 2016 Oct 7; Available from: http://www.ncbi.nlm.nih.gov/pubmed/27717299

[63] Jerne NK. Clonal selection in a lymphocyte network. Soc Gen Physiol Ser. 1974;29:29–48.

[64] Tse BWC, Collins A, Oehler MK, Zippelius A, Heinzelmann-Schwarz VA. Antibody-based immunotherapy for ovarian cancer: where are we at? Ann Oncol Off J Eur Soc Med Oncol [Internet]. 2014 Feb;25(2):322–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24285017

[65] Rustin GJ, Marples M, Nelstrop AE, Mahmoudi M, Meyer T. Use of CA-125 to define progression of ovarian cancer in patients with persistently elevated levels. J Clin Oncol [Internet]. 2001 Oct 15;19(20):4054–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11600607

[66] Bast RC. CA 125 and the detection of recurrent ovarian cancer: a reasonably accurate biomarker for a difficult disease. Cancer [Internet]. 2010 Jun 15;116(12):2850–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20564390

[67] Gubbels JAA, Belisle J, Onda M, Rancourt C, Migneault M, Ho M, et al. Mesothelin-MUC16 binding is a high affinity, N-glycan dependent interaction that facilitates peritoneal metastasis of ovarian tumors. Mol Cancer [Internet]. 2006;5(1):50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17067392

[68] Patankar MS, Jing Y, Morrison JC, Belisle JA, Lattanzio FA, Deng Y, et al. Potent suppression of natural killer cell response mediated by the ovarian tumor marker CA125. Gynecol Oncol [Internet]. 2005 Dec;99(3):704–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16126266

[69] Sabbatini P, Dupont J, Aghajanian C, Derosa F, Poynor E, Anderson S, et al. Phase I study of abagovomab in patients with epithelial ovarian, fallopiant ube, or primary peritoneal cancer. In Vitro. 2006;12:5503–10.

[70] Pfisterer J, du Bois a., Sehouli J, Loibl S, Reinartz S, Reuß A, et al. The anti-idiotypic antibody abagovomab in patients with recurrent ovarian cancer. A phase I trial of the AGO-OVAR. Ann Oncol. 2006;17:1568–77.

[71] Reinartz S, Köhler S, Schlebusch H, Krista K, Giffels P, Renke K, et al. Vaccination of patients with advanced ovarian carcinoma with the anti-idiotype ACA125: immunological response and survival (phase Ib/II). Clin Cancer Res [Internet]. 2004 Mar 1;10(5):1580–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15014007

[72] Sabbatini P, Harter P, Scambia G, Sehouli J, Meier W, Wimberger P, et al. Abagovomab as maintenance therapy in patients with epithelial ovarian cancer: a phase III trial of the AGO OVAR, COGI, GINECO, and GEICO-the MIMOSA study. J Clin Oncol. 2013;31(12):1554–61.

[73] Möbus VJ, Baum RP, Bolle M, Kreienberg R, Noujaim AA, Schultes BC, et al. Immune responses to murine monoclonal antibody-B43.13 correlate with prolonged survival of women with recurrent ovarian cancer. Am J Obstet Gynecol [Internet]. 2003 Jul;189(1):28–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12861134

[74] Noujaim AA, Schultes BC, Baum RP, Madiyalakan R. Induction of CA125-specific B and T cell responses in patients injected with MAb-B43.13 – evidence for antibody-mediated antigen-processing and presentation of CA125 in vivo. Cancer Biother Radiopharm [Internet]. 2001 Jun;16(3):187–203. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11471484

[75] Gordon AN, Schultes BC, Gallion H, Edwards R, Whiteside TL, Cermak JM, et al. CA125- and tumor-specific T-cell responses correlate with prolonged survival in oregovomab-treated recurrent ovarian cancer patients. Gynecol Oncol [Internet]. 2004 Aug;94(2):340–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15297171

[76] Schultes BC, Baum RP, Niesen A, Noujaim AA, Madiyalakan R. Anti-idiotype induction therapy: anti-CA125 antibodies (Ab3) mediated tumor killing in patients treated with Ovarex mAb B43.13 (Ab1). Cancer Immunol Immunother [Internet]. 1998 Jun;46(4):201–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9671143

[77] Ehlen TG, Hoskins PJ, Miller D, Whiteside TL, Nicodemus CF, Schultes BC, et al. A pilot phase 2 study of oregovomab murine monoclonal antibody to CA125 as an immunotherapeutic agent for recurrent ovarian cancer. Int J Gynecol Cancer [Internet]. 15(6):1023–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16343178

[78] Berek JS, Taylor PT, Gordon A, Cunningham MJ, Finkler N, Orr J, et al. Randomized, placebo-controlled study of oregovomab for consolidation of clinical remission in patients with advanced ovarian cancer. J Clin Oncol. 2004;22(17):3507–16.

[79] Braly P, Nicodemus CF, Chu C, Collins Y, Edwards R, Gordon A, et al. The Immune adjuvant properties of front-line carboplatin-paclitaxel: a randomized phase 2 study of alternative schedules of intravenous oregovomab chemoimmunotherapy in advanced ovarian cancer. J Immunother [Internet]. 2009 Jan;32(1):54–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19307994

[80] Matsuzaki J, Gnjatic S, Mhawech-Fauceglia P, Beck A, Miller A, Tsuji T, et al. Tumor-infiltrating NY-ESO-1-specific CD8+ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer. Proc Natl Acad Sci U S A [Internet]. 2010 Apr 27;107(17):7875–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20385810

[81] Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci U S A [Internet]. 2007 Feb 27;104(9):3360–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17360651

[82] Duraiswamy J, Freeman GJ, Coukos G. Therapeutic PD-1 pathway blockade augments with other modalities of immunotherapy T-cell function to prevent immune decline in ovarian cancer. Cancer Res [Internet]. 2013 Dec 1;73(23):6900–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23975756

[83] Brahmer JR, Tykodi SS, Chow LQM, Hwu W-J, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med

[Internet]. 2012 Jun 28;366(26):2455–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22658128

[84] Hamanishi J, Mandai M, Ikeda T, Minami M, Kawaguchi A, Murayama T, et al. Safety and antitumor activity of anti-PD-1 antibody, nivolumab, in patients with platinum-resistant ovarian cancer. J Clin Oncol [Internet]. 2015 Dec 1;33(34):4015–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26351349

[85] Varga A, Piha-Paul SA, Ott PA, Mehnert JM, Berton-Rigaud D, Johnson EA, Cheng JD, Yuan S, Rubin EH. Antitumor activity and safety of pembrolizumab in patients (pts) with PD-L1 positive advanced ovarian cancer: interim results from a phase Ib study. J Clin Oncol. 2015;33(suppl; abstr 5509).

[86] Birkbak NJ, Kochupurakkal B, Izarzugaza JMG, Eklund AC, Li Y, Liu J, et al. Tumor mutation burden forecasts outcome in ovarian cancer with BRCA1 or BRCA2 mutations. PLoS One [Internet]. 2013;8(11):e80023. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24265793

[87] Ku GY, Yuan J, Page DB, Schroeder SEA, Panageas KS, Carvajal RD, et al. Single-institution experience with ipilimumab in advanced melanoma patients in the compassionate use setting: lymphocyte count after 2 doses correlates with survival. Cancer [Internet]. 2010 Apr 1;116(7):1767–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20143434

[88] Liakou CI, Kamat A, Tang DN, Chen H, Sun J, Troncoso P, et al. CTLA-4 blockade increases IFNgamma-producing CD4+ICOShi cells to shift the ratio of effector to regulatory T cells in cancer patients. Proc Natl Acad Sci U S A [Internet]. 2008 Sep 30;105(39):14987–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18818309

[89] Yuan J, Gnjatic S, Li H, Powel S, Gallardo HF, Ritter E, et al. CTLA-4 blockade enhances polyfunctional NY-ESO-1 specific T cell responses in metastatic melanoma patients with clinical benefit. Proc Natl Acad Sci U S A [Internet]. 2008 Dec 23;105(51):20410–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19074257

[90] Hodi FS, Mihm MC, Soiffer RJ, Haluska FG, Butler M, Seiden M V, et al. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci U S A [Internet]. 2003 Apr 15;100(8):4712–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12682289

[91] Hodi FS, Butler M, Oble DA, Seiden M V, Haluska FG, Kruse A, et al. Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. Proc Natl Acad Sci U S A [Internet]. 2008 Feb 26;105(8):3005–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18287062

[92] Disis ML, Patel MR, Pant S, Hamilton EP, Lockhart AC, Kelly K, Beck JT, Gordon MS, Weiss GJ, Taylor MH, Chaves J, Mita AC, Chin KM, von Heydebreck A, Cuiller J-M. Avelumab (MSB0010718C; anti-PD-L1) in patients with recurrent/refractory ovarian

cancer from the JAVELIN Solid Tumor phase Ib trial: safety and clinical activity. J Clin Oncol. 2016;34(suppl; abstr 5533).

[93] Frederick P. Vulvar cancer (squamous cell carcinoma). NCCN Clin Pract Guidel Oncol. Version 1.2017-October 4, 2016; p1–51

[94] Deppe G, Mert I, Belotte J, Winer IS. Chemotherapy of vulvar cancer: a review. Wien Klin Wochenschr [Internet]. 2013 Mar;125(5–6):119–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23519539

[95] Reade CJ, Eiriksson LR, Mackay H. Systemic therapy in squamous cell carcinoma of the vulva: current status and future directions. Gynecol Oncol [Internet]. 2014 Mar;132(3):780–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24296343

[96] Morris VK, Ciombor KK, Salem ME, Nimeiri HS, Iqbal S, Singh PP, Polite BN, Deming DA, Chan E, Wade JL, Bekaii-Saab TS, Uronis HE, Pasia MG, Bland G, Rober T. NCI9673: a multi-institutional eETCTN phase II study of nivolumab in refractory metastatic squamous cell carcinoma of the anal canal (SCCA). J Clin Oncol. 2016;34(suppl; abstr 3503).

[97] Bray F, Ferlay J, Laversanne M, Brewster DH, Gombe Mbalawa C, Kohler B, et al. Cancer Incidence in Five Continents: Inclusion criteria, highlights from Volume X and the global status of cancer registration. Int J Cancer [Internet]. 2015;137(9):2060–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26135522

[98] Koh W-J, Greer BE, Abu-Rustum NR, Apte SM, Campos SM, Chan J, et al. Uterine neoplasms, version 1.2014. J Natl Compr Canc Netw [Internet]. 2014 Feb;12(2):248–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24586086

[99] Colombo N, Preti E, Landoni F, Carinelli S, Colombo A, Marini C, et al. Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol [Internet]. 2013;24(suppl 6):vi33–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24078661

[100] Palisoul M, Mutch DG. The clinical management of inoperable endometrial carcinoma. Expert Rev Anticancer Ther [Internet]. 2016 May;16(5):515–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26999568

[101] Sorbe B, Andersson H, Boman K, Rosenberg P, Kalling M. Treatment of primary advanced and recurrent endometrial carcinoma with a combination of carboplatin and paclitaxel-long-term follow-up. Int J Gynecol Cancer [Internet]. 18(4):803–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17944917

[102] Makker V, Hensley ML, Zhou Q, Iasonos A, Aghajanian CA. Treatment of advanced or recurrent endometrial carcinoma with doxorubicin in patients progressing after paclitaxel/carboplatin: Memorial Sloan-Kettering Cancer Center experience from 1995 to 2009. Int J Gynecol Cancer [Internet]. 2013 Jun;23(5):929–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23598889

[103] Wadler S, Levy DE, Lincoln ST, Soori GS, Schink JC, Goldberg G. Topotecan is an active agent in the first-line treatment of metastatic or recurrent endometrial carcinoma: Eastern Cooperative Oncology Group Study E3E93. J Clin Oncol [Internet]. 2003 Jun 1;21(11):2110–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12775736

[104] Konecny GE, Finkler N, Garcia AA, Lorusso D, Lee PS, Rocconi RP, et al. Second-line dovitinib (TKI258) in patients with FGFR2-mutated or FGFR2-non-mutated advanced or metastatic endometrial cancer: a non-randomised, open-label, two-group, two-stage, phase 2 study. Lancet Oncol [Internet]. 2015;16(6):686–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25981814

[105] Felix AS, Sherman ME, Hewitt SM, Gunja MZ, Yang HP, Cora RL, et al. Cell-cycle protein expression in a population-based study of ovarian and endometrial cancers. Front Oncol [Internet]. 2015;5:25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25709969

[106] Gargiulo P, Della Pepa C, Berardi S, Califano D, Scala S, Buonaguro L, et al. Tumor genotype and immune microenvironment in POLE-ultramutated and MSI-hypermutated endometrial cancers: new candidates for checkpoint blockade immunotherapy? Cancer Treat Rev [Internet]. 2016 Jul;48:61–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27362548

[107] Cancer Genome Atlas Research Network, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. Nature [Internet]. 2013 May 2;497(7447):67–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23636398

[108] Coosemans A, Vanderstraeten A, Tuyaerts S, Verschuere T, Moerman P, Berneman ZN, et al. Wilms' Tumor Gene 1 (WT1)--loaded dendritic cell immunotherapy in patients with uterine tumors: a phase I/II clinical trial. Anticancer Res [Internet]. 2013 Dec;33(12):5495–500. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24324087

[109] Coosemans A, Tuyaerts S, Vanderstraeten A, Vergote I, Amant F, Van Gool SW. Dendritic cell immunotherapy in uterine cancer. Hum Vaccin Immunother [Internet]. 2014;10(7):1822–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25424788

[110] Coosemans A, Vanderstraeten A, Tuyaerts S, Verschuere T, Moerman P, Berneman Z, et al. Immunological response after WT1 mRNA-loaded dendritic cell immunotherapy in ovarian carcinoma and carcinosarcoma. Anticancer Res [Internet]. 2013 Sep;33(9):3855–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24023319

[111] Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med [Internet]. 2015 Jun 25;372(26):2509–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26028255

Chapter 7

Aptamers as a Promising Therapeutic Tool for Cancer Immunotherapy

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Additional information is available at the end of the chapter

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Abstract

Aptamers are single-chained RNA or DNA oligonucleotides (ODNs) with a three-dimensional conformation that provides the ability to fit their targets with high affinity and specificity obtained by a method called SELEX. Cancer immunotherapy has nowadays come back to prominence due to its encouraging results in the clinic with monoclonal antibodies. Aptamers display some important advantages over antibodies at the time of translation into the clinic. They are very suitable for targeting and delivery, reducing off-target side effects, and increasing the therapeutic index of a given strategy. Hundreds of aptamers have been described for very different purposes within biomedical research. Some of the aptamers described recently have been isolated with immunotherapeutic applications to overcome current challenges in cancer immunotherapy. To elicit a specific antitumor immune response, some of these aptamers are engineered to activate co-stimulatory receptors or blocking immunosuppressive signals. Aptamers would hopefully gain an important niche in cancer immunotherapy due to their specific properties.

Keywords: aptamer, oligonucleotide, receptor, cancer, immunotherapy, immune system

1. Introduction

Oligonucleotides (ODNs) are short DNA or RNA oligomers presented as single- or double-stranded molecules containing a specified sequence. This kind of molecules can be generated to be used for a large variety of purposes, such as artificial gene synthesis, DNA sequencing, library construction, molecular probes, and regulation of gene expression, among others. The technical support in terms of detection and analysis that ODNs provide in daily laboratory work is not but a small part of their current use. Nowadays, ODN molecules such as small

interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), CpGs, and aptamers are being currently used as therapeutic agents for the treatment of diseases and malignancies of very different nature.

Aptamers are single-stranded DNA or RNA (ssDNA or ssRNA) oligonucleotides; their three-dimensional conformation provides them with the capability to fit in their targets with high affinity and specificity. The word "aptamer" was coined by Jack Szostak and results from the junction of two words, "aptus" which comes from Latin and means "to fit" and "meros" which comes from Greek and means "particle." The first aptamer was isolated by Andy Ellington and Jack Szostak in 1990 toward organic dyes and paralleled by Craig Tuerk and Larry Gold against the T4-bacteriophage DNA polymerase [1, 2]. Aptamers are isolated through a combinatorial chemical method named SELEX, meaning **s**ystematic **e**volution of **l**igands by **ex**ponential enrichment. The SELEX method (schematically represented in **Figure 1**) is an iterative process that consists in rounds of selection. Each round comprises three main steps: "binding," "partition," and "amplification" [1, 2]. The first step is called "binding" and begins with a complex randomized library of 10^{12} to 10^{15} different sequences to ensure the majority of potential structures. Each sequence comprises two constant regions at

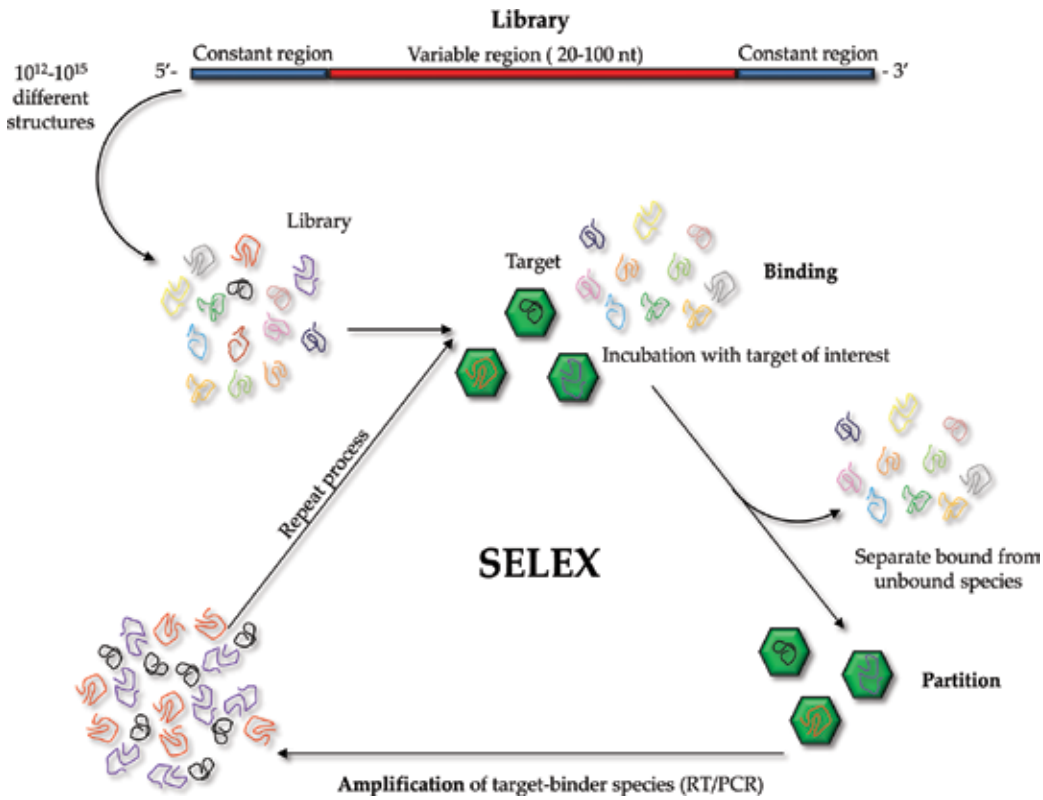

Figure 1. The SELEX procedure. The systematic evolution of ligands by exponential enrichment consists in three main steps: binding, partition, and amplification.

3′ and 5′ ends allowing the primers to anneal flanking a variable region that can vary from 20 to 100 nucleotides (nt). Throughout this step, the library is mixed with the target of interest to allow for some of the sequences to join the target. The following step is known as "partition" and consists in separating the target-binding species from non-binders. Finally, the binder species are amplified by polymerase chain reaction (PCR) in the "amplification" step to serve as library for the next round of selection. It is to note that if the aptamer of interest is an RNA aptamer, in vitro transcription shall be performed before starting each round. The SELEX process usually takes from 9 to 15 rounds, which implies months of work, but new tools such as high-throughput sequencing enable the researchers to identify already enriched sequences at early selection stages, thereby reducing the number of rounds and hence the amount of work to be done [3, 4]. Since the first aptamers isolated by the conventional selection procedure, the SELEX method has evolved and varied through time with the objective of isolating aptamers against targets from every possible nature, including sugars, vitamins, proteins, or even small molecules [5, 6]. Some of the variations are CE-SELEX, which comes from capillary electrophoretic SELEX [7]; cell-SELEX, in which selection is carried out with cells [8]; toggle-SELEX, which on the other hand is used to obtain cross-reactive aptamers [4, 9]; and tailored-SELEX [10], which is used to identify 10-fixed nucleotide aptamers without primer-binding sites. Tailored-SELEX was validated when a Spiegelmer against the migraine-associated target calcitonin gene-related peptide 1 (alpha-CGRP1) was isolated [10]. Spiegelmers are a recently described new class of aptamers, which are "mirror-image" L-conformed enantiomer aptamers [11]. Moreover, in vivo SELEX performs the rounds of selection in animals [12], and genomic SELEX is otherwise used to achieve what are called genomic aptamers directed to bind genomic-encoded functional domains [13].

As mentioned above, aptamers can be isolated against molecule from almost every nature with high affinity and specificity which, in the majority of cases, is comparable or even superior to that of their corresponding monoclonal antibodies (mAbs). Indeed, a DNA aptamer against IL-6 that recognizes this interleukin with a dissociation constant (K_d) of 0.2 nM has been recently described [14]. Following this, line aptamers show several advantages over cell-based products such as antibodies (Abs) or recombinant proteins, as summarized in **Table 1**. Aptamers are smaller than cell-based products, which provide them with an ease to penetrate tissues and therefore make them very suitable for targeting. Thanks to their chemical nature aptamers can be modified to optimize yield and easily customized to add tailored properties to carry cargoes from very diverse nature such as drugs, radioisotopes, proteins, enzymes, RNAs, or even nanostructures, greatly favoring their use for delivery [15–17]. Moreover, aptamers can be easily multimerized to modulate the immune system [18–22]. Throughout the SELEX technique, the process is not interfered with by the toxicity or low immunogenicity of specific antigens as might befall, for example, with Abs [22]. Cell-based products such antibodies or recombinant proteins usually show T-cell-dependent immunity, meaning that an immune response can be directed against these compounds unlike what happens with short ODNs such as aptamers. At the time of translating, the approaches to the clinic aptamers possess a great advantage over other kinds of molecules since they inherently present an antidote [23, 24]. Aptamers are chemical products that can be synthetically manufactured what facilitated their exportation to GMP grade (good manufacturing practices or the practices required

Feature	Aptamer	Cell-based product	Advantage
Nature	Chemically synthesized	Produced by cells	Easy to multimerize for activation of immune receptors
Immunogenicity	Not or very low immunogenic	T-cell-dependent immunity	Do not trigger immune response against them
Size	Small (5–90 KDa)	Big (50–200 KDa)	Easy tissue penetrating, very suitable for targeting
Customization	Easy procedure	Requires specific skills	Tailored properties easy to add, very suitable for delivery
Antidote	Yes	No	Possibility of reversion any undesired effect
GMP grade	Lower cost of manufacturing	Higher complexity and cost of manufacturing	Easier regulatory approval process

Table 1. Advantages of aptamers over cell-based products.

to manufacture and sell any pharmaceutical product). This feature privileges aptamers over antibodies since regulatory approval processes are tougher on cell-based products due to their high complexity and cost of manufacturing.

Aptamers present one disadvantage over other agents currently used in translational medicine—their low plasma stability. Nonetheless, their half-life in plasma can be significantly enhanced by using different approaches, such as selective substitution of HO residues by *O*-methyl or F analogs at the 2′ position of the pyrimidine interactions, thus increasing their resistance to RNA-degrading enzymes [5, 25]. Aptamers can be conjugated to cholesterol to enhance their half-life at the same time that improves their biological activity [26–28]. Another alternative clinically compatible carrier is polyethylene glycol (PEG) [29], which prevents its renal exclusion [5]. PEG conjugation highly increases aptamer survival as exemplified by a PEGylated anti-MUC1 aptamer-doxorubicin conjugate [30]. The PEGylated form of this MUC1-doxorubicin conjugate increased its survival rate to a maximum of sixfold [30]. Furthermore, the addition of nonnatural analogous bases can widen the aptamer-target interactions [5, 25]. This is the case of slow off-rate modified aptamers (SOMAmers), in which aromatic hydrophobic modified nucleotides such as benzyl-dU (Bn-dU) and naphthyl-dU (Nap-dU) are added [31]. Slow off-rate modified aptamers (SOMAmers) show protein-like modified side chains. These substitutions advantage them over conventional aptamers in decreasing the number of exposed polar groups and augmenting their affinity [31].

A tremendous amount of new selected aptamers has been published since the first aptamer isolated in the early 1990s [5, 32, 33]. Some of them are currently undergoing clinical trials for the treatment of several diseases, such as macular edema and age-related macular degeneration as in the case of the antiplatelet-derived growth factor (PDGF) and the anticomplement component (C5) RNA aptamers [34, 35]. Some RNA aptamers including the antifactor IXa of coagulation and the anti-A1 domain for activated von Willebrand factor (vWf) are directed

to control hemostasis [35–37]. For the treatment of diabetes mellitus, one Spiegelmer is being used to target the monocyte chemoattractant protein 1 (MCP-1 also called CCL2) [35]. The two most advanced aptamers for cancer treatment are the anti-nucleolin aptamer AS1411 and the anti-stroma cell-derived factor-1 (SDF-1 also called CXCL12) NOX-A12 [35]. Among every aptamer tested in clinical trials, the first in class was the anti-VEGF RNA aptamer approved in 2004 by the Food and Drug Administration (FDA) which is used for the treatment of age-related macular degeneration and is called MACUGEN.

2. Aptamers in cancer immunotherapy

The cancer burden around the world is extremely growing, to the extent that estimates calculate 21 million new cancer cases and 13 million cancer deaths from now until 2030 [38]. There exist nowadays three main strategies to tackle cancer, namely, chemotherapy, radiotherapy, and surgery in cases of resettable tumors. The elevated relapsing rates and the high toxicity associated with current treatments due to their lack of specificity usually make these conventional treatments not powerful enough in many kinds of tumors. Surgery has the inherent problem of not removing every single malignant cell, causing tumor relapses in the majority of cases. On the other hand, radiotherapy and chemotherapy often show serious side effects, which is not only very harmful but also very uncomfortable for the patient. Immunotherapy is now given prominence thanks to the encouraging results obtained in some clinical trials over the last few years [39] and the use as monotherapy or in combination of several FDA-approved immune-checkpoint blocking Abs such as ipilimumab, nivolumab, or pembrolizumab [40–46]. Nonetheless, due to the severe toxicity associated with the use of agonistic mAbs such 4-1BB Ab or super agonistic CD28 Ab (TGN1412) causing hepatic toxicity and cytokine storm, respectively [47–50], new immunomodulatory ligands with lower associated side effects are strongly needed.

As mentioned above, aptamers have been used in different research fields such as metabolic and cardiovascular diseases or cancer [5]. Among the number of aptamers used in cancer research, some of them have been used to treat cancer within an immunotherapeutic context [51]. This chapter will be focused on aptamers used to date for cancer immunotherapy, which in turn will be subdivided in four main parts: (I) aptamers developed to block immunosuppressive signals, (II) agonistic aptamers directed to trigger activating signals, (III) bi-specific aptamers to target the immune response to the tumor site, and (IV) aptamer-based approaches to enhance tumor immunogenicity.

2.1. Aptamers developed to block immunosuppressive signals

In order to find the first aptamer developed with immunotherapeutic intention, we must go back to 2003. It was then when a CTLA-4 RNA aptamer was isolated and multimerized to block CTLA-4 signaling [18]. Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is a member of the immunoglobulin superfamily expressed by activated T cells, and its engagement with its natural ligand B7 (B7.1=CD80 and B7.2=CD86) leads to T-cell exhaustion [52]. The work published by Gilboa's group laid down the foundations for aptamers as new immunotherapeutic agents. In this work, the multimerized blocking anti-CTLA-4 RNA aptamer showed to bind its target with high affin-

ity and inhibited CTLA-4 action in vitro. This tetrameric form (schematically represented in **Figure 2a**) enhanced its in vitro and in vivo effects similar indeed to that of the mAb [18].

Programmed cell death protein 1 (PD-1) is a protein expressed in several cell types. It is expressed on the surface of CD8+ T lymphocytes especially in tumor-infiltrating lymphocytes (TILs). Its engagement with its ligand PDL-1 expressed on tumor cells induces T-cell exhaustion leading to their dysfunction and therefore tumor progression [53]. An anti-PD-1 DNA aptamer (represented in **Figure 2b**) has been published, which is able to block the PD-1-PDL-1 axis, thereby decreasing tumor burden and increasing survival in murine tumor models [54].

TIM3 is another T-cell exhaustion maker expressed in CD4+ interferon-γ expressing cells and cytotoxic CD8+ T lymphocytes [55]. It is usually expressed on T lymphocytes together with PD-1 [55]. Moreover, the upregulation of TIM3 on a subpopulation of infiltrating Tregs has been correlated with bad prognosis in patients [56]. Our group has recently described a TIM3 RNA aptamer with antagonistic capacity [57]. This aptamer (represented in **Figure 2c**) was able to counteract TIM3 inhibitory signal on T lymphocytes in vitro and reduce tumor burden in a mouse colorectal tumor model in combination with PDL-1 blockade [57] Moreover, we have published at the present time a work that describes the use of in silico and docking studies to predict the mode of action and potential binding site of novel and the already published murine TIM3 RNA aptamer [58].

We have recently selected a murine CD40 RNA aptamer with high affinity for its target (represented in **Figure 2d**). Since CD40 is expressed in several B-cell malignancies, we used it

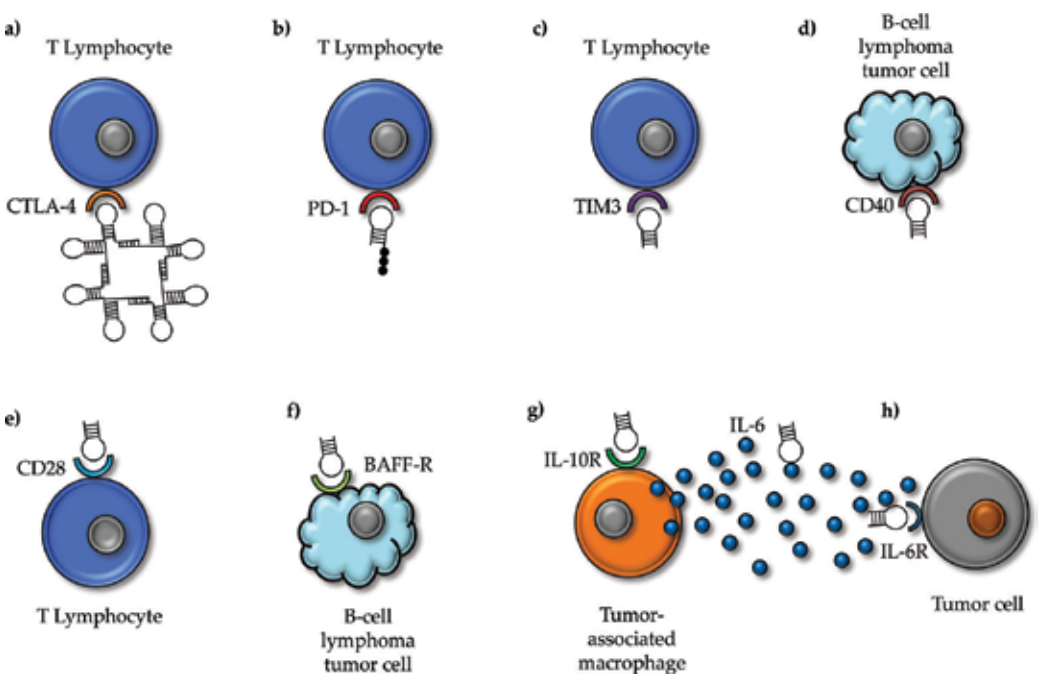

Figure 2. Antagonistic aptamers. (a) CTLA-4, (b) PD-1, (c) TIM3, (d) CD40, (e) CD28, (f) BAFF-R, (g) IL-10R, and (h) IL-6 and IL-6R.

to block the CD40 receptor in vitro and in vivo. The experiments resulted in tumor growth reduction as we increased mice survival by 30% [21]. We had previously described a CD28 antagonistic RNA aptamers which in its monomeric form was able to compete for CD28 ligand B7 and to revert in vitro the co-stimulation induced by B7 in CD4+ T lymphocytes (represented in **Figure 2e**) [20]. This antagonistic aptamer could serve as immunosuppressant in several autoimmune diseases or transplants. For example, in the host-versus-donor immune responses triggered upon transplant engraftment, drug administration is needed to suppress the acute response. Thus, the use of this antagonistic CD28 aptamer might serve as suppressor of the T-cell-guided immune responses against the graft, easing donor engraftment.

The B-cell-activating factor (BAFF) is produced by dendritic cells, monocytes, macrophages, and B cells [59]. The engagement with its receptor has been described to induce B-cell proliferation and survival, and its overexpression has been identified in different B-cell malignancies [60–64]. Aptamers that block BAFF-BAFF-R axis have been selected (represented in **figure 2f**). These aptamers were able to block BAFF-dependent proliferation and survival as well as B-cell malignant proliferation [65].

Several aptamers have been described in the last few years aimed to block cytokine signaling. IL-10 is an immunosuppressive cytokine that promotes immunomodulatory responses favoring tumor growth. It has been shown to be elevated in plasma of cancer patients, which can be used as a prognostic factor in cancer progression [66]. The blockade of its receptor IL-10R has been described to inhibit tumor growth in murine tumor models [67, 68]. An IL-10R-blocking aptamer has been isolated (represented in **figure 2g**). This aptamer was optimized by truncation by removing stearic domains, resulting in an increased affinity for its target [69]. The in vivo experiments revealed that it was able in its truncated monomeric form to inhibit tumor growth at comparable levels to those of the mAb. It was further tetramerized, and this multimeric form was able to block IL-10-IL-10R axis in vitro [69]. Further, a human and murine cross-reactive aptamer against IL-10RA has been recently isolated [4].

A very interesting immunotherapeutic strategy is IL-6-IL-6R axis interruption. IL-6 is a proinflammatory cytokine expressed by B and T cells, monocytes, and fibroblasts [70]. Its presence within the tumor microenvironment leads to immunoregulatory responses favoring tumor growth. Two SOMAmers (represented in **Figure 2h**) have been selected that bind IL-6 with high affinity and inhibit IL-6-mediated signaling by blocking its interaction with IL-6R [71]. The in vitro experiments revealed a similar effect to that obtained by the corresponding IL-6 mAb tocilizumab [71]. Furthermore, an RNA aptamer toward IL-6 (represented in **Figure 2h**) has been selected which showed no blocking activity but was able to effectively deliver cargoes to IL-6 expressing cells [14].

Aptamers toward other cytokines, such as IL-4R or tumor necrosis factor alpha (TNF-α), have been selected. A human and murine RNA cross-reactive aptamer was isolated toward IL-4R able to induce apoptosis in myeloid-derived suppressor cells (MDSCs). The IL-4R-mediated signaling in MDSCs and tumor-associated macrophages (TAMs) resulted in an increased number of TILs and reduction of tumor burden in a mammary carcinoma tumor model [72]. Finally, a TNF-α DNA-blocking aptamer has been isolated that is able to inhibit its activity in vitro [73]. These antagonistic and the remaining aptamers used to date in cancer immunotherapy are summarized in **Table 2**.

Type	Function	Target/s	Nature	Specie/s	Treatment/s	Reference
T-cell exhaustion markers	Antagonists	CTLA-4	RNA	Murine	Melanoma	Santulli-Marotto [18]
		PD-1	DNA	Murine	Colon carcinoma	Prodeus [54]
		TIM3	RNA	Murine	Colon carcinoma in combination with PD-1 blockade	Hervas-Stubbs [57]
Cytokines		IL-10R	RNA	Murine	Colon carcinoma	Vicari [68]
			RNA	Human and murine	Not described (*)	Levay [4]
		IL-6	DNA	Human	Glioma and hepatoma (in vitro)	Gupta [14]
		IL-6R	RNA	Human	Not described (*)	Scheller [70]
		IL-4R	RNA	Human and murine	Mammary carcinoma	Meyer [71]
		TNF-α	DNA	Human	Prevention of TNF-α-induced apoptosis (in vitro)	Roth [72]
Immune receptors		CD28	RNA	Murine	Reversion of CD4 T-cell proliferation (in vitro)	Pastor [20]
		CD40	RNA	Murine	B-cell lymphoma	Soldevilla [21]
		BAFF-R	RNA	Human	Mantle cell lymphoma	Kern [64]
	Agonists	CD28	RNA	Murine	B-cell lymphoma	Pastor [20]
		4-1BB	RNA	Murine	Mastocytoma	McNamara [19]
			RNA	Human and murine	Not described (*)	Levay [4]
		OX-40	RNA	Human	CD4 proliferation (in vitro)	Vinay [74]
			RNA	Murine	Melanoma	Dollins [75]
		CD40	RNA	Murine	Aplasia recovery	Soldevilla [21]

Type	Function	Target/s	Nature	Specie/s	Treatment/s	Reference
Bi-specific immune receptor-tumor marker	Tumor-targeted co-stimulation	4-1BB-PSMA	RNA-RNA	Murine	Colon carcinoma	Niu [76]
		4-1BB-VEGF	RNA-RNA	Murine	Breast carcinoma	Pastor [77]
		CD28-MRP1	RNA-RNA	Murine	Chemotherapy-resistant melanoma	Gilboa [78]
	Tumor-targeted ADCC	CD16α-c-Met	RNA-RNA	Human	ADCC of gastric carcinoma (in vitro)	Dean [79]
Increase tumor antigenicity	Immunomodulating aptamer-based approaches	PSMA-NMD	RNA-siRNA	Murine	Colon carcinoma	Pastor [20]
		CD40-NMD	RNA-shRNA	Murine	B-cell lymphoma	Soldevilla [21]
Increase tumor immunogenicity		CTLA-4-STAT-3	RNA-siRNA	Murine	T-cell lymphoma	Kortylewski [80]
		4-1BB-mTORC1	RNA-siRNA	Murine	Melanoma	Herrmann [81]
		CD28-FOXP3	RNA-peptide	Murine	Colon carcinoma	Casares [82]
		DEC205	RNA	Murine	Melanoma	Berezhnoy [3]

Aptamers are divided into categories depending on type and function. Types: T-cell exhaustion markers, cytokines, immune receptors, bi-specific immune receptor-tumor marker, increase tumor antigenicity, and increase of tumor immunogenicity. Functions: antagonists, agonists, tumor-targeted co-stimulation, tumor-targeted ADCC, and immunomodulating aptamer-based approaches.
Are not described as antagonists.

Table 2. Summary of aptamers used in cancer immunotherapy.

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2.2. Agonistic aptamers directed to trigger activating signals

T lymphocytes need at least two signals to be properly activated. The first one comes from the engagement of major histocompatibility complex (MHC) class I in the case of CD8+ and MHC class II in CD4+ cells with the T-cell receptor (TCR) along with CD3. The second signal, well known as co-stimulatory signal, comes mainly from the engagement of CD28 expressed on the surface of T lymphocytes and its ligand B7 expressed on antigen-presenting cells (APCs). Within the tumor microenvironment, lack of co-stimulatory ligands leads to T-cell exhaustion, what turns them into anergic cells unable to trigger an immune response. Thus, the search for agonistic agent has always been of great interest in cancer immunotherapy. The first agonistic aptamer was not described until 2008. It was isolated by conventional SELEX toward murine 4-1BB, which is one of the major co-stimulatory receptors expressed in T lymphocytes. Its ligand 4-1BBL is expressed on the surface of APCs, and their engagement leads to T-cell proliferation [74, 83]. This aptamer was dimerized with the intention of displaying agonistic functions. To that end the agonistic aptamer was generated by adding a complementary 21 nt length to the 3′ end of each monomer using polymerase chain reaction (PCR). After in vitro transcription, monomers were hybridized by pair-wise fashion generating a dimer with a double-stranded linker, which provides a more rigid structure and mirrors the average distance of the two Fv of an IgG (represented on **Figure 3a**). The use of this 4-1BB agonistic RNA aptamer in murine tumor models resulted in inhibition of tumor growth [19]. This work strengthened the idea of using aptamers as novel agents for cancer immunotherapy. Moreover, a human and murine cross-reactive aptamer has been recently published [4]. In this work, they describe a parallel both human- and murine-specific target selection against IL-10RA and 4-1BB followed by identification of common sequences by HTS. This is a "toggle-type" SELEX, which shows a very feasible manner to isolate cross-reactive species aptamers [4].

CD28 is one of the main co-stimulatory receptors with a very important role in immunotherapy. Our research team has generated multimerized CD28 agonistic RNA aptamers able to provide proper CD28 what lead CD4+ and CD8+ proliferation in vitro [20]. Two different dimeric structures were generated to evaluate their effect on T-cell co-stimulation and therefore optimize the strategy. The first dimer was generated as previously described [19] by pair-wise annealing fashion. However, the dimeric structure generated by in vitro transcribing two contiguous monomer units exerted in this case the highest co-stimulatory capacity in CD4+ and CD8+ T lymphocytes [20]. This dimer provides a shorter linker reducing the distance to the minimum and a more flexible structure (represented in **Figure 3b**). This agonistic CD28 aptamer was able to induce both cellular and humoral response in mice in a context of idiotypic vaccination. The use of this agonistic aptamer as adjuvant in an already established idiotypic vaccination protocol to treat B-cell lymphoma resulted in decreased tumor growth and increased survival rate. This aptamer showed a similar effect to that of the mAb [20].

OX-40 is another co-stimulatory receptor upregulated on the surface of CD4+ T cells upon activation, and the engagement with its natural ligand OX-40L expressed on APCs promotes T-cell proliferation, increased cytokine release, and long-term survival [75]. An RNA aptamer toward murine OX-40 was isolated and engineered to exert agonistic functions (represented

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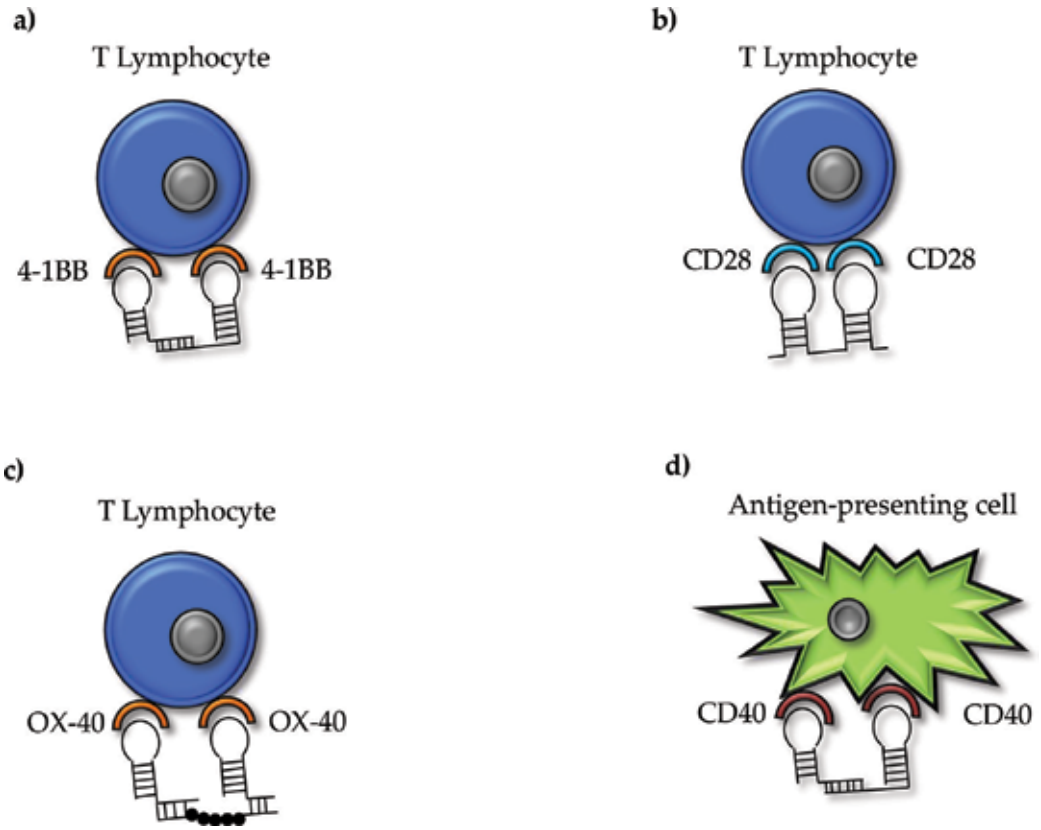

Figure 3. Agonistic aptamers. (a) 4-1BB, (b) CD28, (c) OX-40, and (d) CD40.

in **Figure 3c**) [75]. It was generated as a two-copy scaffold with 18 nt length polycarbon units between the two 3′ aptamer end of the complementary sequences that will anneal by pair-wise fashion [75, 84]. Further, a human OX-40 aptamer has been described which in its dimeric form provided a co-stimulatory effect as demonstrated by cellular proliferation and increased INF-γ production [84].

CD40 is a receptor expressed on the surface of APCs, and its ligand is expressed on T lymphocytes. Their engagement promotes clonal expansion, isotype switching, maturation, proliferation, generation of plasma cells in the case of B cells, and increased antigen presentation on dendritic cells [85, 86]. We have recently published two different agonistic CD40 RNA aptamer-based constructs able to recover bone marrow aplasia while increasing antigen presentation [21]. As mentioned above, in this work, one of the isolated aptamers acted as an antagonist which by simple dimerization was turned into agonist (represented in **Figure 3d**).

2.3. Bi-specific aptamers to target the immune response to the tumor site

The use of agonistic mAbs has been demonstrated to exert severe toxicities as happened with the CD28 superagonistic mAb TGN1412, which resulted in cytokine storm leading a multi-

organ failure forcing the clinical trial to be concluded [47]. Another example is the elevated liver toxicity displayed by the 4-1BB mAb [48]. Moreover, the use of immune-checkpoint blockade mAbs raises the major concern of the appearance of several side effects such as hepatotoxicity, lymphopenia, and thrombocytopenia [76]. Thus, targeting the immune response to the tumor site would reduce the toxicity owed to the off-target effects while increasing the therapeutic index. This approach was published for the first time in 2011 by generating the first bi-specific aptamer, which consisted of both the PSMA and the agonistic 4-1BB aptamers (represented in **Figure 4a**) [77]. This new approach displayed a more potent antitumor immunity at lower doses than that of the corresponding mAbs or the nontargeted co-stimulation. Targeting the immune response to the tumor site is a strategy that requires less amount of reagent as demonstrated in this work, which shows that targeted co-stimulation works as effectively as 10-fold levels of the corresponding controls [77]. A new bi-specific aptamer has been published with the intention of targeting 4-1BB co-stimulation to the vascular endothelial growth factor (VEGF). This new construct consisting of both the VEGF and the agonistic 4-1BB aptamers (represented in **Figure 4b**) showed less toxicity than the rest of the controls while obtaining the same therapeutic effect [87]. In fact, this therapeutic index widening was mirrored in a similar antitumor effect while reducing CD8+ T-cell hyperplasia as well as spleen, lymph node, lung, and liver weights [78, 88]. These described works demonstrate the feasibility of the strategies based on targeting co-stimulation to the tumor site using aptamers.

We have recently published a bi-specific CD28-MRP1 aptamer to target CD28 co-stimulation to cancer stem cells (represented in **Figure 4c**) [89]. The targeted co-stimulation to cancer stem cells which imply chemotherapy resistance would exert a selection pressure on these cells usually responsible for tumor metastasis and tumor relapses [79, 90]. We isolated an aptamer that recognizes multidrug-resistant protein 1 (MRP1) with high affinity, and we used it to generate the

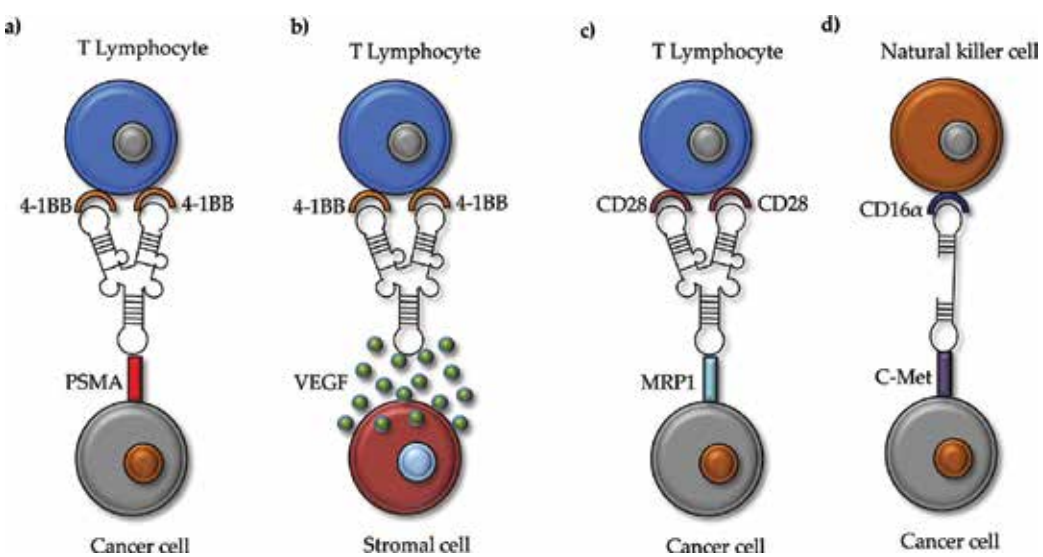

Figure 4. Bi-specific aptamers. (a) 4-1BB-PSMA, (b) 4-1BB-VEGF, (c) CD28-MRP1, and (d) CD16α-c-Met.

CD28-MRP1 bi-specific aptamer together with the already published agonistic CD28 aptamer. This bi-specific aptamer was able to target and properly provide co-stimulation signal to MRP1-overexpressing cells in vitro and in vivo. It was tested in a murine tumor model in the presence of a vaccine (Gvax) and a transient inhibitor peptide of FOXP3 resulting in higher T-lymphocyte tumor infiltration, slower tumor growth, and longer survival [89]. Further, we developed a new vaccination strategy consisting in irradiated MRP1-overexpressing cells coated ex vivo with the CD28-MRP1 bi-specific aptamer termed CD28 Aptvax. CD28 Aptvax exerted delay in MRP1-expressing tumors as well as 50% survival after 50 days of follow-up [89].

Finally, targeted antibody-dependent cell-mediated cytotoxicity (ADCC) can be achieved as well. To that purpose, a DNA aptamer against the Fcγ receptor III (CD16α) was developed to generate a bi-specific aptamer to target ADCC to c-Met-overexpressing tumor cells (represented in **Figure 4d**) [91]. This bi-specific aptamer was tested in both human gastric and lung cancer cell lines resulting in specific c-Met-targeted ADCC [91].

2.4. Aptamer-based approaches to enhance tumor immunogenicity

Despite efforts invested in blocking immunosuppressive signals and activating positive signals, tumor antigenicity is a challenge that remains mostly unsolved. In 2010, a feasible approach was described to increase tumor antigenicity by expressing new tumor antigens. In this work published by Pastor et al. [92], new and therefore more potent antigens are expressed by the tumor triggering a powerful immune response [92, 93]. This approach was based on generating an aptamer-based chimera consisting of the PSMA aptamer and an siRNA for one of the NMD factors. Nonsense-mediated RNA decay (NMD) is a mechanism that controls abnormal transcripts in charge of deleting mRNAs that encode premature termination codons (PTCs). This targeted NMD inhibition resulted in triggering an increased tumor antigenicity leading to a potent immune response in vivo, thereby reducing tumor growth [92]. Moreover, it has been demonstrated that higher lymphocyte infiltration in the tumor is correlated with lower NMD expression, as was shown by the inverse correlation between the accumulation of CD3+ and the expression of the NMD in colorectal cancer with microsatellite instability [94]. Since tumor regression has been demonstrated by the expression of new antigens in the tumor by NMD inhibition [92–94], we decided to apply this strategy to B-cell lymphoma. In this work recently published by our research team, we generated a chimera with the CD40 agonistic aptamer coupled with an shRNA aimed at inhibiting the NMD [21]. In this work, the optimized chimera led to the expression of new powerful antigens, thus triggering an immune response against the tumor. This chimera was able to generate higher lymphocyte infiltration, decreasing tumor growth and increasing mice survival in a B-cell lymphoma tumor model [21].

The expression of new antigens is in some cases insufficient due to the immunosuppressive microenvironment. In fact, the expression of new antigens induces regulatory T-cell (Treg) infiltration indicating that the combination with other aptamer-based strategies would serve to optimize the antitumor immune responses. Signal transducer and activator of transcription 3 (STAT-3)-targeted inhibition can be achieved using Toll-like receptor 9 (TLR9) natural ligands such as CpG. It has been demonstrated that this targeted inhibition triggers a strong antitumor immune response mediated by the activation of tumor-associated immune cells

[80, 95]. In addition, STAT-3 is upregulated in immunosuppressive cells and favors $CD4^+$ Treg expansion. Aptamer-based CTLA-4 delivery strategies have been demonstrated to target both $CD4^+$ Tregs and $CD8^+$ infiltrated lymphocytes [81]. CTLA-4 aptamer-based-targeting delivery of STAT-3 siRNA to T lymphocytes results in inhibition of tumor growth and of metastasis [81]. STAT-3 promotes tumor cell survival and proliferation in tumor cells, as well as invasion and immunosuppression [81]. This work shows an increase of CD8+ T-effector response in vivo thanks to the blockade of CTLA-4 in the first place and subsequently to STAT-3 silencing. STAT-3 inhibition provided a systemic antitumor response leading to inhibition of tumor growth in various cancer cell lines as well as metastasis [81].

mTOR is an intracellular mediator associated with the presence of immune-system shod-living cells [3]. A strategy that demonstrated the agonistic 4-1BB optima coupled with an siRNA for a key factor of the mTOR complex 1(mTORC1) was called raptor [3]. This strategy resulted in mTORC1 downregulation in vitro, and its combination with an already established vaccination protocol promoted a protective immunity in a murine tumor model. This achieved antitumor response showed memory features with cytotoxic effect function [3].

Moreover, an RNA aptamer toward DEC205 has been recently published able to delivery in vitro deliver-specific cargoes for cross-presentation. DEC205 is a surface receptor expressed on CD8+α dendritic cells, which promotes antigen cross-presentation and the subsequent CD8+ activation. The use of this aptamer in vivo displayed strong T-cell-mediated tumor immunity [96].

Our research team has recently shown a new strategy to increase tumor immunogenicity by targeting the inhibition of FOXP3. We generated a CD28 aptamer chimera coupled with the already published FOXP3 transient inhibitory peptide P60 [82, 97]. This peptide is able to penetrate into Tregs and inhibits its function [82]. Due to the absence of specificity of the P60 peptide, we decided to couple it with one of our CD28 described aptamers and therefore target FOXP3 inhibition to CD28-expressing cells. This targeted inhibition counteracted Treg immunosuppression activity while reducing the concentration hundreds of times up to 0.5 mictoM [82, 97]. A very similar antitumor effect in a colon carcinoma tumor model was achieved using 625 pmol of the CD28-P60 chimera compared with 500 nmol of the P60 control [97].

3. Conclusion

Aptamers have gained a large spot among therapeutic agents [5] in several research fields. They have colonized experimental approaches for the treatment of several metabolic and vascular diseases, and their preclinical use in cancer treatments has been widely used. Aptamers can be used to face the three major challenges that immunotherapy poses today [51]. To address the blockade of immunosuppressing signals, aptamers toward CTLA-4, PD1, TIM3, IL-10R, or IL-6 can be used. With the purpose of activating positive signals, agonistic aptamers directed to trigger CD28, 4-1BB, OX40, or CD40 receptors can be utilized. In order to increase tumor immunogenicity, several aptamer-based strategies can be used, such as targeting the NMD inhibition to the tumor, STAT-3-targeted inhibition in TILs, or FOXP3-targeted inhibition in Tregs. Finally, it is to be noted that bi-specific aptamers such as 4-1BB-PSMA, 4-1BB-VEGF, CD28-MRP1, or CD16α-c-MET can be utilized to direct the immune response

to the tumor site. To conclude, despite the youth of this emerging platform, aptamers might feasibly serve as a promising therapeutic tool for cancer immunotherapy.

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References

[1] Ellington AD, Szostak JW. *In vitro* selection of RNA molecules that bind specific ligands. Nature. 1990;346(6287):818–822.

[2] Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990;249(4968):505–510.

[3] Berezhnoy A, Castro I, Levay A, Malek TR, Gilboa E. Aptamer-targeted inhibition of mTOR in T cells enhances antitumor immunity. J Clin Invest. 2014;124(1):188–197.

[4] Levay A, Brenneman R, Hoinka J, Sant D, Cardone M, Trinchieri G, et al. Identifying high-affinity aptamer ligands with defined cross-reactivity using high-throughput guided systematic evolution of ligands by exponential enrichment. Nucleic Acids Res. 2015;43(12):e82.

[5] Keefe AD, Pai S, Ellington A. Aptamers as therapeutics. Nat Rev Drug Discov. 2010;9(7):537–550.

[6] Cerchia L, de Franciscis V. Nucleic acid-based aptamers as promising therapeutics in neoplastic diseases. Methods Mol Biol. 2007;361:187–200.

[7] Mosing RK, Bowser MT. Isolating aptamers using capillary electrophoresis-SELEX (CE-SELEX). Methods Mol Biol. 2009;535:33–43.

[8] Daniels DA, Chen H, Hicke BJ, Swiderek KM, Gold L. A tenascin-C aptamer identified by tumor cell SELEX: systematic evolution of ligands by exponential enrichment. Proc Natl Acad Sci U S A. 2003;100(26):15416–15421.

[9] White R, Rusconi C, Scardino E, Wolberg A, Lawson J, Hoffman M, et al. Generation of species cross-reactive aptamers using "toggle" SELEX. Mol Ther. 2001;4(6):567–573.

[10] Vater A, Jarosch F, Buchner K, Klussmann S. Short bioactive spiegelmers to migraine-associated calcitonin gene-related peptide rapidly identified by a novel approach: tailored-SELEX. Nucleic Acids Res. 2003;31(21):e130.

[11] Oberthur D, Achenbach J, Gabdulkhakov A, Buchner K, Maasch C, Falke S, et al. Crystal structure of a mirror-image L-RNA aptamer (Spiegelmer) in complex with the natural L-protein target CCL2. Nat Commun. 2015;6:6923.

[12] Cooper TA. *In vivo* SELEX in vertebrate cells. Methods Mol Biol. 1999;118:405–417.

[13] Kim S, Shi H, Lee DK, Lis JT. Specific SR protein-dependent splicing substrates identified through genomic SELEX. Nucleic Acids Res. 2003;31(7):1955–1961.

[14] Gupta S, Hirota M, Waugh SM, Murakami I, Suzuki T, Muraguchi M, et al. Chemically modified DNA aptamers bind interleukin-6 with high affinity and inhibit signaling by blocking its interaction with interleukin-6 receptor. J Biol Chem. 2014;289(12):8706–8719.

[15] McNamara JO 2nd, Andrechek ER, Wang Y, Viles KD, Rempel RE, Gilboa E, et al. Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. Nat Biotechnol. 2006;24(8):1005–1015.

[16] Orava EW, Cicmil N, Gariepy J. Delivering cargoes into cancer cells using DNA aptamers targeting internalized surface portals. Biochim Biophys Acta. 2010;1798(12):2190–2200.

[17] Kanwar JR, Roy K, Kanwar RK. Chimeric aptamers in cancer cell-targeted drug delivery. Crit Rev Biochem Mol Biol. 2011;46(6):459–477.

[18] Santulli-Marotto S, Nair SK, Rusconi C, Sullenger B, Gilboa E. Multivalent RNA aptamers that inhibit CTLA-4 and enhance tumor immunity. Cancer Res. 2003;63(21):7483–7489.

[19] McNamara JO, Kolonias D, Pastor F, Mittler RS, Chen L, Giangrande PH, et al. Multivalent 4-1BB binding aptamers costimulate CD8+ T cells and inhibit tumor growth in mice. J Clin Invest. 2008;118(1):376–386.

[20] Pastor F, Soldevilla MM, Villanueva H, Kolonias D, Inoges S, de Cerio AL, et al. CD28 aptamers as powerful immune response modulators. Mol Ther Nucleic Acids. 2013;2:e98.

[21] Soldevilla MM, Villanueva H, Bendandi M, Inoges S, Lopez-Diaz de Cerio A, Pastor F. 2-fluoro-RNA oligonucleotide CD40 targeted aptamers for the control of B lymphoma and bone-marrow aplasia. Biomaterials. 2015;67:274–285.

[22] Gilboa E, McNamara J 2nd, Pastor F. Use of oligonucleotide aptamer ligands to modulate the function of immune receptors. Clin Cancer Res. 2013;19(5):1054–1062.

[23] Oney S, Lam RT, Bompiani KM, Blake CM, Quick G, Heidel JD, et al. Development of universal antidotes to control aptamer activity. Nat Med. 2009;15(10):1224–1228.

[24] Bompiani KM, Woodruff RS, Becker RC, Nimjee SM, Sullenger BA. Antidote control of aptamer therapeutics: the road to a safer class of drug agents. Curr Pharm Biotechnol. 2012;13(10):1924–1934.

[25] Schmidt KS, Borkowski S, Kurreck J, Stephens AW, Bald R, Hecht M, et al. Application of locked nucleic acids to improve aptamer *in vivo* stability and targeting function. Nucleic Acids Res. 2004;32(19):5757–5765.

[26] de Smidt PC, Le Doan T, de Falco S, van Berkel TJ. Association of antisense oligonucleotides with lipoproteins prolongs the plasma half-life and modifies the tissue distribution. Nucleic Acids Res. 1991;19(17):4695–4700.

[27] Lee CH, Lee YJ, Kim JH, Lim JH, Kim JH, Han W, et al. Inhibition of hepatitis C virus (HCV) replication by specific RNA aptamers against HCV NS5B RNA replicase. J Virol. 2013;87(12):7064–7074.

[28] Lee CH, Lee SH, Kim JH, Noh YH, Noh GJ, Lee SW. Pharmacokinetics of a cholesterol-conjugated aptamer against the hepatitis C virus (HCV) NS5B protein. Mol Ther Nucleic Acids. 2015;4:e254.

[29] Da Pieve C, Blackshaw E, Missailidis S, Perkins AC. PEGylation and biodistribution of an anti-MUC1 aptamer in MCF-7 tumor-bearing mice. Bioconjug Chem. 2012;23(7):1377–1381.

[30] Tan L, Neoh KG, Kang ET, Choe WS, Su X. PEGylated anti-MUC1 aptamer-doxorubicin complex for targeted drug delivery to MCF7 breast cancer cells. Macromol Biosci. 2011;11(10):1331–1335.

[31] Rohloff JC, Gelinas AD, Jarvis TC, Ochsner UA, Schneider DJ, Gold L, et al. Nucleic acid ligands with protein-like side chains: modified aptamers and their use as diagnostic and therapeutic agents. Mol Ther Nucleic Acids. 2014;3:e201.

[32] Bunka DH, Stockley PG. Aptamers come of age—at last. Nat Rev Microbiol. 2006;4(8):588–596.

[33] Zhou G, Wilson G, Hebbard L, Duan W, Liddle C, George J, et al. Aptamers: apromising chemical antibody for cancer therapy. Oncotarget. 2016; 7(12):13446–13463.

[34] Ricklin D, Lambris JD. Complement-targeted therapeutics. Nat Biotechnol. 2007;25(11):1265–1275.

[35] Sundaram P, Kurniawan H, Byrne ME, Wower J. Therapeutic RNA aptamers in clinical trials. Eur J Pharm Sci. 2013;48(1–2):259–271.

[36] Gilbert JC, DeFeo-Fraulini T, Hutabarat RM, Horvath CJ, Merlino PG, Marsh HN, et al. First-in-human evaluation of anti von willebrand factor therapeutic aptamer ARC1779 in healthy volunteers. Circulation. 2007;116(23):2678–2686.

[37] Jilma B, Paulinska P, Jilma-Stohlawetz P, Gilbert JC, Hutabarat R, Knobl P. A randomised pilot trial of the anti-von willebrand factor aptamer ARC1779 in patients with type 2b von willebrand disease. Thromb Haemost. 2010;104(3):563–570.

[38] Siegel RL, Miller KD, Jemal A. Cancer Facts & Figures 2015. Atlanta: American Cancer Society; 2015.

[39] Kyi C, Postow MA. Checkpoint blocking antibodies in cancer immunotherapy. FEBS Lett. 2014;588(2):368–376.

[40] No authors listed. Ipilimumab. Drugs R D. 2010;10(12):97–110

[41] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–723.

[42] Lipson EJ, Drake CG. Ipilimumab: an anti-CTLA-4 antibody for metastatic melanoma. Clin Cancer Res. 2011;17(22):6958–6962.

[43] Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364(26):2517–2526.

[44] Raedler LA. Keytruda (pembrolizumab): first PD-1 inhibitor approved for previously treated unresectable or metastatic melanoma. Am Health Drug Benefits. 2015;8(Spec Feature):96–100.

[45] Raedler LA. Opdivo (nivolumab): second PD-1 inhibitor receives FDA approval for unresectable or metastatic melanoma. Am Health Drug Benefits. 2015;8(Spec Feature):180–183.

[46] Melero I, Berman DM, Aznar MA, Korman AJ, Perez Gracia JL, Haanen J. Evolving synergistic combinations of targeted immunotherapies to combat cancer. Nat Rev Cancer. 2015;15(8):457–472.

[47] Attarwala H. TGN1412: from discovery to disaster. J Young Pharm. 2010;2(3):332–336.

[48] Bartkowiak T, Curran MA. 4-1BB agonists: multi-potent potentiators of tumor immunity. Front Oncol. 2015;5:117.

[49] Naidoo J, Page DB, Li BT, Connell LC, Schindler K, Lacouture ME, et al. Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. Ann Oncol. 2015;26(12):2375–2391.

[50] Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. J Clin Oncol. 2015;33(17):1974–1982.

[51] Soldevilla MM, Villanueva H, Pastor F. Aptamers: a feasible technology in cancer immunotherapy. J Immunol Res. 2016;2016:1083738.

[52] Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. 1995;182(2):459–465.

[53] Gros A, Robbins PF, Yao X, Li YF, Turcotte S, Tran E, et al. PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors. J Clin Invest. 2014;124(5):2246–2259.

[54] Prodeus A, Abdul-Wahid A, Fischer NW, Huang EH, Cydzik M, Gariepy J. Targeting the PD-1/PD-L1 immune evasion axis with DNA aptamers as a novel therapeutic strategy for the treatment of disseminated cancers. Mol Ther Nucleic Acids. 2015;4:e237.

[55] Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, et al. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. Nature. 2002;415(6871):536–541.

[56] Sakuishi K, Ngiow SF, Sullivan JM, Teng MW, Kuchroo VK, Smyth MJ, et al. TIM3+FOXP3+ regulatory T cells are tissue-specific promoters of T-cell dysfunction in cancer. Oncoimmunology. 2013;2(4):e23849.

[57] Hervas-Stubbs S, Soldevilla MM, Villanueva H, Manchero U, Bendandi M, Pastor F. Identification of TIM3 2′-fluoro oligonucleotide aptamer by HT-SELEX for cancer immunotherapy. Oncotarget. 2015;7(4):4522–4530.

[58] Rabal O, Pastor F, Villanueva H, Soldevilla MM, Hervas-Stubbs S, Oyarzabal J. In silico aptamer docking studies: from a retrospective validation to a prospective case study-TIM3aptamers binding. Mol Ther Nucleic Acids. 2016;5(10):e376. doi: 10.1038/mtna.2016.84.

[59] Craxton A, Magaletti D, Ryan EJ, Clark EA. Macrophage- and dendritic cell—dependent regulation of human B-cell proliferation requires the TNF family ligand BAFF. Blood. 2003;101(11):4464–4471.

[60] Shulga-Morskaya S, Dobles M, Walsh ME, Ng LG, MacKay F, Rao SP, et al. B cell-activating factor belonging to the TNF family acts through separate receptors to support B cell survival and T cell-independent antibody formation. J Immunol. 2004;173(4):2331–2341.

[61] Batten M, Groom J, Cachero TG, Qian F, Schneider P, Tschopp J, et al. BAFF mediates survival of peripheral immature B lymphocytes. J Exp Med. 2000;192(10):1453–1466.

[62] He B, Chadburn A, Jou E, Schattner EJ, Knowles DM, Cerutti A. Lymphoma B cells evade apoptosis through the TNF family members BAFF/BLyS and APRIL. J Immunol. 2004;172(5):3268–3279.

[63] Novak AJ, Grote DM, Stenson M, Ziesmer SC, Witzig TE, Habermann TM, et al. Expression of BLyS and its receptors in B-cell non-Hodgkin lymphoma: correlation with disease activity and patient outcome. Blood. 2004;104(8):2247–2253.

[64] Kern C, Cornuel JF, Billard C, Tang R, Rouillard D, Stenou V, et al. Involvement of BAFF and APRIL in the resistance to apoptosis of B-CLL through an autocrine pathway. Blood. 2004;103(2):679–688.

[65] Zhou J, Tiemann K, Chomchan P, Alluin J, Swiderski P, Burnett J, et al. Dual functional BAFF receptor aptamers inhibit ligand-induced proliferation and deliver siRNAs to NHL cells. Nucleic Acids Res. 2013;41(7):4266–4283.

[66] Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001;19:683–765.

[67] Guiducci C, Vicari AP, Sangaletti S, Trinchieri G, Colombo MP. Redirecting *in vivo* elicited tumor infiltrating macrophages and dendritic cells towards tumor rejection. Cancer Res. 2005;65(8):3437–3446.

[68] Vicari AP, Chiodoni C, Vaure C, Ait-Yahia S, Dercamp C, Matsos F, et al. Reversal of tumor-induced dendritic cell paralysis by CpG immunostimulatory oligonucleotide and anti-interleukin 10 receptor antibody. J Exp Med. 2002;196(4):541–549.

[69] Berezhnoy A, Stewart CA, Mcnamara JO 2nd, Thiel W, Giangrande P, Trinchieri G, et al. Isolation and optimization of murine IL-10 receptor blocking oligonucleotide aptamers using high-throughput sequencing. Mol Ther. 2012;20(6):1242–1250.

[70] Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta. 2011;1813(5):878–888.

[71] Meyer C, Eydeler K, Magbanua E, Zivkovic T, Piganeau N, Lorenzen I, et al. Interleukin-6 receptor specific RNA aptamers for cargo delivery into target cells. RNA Biol. 2012;9(1):67–80.

[72] Roth F, De La Fuente AC, Vella JL, Zoso A, Inverardi L, Serafini P. Aptamer-mediated blockade of IL4Ralpha triggers apoptosis of MDSCs and limits tumor progression. Cancer Res. 2012;72(6):1373–1383.

[73] Orava EW, Jarvik N, Shek YL, Sidhu SS, Gariepy J. A short DNA aptamer that recognizes TNF alpha and blocks its activity *in vitro*. ACS Chem Biol. 2013;8(1):170–178.

[74] Vinay DS, Kwon BS. Role of 4-1BB in immune responses. Semin Immunol. 1998;10(6):481–489.

[75] Dollins CM, Nair S, Boczkowski D, Lee J, Layzer JM, Gilboa E, et al. Assembling OX40 aptamers on a molecular scaffold to create a receptor-activating aptamer. Chem Biol. 2008;15(7):675–682.

[76] Niu L, Strahotin S, Hewes B, Zhang B, Zhang Y, Archer D, et al. Cytokine-mediated disruption of lymphocyte trafficking, hemopoiesis, and induction of lymphopenia, anemia, and thrombocytopenia in anti-CD137-treated mice. J Immunol. 2007;178(7):4194–4213.

[77] Pastor F, Kolonias D, McNamara JO 2nd, Gilboa E. Targeting 4-1BB costimulation to disseminated tumor lesions with bi-specific oligonucleotide aptamers. Mol Ther. 2011;19(10):1878–1886.

[78] Gilboa E, Berezhnoy A, Schrand B. Reducing toxicity of immune therapy using aptamer-targeted drug delivery. Cancer Immunol Res. 2015;3(11):1195–1200.

[79] Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer. 2005;5(4):275–284.

[80] Kortylewski M, Swiderski P, Herrmann A, Wang L, Kowolik C, Kujawski M, et al. *In vivo* delivery of siRNA to immune cells by conjugation to a TLR9 agonist enhances antitumor immune responses. Nat Biotechnol. 2009;27(10):925–932.

[81] Herrmann A, Priceman SJ, Swiderski P, Kujawski M, Xin H, Cherryholmes GA, et al. CTLA4 aptamer delivers STAT3 siRNA to tumor-associated and malignant T cells. J Clin Invest. 2014;124(7):2977–2987.

[82] Casares N, Rudilla F, Arribillaga L, Llopiz D, Riezu-Boj JI, Lozano T, et al. A peptide inhibitor of FOXP3 impairs regulatory T cell activity and improves vaccine efficacy in mice. J Immunol. 2010;185(9):5150–5159.

[83] Sica G, Chen L. Modulation of the immune response through 4-1BB. Adv Exp Med Biol. 2000;465:355–362.

[84] Pratico ED, Sullenger BA, Nair SK. Identification and characterization of an agonistic aptamer against the T cell costimulatory receptor, OX40. Nucleic Acid Ther. 2013;23(1):35–43.

[85] Foy TM, Laman JD, Ledbetter JA, Aruffo A, Claassen E, Noelle RJ. gp39-CD40 interactions are essential for germinal center formation and the development of B cell memory. J Exp Med. 1994;180(1):157–163.

[86] Arpin C, Dechanet J, Van Kooten C, Merville P, Grouard G, Briere F, et al. Generation of memory B cells and plasma cells *in vitro*. Science. 1995;268(5211):720–722.

[87] Schrand B, Berezhnoy A, Brenneman R, Williams A, Levay A, Kong LY, et al. Targeting 4-1BB costimulation to the tumor stroma with bispecific aptamer conjugates enhances the therapeutic index of tumor immunotherapy. Cancer Immunol Res. 2014;2(9):867–877.

[88] Schrand B, Berezhnoy A, Brenneman R, Williams A, Levay A, Gilboa E. Reducing toxicity of 4-1BB costimulation: targeting 4-1BB ligands to the tumor stroma with bi-specific aptamer conjugates. Oncoimmunology. 2015;4(3):e970918.

[89] Soldevilla MM, Villanueva H, Casares N, Lasarte JJ, Bendandi M, Inoges S, et al. MRP1-CD28 bi-specific oligonucleotide aptamers: target costimulation to drug-resistant melanoma cancer stem cells. Oncotarget 2016;7(17):23182–23196.

[90] Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer. 2002;2(6):442–454.

[91] Boltz A, Piater B, Toleikis L, Guenther R, Kolmar H, Hock B. Bi-specific aptamers mediating tumor cell lysis. J Biol Chem. 2011;286(24):21896–21905.

[92] Pastor F, Kolonias D, Giangrande PH, Gilboa E. Induction of tumour immunity by targeted inhibition of nonsense-mediated mRNA decay. Nature. 2010;465(7295):227–230.

[93] Holbrook JA, Neu-Yilik G, Hentze MW, Kulozik AE. Nonsense-mediated decay approaches the clinic. Nat Genet. 2004;36(8):801–808.

[94] El-Bchiri J, Guilloux A, Dartigues P, Loire E, Mercier D, Buhard O, et al. Nonsense-mediated mRNA decay impacts MSI-driven carcinogenesis and anti-tumor immunity in colorectal cancers. PLoS One. 2008;3(7):e2583.

[95] Kortylewski M, Kujawski M, Herrmann A, Yang C, Wang L, Liu Y, et al. Toll-like receptor 9 activation of signal transducer and activator of transcription 3 constrains its agonist-based immunotherapy. Cancer Res. 2009;69(6):2497–2505.

[96] Wengerter BC, Katakowski JA, Rosenberg JM, Park CG, Almo SC, Palliser D, et al. Aptamer-targeted antigen delivery. Mol Ther. 2014;22(7):1375–1387.

[97] Lozano T, Soldevilla MM, Casares N, Villanueva H, Bendandi M, Lasarte JJ, et al. Targeting inhibition of Foxp3 by a CD28 2′-Fluro oligonucleotide aptamer conjugated to P60-peptide enhances active cancer immunotherapy. Biomaterials. 2016;91:73–80.

Chapter 8

Antigen-Presenting Cell/Tumour Cell Hybrid Vaccines in Cancer Immunotherapy

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Additional information is available at the end of the chapter

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Abstract

In recent years, there has been a considerable interest in the development of immunotherapeutic approaches for treating cancers, including strategies for inducing antigen-specific cytotoxic T cells (CTLs) capable of killing tumour cells *in situ*. These approaches include both the active induction of CTLs by vaccination of tumour bearing patients, and the *ex vivo* expansion of tumour-specific CTLs for adoptive cellular transfer. One promising approach has been through the generation of hybrid cells, formed by fusion of professional antigen presenting cells (pAPCs) with tumour cells expressing relevant tumour associated antigens. Dendritic cells (DCs) represent the most potent form of pAPCs, and have been widely used in the generation of APC/tumour cell hybrid vaccines, in the context of a range of tumour types. Studies of fusion cell vaccines in animals have demonstrated not only the induction of tumour-specific CTLs, but also protection against subsequent tumour challenge and regression of established tumours. Results of clinical trials in patients have been less dramatic, but have shown the ability of hybrid vaccines to induce tumour-specific T cell responses, in some instances associated with disease stabilization or tumour regression. In addition to dendritic cell fusion vaccines, a number of non-DC fusion vaccines have been described.

Keywords: antigen-presenting cell, cancer, tumour, hybrid, fusion, vaccine

1. Introduction

Recently, there has been significant interest in the development of immunotherapeutic approaches for cancer management. This has been strengthened by the approval of the first therapeutic dendritic cell-based vaccine explicitly prepared for the management of cancer by the Food and Drug Administration (FDA) in 2010 [1]. Many experimental cancer immunotherapy

studies depend on the use of professional antigen-presenting cells (pAPCs), such as dendritic cells, as inducers of tumour-specific immune responses, in particular for inducing tumour antigen-specific cytotoxic T-lymphocytes (CTLs) capable of targeting and killing tumour cells. One such strategy has been the development of APC/tumour fusion cells as candidate cancer vaccines. The approach was first described by Guo and colleagues [2], who showed that a vaccine made by fusion of hepatoma cells and activated B-cells protected rats against subsequent tumour challenge, and induced rejection of established tumours, by a mechanism that was mediated by CD4+ and CD8+ T-cells. In this chapter, we shall review the status, prospects and limitations of APC/tumour cell fusion vaccines for immunotherapy of cancer.

2. The concept of APC/tumour cell hybrids

The idea behind APC/tumour cell fusion hybrids as immunotherapeutic agents is relatively straightforward (**Figure 1**). Tumour cells express mutated proteins or overexpress proteins that the immune system recognizes as antigenic, and which differentiate them from normal somatic cells. However, they fail to present these to the host immune system in a way that elicits an effective anti-tumour immune response. In addition, many tumours evade immune responses [3] by a number of mechanisms, including downregulation of antigen processing, reduced or failure to express major histocompatibility complex (MHC) molecules, and failure to express co-stimulatory molecules. By contrast, professional APCs are potent inducers of antigen-specific T-cell responses, due to a high level of expression of MHC class I and MHC class II molecules, efficient antigen processing and expression of T-cell co-stimulatory molecules. *In vitro* fusion of tumour cells and professional APCs produces hybrid cells that express tumour-associated antigens (TAAs), and process and present them in a way that induces tumour-specific immunity (**Figure 1**). Fusions of tumour cells and APCs therefore represent potential agents for cancer immunotherapy, as they express multiple tumour antigens, process and present them to CD4+ and CD8+ T-cells, and provide effective T-cell co-stimulation [4].

3. Sources of tumour antigens—why use whole tumour cells

The first step in developing a tumour vaccine is to provide a source of tumour-specific antigens. There are a variety of tumour antigen sources, including peptides, exosomes, dead or dying tumour cells, recombinant viruses, DNA or RNA transfection or whole tumour cells. The latter represents an effective way for pAPC to present the entire range of antigens expressed in a given tumour, stimulating anti-tumour responses against a broad array of antigens, including mutations relevant to the oncogenic process [5]. Dendritic cells (DCs) represent the most potent form of pAPCs, and DCs pulsed with whole tumour cells or their derivatives have been used in clinical trials of cancer immunotherapy. Unlike vaccines using known tumour-associated peptides or antigens, whole tumour cell-derived vaccines may also present undefined tumour-specific antigens, extending the range of potential targets for the

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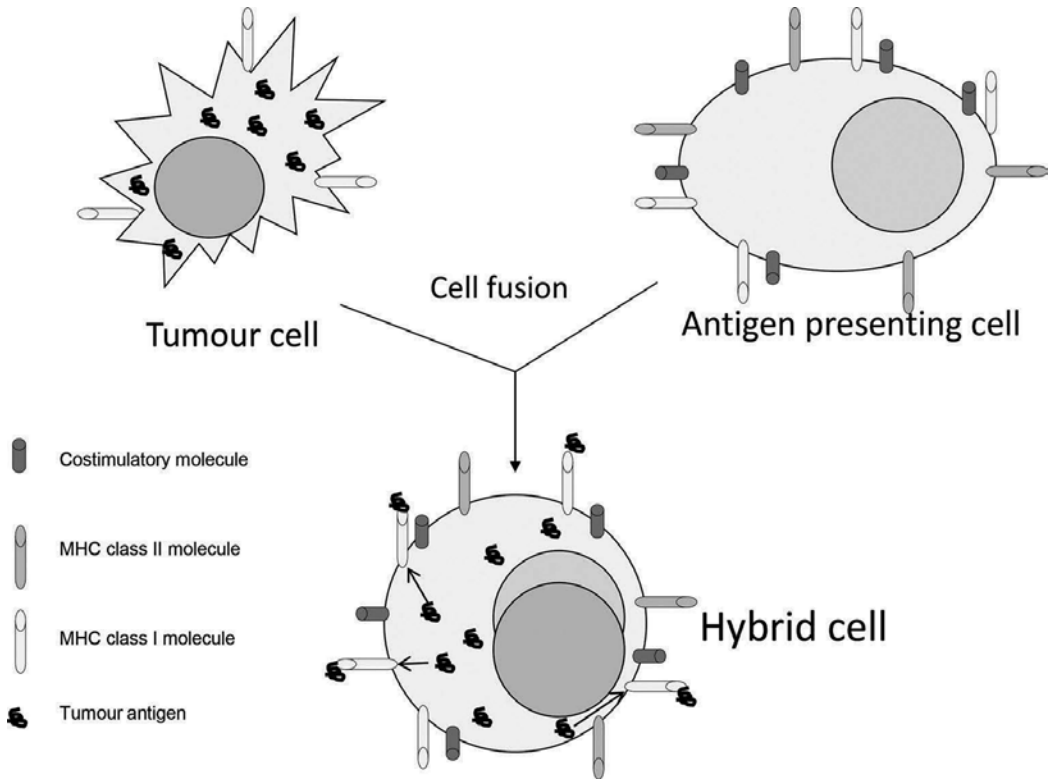

Figure 1. Principle of APC/tumour cell hybridization. (Figure adapted with permission from Ref. [4].).

immune system, resulting in the polyvalent stimulation of both CD8+ CTLs and CD4+ T-cells against a range of tumour antigens. Thus, by using whole tumour cells as a source of tumour antigens, a multi-antigenic response will be produced, and the probability of tumour escape *via* loss of antigens should be reduced.

4. Choice of pAPCs—the role of dendritic cells

Dendritic cells are the most potent antigen-presenting cells for naive T-cell activation. To understand the therapeutic use of DC vaccination strategies, it is important to understand the biology of DCs and how they regulate the innate and the adaptive immune systems—particularly in the context of the tumour microenvironment [6].

DCs are bone marrow-derived cells, which are found in a resting or immature state in non-lymphoid tissues, where they capture antigens. Stimulation of the immature DCs with a range of factors, including microbial products, inflammatory cytokines or cognate receptor-ligand interactions, induces the DCs to undergo maturation, resulting in increased antigen presentation, increased expression of MHC and co-stimulatory molecules, and migration to

secondary lymphoid organs, where they present the antigens to naive, antigen-specific T-cells [7]. DCs present the captured antigen to the T-cells in the form of peptide bound to self-MHC molecules in lymphoid tissues. DCs are the most potent type of pAPC, and can elicit immune responses even where very low numbers of antigen-specific T-cells are present. In mice and humans, there are two major subsets of DCs: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). The majority of studies of cancer immunotherapy have focussed on the use of mDCs.

DCs capture antigens in the periphery by a variety of mechanisms. The DCs then migrate into the lymph nodes (LNs), whilst processing the protein antigens into peptides that bind to MHC class I and MHC class II molecules. Antigens can also reach DCs resident in the lymphoid tissues through the lymphatic system [8].

On interaction with DCs, naive CD4+ and CD8+ T-cells (expressing appropriate T-cell receptors, with specificity for the peptide-MHC complex presented by the DCs) are activated to differentiate into effector T-cells with a variety of functions. Depending on additional signals that they receive, CD4+ T-cells can differentiate into helper T-cells with different patterns of cytokine release (TH1, TH2, TH9 or TH17 cells), or into T-follicular helper (TFH) cells that help B-cells to differentiate into antibody-secreting cells, or regulatory T (T_{Reg})-cells that have suppressive effects on the functions of other lymphocytes. Naïve, antigen-specific CD8+ T-cells differentiate into effector cytotoxic T-lymphocytes on activation. The nature of the T-cell response produced is dependent at least in part on the subset and differentiation status of DCs presenting the antigen [9].

DCs also play a role in controlling antibody responses. They do so by interacting both directly with B-cells and indirectly by activating cytokine-releasing CD4+ helper T-cells. The mechanism of direct presentation of (unprocessed) antigens by DCs to B-cells is incompletely understood [8]. Through these properties of DCs, activating both T-cell and B-cell arms of the immune response, DCs and their derivatives represent ideal candidates for cancer vaccines [9].

5. DCs in the tumour microenvironment

DCs are found in most tumours in humans and mice. Tumours, however, can avert antigen presentation and the establishment of tumour-specific immune responses through a variety of mechanisms, causing an imbalance between immunity and tolerance [10]. By switching the differentiation of monocytes to macrophages, rather than DCs (through the interplay of interleukin (IL)-6 and macrophage colony-stimulating factor; [11]), tumours can prevent the induction of tumour-specific T-cell responses. In addition, tumour glycoproteins such as carcinoembryonic antigen (CEA) and mucin 1 (MUC1) are endocytosed by DCs into early endosomes, bypassing the normal pathway of processing and presentation of antigens to T-cells [12]. Tumours also interfere with DC maturation. Firstly, they can inhibit DC maturation through the secretion of IL 10 [13], leading to antigen-specific anergy. Secondly, tumour-derived factors may subvert the normal maturation of mDCs, in ways that lead to the promotion of tumour growth ('pro-tumour' DCs). For example, tumour-derived TSLP induces OX40 ligand expression by DCs, which supports the differentiation of CD4+ T-cells

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into TH2 cells, and promotes tumour development through the secretion of IL 4 and IL 13, inhibiting tumour cell apoptosis and stimulating tumour-associated macrophages to secrete factors that promote tumour growth, such as epidermal growth factor (EGF) [14, 15].

Thus, DCs can have direct pro-tumour effects by promoting the survival and progression of tumour cells in a variety of ways [16]. The complex interactions between DCs and the tumour microenvironment can lead to the dysfunction of endogenous DCs in cancer-bearing patients. However, culture conditions have been defined by which DCs can be differentiated *in vitro* to optimize their APC functions, allowing such cells to be used effectively as cancer-immunotherapeutic agents.

6. *In vitro* differentiation of DCs for use as vaccines

The goal of cancer immunotherapy is to elicit tumour-specific CD8+ T-cell-mediated immune responses that will be sufficiently robust and long-lasting to generate durable tumour regression and/or eradication. The application of *ex vivo*-educated DCs emerged in an effort to avoid possible interferences in therapeutic efficacy due to the dysfunction of endogenous DCs commonly observed in cancer patients [17]. *Ex vivo* DCs are mainly generated through *in vitro* differentiation of peripheral blood mononuclear cells (PBMCs) in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 or IL-13 [18]. DC-based vaccines should present a 'mature' phenotype in order to activate an antigen-specific immune response upon T-cell encounter. This differentiated state is characterized by the expression of several co-stimulatory molecules (such as CD80 and CD86, CD40, CD70, or inducible T-cell co-stimulator ligand), the necessary activating 'second signals' in the immunological synapse [19]. Mature DCs also have high levels of expression of the antigen-presenting molecules, MHC class I and MHC class II (and CD1 for presentation of lipid antigens). In addition, a third signal is required to trigger an efficient $CD8^+$ T-cell response, which is the presence of an immunostimulatory cytokine profile [17]. This process is accompanied by an augmented chemokine-driven migratory capacity, with increased chemokine receptor 7 (CCR7) expression, which favours lymph node homing and T-cell encounter, and allows antigen presentation and T-cell activation [20]. This complex context has required the exploration of various strategies. A 'standard' maturation cocktail, composed of tumour necrosis factor (TNF)-α, IL-1β, IL-6, and prostaglandin E2 [21], has been extensively used to develop conventional DCs. This 'standard' mature DCs acquire an activated phenotype, respond to LN-homing signals and secrete moderate amounts of T helper (Th)1 cytokine IL-12p70, but with low immunoregulatory cytokine production [21]. Targeting the innate danger signal pathway of Toll-like receptors (TLRs) improved migration, cytokine profiles and immune responses [22]. Alternative approaches use type-1 polarized DCs, generated in the presence of interferon (IFN)-γ, which show a mature state with IL-12 release, chemotactical response to the LN-homing chemokine CCL19 and generate Antigen-specific T-effector cells [23]. Alternative strategies for the production of 'clinical grade' DCs include '*Fast* DCs', which are generated in a 3-day culture, show similar performance [24, 25], and DCs derived from CD34+ blood progenitor cells [26]. Taken together, considerable progress has been made over the years in

generating DCs suitable for use as cancer-immunotherapeutic agents, although the potential impact of *ex vivo*-generated DCs on immunotherapy requires additional studies to be fully understood.

7. DC/tumour cell fusion approaches

Several approaches have been used to generate APC/tumour cell hybrids, including electroporation, chemical fusion using polyethylene glycol or viral fusion. Fusion efficiencies can vary greatly. Other limitations of DC/tumour cell hybrids include a lack of replicative capacity of the fusion cells, and poor standardization of the resulting fusion products [27]. Methods of separating heterokaryons of the APC and tumour cells from unfused cells and cellular debris are an important consideration following the fusion. In many studies describing DC/tumour fusion vaccines, no definitive evidence of heterokaryonic fusion cell formation was given, and the effects described cannot, therefore, be directly ascribed to the hybrid cells themselves. Corroboration of this conclusion comes from reports that fusion hybrids generated from autologous (syngeneic) and allogeneic DCs displayed equivalent immunological function and therapeutic effects *in vitro* and *in vivo*. This suggests that at least part of the therapeutic effect of the DC/tumour fusion vaccines in these studies may depend on tumour antigen scavenging and presentation by antigen-presenting cells of host origin within the vaccine preparation. In support of this, a recent study showed that the presence of unfused (syngeneic) DCs in the vaccine preparation enhanced the immunogenicity of the vaccine, possibly by a combination of uptake and processing of necrotic tumour cells by the DCs and their differentiation to mature DCs following the electrofusion process [28].

8. Dendritic cell/tumour fusion hybrids and their utility in cancer immunotherapy

DC/tumour hybrid fusion cells may be more effective in cancer immunotherapy than other DC-based vaccine approaches. DC-tumour cell fusion potentially confers not only the DCs' professional APC capacity but also the endogenous expression of a range of TAAs for processing and MHC-restricted T-cell sensitization. Many investigators have shown, in animal models, that vaccination with DC/tumour fusion hybrids protected against challenge with the relevant tumour and mediated the regression of established tumours of a wide range of tumour types, including renal, colon, lung, breast, hepatic and cervical carcinomas, melanoma, sarcoma, neurological and haematological tumours [29–35]. In addition, studies in tumour-prone mouse strains vaccinated with fusion cell vaccines showed protection against, or delay in the development of, tumours [36–38]. Both syngeneic and allogeneic DCs were shown to be effective as APCs for fusion hybrids for vaccination, and the mechanisms of protective immunity induced by DC/tumour fusion vaccines depended on their ability to induce both CD4+ and CD8+ T-cells, with CD8+ antigen-specific CTLs representing the major mediators of tumour rejection [30, 31, 33, 34, 37].

Appropriate antigen loading is a crucial parameter for optimizing the efficacy of anti-tumour immunotherapy. Using a murine colon cancer model, Yasuda and his colleagues evaluated

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the anti-tumour efficacy of four different preparations of DC vaccines, including DCs pulsed with tumour lysate, DCs pulsed with necrotic tumour cells, DCs pulsed with apoptotic tumour cells and DC/tumour fusion hybrid cells. Their results showed that DC/tumour cell fusion hybrids and DCs pulsed with apoptotic tumour cells induced stronger anti-tumour protection than DCs pulsed with necrotic tumour cells, whilst vaccination of DCs pulsed with tumour lysate failed to elicit any anti-tumour effect [35]. DC/tumour fusion hybrid cells induced the most effective anti-tumour response in animals receiving higher doses of tumour-cell challenge. DC/tumour cell fusion hybrids also induced the strongest cytotoxic T-lymphocyte activity and *in vitro* production of IFN-gamma of the preparations tested. These results suggest that DC/tumour fusion hybrids are stronger stimulators of protective immunity against solid tumours than other antigen-loading strategies using whole tumour cell materials [35]. Furthermore, DC/tumour cell fusion hybrids have been shown to demonstrate superior efficacy for the treatment of murine tumour models than other DC-based vaccination strategies in other studies [39–43]. Parameters that may require further adjustment to maximize the anti-tumour effect of DC/tumour cell fusion hybrids include the DC maturation state, fusion efficiency between DC and tumour cells, and the use of appropriate adjuvants.

In clinical trials for patients with a variety of metastatic diseases, fusion hybrid vaccines were well tolerated, but the overall objective response rate was less might have been expected from the animal studies. For example, in a study of DC/tumour cell hybrid vaccination in patients with stage III/IV melanoma, Trefzer et al. reported 1 complete clinical remission, 1 partial response and 6 cases of disease stabilization in 17 patients studied, with 11 of 14 patients analysed demonstrating T-cell responses to tumour-associated T-cell epitopes [44, 45]. Similarly, in a study of 21 renal cell cancer patients vaccinated with autologous tumour/allogeneic dendritic cell fusions, 2 showed partial clinical responses and 8 showed disease stabilization [46]. In this study, of the 21 patients included, 10 showed increased anti-tumour immune responses in response to the vaccine, with increased CD4 and/or CD8 T-cell expression of interferon-gamma on stimulation of cells with tumour cell lysate [46]. Avigan showed disease regression in 2 patients with breast cancer, and disease stabilization in 6 more of the vaccinated patients, in a study of 23 patients with breast or renal cancer, vaccinated with autologous DC/tumour cell fusions [47]. Finally, in 17 patients with multiple myeloma, immunized with autologous DC/tumour cell fusion vaccines, T-cell responses to autologous tumour cells was seen in 11 patients, with disease stabilization seen in the majority of evaluable patients [48]. Although the clinical responses seen in these phase I/II clinical trials have been less dramatic than the responses seen in the animal studies, the vaccines have proved to be safe, and larger, placebo-controlled studies are needed to demonstrate whether these DC/tumour cell vaccines offer significant therapeutic benefit.

9. Future cancer regimens using DC/tumour fusion cells

Effective and selective targeted therapies with little toxicity are urgently needed for patients with advanced cancer. Treatment of cancer patients with DC/tumour fusion cells alone may be limited by the induction of immunosuppressive mechanisms. DC/tumour fusion cells can induce not only antigen-specific CTLs but also Tregs, which may counteract their

therapeutic effects [49]. Some chemotherapeutic agents, such as cyclophosphamide and gemcitabine, can activate anti-tumour immunity by depleting Tregs and myeloid-derived suppresser cells (MDSCs) [50], leading to improved clinical outcomes. Recent reports have shown that CTLs induced by vaccination may express the marker programmed death 1 (PD1, and that its ligand, PD-L1, is upregulated in tumour cells by IFN-γ produced by activated CTLs) [51]. The interaction of PD1 (on the CTL) and PD-L1 (on the tumour) leads to impaired CTL function. In a recent preclinical study, it was shown that the use of an anti-PD1 antibody was associated with enhanced CTL activity, and decreased Tregs [52]. Moreover, inactivation of CD4+CD25+Foxp3+ Tregs by an anti-CD25 antibody following DC/tumour fusion cell vaccination significantly improved anti-tumour immunity in a murine model [53]. Therefore, the inhibition of immune checkpoint blockade may enhance CTL activity and reduce induction of T-cell anergy in DC cancer vaccination strategies, and a therapeutic regimen combining DC/tumour fusion hybrid cells, chemotherapy, Treg depletion and/or antibody blockade of PD1-PD-L1 signalling may have potential in advanced cancer patients [54]. Further work will be required to identify which combinations of such strategies will provide optimum benefit, and in which patients and tumour types [55].

10. Fusion of DCs and cancer stem cells

It is well accepted that cancer stem cells (CSCs) are resistant to standard therapies, such as chemotherapy and irradiation [56]. Therefore, small populations of chemoresistant CSCs may result in tumour relapse and growth, following conventional cancer therapies [57]. Importantly, chemoresistant CSCs preferentially express stem cell markers, including OCT3/4, ABCG2, nestin, SOX2, Bmi-1, Notch-1, CD44, CD133 and CD177 [56]. CSCs also overexpress a range of known tumour-associated antigens, such as survivin, MUC1, hTERT, HER2, CERP55, COA-1 and WT1 [58]. In addition, MUC1 expression is upregulated in chemoresistant CSCs which are efficiently lysed by MUC1-specific CTLs in mice [59].

Thus, CSCs remain potential targets for cancer vaccines, and the success of cancer vaccines may at least partly depend on the efficient induction of anti-CSC immunity. Fusions of DCs with pancreatic tumour cells with CSC characteristics were shown to process and present multiple endogenous CSC-specific antigenic peptides on MHC class I and II molecules, and to induce CSC-specific CTL responses [57]. Moreover, fusions of DCs and both CD133+ and CD133- glioma tumour cells were equally effective at inducing cytotoxic anti-tumour immunity against autologous glioma cells [60]. These data suggest that fusion cells generated with DCs and CSCs (DC/CSC-FCs) can process and present CSC antigens, and induce CSC-specific CTLs, without the need to identify CSC-specific antigens. Therefore, DC/CSC fusion cell vaccines may provide an approach capable of eliminating residual, chemoresistant CSCs that would otherwise result in disease relapse following conventional cancer therapies.

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11. Other APC/tumour cell hybrids

Most studies of hybrid cell vaccines have used autologous or allogeneic DCs as the APC to fuse with tumour cells. However, non-DC APCs have also been used to generate hybrid cell vaccines. As mentioned above, Guo et al. used activated B-cells as APCs in their study of fusion cell vaccines against hepatocellular cancer in rats [2]. Several phase I clinical trials have been reported using non-DC APCs as fusion cell vaccine partners, including activated autologous B-cells, and activated allogeneic peripheral blood lymphocytes [61, 62]. As with the clinical trials using DC/tumour fusion cell vaccines, clinical or immunological responses were reported in individual patients, and the approaches were safe with minimal toxicity.

11.1. The use of EBV B-lymphoblastoid cells as pAPC

It is important that the hybrids express multiple tumour antigens in the context of MHC class I and/or class II molecules as well as co-stimulatory molecules essential for T-cell activation, and that careful characterization of the vaccine cell lines should be carried out prior to their use as immunotherapeutic agents. To address the limitations of poor standardization and replicative capacity of DC/tumour cell fusion hybrids, we have used an EBV B-lymphoblastoid cell line (B-LCL) as APCs in generating APC/tumour hybrid cell lines. EBV B-LCLs show many of the characteristic features of professional APCs, including high levels of expression of MHC class I and class II molecules, and important T-cell co-stimulatory molecules, such as CD80, CD86 and CD40 [63], and are immortalized for growth in cell culture. They therefore represent an attractive alternative to DCs as the APC partner in APC/tumour hybrid vaccine cells. The LCL that we have used (HMy2; [64]) has been modified to allow for double chemical selection of the fusion cells, facilitating the selection of stable, self-replicating LCL/tumour hybrid cell lines following fusion. Fusion of HMY2 with a range of haematological tumour cells and cell lines resulted in hybrid cell lines that expressed high levels of MHC class I and class II molecules, as well as relevant T-cell co-stimulatory molecules [27, 63, 65, 66]. Interestingly, these hybrid cell lines expressed TAAs not only associated with haematological malignancies, including TAAs that are commonly expressed in solid tumour cells, and widely expressed tumour antigens such as hTERT and survivin [27, 63, 65, 66; Khalaf et al., unpublished]. Stimulation of peripheral blood T-cells from both healthy donors and tumour-bearing patients *in vitro* using LCL/tumour hybrid cell lines induced tumour antigen-specific CTLs that secreted interferon-gamma and killed tumour cells presenting the relevant antigen(s), demonstrating the potential of these hybrid cell lines to induce tumour-specific immune responses in humans, *in vitro* at least [27, 66, 67; Khalaf et al., unpublished]. An important feature of the HMy2/tumour cell fusion system is that it produces stable hybrid cell lines, which proliferate spontaneously in tissue culture. This means that detailed phenotypic and antigenic characterization of the hybrid cells can be carried out, and that large numbers of standardized cells can be produced. So far, however, there have been no clinical trials of LCL/tumour hybrid cell vaccines.

12. Adoptive immunotherapy

An alternative use of APC/tumour fusion cells in cancer immunotherapy is as *in vitro* stimulators of tumour-specific CTLs for adoptive cellular immunotherapy [27, 48, 68]. Adoptive CTL therapy has been used with clinical benefit in a range of malignancies, including haematological and non-haematological tumours [69–71]. As outlined above, we have shown that LCL/tumour hybrid cell lines can induce tumour antigen-specific CTLs in PBMCs from both healthy individuals and tumour-bearing patients [27, 66, 67]. Given some of the current uncertainties of APC/tumour cells as therapeutic cancer vaccines in humans, and the demonstrated ability of APC/tumour hybrid cells to induce tumour antigen-specific CTL *in vitro*, [27, 66, 72–76], the use of APC/tumour hybrid cells as inducers of tumour-specific CTLs for adoptive immunotherapy merits further investigation.

13. Future challenges and directions

Many animal studies over the previous two decades have shown the ability of APC/tumour cell fusion vaccines to protect against tumour challenge, to eliminate established tumours, and to prevent tumour development in genetically prone animals. Phase I/II clinical trials have shown that vaccination with APC/tumour hybrid vaccines is both safe and well tolerated, although the efficacy of the approach in human subjects remains to be established. Outstanding questions remain in relation to the optimization of the approach for use in humans, the nature of the APC used for the production of hybrids, tumour types where it may be effective, and whether it should be used in conjunction with other forms of cancer therapy. Further investigation is required to address these questions. In addition, APC/tumour fusion cells have been shown to induce tumour antigen-specific CTL *in vitro*, and in this capacity they may have a role in generating antigen-specific effector T-cells for adoptive T-cell cancer immunotherapy [4].

The limited efficacy of DC/tumour cell hybrid vaccines in clinical trials may be due to a number of factors. Firstly, genuine DC/tumour fusion cells need to be verified and isolated or selected. A number of clinical studies did not demonstrate clear verification of DC/tumour fusion cells, making assessment of resultant clinical impact difficult. Secondly, the optimal-dosing schedule and number of fusion cells per injection remain uncertain. This may differ in patients with different tumour types and burdens, and immunological status. Furthermore, the site of vaccine delivery may affect the treatment response. In a clinical study, the intradermal injection of DC-tumour hybrid vaccine resulted in superior antitumour response compared to other routes [77]. Other pre-clinical data suggest that the provision of a third signal with the hybrid vaccines may generate a better response rate [78, 79]. Finally, the use of DC/tumour fusion vaccines may demonstrate significant efficacy when combined with other treatment modalities. There is evidence that radiation as well as chemotherapy combination synergizes with immunotherapy vaccines [80]. Moreover, targeting the

immunosuppressive immune mechanisms such as regulatory T-cells and myeloid-derived suppressor cells may also improve the vaccine efficiency [81]. The immune system, and its interaction with normal and tumour cells, is complex and the advances that are being made in immunotherapy field are numerous. Although these advances have been fewer to date in the field of cancer vaccination than in other forms of cancer immunotherapy, such as monoclonal antibodies against immune checkpoint control molecules, the potential of harnessing and directing the immune system to eliminate tumours through antigen-specific immunotherapy remains the goal of many researchers. APC/tumour fusion cells represent a promising approach to realizing this goal.

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References

[1] Sims, R. B. Development of sipuleucel-T: autologous cellular immunotherapy for the treatment of metastatic castrate resistant prostate cancer. Vaccine, v. 30, n. 29, pp. 4394–7, Jun 19 2012.

[2] Guo, Y., Wu, M., Chen, H., Wang, X., Liu, G., et al. Effective tumor vaccine generated by fusion of hepatoma cells with activated B cells. Science, v. 263, n. 5146, pp. 518–20, Jan 28 1994.

[3] Dunn, G. P., Bruce, A.T., Ikeda, H., Old, L.J., Schreiber, R.D. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol, v. 3, n. 11, pp. 991–8, Nov 2002.

[4] Browning, M. J. Antigen presenting cell/tumor cell fusion vaccines for cancer immunotherapy. Hum Vaccin Immunother, v. 9, n. 7, pp. 1545–8, Jul 2013.

[5] Dubey, P., Hendrickson, R.C., Meredith, S.C., Siegel, C.T., Shabanowitz, J., et al. The immunodominant antigen of an ultraviolet-induced regressor tumor is generated by a somatic point mutation in the DEAD box helicase p68. J Exp Med, v. 185, n. 4, pp. 695–705, Feb 17 1997.

[6] Fuertes, M. B., Kacha, A.K., Kline, J., Woo, S.R., Kranz, D.M., et al. Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells. J Exp Med, v. 208, n. 10, pp. 2005–16, Sep 26 2011.

[7] Cella, M., Sallusto, F., Lanzavecchia, A. Origin, maturation and antigen presenting function of dendritic cells. Curr Opin Immunol, v. 9, n. 1, pp. 10–6, Feb 1997.

[8] Palucka, K., Banchereau, J. Cancer immunotherapy via dendritic cells. Nat Rev Cancer, v. 12, n. 4, pp. 265–77, Apr 2012.

[9] Steinman, R. M., Banchereau, J. Taking dendritic cells into medicine. Nature, v. 449, n. 7161, pp. 419–26, Sep 27 2007.

[10] Dhodapkar, M. V., Dhodapkar, K. M., Palucka, A. K. Interactions of tumor cells with dendritic cells: balancing immunity and tolerance. Cell Death Differ, v. 15, n. 1, pp. 39–50, Jan 2008.

[11] Chomarat, P., Banchereau, J., Davoust, J., Palucka, A.K. IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. Nat Immunol, v. 1, n. 6, pp. 510–4, Dec 2000.

[12] Hiltbold, E. M., Vlad, A.M., Ciborowski, P., Watkins, S.C., Finn, O.J. The mechanism of unresponsiveness to circulating tumor antigen MUC1 is a block in intracellular sorting and processing by dendritic cells. J Immunol, v. 165, n. 7, pp. 3730–41, Oct 1 2000.

[13] Steinbrink, K., Wolfl, M., Jonuleit, H., Knop, J., Enk, A.H. Induction of tolerance by IL-10-treated dendritic cells. J Immunol, v. 159, n. 10, pp. 4772–80, Nov 15 1997.

[14] Aspord, C., Pedroza-Gonzalex, A., Gallegos, M., Tindle, S., Burton, E.C., *et al*. Breast cancer instructs dendritic cells to prime interleukin 13-secreting CD4+ T cells that facilitate tumor development. J Exp Med, v. 204, n. 5, pp. 1037–47, May 14 2007.

[15] De Monte, L., Reni, M., Tassi, E., Clavenna, D., Papa, I., *et al*. Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer. J Exp Med, v. 208, n. 3, pp. 469–78, Mar 14 2011.

[16] Coukos, G., Benencia, F., Buckanovich, R.J., Conejo-Garcia, J. R. The role of dendritic cell precursors in tumour vasculogenesis. Br J Cancer, v. 92, n. 7, pp. 1182–7, Apr 11 2005. I

[17] Kalinski, P., Edington, H., Zeh, H.J., Okada, H., Butterfield, L.H., *et al*. Dendritic cells in cancer immunotherapy: vaccines or autologous transplants? Immunologic research, v. 50, n. 0, pp. 235–47, 2011.

[18] Alters, S. E., Gadea, J.R., Holm, B., Lebkowski, J, Philip, R. IL-13 can substitute for IL-4 in the generation of dendritic cells for the induction of cytotoxic T lymphocytes and gene therapy. J Immunother, v. 22, n. 3, pp. 229–36, May 1999.

[19] Reichardt, P., Dornbach, B., Gunzer, M. APC, T cells, and the immune synapse. Curr Top Microbiol Immunol, v. 340, pp. 229–49, 2010.

[20] Delamarre, L., Mellman, I. Harnessing dendritic cells for immunotherapy. Semin Immunol, v. 23, n. 1, pp. 2–11, Feb 2011.

[21] Jonuleit, H., Kuhn, U., Muller, G., Steinbrink, K., Paragnik, L., et al. Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. Eur J Immunol, v. 27, n. 12, pp. 3135–42, Dec 1997.

[22] Marongiu, L., Donni, M., Toffali, L., Zenaro, E., Dusi, S. ESAT-6 and HspX improve the effectiveness of BCG to induce human dendritic cells-dependent Th1 and NK cells activation. PLoS One, v. 8, n. 10, pp. e75684, 2013.

[23] Hansen, M., Met, O., Svane, I.M., Andersen, M.H. Cellular based cancer vaccines: type 1 polarization of dendritic cells. Curr Med Chem, v. 19, n. 25, pp. 4239–46, 2012.

[24] Dauer, M., Obermaier, B., Herten, J. Haerle, C., Pohl, K., *et al*. Mature dendritic cells derived from human monocytes within 48 hours: a novel strategy for dendritic cell differentiation from blood precursors. J Immunol, v. 170, n. 8, pp. 4069–76, Apr 15 2003.

[25] Jarnak-Jankovic, S., Hammerstad, H., Saeboe-Larssen, S., Kvalheim, G., Gaudernack, G. A full scale comparative study of methods for generation of functional dendritic cells for use as cancer vaccines. BMC Cancer, v. 7, p. 119, Jul 2007.

[26] Mu, L.J., Lazarova, P., Gaudernack, G., Saeboe-Larssen, S., Kvalheim G. Development of a clinical grade procedure for generation of mRNA transfected dendritic cells from purified frozen CD34(+) blood progenitor cells. Int J Immunopathol Pharmacol, v. 17, n. 3, pp. 255–63, 2004.

[27] Mohamed, Y. S., Dunnion, D.J., Teobald, I., Walewska, R., Browning, M.J. Long-lived fusions of human haematological tumour cells and B-lymphoblastoid cells induce tumour antigen-specific cytotoxic T-cell responses in vitro. Immunobiology, v. 217, n. 7, pp. 719–29, Jul 2012.

[28] Zhang, Y., Luo, W., Wang, Y., Liu, Y., Zheng, L. Purified dendritic cell-tumor fusion hybrids supplemented with non-adherent dendritic cells fraction are superior activators of antitumor immunity. PLoS One, v. 9, n. 1, pp. e86772, 2014.

[29] Wang J, Saffold S, Cao X, Krauss J, Chen W. Eliciting T cell immunity against poorly immunogenic tumors by immunization with dendritic cell-tumor fusion vaccines. J Immunol, v. 161, pp. 5516–24, 1998.

[30] Gong J, Koido, S., Chen, D., Tanaka, Y., Huang, L., *et al*. Immunization against murine multiple myeloma with fusions of dendritic and plasmacytoma cells is potentiated by IL-12. Blood, v. 99, pp. 2512–7, 2002.

[31] Siders WM, Vergilis KL, Johnson C, Shields J, Kaplan JM. Induction of specific antitumor immunity in the mouse with the electrofusion product of tumor cells and dendritic cells. Mol Ther, v. 7, pp. 498–505, 2003.

[32] Suzuki T, Fukuhara, T., Tanaka, M., Nakamura, A., Akiyama, K., *et al*. Vaccination of dendritic cells loaded with interleukin-12-secreting cancer cells augments in vivo antitumor immunity: characteristics of syngeneic and allogeneic antigen-presenting cell cancer hybrid cells. Clin Cancer Res, v. 11, pp. 58–66, 2005.

[33] Tanaka Y, Koido S, Ohana M, Liu C, Gong J. Induction of impaired antitumor immunity by fusion of MHC class II-deficient dendritic cells with tumor cells. J Immunol, v. 174, pp. 1274–80, 2005.

[34] Savai, R., Schermuly, R.T., Schneider, M., Pullamsetti, S.S., Grimminger, F., *et al*. Hybrid-primed lymphocytes and hybrid vaccination prevent tumor growth of Lewis lung carcinoma in mice. J Immunother, v. 29, pp. 175–87, 2006.

[35] Yasuda, T., Mamigaki, T., Nakamura, T., Imanishi, T., Hayashi, S., *et al*. Dendritic cell-tumor cell hybrids enhance the induction of cytotoxic T lymphocytes against murine colon cancer: a comparative analysis of antigen loading methods for the vaccination of immunotherapeutic dendritic cells. Oncol Rep, v. 16, n. 6, pp. 1317–24, Dec 2006.

[36] Chen, D., Xia, J., Tanaka, Y., Chen, H., Koido, S., *et al*. Immunotherapy of spontaneous mammary carcinoma with fusions of dendritic cells and mucin 1-positive carcinoma cells. **Immunol**, v. 109, pp. 300–07, 2003.

[37] Xia, J., Tanaka, Y., Koido, S., Liu, C., Mukherjee, P., *et al*. Prevention of spontaneous breast carcinoma by prophylactic vaccination with dendritic/tumor fusion cells. J Immunol, v. 170, pp. 1980–6, 2003.

[38] Iinuma T, Homma S, Noda T, Kufe D, Ohno T, Toda G. Prevention of gastrointestinal tumors based on adenomatous polyposis coli gene mutation by dendritic cell vaccine. J Clin Invest, v. 113, pp. 1307–17, 2004.

[39] Galea-Lauri, J., Darling, D., Mufti, G., Harrison, P., Farzaneh, F. Eliciting cytotoxic T lymphocytes against acute myeloid leukemia-derived antigens: evaluation of dendritic cell-leukemia cell hybrids and other antigen-loading strategies for dendritic cell-based vaccination. Cancer Immunol Immunother, v. 51, n. 6, pp. 299–310, Aug 2002.

[40] Galea-Lauri, J., Wells, J.W., Darlind, D., Harrison, P., Farzaneh, F. Strategies for antigen choice and priming of dendritic cells influence the polarization and efficacy of antitumor T-cell responses in dendritic cell-based cancer vaccination. Cancer Immunol Immunother, v. 53, n. 11, pp. 963–77, Nov 2004.

[41] Shimizu, K., Kuriyama, H., Kjaergaard, J., Lee, W., Tanaka, H., Shu, S. Comparative analysis of antigen loading strategies of dendritic cells for tumor immunotherapy. J Immunother, v. 27, n. 4, pp. 265–72, Jul-Aug 2004.

[42] Kao, J. Y., Zhang, M., Chen, C.M., Chen, J.J. Superior efficacy of dendritic cell-tumor fusion vaccine compared with tumor lysate-pulsed dendritic cell vaccine in colon cancer. Immunol Lett, v. 101, n. 2, pp. 154–9, Nov 15 2005.

[43] Chen, X., Liu, Z., Huang, Y., Li, R., Zhang, H., *et al*. Superior anti-tumor protection and therapeutic efficacy of vaccination with dendritic cell/tumor cell fusion hybrids for murine Lewis lung carcinoma. Autoimmunity, v. 47, n. 1, pp. 46–56, 2014.

[44] Trefzer, U., Herberth, G., Wohlan, K., Milling, A., Thiemann, M., *et al*. Vaccination with hybrids of tumor and dendritic cells induces tumor-specific T-cell and clinical responses in melanoma stage III and IV patients. Int J Cancer, v. 110, pp. 730–40, 2004.

[45] Trefzer, U., Herberth, G., Wohlan, K., Milling, A., Thiemann, M., *et al*. Tumour-dendritic hybrid cell vaccination for the treatment of patients with malignant melanoma: immunological effects and clinical results. Vaccine, v. 23, n. 17–18, pp. 2367–73, Mar 18 2005.

[46] Avigan, D. E., Vasir, B., George, D. T., Oh, W. K., Atkins, M.B., *et al*. Phase I/II study of vaccination with electrofused allogeneic dendritic cells/autologous tumor-derived cells in patients with stage IV renal cell carcinoma. J Immunother, v. 30, n. 7, pp. 749–61, Oct 2007.

[47] Avigan, D. Dendritic cell-tumor fusion vaccines for renal cell carcinoma. **Clin Cancer Res**, v. 10, n. 18 Pt 2, pp. 6347s–52s, Sep 15 2004.

[48] Rosenblatt, J., Vasir, B., Uhl, L., Blotta, S., Macnamara, C., *et al*. Vaccination with dendritic cell/tumor fusion cells results in cellular and humoral antitumor immune responses in patients with multiple myeloma. Blood, v. 117, n. 2, pp. 393–402, Jan 13 2011.

[49] Koido, S., Homma, S., Hara, E., Mitsunaga, M., Namiki, Y., *et al*. In vitro generation of cytotoxic and regulatory T cells by fusions of human dendritic cells and hepatocellular carcinoma cells. Journal of Translational Medicine, v. 6, n. 1, pp. 1–19, 2008.

[50] Zitvogel, L., Apetoh, L., Ghiringhelli, F., Kroemer, G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol, v. 8, n. 1, pp. 59–73, Jan 2008.

[51] Pardoll, D. M. The blockade of immune checkpoints in cancer immunotherapy. **Nat Rev Cancer**, v. 12, n. 4, pp. 252–64, Apr 2012.

[52] Rosenblatt, J., Glotzbecker, B., Mills, H., Vasir, B., Tzachanis, D., *et al*. PD-1 blockade by CT-011, anti-PD-1 antibody, enhances ex vivo T-cell responses to autologous dendritic cell/myeloma fusion vaccine. J Immunother, v. 34, n. 5, pp. 409–18, Jun 2011.

[53] Tan, C., Reddy, V., Dannull, J., Ding, E., Nair, S.K., *et al*. Impact of anti-CD25 monoclonal antibody on dendritic cell-tumor fusion vaccine efficacy in a murine melanoma model. J Transl Med, v. 11, p. 148, 2013.

[54] Avigan, D., Rosenblatt, J., Kufe, D. Dendritic/tumor fusion cells as cancer vaccines. Semin Oncol, v. 39, n. 3, pp. 287–95, Jun 2012.

[55] Mahoney, K. M., Rennert, P. D., Freeman, G. J. Combination cancer immunotherapy and new immunomodulatory targets. Nat Rev Drug Discov, v. 14, n. 8, pp. 561–84, Aug 2015.

[56] Hirohashi, Y., Torigoe, T., Inoda, S., Takahashi, A., Morita, R., *et al*. Immune response against tumor antigens expressed on human cancer stem-like cells/tumor-initiating cells. Immunotherapy, v. 2, n. 2, pp. 201–11, Mar 2010.

[57] Wang, Y. H., Li, F., Luo, B., Wang, X.H., Sun, H.C., *et al*. A side population of cells from a human pancreatic carcinoma cell line harbors cancer stem cell characteristics. Neoplasma, v. 56, n. 5, pp. 371–8, 2009.

[58] Hirohashi, Y., Torigoe, T., Tsukahara, T., Kanaseki, T., Kochin, V., Sato, N. Immune responses to human cancer stem-like cells/cancer-initiating cells. Cancer Sci, v. 107, n. 1, pp. 12–7, Jan 2016.

[59] Engelmann, K., Shen, H., Finn, O. J. MCF7 side population cells with characteristics of cancer stem/progenitor cells express the tumor antigen MUC1. Cancer Res, v. 68, n. 7, pp. 2419–26, Apr 1 2008.

[60] Qin, K., Tian, G., Li, P., Chen, Q., Zhang, R., *et al*. Anti-glioma response of autologous T cells stimulated by autologous dendritic cells electrofused with CD133+ or CD133- glioma cells. J Neuroimmunol, v. 242, n. 1–2, pp. 9–15, Jan 18 2012.

[61] Moviglia, G. A. Development of tumor B-cell lymphocyte hybridoma (TBH) autovaccination. Results of a phase I-II clinical trial. Transfus Sci, v. 17, n. 4, pp. 643–9, Dec 1996.

[62] Kugler, A., Seseke, F., Thelen, P., Kallerhof, M., Muller, G.A., *et al*. Autologous and allogenic hybrid cell vaccine in patients with metastatic renal cell carcinoma. Br J Urol, v. 82, n. 4, pp. 487–93, Oct 1998.

[63] Dunnion, D. J., Cywinski, A.L., Tucker, V.C., Murray, A.K., *et al*. Human antigen-presenting cell/tumour cell hybrids stimulate strong allogeneic responses and present tumour-associated antigens to cytotoxic T cells in vitro. Immunology, v. 98, n. 4, pp. 541–50, 1999.

[64] Edwards PA, Smith CM, Neville AM, O'Hare MJ. A human-hybridoma system based on a fast growing mutant of the ARH-77 plasma cell leukaemia-derived line. Eur J Immunol, v. 12, pp. 641–8, 1982.

[65] Walewska, R., Teobald, I., Dunnion, D., Abdulmajed, H., Aldred, M., *et al*. Preclinical development of hybrid cell vaccines for multiple myeloma. Eur. J. Immunol. v. 78, pp. 11–20, 2007.

[66] Mohamed, Y. S., Dunnion, D.J., Teobald, I., Walewska, R., Browning, M.J. In vitro evaluation of human hybrid cell lines generated by fusion of B-Iymphoblastoid cells and ex vivo tumour cells as candidate vaccines for haematological malignancies. Vaccine, v. 30, n. 46, pp. 6578–6587, Oct 2012.

[67] Mohamed, Y. S., Bashawri, L.A., Vatte, C., Abu-Rish, E.Y., Cyrus, C., *et al*. The in vitro generation of multi-tumor antigen-specific cytotoxic T cell clones: Candidates for leukemia adoptive immunotherapy following allogeneic stem cell transplantation. Mol Immunol, v. 77, pp. 79–88, Aug 1 2016.

[68] Mohamed, Y. S., El Ghareeb, K.A., Gomaa, F.A., Abu-Rish, E.Y. In Vitro Generated Tumor/APC Hybrids Induce Allogeneic Tumor-Killer T Cells. EC Microbiology, v. 2, n. 3, pp. 296–306, 2015.

[69] Heslop, H. E., Slobold, K.S., Pule, M.A., Hale, G.A., Rousseau, A., *et al*. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood, v. 115, n. 5, pp. 925–35, Feb 2010.

[70] Warren, E. H., Fujii, N., Akatsuka, Y., Chaney, C.N., Mito, J.K., *et al*. Therapy of relapsed leukemia after allogeneic hematopoietic cell transplantation with T cells specific for minor histocompatibility antigens. Blood, v. 115, n. 19, pp. 3869–78, May 2010.

[71] Montagna, D., Turin, I., Schiavo, R., Montini, E., Zaffaroni, M., *et al*. Feasibility and safety of adoptive immunotherapy with ex vivo-generated autologous, cytotoxic T lymphocytes in patients with solid tumor. Cytotherapy, v. 14, n. 1, pp. 80–90, Jan 2012.

[72] Gong, J., Koido, S., Kato, Y., Tanaka, Y., Chen, D., *et al*. Induction of anti-leukemic cytotoxic T lymphocytes by fusion of patient-derived dendritic cells with autologous myeloblasts. Leuk Res, v. 28, n. 12, pp. 1303–12, Dec 2004.

[73] Raje, N., Hideshima, T., Davies, F.E., Chauhan, D., Treon, S.P., *et al*. Tumour cell/dendritic cell fusions as a vaccination strategy for multiple myeloma. Br J Haematol, v. 125, n. 3, pp. 343–52, May 2004.

[74] Klammer, M., Waterfall, M., Samuel, K., Turner, M.L., Roddie, P.H. Fusion hybrids of dendritic cells and autologous myeloid blasts as a potential cellular vaccine for acute myeloid leukaemia. Br J Haematol, v. 129, n. 3, pp. 340–9, May 2005.

[75] Imura, K., Ueda, Y., Hayashi, T., Itoh, T., Shimizu, K., *et al*. Induction of cytotoxic T lymphocytes against human cancer cell lines using dendritic cell-tumor cell hybrids generated by a newly developed electrofusion technique. Int J Oncol, v. 29, n. 3, pp. 531–9, Sep 2006.

[76] Rosenblatt, J., Wu, Z., Vasir, B., Zarwan, C., Stone, R., *et al*. Generation of tumor-specific T lymphocytes using dendritic cell/tumor fusions and anti-CD3/CD28. J Immunother, v. 33, n. 2, pp. 155–66, Feb–Mar 2010.

[77] Lesterhuis, W. J., de Vries, I.J., Schreibelt, G., Lambeck, A.J., Aarntzen, E.H., *et al*. Route of administration modulates the induction of dendritic cell vaccine-induced antigen-specific T cells in advanced melanoma patients. Clin Cancer Res, v. 17, n. 17, pp. 5725–35, Sep 1 2011.

[78] Dittmar, T., Zanker, K. S. Cell fusion in health and disease. Volume II: cell fusion in disease. Introduction. Adv Exp Med Biol, v. 714, pp. 1–3, 2011.

[79] Pizzurro, G. A., Barrio, M. M. Dendritic cell-based vaccine efficacy: aiming for hot spots. Frontiers in Immunology, v. 6, p. 91, 2015.

[80] Hodge, J. W., Ardiani, A., Farsaci, B., Kwilas, A.R., Gameiro, S.R. The tipping point for combination therapy: cancer vaccines with radiation, chemotherapy, or targeted small molecule inhibitors. Seminars in Oncology, v. 39, n. 3, pp. 323–339, 2012.

[81] Baxevanis, C. N., Perez, S. A., Papamichail, M. Combinatorial treatments including vaccines, chemotherapy and monoclonal antibodies for cancer therapy. Cancer Immunol Immunother, v. 58, n. 3, pp. 317–24, Mar 2009.

Chapter 9

CARs on the Highway: Chimeric Antigen Receptor Modified T Cells for the Adoptive Cell Therapy of Malignant Diseases

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Additional information is available at the end of the chapter

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Abstract

Adoptive therapy of malignant diseases by chimeric antigen receptor (CAR) redirected T cells takes advantage of the patient's own immune system to recognize and destroy cancer cells. This is impressively demonstrated by the induction of complete and lasting remissions of leukemia with CAR-engineered T cells in early phase trials. Recent developments in optimizing the CAR design, in the recognition of target cells and the production of modified cells for clinical use, have paved the path for a broader application than currently explored. The chapter reviews the differences in CAR design, the success in the treatment of hematologic malignancies, the challenges in treating solid cancer, the treatment-related toxicities, and strategies to improve safety of CAR T cell therapy. Challenges for future applications are discussed.

Keywords: adoptive cell therapy, chimeric antigen receptor, T cell, clinical trial, costimulation, cancer

1. Synopsis

Adoptive cell therapy with redirected T cells has recently shown spectacular success in the treatment of hematologic malignancies supporting the concept that patient's own T cells can control cancer in the long term. A current strategy to specifically redirect patient's immune cells toward cancer is based on the adoptive transfer of cytolytic T cells which are ex vivo engineered with a chimeric antigen receptor (CAR) to provide both targeting specificity and T cell activation upon cancer cell recognition. The CAR is a composite transmembrane receptor molecule with a single-chain fragment of variable region (scFv) antibody binding domain in the extracellular part for recognizing a "tumor-associated antigen" on the surface of the

targeted cancer cell (**Figure 1**). The CAR transmits a T cell activation upon cancer cell recognition. The CAR is a composite transmembrane–activating signal through its intracellular part which is mostly derived from the T cell activation upon cancer cell recognition. The CAR is a composite transmembrane receptor (TCR)/CD3ζ signaling moiety with or without a costimulatory domain. Engagement of the cognate antigen on cancer cells initiates immune cell activation resulting in a lasting anti-tumor cell response [1, 2].

The prototype CAR for redirecting T cell activation has several advantages which are due to the modular design, in particular the combination of the antigen recognition by an antibody with the T cell activating machinery of the TCR. The antibody-mediated CAR recognition is independent of MHC presentation of antigen, which is in contrast to the TCR, and allows recognizing any target for which an antibody is available, including carbohydrates, lipids, or structural variants of an antigen. In contrast to the TCR recognition of antigen presented by the MHC, the CAR recognizes only antigens on the cell surface. However, by using an antibody which recognizes a specific peptide in the context of MHC, CARs can also gain TCR-like specificity; one example is a CAR recognizing a MHC class I presented NY-ESO-1 peptide [3, 4].

Adoptive therapy with CAR-modified T cells takes advantage of the power of cytolytic T cells that actively migrate through vascular endothelia and penetrate tissues, are activated upon antigen recognition, amplify, eliminate cognate target cells and have the capacity for repetitive killing. Once activated CAR T cells can moreover induce a secondary immune response by the release of a variety of pro-inflammatory factors which attract innate immune cells to the targeted tissue. After target elimination and without further re-stimulation, most CAR T cells undergo apoptosis; however, some CAR T cells can persist over years and provide an antigen-specific memory.

The efficiency of CAR-mediated T cell activation depends on various parameters; most of them are empirically defined, including the CAR design, the CAR primary and costimulatory moieties, the binding affinity, the targeted antigen epitope and its accessibility, the density of the cognate antigen on the target cell, and others. Bispecific CARs were engineered to target cancer cells which lost one antigen but retained the other. Several other modifications of the prototype CAR design were explored to increase treatment safety, targeting selectivity, and clinical efficacy. T cells were engineered with two CARs which recognize defined patterns of target antigens and complement in signaling to provide only full T cell activation when both antigens are engaged. Inhibitory CARs (iCARs) provide an inhibitory signal when engaging an antigen on healthy cells, thereby preventing unintended T cell activation against healthy tissues. Switch CARs provide an activating signal while engaging an inhibitory ligand on the target cell, thereby "switching" a suppressor signal to an activating signal for the engineered T cell.

In clinical applications, patient's T cells are ex vivo engineered with the CAR, mostly by lenti- or retroviral gene transfer, amplified to relevant numbers and re-administered to the patient who, prior T cell therapy, received a non-myeloablative lymphodepleting treatment to provide favorite conditions for the transferred CAR T cells. While the genetic modification of T cells by viral transduction is permanent, T cells can be transiently modified by RNA transfection in order to display the CAR for short term on the cell surface. In the majority of trials, the entire population of T cells is genetically modified; apart thereof, T cell subsets such as $CD8^+$ T cells, $CD4^+$ T cells, γδ T cells, cytokine activated killer (CIK) cells, or NK cells are also used. CAR-engineered regulatory T cells (Treg cells) are explored in experimental models to treat autoimmune diseases.

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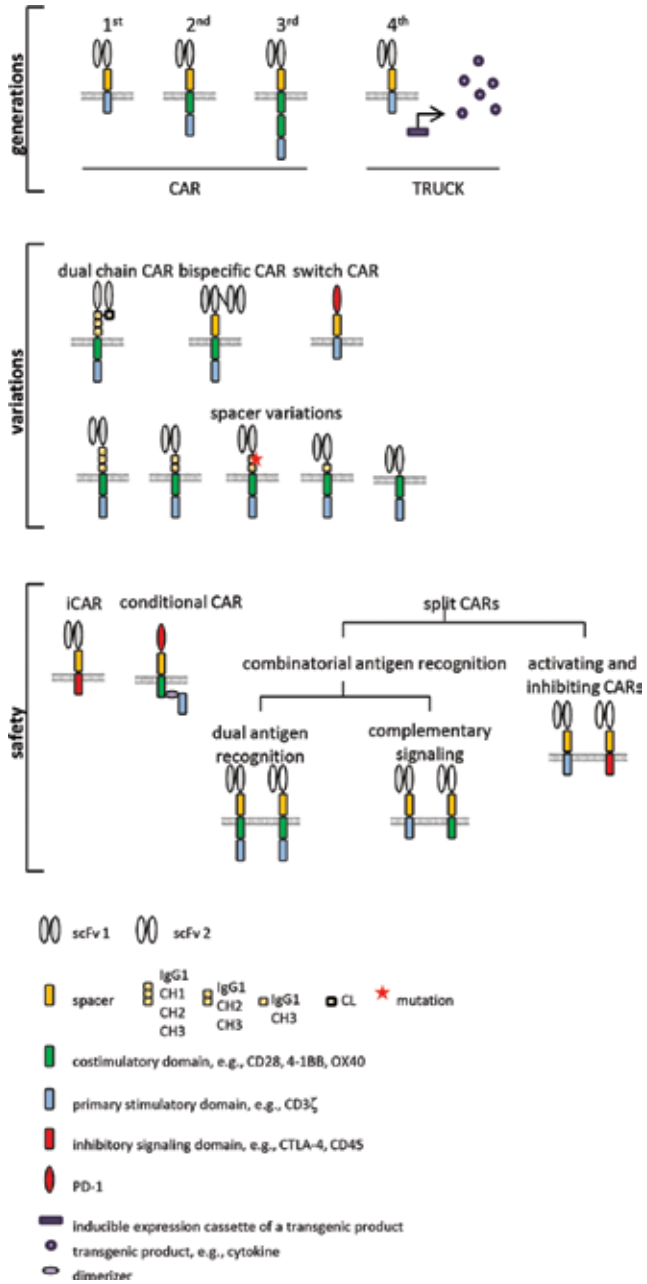

Figure 1. Modularity of the Chimeric Antigen Receptor (CAR) design. The CAR is an artificial composite receptor in order to bind a target in a specific fashion and to provide host cell activation in a predictable manner. The extracellular CAR binding domain, the spacer, the transmembrane and the intracellular signaling domains can be swapped with diverse other domains. On the extracellular side, various recognition domains were used, mostly single chain fragment of variable region (scFv) antibodies, or receptor derived binding modules. On the intracellular side, a panel of signaling domains can be used; the primary activating signaling domain is mostly derived from the TCR CD3ζ, Fc ε receptor-I (FcεRI) or downstream kinases. The costimulatory domain providing the secondary activating signal is linked to the primary signaling domain. Alternatively, inhibitory signals can be used to block T cell activation.

Currently, an overwhelming number of clinical trials has been initiated, and most of them are extraordinary successful in the treatment of hematologic malignancies [5]. As a treatment with "living drugs", the CAR T cell therapy provides a significant advancement toward a specific and individualized cell therapy of cancer which is going to establish in clinical practice. However, the CAR T cell treatment of solid tumors is still challenging demanding further developments of the basic strategy. CAR T cells with the inducible release of a transgenic payload, so-called TRUCKs, are envisaged to overcome some of the current hurdles [6]. In the following, recent developments are reviewed in the context of clinical applications, safety concerns, and challenges for future applications.

2. The prototype CAR

A CAR of the classical design consists of an antibody-derived binding domain, a spacer, a transmembrane domain, and one or more signaling domains; the attributes for an optimal CAR function are so far empirically defined and multiple variations of the prototype CAR design were described during the last two decades (**Figure 1**). Most CARs use an antibody-derived binding domain in a single-chain format, the so-called single-chain fragment of variable region (scFv) antibody. The scFv is engineered by joining the heavy and light immunoglobulin (Ig) variable regions by a flexible peptide linker, for example, $(Gly_4Ser)_3$, resulting in a continuous polypeptide chain in the order V_H-linker-V_L or V_L-linker-V_H. Such an antibody format facilitates the combination with the transmembrane polypeptide chain for membrane anchoring and the intracellular signaling chain. Since the conversion of a natural antibody into a scFv format does not always conserve the binding affinity and specificity of the antibody, a CAR format was recently reported which uses the entire Ig heavy and light chains with their constant domains to form a natural antibody on the surface of the engineered T cell; the Ig heavy chain is at the N terminus linked to the transmembrane and intracellular CAR domain [7]. The two CAR chains form a stable heterodimer, a so-called dual chain CAR (dcCAR), and bind with high affinity and in a specific fashion to their cognate antigen. The dual chain CAR format seems to be universally applicable and broadens the CAR T cell therapy toward a variety of antigens for which a scFv antibody is not available. Instead of an antibody, a naturally occurring receptor or ligand can also be used for binding. For instance, a CAR with a mutated IL-13 extracellular domain was designed to selectively bind to IL-13 receptor-α2 which is over-expressed by a broad variety of solid tumors but less by healthy tissues [8–10].

The CAR extracellular domain additionally incorporates a spacer of various lengths and a hinge domain to provide some flexibility. Various spacers were explored in the context of various antigens since the design and length of the spacer domain can be decisive for optimal CAR expression and function (**Figure 1**). This became obvious through discrepancies in the potency of CARs to activate T cells; some CARs are expressed and are active when the scFv is directly fused onto the signaling domain, and others are only active with a spacer [11]. Targeting of some antigens requires a spacer region between the antigen recognition and signaling domains implying some impact of the antigen itself. The spacer is typically

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derived from the IgG1 or IgG4 constant domain; other spacers such as CD4 or CD8 are also used [2, 12]. The spacer length can be varied by using different moieties, for example, IgG1 CH1-CH2-CH3 vs CH2-CH3 vs CH3; for each antigen, there seems to be an optimal CAR design providing the best suitable distance between the interacting CAR T cell and target cell [13]. The optimal distance moreover depends on the position of the targeted epitope on the antigen; higher order structural requirements and multimerization driven by the extracellular spacer domain may additionally apply demanding a more thorough exploration in the context of a particular antigen.

Apart thereof, the commonly used IgG1 constant domain in the extracellular CAR moiety can bind to Fc γ receptors (FcγR) (CD64) on myeloid cells, thereby initiating an unintended "off-target" activation of both T cells and innate cells. It is therefore essential to abrogate binding to Fc receptors by either deleting the IgG1 CH2 domain or by replacing through the IgG4 domain. Alternatively, the FcγR binding motif within IgG1 CH2 was modified by deleting the Asn_{297} glycosylation site [12, 14].

CARs typically have a membrane spanning region consisting of 20–23 hydrophobic amino acids, rich in leucines, isoleucines, and valines; a variety of membrane spanning receptor domains have been used in CAR design, including those of CD3ζ, CD4, CD8, CD28, or OX40 [2]. The choice for a transmembrane region is so far empiric, and some evidences imply that CARs with CD3ζ transmembrane domain incorporate into the endogenous TCR/CD3 complex and may be more robust in expression and signaling than others [15].

"First generation" CARs used the TCR-derived intracellular CD3ζ or the Fc ε receptor-I (FcεRI) derived γ chain for signaling; CD3ζ has become the most widely used signaling component [16, 17]. The CD3ζ harbors three immunoreceptor tyrosine activation motifs (ITAMs), the γ chain one ITAM. Upon CAR engagement of antigen, the ITAMs become phosphorylated and serve as specific adaptors for a panel of signaling proteins, thereby utilizing the endogenous TCR downstream signaling machinery for initiating the cascade of activation events. In this context, downstream kinases like lck or fyn can also be used as CAR activation domains.

Based on the "two signal hypothesis" that sustained T cell activation requires both the primary TCR-derived signal and a costimulatory signal, researchers added a costimulatory moiety to the primary signaling moiety to improve T cell activation in the long term [18–20]. Such "second generation CARs" harbor combined primary and costimulatory signaling moieties within the same polypeptide chain (**Figure 1**). CD28 was initially incorporated as a costimulatory domain, and alternative costimulatory molecules are also used including 4-1BB (CD137) and OX40 (CD134). The specific order of signaling domains within the CAR appears to be important for optimal activity; the CD28 domain is located in the membrane proximal and the CD3ζ domain in the distal position; the same applies for 4-1BB while OX40 is also active in a membrane distal position. Due to the modular composition of signaling moieties, two costimulatory domains can also be combined within the same CAR providing a more complex signaling signature to the engineered cell. Such "third generation" CARs incorporating CD28 and OX40, for instance, may be of benefit for T cells during late stages of maturation [21].

The individual costimulatory signals drive T cell activation in a different fashion resulting in a fine tuning of the T cell response with respect to T cell amplification, secretion of pro-inflammatory cytokines, and cytolysis of antigen-positive target cells [22]. CAR T cells with 4-1BB costimulation persisted for more than 6 months in the blood of most patients, whereas CD28 CAR T cells were mostly undetectable beyond 3 months [23]. CD28 CAR T cells show a reprogramming toward $CD45RO^{+}$ $CCR7^{-}$ effector memory maturation while 4-1BB CAR T cells predominantly show a $CD45RO^{+}$ $CCR7^{+}$ central memory cell differentiation [24]. Costimulation moreover orchestrates the metabolism of the CAR T cells; CD28 signals through the PI3K/Akt pathway increase glucose uptake through the Glut1 transporter and PDK1 which inhibits the pyruvate decarboxylation, all resulting in an increased ATP generation. In contrast, T cells with the 4-1BB CAR show an enhanced catabolic activity and oxidative metabolism together with an enhanced mitochondrial respiratory capacity. With that respect, the 4-1BB CAR initiates a long-lasting central memory and the CD28 CAR a more short-lived effector cell response. Both CD28 and 4-1BB CAR T cells in high doses eradicates large established tumors in preclinical models; at lower doses, CD28 CAR T cells show larger degree of exhaustion than the 4-1BB CAR T cells and treatment with 4-1BB CAR T cells more efficiently eradicated tumors [25]. T cells with 4-1BB CAR are still sensitive to tumor-mediated inhibition, however, show less exhaustion and decline in cytolytic capacities and cytokine secretion upon repeated antigen encounter than CD28 CAR T cells. Therefore, the criteria for selecting a CAR design depend on multiple parameters including T cell persistence, resistance to repression, the pattern of costimulatory and co-inhibitory ligands on targeted tumor cells and CAR T cells, the CAR density on the modified T cell, and require variations in CAR design such as affinity, the spacer, and transmembrane domains among others.

3. TRUCK: a CAR T cell releasing a transgenic product

CAR T cells of the "fourth generation," so-called TRUCKs, are T cells engineered with a CAR and an additional "payload", that is, a transgenic product (**Figure 1**) [6]. TRUCKs are aimed at depositing a protein in the CAR-targeted tissue in order to locally achieve therapeutic effective concentrations of the transgenic protein while avoiding systemic toxicity. The transgenic product may by a cytokine or any other protein which is produced and released upon CAR signaling. Technically, the CAR T cells are engineered with an additional expression construct for the transgenic protein directed by a constitutive or a CAR responsive promoter. Such TRUCKs deposit the transgenic product at the place of activation as long as the CAR T cell remains activated [26, 27]. In a specific development, TRUCKs were engineered to release a transgenic immune modifier upon CAR signaling to shape the targeted tumor environment in a specific fashion without causing systemic toxicity. In an example, IL-12 TRUCKs were shown to release IL-12 upon CAR activation into the targeted tumor tissue where the accumulated IL-12 recruited innate immune cells, such as NK cells and macrophages, which in turn drive a secondary immune response [28]. The strategy is of particular interest with respect to the fact that cancer cells which have down-regulated the cognate target are invisible to CAR T cells and may give rise to tumor relapse despite the presence of CAR T cells. Tumor relapse through antigen loss cancer cell variants is becoming an increasing obstacle when solid cancer

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lesions with a substantial genetic or phenotypic heterogeneity are treated by mono-specific CAR T cells. Combining the CAR T cell attack with the local deposition of an immune modifier represents a strategy to initiate a broader immune response. Beyond the treatment of cancer, TRUCKs may be envisioned for the therapy of virus infections, autoimmune diseases, or metabolic disorders delivering a therapeutic protein into the diseased tissue.

So-called armored CARs include the 4-1BB ligand in addition to the CAR in order to provide increased costimulation through the 4-1BB pathway [29]. Recently described examples are TRUCKs that co-express catalase to protect T cells from oxidative stress-mediated repression [30] and heparanase to improve T cell penetration through tumor stroma [31]. T cells engineered to secrete Toll-like receptor (TLR) ligands including TLR5 ligand can stimulate the TLR on T cells and on antigen-presenting cells which then activate a broad panel of tumor-reactive T cells [32, 33].

4. CAR targetable antigens: neo-antigen, tumor-associated antigen, and activation-induced antigen

The CAR T cell treatment of tumors demands both specificity in antigen recognition and selectivity in cancer cell targeting to avoid destruction of healthy tissues. Ideally, the targeted antigen is required for cancer cell survival and harbors mutations that are large enough to produce new epitopes, so-called neo-antigens, which then can be specifically recognized by the CAR [34]. Although mutations are thought to occur frequently enough in cancer cells to provide multiple new targetable epitopes, the identification of neo-antigens requires deep sequencing of the tumor material. Only a subset of such neo-antigens may be presented by the MHC to be recognized by the TCR; neo-antigens on the cell surface to be recognized by the CAR occur less frequently. Currently, such neo-antigens are rarely identified, asking whether the technical ability to predict relevant neo-antigens are sufficiently advanced and whether antibody-based binders for the use in a CAR can be engineered in due time. The situation is even more complex since the tumor lesion is genetically extremely heterogeneous which requires targeting more than one antigen in order to deplete the majority of cancer cells and to minimize the risk for a tumor relapse by remaining cancer cells.

Since the peptide processing is frequently impaired in cancer cells, T cell epitopes associated with impaired peptide processing and derived from broadly expressed self-antigens are potential targets for cell therapy since they are not restricted by central tolerance [35]. Since such antigens do not require the cellular peptide transporter, tumors defective in TAP transporter remain targetable by CAR T cells despite downregulation of the antigen presentation machinery.

Basically, subpopulations of tumor cells with “stem cell–like” properties, so-called cancer stem cells (CSCs), and the expression of stem cell–associated antigens may be good targets since CSCs are thought to trigger disease progression and tumor relapse and to be responsible for the resistance to conventional therapy. CAR T cells are capable to eliminate those CSCs in experimental models including melanoma; one trial is going to explore the clinical efficacy [36–40]. CAR T cell targeting of activation antigens expressed by stem cells may be superior to targeting

lineage-associated antigens since activation associated antigens are transiently expressed during maturation. For instance, CAR T cells targeting CD30 spare $CD30^+$ hematopoietic stem and progenitor cells while eliminating $CD30^+$ lymphoma cells in an experimental model [41]. In contrast, lineage-associated antigens increase in expression during cell maturation, like folate receptor-β and CD123; targeting those antigens increases the risk to destruct tissue stem cells [42].

In the absence of truly tumor-specific neo-antigens, so-called tumor-associated antigens are preferred; some of those antigens are expressed in a polarized fashion by healthy cells and topologically sequestered from redirected T cells, while uniformly distributed on cancer cells. An example of such antigen is carcinoembryonic antigen (CEA) which is expressed on the luminal surface of gastrointestinal and lung epithelia but homogenously by cancer cells where it can be recognized by CAR T cells.

For the treatment of B cell malignancies, CD19 and CD20 are most frequently targeted by CAR T cells with the consequence that healthy $CD19^+$ B cells are also depleted. Although B cell deficiency is clinically manageable, researchers are looking for alternative targets to avoid CAR T cell–induced immune deficiency in the long term. In the case of a malignant B cell clone with an immunoglobulin κ (Igκ) light chain, CAR T cells targeting Igκ may be of benefit since the Igλ B cells and plasma cells remain untouched [43]. Moreover, Igκ B cell deficiency does not increase the risk of infection making Igκ a good candidate target for treatment. An alternative target is the receptor tyrosine kinase-like orphan receptor-1 (ROR1) which is expressed by cells of chronic lymphocytic leukemia (CLL), mantle cell lymphoma, B-ALL, and numerous types of solid tumors [44–47], however, also by many healthy tissues.

5. CAR T cells recognizing multiple antigens

Cancer cells may lose the expression of particular antigens due to various mechanisms during tumor progression making them invisible to specific CAR T cells demanding targeting of multiple antigens on the cancer cells. Instead of engineering a panel of CARs with different specificities and applying a panel of T cells with different CARs, a CAR with multiple specificities can be engineered by linking the scFvs to each other (**Figure 1**). A so-called TanCAR is a bispecific CAR which harbors two linked scFvs of different specificities in the same CAR polypeptide chain and is aimed at targeting two antigens in order to control tumors with a growing number of antigen loss cancer cell variants [48]. Such TanCAR induces a T cell response upon engagement of either antigen; both antigens are not needed to be simultaneously present on the same cell to initiate CAR T cell activation. With this respect, CD19-CD20 bispecific CARs were engineered in order to mitigate a B cell leukemia relapse through cells which lost either antigen [49]. In particular, leukemia relapses occurred upon therapy with CD19 CAR T cells, and the relapse is predominantly driven by $CD19^-$ $CD20^+$ leukemic cells which are likely recognized by CD19- and CD20-specific TanCAR T cells. Simultaneous engagement of two antigens by a bispecific CAR has moreover the advantage to increase the avidity and the interaction of the CAR T cell with the respective target cell which stabilizes the formation of the synapse and improves T cell activation toward target cells with low antigen levels. Apart from tandem scFvs, diabodies, two-in-one antibodies, and dual variable domain antibodies may also be used as bispecific binding domains in the context of a CAR.

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Strategies were developed to increase selectivity in cancer cell targeting. The sensing of an antigen pattern is more selective in cancer cell recognition as long as the targeted antigens are co-expressed by the cancer cells and less by healthy cells. The antigen pattern is recognized by a pair of cooperating CARs; each CAR specific for a different antigen and providing different signals, one CAR the primary activating, the other CAR the costimulatory signal; both signals need to complement in order to induce a T cell response (**Figure 1**). CAR T cell targeting of an antigen pattern is thought to minimize off-tumor toxicities toward healthy tissues. The design is in contrast to a second generation CAR which provides both the primary and costimulatory signal through the linked signaling moieties upon engaging the one cognate antigen.

Some examples of combinatorial antigen recognition were reported, for instance, targeting ErbB2 by the CD3ζ CAR and Muc1 by the CD28 CAR [50], or targeting mesothelin by the CD3ζ CAR and folate receptor-α by the CD28 CAR [51]. T cell activation by combinatorial antigen recognition requires a subthreshold primary signaling to ensure a dependence on costimulation for a productive T cell response. Such fine-tuning of signaling strength is required to avoid unintended activation against cells with one antigen only and to achieve a complete T cell response against cells with both antigens. In this situation, de-tuning of the primary activation signal can be achieved by using binding domains of lower affinities [52].

6. CARs with exchangeable antigen recognition

In the current situation, each CAR has specificity for a defined antigen; changing specificity requires engineering T cells with a new CAR. To obtain some exchangeability in targeting specificity once the CAR T cells are applied to the patient, strategies were developed which use the CAR for binding a "linker" molecule which targets the cancer cell. In particular, the CAR binds, for example, via CD16, to the immunoglobulin Fc region of an antibody which binds to the cancer cell; the CAR will bind the antibody and in turn gains specificity for the targeted antigen [53]. The strategy allows using an universal CAR which is grafted with tumor specificity by an applied antibody. In an alternative approach, the CAR binds to a protein epitope, which is not encoded by the human genome and which is linked to cancer-targeting antibodies [54]. In another example, folate receptor-positive cancer cells were marked by a fluorescein isothiocyanate (FITC)-conjugated folate which binds to the cancer cells and is recognized by a FITC-specific CAR [55, 56]. By using more than one FITC-labeled molecule which mark the cancer cells, the same CAR T cell can target multiple types of cells within the tumor lesion. However, the strategy requires sufficient concentrations of the marking molecule and adequate numbers of CAR T cells in the tumor lesion for productive T cell activation.

7. Conditional CARs to control toxicity

In order to combine primary and costimulatory signal only "on demand", a conditional CAR was designed which consists of two chains: one chain providing the extracellular and transmembrane moiety together with the primary signaling moiety and the second chain providing the costimulatory signaling moiety (**Figure 1**). Both chains keep "switched off" and are

only "switched on" upon adding a small dimerizer molecule which enables the formation of a functional CAR heterodimer synapse and the delivery of both signals to the T cell in a temporally limited fashion [57]. Withdrawal of the dimerizer terminates switch-on of the CAR. The strategy is thought to be safe as in the absence of a dimerizer no signaling occurs. Moreover, the T cell activity may be fine-tuned by titrating the dose of the dimerizer.

An alternative strategy is based on synthetic Notch (synNotch) receptors which enable the conditional expression of a targeting receptor upon engagement with a tissue-specific ligand [58, 59]. The strategy is based on Notch, which is composed of an extracellular receptor, a transmembrane domain, and an intracellular transcription regulator, and upon activation mediates proteolysis of the internal domain which is releasing the intracellular transcription regulator. The synNotch receptor activity is cell contact dependent and controls cellular responses in a spatially defined fashion [58]. These properties can be used in a CAR-like synNotch receptor which controls the transcription of an authentic CAR and thereby uses combinatorial antigen recognition to spatially control CAR T cell function [59]. The CD19-specific synNotch receptor was tested in a model recognizing CD19 and releasing a transcription regulator to induce the expression of a CAR against mesothelin. The described synNotch receptor is composed of a CD19-specific scFv and of the transcriptional effector domains Gal4-VP64 or TetR-VP64 required to induce CAR expression; the engineered T cell is only activated when both the synNotch ligand and the CAR ligand were engaged on the target cell. There is a dose-response relationship between ligand concentration and CAR engagement. The kinetics of the "on-switch" determines the selectivity in targeting cancer cells while protecting healthy tissues; the induction of CAR expression by synNotch ligand binding needs to be fast enough to engage the tumor while the decayed CAR expression needs to be timely enough to spare healthy tissues expressing the cognate antigen.

8. Switch CARs: converting a suppressor into an activator

Solid tumor lesions display a plethora of inhibitory ligands to T cells, thereby actively suppressing the T cell anti-tumor attack; programed cell death ligand-1 (PD-L1) and PD-L2 binding to programed cell death-1 (PD-1) expressed by activated T cells are a major mechanism in this scenario. Kobold et al. [60] and Liu et al. [61] explored the concept to switch the inhibitory signal provided upon PD-1—ligand interaction into a T cell activating signal by a "switch CAR" which consists of the PD-1 extracellular domain for ligand binding and the CD28 intracellular domain for activation (**Figure 1**). The PD-1:CD28 "switch" CAR increased ERK phosphorylation, the release of pro-inflammatory cytokines such as IL-2, IFN-γ and TNF-α, the T cell proliferation, and the expression of the cytolytic molecule granzyme B upon PD-L1 binding [62]. Obviously, the switch CAR provided CD28 costimulation overruns the PD-1 mediated suppressive signal in the engineered T cell. The switch receptor moreover competes for available PD-1 ligands on the tumor cells and thereby reduces the number of repressive PD-1 interactions. However, other suppressive mechanisms are still in place in the tumor lesion, for instance through T cell immunoglobulin and mucin-domain containing protein-3 (TIM-3), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), B and T lymphocyte

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attenuator (BTLA), or lymphocyte-activation gene-3 (LAG-3), which all need to be overrun in a sufficient fashion to sustain the T cell anti-tumor attack in the long term.

9. CARs providing inhibitory signals: iCARs

Based on the modular composition of the prototype CAR, the intracellular activating domain can be exchanged by a moiety which delivers inhibitory signals to the T cell, including PD-1, CTLA-4, or CD45 [63]. Such an inhibitory CAR (iCAR) represses T cell activation upon binding to cognate antigen (**Figure 1**). The rationale for iCARs in the context of anti-tumor immunotherapy is to suppress T cell activation when engaging an antigen expressed by healthy cells. T cells which co-express a dominant iCAR along with an activating CAR recognizing a tumor-associated antigen are activated when engaging the cancer cells and repressed upon contact with healthy cells. In this context, the inhibitory CAR signal is aimed at avoiding off-tumor toxicities by overrunning the activating CAR signal when engaging healthy tissues.

10. Universal CAR T cells

The fundamental idea of an "off-the-shelf" CAR T cell is a genetically edited CAR T cell with deleted endogenous $\alpha\beta$ TCR and HLA molecules which can be achieved by the zinc finger nuclease technology [64]. Such CAR T cells are expected to be applied to a number of patients without causing graft-versus-host disease (GvHD) and without being eliminated by the host immune cells while providing CAR-mediated effector functions against cancer cells. Based on the same rationale, T cells were genetically edited by TALEN technology in the TCRα and CD52 locus [65] for the treatment of ALL. In a first in-human application, gene-edited CAR T cells were administered for the treatment of pediatric $CD19^+$ ALL in a patient for whom autologous CAR T cells could not be produced. CAR T cells from an unrelated donor were genetically edited by deleting the endogenous TCR to prevent GvHD and by deleting CD52, present on the patient's malignant B cells, which allowed to eliminate recipient lymphocytes while sparing the infused CD52-negative CAR T cells. Donor-derived, gene-edited allogeneic T cells may have the potential to provide CAR T cell products for numerous recipients; however, the safe treatment with allogeneic CAR T cells still requires additional editing of self-recognition molecules.

11. CAR T cells are successful in first clinical explorations

Almost 100 early phase trials in the adoptive therapy of cancer with second generation CAR T cells have been initiated pushing the field into a new era [66]. During the last years, good manufacturing practice (GMP) procedures have been developed for genetically engineering patient's T cells from autologous leukapheresis products and for amplifying the modified cells by CD3/CD28 bead stimulation during a 10-day period to clinically relevant numbers. In the majority of trials, retro- or lentivirus transductions are used to genetically modify the

T cells; electroporation-mediated DNA or RNA transfer is also applied in some trials. DNA transposons have been used to efficiently insert gene cassettes into the host genomic DNA [67–69]. DNA transposon-based systems, such as the Sleeping Beauty (SB) and the PiggyBac transposon, have been used to engineer CAR T cells for clinical applications [70–72]. Although there is a theoretical risk of insertional oncogenesis, no transforming event in mature T cells after viral or non-viral gene transfer was so far observed.

The ex vivo amplification of CAR T cells is currently performed in the presence of IL-7 and IL-15 or IL-21 [73] which favors a more rapid expansion of less matured T cells which provide a more robust persistence, cytokine release, and cytolytic activity compared with anti-CD3 antibody and IL-2-amplified T cells. While static culture systems have been traditionally used, shaking reactors or bags, and gas-permeable rapid expansion culture-ware (G-Rex) [74] are sustaining T cell expansion to much higher densities. The currently entirely manual process is going to be translated into a closed, fully automated and supervised system. Automated systems will allow the production of multiple batches in parallel which will be required when clinical exploration in trials transforms to standard applications to a huge number of patients.

In a number of trials, CAR T cells targeting CD19 produced significant therapeutic efficacy in the treatment of B cell malignancies, including so far refractory B cell chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), and various other types of B cell malignancies [5, 66]. The success, however, is rather heterogeneous; CAR T cell therapy of pediatric and adult ALL achieved complete remission rates of about 90% and sustained remissions for more than 4 years [75]; in CLL, about 63% overall remissions and 19% complete remissions were achieved [76]. T cells were effective upon one application only and even at low dosage levels of about 1.5×10^5 cells per kg. CAR T cells were capable to expand more than 1000-fold after administration and to persist in the peripheral blood and bone marrow for months and in some patients for years. Clinical trials targeting B cell leukemia/lymphoma are currently entering phase II development by pharmaceutical companies.

The trials have been performed by academic centers and major pharmaceutical companies; some of the CAR T cells provided by Novartis, Juno Therapeutics, and Kite Pharma received "breakthrough designation" by the US Food and Drug Administration in 2014 and 2015. More than 50 trials are currently open to treat B cell malignancies with CD19-specific CAR T cells [66]. While the anti-CD19 CAR used in clinical exploration contains a murine scFv domain, fully human CARs are currently developed to avoid an anti-CAR immune response and finally depletion of CAR T cells through the host immune system. Most currently active trials use CARs with CD28 or 4-1BB costimulatory domain, alternative costimulation by OX40 [77], ICOS [78, 79], CD27 [80], CD40-MyD88 [81], CD2 [82], CD244 [83], and others are currently being studied.

Current efforts are aiming at sustaining engraftment and improving CAR T cell amplification and persistence in vivo. A key factor is thought to be the "preconditioning" of the patient's immune system through non-myeloablative lymphodepletion. A number of trials are exploring modifications of the basic regimens, and only a small minority of them does not perform preconditioning. Patient treatment furthermore includes reducing the bulk of tumor mass prior therapy, CAR T cell administration, systemic cytokine supplementation, and clinical managing of comorbidities and toxicities.

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While a number of centers are performing clinical trials, a direct comparison of therapeutic efficacies is difficult to make due to a number of differences in the CAR design and study protocols. However, a recent meta-analysis of CD19 CAR T cell trials confirmed lymphodepletion and CAR T cell dose as key factors for successful treatment, while IL-2 co-administration is not recommended [23]. Most patients with 4-1BB-CD3ζ CAR T cell therapy did not receive further treatment; patients treated with CD28-CD3ζ CAR T cells frequently underwent subsequent allogeneic stem cell transplantation; the clinical decision is partly based on the observation that 4-1BB-CD3ζ CAR T cells persist over years while CD28-CD3ζ CAR T cells persist only for a few months.

CAR T cell persistence is crucial to obtain lasting remission of the disease; no patients with B-ALL relapsed 1 year after CAR T cell infusion. If the CAR T cells do not persist that long, a consolidation approach such as allogeneic stem cell transplantation may be required. To improve CAR T cell persistence in vivo, virus-specific T cells are applied which engage viral antigens through their TCR in a repetitive fashion and thereby obtain survival signals independently of CAR engagement of tumor target. For instance, Epstein-Barr virus (EBV) specific, autologous CAR T cells persisted longer after infusion than non-virus specific CAR T cells from the same patient [84]. T cells of other specificities toward endogenous virus antigens are also envisaged. In addition, the stage of maturation impacts the T cell persistence. Less differentiated T cells such as naïve, stem cell memory, and central memory T cells seem to provide a more persistent anti-tumor response as compared with effector T cells [85–87]. In particular, $CD4^+$ $CD45RO^+$ $CD62L^+$ memory T cells seem to be superior in the long-term providing the rationale to explore $CD62L^+$-enriched CAR T cells for clinical application.

Apart from CD19, alternative targets for B-cell malignancies such as CD20, CD22, the Igκ light chain, ROR-1 for B-NHL and B-ALL, and CD30 for Hodgkin's lymphoma are being actively studied as CAR T cell targets. The Fcμ receptor seems to be a more selective candidate target for the treatment of CLL in experimental settings in order to spare healthy B cells from a CAR T cell attack [88].

12. CAR T cell therapy of solid cancer is still challenging

The capability of T cells to home to specific targets throughout the body basically allows the elimination of widespread and metastatic tumor lesions. However, the treatment of larger solid cancer lesions is challenging due to multiple reasons.

First, CAR T cells need to traffic to the tumor lesion in the periphery which depends on a number of soluble and cell bound factors, in particular chemokines. Extensively amplified CAR T cells often express an altered panel of chemokine receptors; transgenic co-expression of chemokine receptors can enforce specific trafficking, for example, transgenic CXCR2 (CXCL1 receptor) improves trafficking to melanoma [89] and CCR2b to neuroblastoma [90]. On the other hand, blocking of migration inhibitory receptors like endothelin-B receptor improves T cell infiltration into the tumor lesion [91]. Targeting vascular endothelial growth factor (VEGF) receptor-2, which is over-expressed by tumor endothelial cells, improves vascular evasion of

CAR T cells [92]. Normalization of vasculature by low-dose angiogenesis inhibitors may also be efficacious in the long term [93]. T cells can penetrate the central nervous system (CNS) by migrating through the blood-brain barrier [94]; T cells also infiltrate other immune-privileged sites such as the testes and eyes [95]. The profound migratory capacity of T cells allows the treatment of tumors which are otherwise difficult to access like brain tumors and prostate cancer. However, T cell extravasation and migration are frequently inhibited by various means including the loss of adhesion molecules on vascular endothelial cells [96–98], an altered chemokine milieu [99, 100] and immune suppression by a plethora of inhibitory molecules. Thus, the risk of targeting healthy tissues and the localization of bulky tumor mass demands to decide between local and systemic application of T cells. In some trials, CAR T cells are locally applied by endoscopy or puncture into or in near vicinity of the tumor lesion [101].

Second, tumor tissues execute immune suppression by various means through cells like regulatory T (Treg) cells or myeloid-derived suppressor cells (MDSCs), suppressive cytokines such as IL-10 or TGF-β, or other factors such as IDO, glucose depletion, nutrient deprivation, and acidosis. The inhibitory ligands in tumors are furthermore increased upon an immune attack. On the other hand, CAR T cells express immune repressive receptors upon activation, including programed cell death-1 (PD-1), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), or Fas, which upon ligand interaction repress the T cell response. To overcome the situation, a growing number of strategies are explored to make CAR T cells more resistant to repression. For instance, the anti-tumor activity of CAR T cells in the presence of Treg cells is improved by abrogation of IL-2 release through deletion of the lck binding site in the CD28 CAR signaling domain [102]. On the other hand, CAR-mediated CD28 costimulation overcomes TGF-β repression resulting in an improved tumor cell killing [103]. Suppression by TGF-β can moreover be prevented by engineering T cells with a dominant negative mutant of TGF-β [104, 105]. PD-1 upregulation within the tumor suppresses the T cell anti-tumor response; blocking the PD-1/PD-1 ligand pathway through PD-1 antibody checkpoint blockade, cell-intrinsic PD-1 shRNA blockade, or a PD-1 dominant negative receptor, improves CAR T cell activity in a preclinical model [25].

PD-1 expression correlated with exhaustion is moreover triggered by the CAR provided costimulation. In particular, CAR T cells with 4-1BB costimulation are less exhausted upon repetitive re-stimulation and retained their cytotoxic and cytokine secretion functions longer than CD28 CAR T cells. PD-1/PD-L1 blockade may be an effective strategy for improving the potency of CAR T cell therapies. Accordingly, antibody-mediated checkpoint blockade is currently explored in a clinical trial; PD-1 and CTLA-4 blockade are also explored in combination. In addition, antibodies to neutralize immune suppressive cytokines including GM-CSF, IL-6, IL-10, and VEGF may also improve the CAR T cell response. In the end, the best combination of checkpoint blockades to tackle the complex network of immune repression needs to be explored in clinical trials.

Third, some solid tumors have a strong stroma barrier which hampers the penetration of CAR T cells into the lesion. Evidences are increasing that successful treatment of advanced tumors requires breaking the barrier and eliminating the stroma cells; the latter is mediated by IFN-γ accompanied by M1 macrophage infiltration [106]. Costimulation increased IFN-γ release

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into the targeted tumor lesion which then acted on stroma cells in an antigen-independent fashion by both stroma destruction and activating the non-T cell immune compartment.

13. CAR T cell therapy-associated toxicities

CAR redirected T cell targeting is antigen specific, however, mostly not tumor selective as long as antigens are targeted which are also expressed by healthy cells. For instance, targeting CD19 produced a lasting depletion of healthy B cells which is clinically manageable by substitution with immunoglobulins and by antibiotic protection. In addition, antigen-independent toxicities may occur when a huge number of CAR T cells are heavily activated. The following toxicities are frequently observed (**Table 1**).

Limitations & challenges	Potential solutions
CAR design	Identify best suitable combination of CAR domains for targeting, T cell activation and counter-acting suppression in the specific tumor tissue by evaluating a panel of results effective variables, including the targeted epitope, the distance of the epitope from the membrane, the affinity of the targeting scFv, the spacer and transmembrane domains, the primary and co-stimulatory signaling, the number of CARs on T cell surface
T cell suppression	Make CAR T cells more resistant toward repression, for instance by secreting inhibitors of immunosuppressive cytokines such as IL-6, IL-10, and TGF-β; by secreting inhibitors of factors involved in the induction of MDSCs or Treg cells; by modified CD28 signaling deficient in IL-2 induction
PD-1/PD-L1 upregulation within the tumor tissue	Interfere PD-1/PD-L1 pathway through antibody checkpoint blockade; PD-1 suppression by shRNA; coexpression of a PD-1 dominant negative receptor
Impaired T cell migration and trafficking	Engineer CAR T cells to express chemokine receptors such as CXCR2 or CCR2b
Cytokine release syndrome (CRS)	Apply fractionated T cell doses; neutralize IL-6 function by application of tocilizumab
Vascular leakage syndrome (VLS)	Substitute peripheral blood volume; deplete serum from cytokines by plasmapheresis
Tumor lysis syndrome (TLS)	Reduce CAR T cell dose; split CAR T cell dosing; de-bulk tumor mass before CAR T cell therapy
Macrophage activation syndrome (MAS)	Neutralize IL-6 function by application of tocilizumab
Neurotoxicity	No specific treatment so far available; toxicity is transient and fully reversible
Anti-CD19 CAR T cell induced B cell aplasia	Replace immunoglobulins; provide antibiotic prophylaxis; co-express an inhibitory CAR to protect normal B cells; target an alternative, more selective antigen

Limitations & challenges	Potential solutions
"on-target off-tumor" toxicities	Identify more tumor-selective antigens, e.g., tumor-specific antigens, neo-antigens, antigens expressed only by non-essential healthy tissues, antigens physiologically expressed on apical surfaces, activation associated antigens; block target antigen with high dose of a specific antibody; co-express iCARS to prevent activation against healthy tissues; use combinatorial antigen recognition by two complementing CARs; use CARs with optimized recognition of cancer cell associated antigens; express the CAR transiently by RNA transfer; activate the CAR conditionally by a dimerizer; administer CAR T cells intratumorally
CAR T cell elimination	Co-express suicide genes, e.g., HSV-TK, iCasp9; deplete epitope marked CAR T cells by antibodies, e.g., targeting truncated CD34, EGFR, CD20; use a conditional CAR which is inactive in the absence of a dimerizer
GvHD after allogeneic T cell therapy	Engineer T cells with genetically edited endogenous TCR and HLA molecules
Commercialization	Provide "off-the-shelf" CAR T cell products with genetically edited allogeneic T cells
Tumor relapse by antigen escape cancer cells	Target more than one antigen by applying a CAR T cell mixture or T cells engineered with bispecific CARs
Poor in vivo expansion	Improve patient's pre-conditioning and/or cytokine supplementation

Table 1. Limitations, challenges, and potential solutions of CAR T cell therapy.

(i) "On-target on-tumor" toxicity describes a tumor lysis syndrome which is mediated through the rapid destruction of a large tumor mass in response to therapy. The release of tumor cell components into the circulation causes electrolyte and metabolic disturbances which can induce multi-organ failure.

(ii) "On-target off-tumor" toxicities occur when CAR T cells engage their cognate antigen on healthy tissue. Such autoimmune toxicity can be life-threatening, in particular, when targeting lung, heart, liver, or other essential organs. In the case of anti-CD19 CAR T cell therapy of B-cell malignancies, "on-target off-tumor" toxicity consistently causes lasting B cell aplasia and hypo-gammaglobulinemia which are clinically manageable and considered as biomarkers for the anti-CD19 CAR T cell function. "On-target off-tumor" toxicity is more serious when targeting ErbB2 expressed by a broad variety of epithelia resulting in fatal cardio-pulmonary failure [107]. The strength of T cell activation clearly impacts the severity of "on-target off-tumor" symptoms; reducing CAR signaling and a more cautious dose-escalation regimen lowers the risk of toxicities [108].

(iii) "Off-target off-tumor" toxicity can be induced by CAR T cells independently of cognate target recognition. For instance, the extracellular IgG1 Fc spacer in the CAR can activate cells of the innate immune system such as NK cells and macrophages through binding to the IgG Fc receptor (FcγR) resulting in a systemic inflammatory reaction. Modification of the CAR IgG1 Fc domain [14] or the use of the IgG4 domain reduces the risk of this type of side effects.

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(iv) Activation of a huge number of T cells results in the release of extensive amounts of pro-inflammatory cytokines, in particular IFN-γ and TNF-α, and the release of IL-6 upon activation of monocytes or macrophages, causing a cytokine release syndrome (CRS) with the risk of multiple organ failures. CRS is clinically characterized by fever, nausea, and supra-physiological serum levels of pro-inflammatory cytokines and is closely associated with the systemic macrophage activation syndrome, resembling hematophagocytic lymphohistiocytosis. CRS may also occur together with the vascular leakage syndrome (VLS). The occurrence of CRS is associated with clinical efficacy, a high tumor burden and the dose and potency of applied CAR T cells. However, CRS constitutes a major limitation of CAR T cell therapy, and the clinical management of cytokine-related toxicities is still challenging. The clinical symptoms can be reduced without diminishing the therapeutic efficacy by applying the anti-IL-6 receptor antibody tocilizumab which blocks the IL-6 receptor without eliminating the CAR T cells [109]. An algorithm of treatment has recently been proposed based on tumor burden, age, comorbidities, and other factors to standardize grading of CRS and to develop clinical guidelines for treatment [110, 111].

(v) Apart from CRS, neurotoxicity with aphasia, hallucinations, and delirium, is observed in about 40% of treated patients after CAR T cell application [112]. Neurotoxicity was mostly reversible and may be due to a diffuse encephalopathy caused by IL-6 released by brain infiltrating CAR T cells.

14. Strategies to improve safety of the CAR T cell therapy

Strategies were developed to improve safety while maintaining efficacy against tumors; these include target recognition, CAR design and expression as well as CAR T-cell elimination.

(i) Combinatorial antigen recognition

While truly tumor-specific antigens are rare, a pattern of antigens may be more indicative for cancer cells than the expression of a single marker. Redirecting CAR T cells specifically toward such an antigen signature is thought to provide more selectivity for cancer cells while sparing healthy cells. Therefore, two CARs recognizing two different antigens on cancer cells are co-expressed as follows: one CAR providing the primary activating signal and the other CAR providing the costimulatory signal [50–52]. Since both signals are required, only simultaneous engagement of both antigens initiates a productive T cell anti-tumor response while binding to one antigen is not sufficient.

(ii) Inhibitory CARs

A co-expressed inhibitory CAR (iCAR) is aimed at avoiding T cell activation when engaging healthy cells. The iCAR inhibitory signaling moiety is derived from the intracellular PD-1 or CTLA-4 signaling domain, provides a suppressor signal to the T cell upon recognition of an antigen present on healthy, but not on tumor cells, and is dominant over the activating signal provided by tumor-specific CAR, thereby preventing T cell activation against healthy tissues

[63]. The inhibitory effect by iCARs is present as long as the iCAR engages its cognate target; without iCAR signaling the co-expressed activating CAR triggers the T cell response toward the cognate cancer cell.

(iii) Optimized antigen recognition domain

To make a CAR more tumor selective the antigen binding domain was mutated with respect to improve binding to a variant antigen which is expressed by the cancer cells and less by healthy cells. For instance, a CAR was optimized in binding to the IL-13 receptor-α2 of cancer cells but less to the IL-13 receptor-α1 on healthy tissues [8, 9].

(iv) Transient CAR expression

The transient expression of the CAR by T cells transfected with in vitro transcribed RNA limits the CAR T cell response. The transfected RNA is diluted upon T cell division and degraded with time resulting in a half-life of CARs on the T cell surface in the order of several days. With this rationale, RNA-modified CAR T cells were applied with some anti-tumor efficacy so far [113]; however, repeated doses of CAR T cells produced an anti-CAR response due to xenogenic CAR components [114]. Upon activation of CAR T cells, the time of CAR expression is moreover shortened thereby limiting a potential side effect on healthy tissues [115].

(v) CAR T cell elimination

In case of non-controlled toxicity, CAR T cells can be eliminated by various means including a high-dose steroid treatment as applied in a trial with carboanhydrase IX-specific CAR T cells [116]. Another strategy takes an advantage of marking CAR T cells with a unique cell surface molecule to which an approved therapeutic antibody binds. For instance, the truncated EGFR, co-expressed with the CAR by the same T cell, can be targeted by the antibody cetuximab which efficiently eliminates those marked cells [117]. Efforts are also being undertaken to co-express the targetable epitope within the extracellular part of the CAR, thereby making the CAR itself a target of a depleting antibody [118]. An anti-idiotypic antibody directed against the scFv of the CAR itself may be used for depleting CAR T cells as well [119]. Alternatively, CAR T cells can be eliminated by the action of suicide genes. Basically, two strategies are currently explored, the co-expression of the herpes simplex virus thymidine kinase (HSV-tk) which phosphorylates the guanosine analog gancyclovir into a toxic derivative, or the co-expression of a truncated caspase-9 and a mutated FK506 binding protein which mediates dimerization through a non-toxic synthetic drug, thereby initiating the caspase-9 apoptotic cascade [120].

(vi) Routes of T cell administration

CAR T cell–associated toxicities are mitigated by applying the T cells in tumor burden-adapted doses or in fractionated doses. Usually CAR-modified T cells are administered by i.v. injection upon which the cells accumulate in the lung within 30 min and later on in the liver and spleen [121, 122]. Where possible, more localized routes of CAR T cell administration may avoid off-tumor T cell activation to some extent. For instance, CAR T cells are administered by endoscopic injection into the tumor lesion or liver metastases in some trials [123, 124].

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15. Future perspectives

CAR T cell therapy of hematological malignancies is likely to become established in clinical practice within the next years; CAR T cell therapy for the treatment of solid tumor lesions or for the elimination of residual cancer cells is still in its infancy. The situation is even more complex since the currently accumulating clinical results are difficult to compare due to a number of differences in CAR T cell engineering, clinical protocols, preconditioning of patients, and other relevant factors, demanding a more rigorous standardization of CAR T cell trials in the near future.

(i) Identification of the most suitable target

Tumor-selective antigens are preferred targets, however, are rare demanding the use of tumor-associated antigens as targets for a redirected T cell therapy. An example of a tumor-selective antigen is a glycosylation variant, like Muc1, or a specific mutation of protein identified by deep sequencing the cancer cell transcriptome. By decreasing selectivity, the risk of targeting healthy tissues with life-threatening toxicities increases demanding a thorough preclinical evaluation of potential other target cells and a cautious dose-escalation regimen when entering clinical exploration. Combinatorial antigen recognition, transient CAR expression, and inhibitory CARs are some examples which are expected to increase the safety in CAR T cell targeting, however, still need clinical exploration in a specific tumor context.

(ii) Optimizing the CAR design

There is obviously no universal CAR design which equally fits to each potential antigen; each CAR needs to be optimized with respect to both the particular target and to the T cell subset which is used for the anti-tumor attack. So far, the binding affinity, the targeted antigen epitope, and the extracellular length and transmembrane domain of the CAR as well as the provided costimulation were identified to be crucial for optimal T cell activation in the specific context.

(iii) T cell stage of maturation

CAR T cells with 4-1BB costimulation are primed toward a central memory phenotype, have a more enhanced catabolic activity and oxidative metabolism together with an enhanced mitochondrial respiratory capacity. With that respect, the 4-1BB CAR initiates a long-lasting central memory response while the CD28 CAR mediates a more short-lived effector cell response with enhanced glycolysis [24]. Moreover, the required co-signaling for optimal activation seems to be different for T cells in various stages of maturation; different co-stimuli are needed in different stages of maturation upon repetitive re-stimulation. For instance, T cells in the $CCR7^{-}$ maturation stage benefit from CD28-OX40 costimulation while CD28 costimulation is sufficient in younger stages of maturation [21].

(iv) Patient preconditioning

Non-myeloablative lymphodepletion of patients prior to T cell therapy seems to be mandatory to allow extensive amplification of CAR T cells after application. There may also be an additional impact of the preconditioning chemotherapy on the tumor milieu by depleting suppressor cells and/or releasing antigens recognized by patient's T cells. With that respect,

the clinical protocols substantially differ and need further standardization to allow conclusions with relevance for future trials.

(v) Clinical exploration and control of side effects

CAR T cell therapy-associated toxicities are life-threatening and need intensive care treatment. With the establishment of a CRS screening and grading protocol [110–112], first steps toward a more standardized clinical management are being made which need further attention in future trials. With the progression in the clinical exploration of CAR T cells, there is a need for novel pharmacokinetic and pharmacodynamic models to sustain the development of optimized mono- and combo-immunotherapies in the near future.

(vi) Hematopoietic stem cell transplantation

A number of patients in clinical remission after CAR T cell therapy were subsequently treated by hematopoietic stem cell transplantation, in particular patients treated with CD28 CAR T cells. While the anti-leukemia efficacy of CAR T cells is clearly documented, the benefit of stem cell transplantation to control the disease in the long term needs to be established.

(vii) SynNotch receptor-mediated immune cell activation

In a broader sense CARs are tools in the growing field of synthetic biology to engineer immune cells with defined specificity and to redirect a cellular response. As such, a modular receptor was designed on the basis of the Notch. At the extracellular side, the receptor binds to targets by an scFv or any other binding domain and at intracellular side effector domains such as transcriptional activators or repressors are released upon proteolytic cleavage which enter the nucleus for function [59, 125, 126]. Such receptors can be used in a broad variety of cells including immune cells to direct the induction of complex cellular responses [59]. The benefit and potential of such receptor types in the adoptive T cell therapy need further exploration.

(viii) CAR T cell–induced anti-tumor immunity

The CAR T cell response against targeted cancer cells can induce a second wave of anti-tumor immune response which is potentially capable of eliminating non-targeted cancer cells. In particular, mice treated with anti-EGFRvIII CAR T cells were shown to resist EGFRvIII negative tumor challenge [127]. The perception rises that a redirected T cell anti-tumor response may additionally have substantial impact on the host immune response itself than rather targeting the cognate cancer cells. Such secondary host response needs to be explored and improved toward a long-term control of cancer.

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Abbreviations

ACT	adoptive cell therapy
CAR	chimeric antigen receptor
CRS	cytokine release syndrome
CTLA-4	cytotoxic T lymphocyte-associated antigen-4
CSC	cancer stem cell
GMP	Good Manufacturing Practice
IFN	interferon
IL	interleukin
ITAM	immunoreceptor tyrosine activation motif
MHC	major histocompatibility complex
PD-1	programed cell death-1
scFv	single-chain fragment of variable region
TCR	T cell receptor

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References

[1] Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc Natl Acad Sci USA. 1993;90(2):720–4.

[2] Bridgeman JS, Hawkins RE, Hombach AA, Abken H, Gilham DE. Building better chimeric antigen receptors for adoptive T cell therapy. Curr Gene Ther. 2010;10(2):77–90.

[3] Stewart-Jones G, Wadle A, Hombach A, Shenderov E, Held G, Fischer E, et al. Rational development of high-affinity T-cell receptor-like antibodies. Proc Natl Acad Sci USA. 2009;106(14):5784–8.

[4] Ma Q, Garber HR, Lu S, He H, Tallis E, Ding X, et al. A novel TCR-like CAR with specificity for PR1/HLA-A2 effectively targets myeloid leukemia in vitro when expressed in human adult peripheral blood and cord blood T cells. Cytotherapy. 2016;18(8):985–94.

[5] Ruella M, June CH. Chimeric antigen receptor T cells for B cell neoplasms: choose the right CAR for you. Curr Hematol Malig Rep. 2016;11(5):368–84.

[6] Chmielewski M, Hombach AA, Abken H. Of CARs and TRUCKs: chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. Immunol Rev. 2014;257(1):83–90.

[7] Faitschuk E, Nagy V, Hombach AA, Abken H. A dual chain chimeric antigen receptor (CAR) in the native antibody format for targeting immune cells towards cancer cells without the need of an scFv. Gene Ther. 2016;23(10):718–26.

[8] Kahlon KS, Brown C, Cooper LJN, Raubitschek A, Forman SJ, Jensen MC. Specific recognition and killing of glioblastoma multiforme by interleukin 13-zetakine redirected cytolytic T cells. Cancer Res. 2004;64(24):9160–6.

[9] Kong S, Sengupta S, Tyler B, Bais AJ, Ma Q, Doucette S, et al. Suppression of human glioma xenografts with second-generation IL13R-specific chimeric antigen receptor-modified T cells. Clin Cancer Res. 2012;18(21):5949–60.

[10] Krebs S, Chow KKH, Yi Z, Rodriguez-Cruz T, Hegde M, Gerken C, et al. T cells redirected to interleukin-13Rα2 with interleukin-13 mutein--chimeric antigen receptors have anti-glioma activity but also recognize interleukin-13Rα1. Cytotherapy. 2014;16(8):1121–31.

[11] Hombach A, Heuser C, Gerken M, Fischer B, Lewalter K, Diehl V, et al. T cell activation by recombinant FcepsilonRI gamma-chain immune receptors: an extracellular spacer domain impairs antigen-dependent T cell activation but not antigen recognition. Gene Ther. 2000;7(12):1067–75.

[12] Hudecek M, Sommermeyer D, Kosasih PL, Silva-Benedict A, Liu L, Rader C, et al. The nonsignaling extracellular spacer domain of chimeric antigen receptors is decisive for in vivo antitumor activity. Cancer Immunol Res. 2015;3(2):125–35.

[13] Srivastava S, Riddell SR. Engineering CAR-T cells: design concepts. Trends Immunol. 2015;36(8):494–502.

[14] Hombach A, Hombach AA, Abken H. Adoptive immunotherapy with genetically engineered T cells: modification of the IgG1 Fc "spacer" domain in the extracellular moiety of chimeric antigen receptors avoids "off-target" activation and unintended initiation of an innate immune response. Gene Ther. 2010;17(10):1206–13.

[15] Bridgeman JS, Hawkins RE, Bagley S, Blaylock M, Holland M, Gilham DE. The optimal antigen response of chimeric antigen receptors harboring the CD3 transmembrane domain is dependent upon incorporation of the receptor into the endogenous TCR/CD3 complex. J Immunol. 2010;184(12):6938–49.

http://dx.doi.org/10.5772/66496

[16] Irving BA, Weiss A. The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. Cell. 1991;64(5): 891–901.

[17] Geiger TL, Leitenberg D, Flavell RA. The TCR zeta-chain immunoreceptor tyrosine-based activation motifs are sufficient for the activation and differentiation of primary T lymphocytes. J Immunol. 1999;162(10):5931–9.

[18] Alvarez-Vallina L, Hawkins RE. Antigen-specific targeting of CD28-mediated T cell co-stimulation using chimeric single-chain antibody variable fragment-CD28 receptors. Eur J Immunol. 1996;26(10):2304–9.

[19] Finney HM, Lawson AD, Bebbington CR, Weir AN. Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. J Immunol. 1998;161(6):2791–7.

[20] Hombach A, Sent D, Schneider C, Heuser C, Koch D, Pohl C, et al. T-cell activation by recombinant receptors CD28 costimulation is required for interleukin 2 secretion and receptor-mediated T-cell proliferation but does not affect receptor-mediated target cell lysis. Cancer Res. 2001;61(5):1976–82.

[21] Hombach AA, Chmielewski M, Rappl G, Abken H. Adoptive immunotherapy with redirected T cells produces CCR7-cells that are trapped in the periphery and benefit from combined CD28-OX40 costimulation. Hum Gene Ther. 2013;24(3):259–69.

[22] Hombach A, Abken H. Costimulation tunes tumor-specific activation of redirected T cells in adoptive immunotherapy. Cancer Immunol Immunother. 2007;56(5):731–7.

[23] Zhang T, Cao L, Xie J, Shi N, Zhang Z, Luo Z, et al. Efficiency of CD19 chimeric antigen receptor-modified T cells for treatment of B cell malignancies in phase I clinical trials: a meta-analysis. Oncotarget. 2015;6(32):33961–71.

[24] Kawalekar OU, O'Connor RS, Fraietta JA, Guo L, McGettigan SE, Posey AD, et al. Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. Immunity. 2016;44(2):380–90.

[25] Cherkassky L, Morello A, Villena-Vargas J, Feng Y, Dimitrov DS, Jones DR, et al. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. J Clin Invest. 2016;126(8):3130–44.

[26] Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. Expert Opin Biol Ther. 2015;15(8):1145–54.

[27] Pegram HJ, Park JH, Brentjens RJ. CD28z CARs and armored CARs. Cancer J. 2014; 20(2):127–33.

[28] Chmielewski M, Kopecky C, Hombach AA, Abken H. IL-12 release by engineered T cells expressing chimeric antigen receptors can effectively Muster an antigen-independent macrophage response on tumor cells that have shut down tumor antigen expression. Cancer Res. 2011;71(17):5697–706.

[29] Yeku OO, Brentjens RJ. Armored CAR T-cells: utilizing cytokines and pro-inflammatory ligands to enhance CAR T-cell anti-tumour efficacy. Biochem Soc Trans. 2016;44(2):412–8.

[30] Ligtenberg MA, Mougiakakos D, Mukhopadhyay M, Witt K, Lladser A, Chmielewski M, et al. Coexpressed catalase protects chimeric antigen receptor-redirected T cells as well as bystander cells from oxidative stress-induced loss of antitumor activity. J Immunol. 2016;196(2):759–66.

[31] Caruana I, Savoldo B, Hoyos V, Weber G, Liu H, Kim ES, et al. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. Nat Med. 2015;21(5):524–9.

[32] Kaczanowska S, Joseph AM, Davila E. TLR agonists: our best frenemy in cancer immunotherapy. J Leukoc Biol. 2013;93(6):847–63.

[33] Geng D, Kaczanowska S, Tsai A, Younger K, Ochoa A, Rapoport AP, et al. TLR5 ligand-secreting T cells reshape the tumor microenvironment and enhance antitumor activity. Cancer Res. 2015;75(10):1959–71.

[34] Gross G, Eshhar Z. Therapeutic potential of T cell chimeric antigen receptors (CARs) in cancer treatment: counteracting off-tumor toxicities for safe CAR T cell therapy. Annu Rev Pharmacol Toxicol. 2016;56:59–83.

[35] van der Burg SH, Arens R, Ossendorp F, van Hall T, Melief CJM. Vaccines for established cancer: overcoming the challenges posed by immune evasion. Nat Rev Cancer. 2016;16(4):219–33.

[36] Ahmed N, Salsman VS, Kew Y, Shaffer D, Powell S, Zhang YJ, et al. HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors. Clin Cancer Res. 2010;16(2):474–85.

[37] Brown CE, Starr R, Aguilar B, Shami AF, Martinez C, D'Apuzzo M, et al. Stem-like tumor-initiating cells isolated from IL13Rα2 expressing gliomas are targeted and killed by IL13-zetakine-redirected T Cells. Clin Cancer Res. 2012;18(8):2199–209.

[38] Morgan RA, Johnson LA, Davis JL, Zheng Z, Woolard KD, Reap EA, et al. Recognition of glioma stem cells by genetically modified T cells targeting EGFRvIII and development of adoptive cell therapy for glioma. Hum Gene Ther. 2012;23(10):1043–53.

[39] Zhu X, Prasad S, Gaedicke S, Hettich M, Firat E, Niedermann G. Patient-derived glioblastoma stem cells are killed by CD133-specific CAR T cells but induce the T cell aging marker CD57. Oncotarget. 2015;6(1):171–84.

[40] Schmidt P, Kopecky C, Hombach A, Zigrino P, Mauch C, Abken H. Eradication of melanomas by targeted elimination of a minor subset of tumor cells. Proc Natl Acad Sci USA. 2011;108(6):2474–9.

[41] Hombach AA, Görgens A, Chmielewski M, Murke F, Kimpel J, Giebel B, et al. Superior therapeutic index in lymphoma therapy: CD30(+) CD34(+) hematopoietic stem cells resist a chimeric antigen receptor T-cell attack. Mol Ther. 2016;24(8):1423–34.

http://dx.doi.org/10.5772/66496

[42] Hombach AA, Abken H. Shared target antigens on cancer cells and tissue stem cells: go or no-go for CAR T cells? Expert Rev Clin Immunol. 2016;1–5.

[43] Vera J, Savoldo B, Vigouroux S, Biagi E, Pule M, Rossig C, et al. T lymphocytes redirected against the kappa light chain of human immunoglobulin efficiently kill mature B lymphocyte-derived malignant cells. Blood. 2006;108(12):3890–7.

[44] Baskar S, Kwong KY, Hofer T, Levy JM, Kennedy MG, Lee E, et al. Unique cell surface expression of receptor tyrosine kinase ROR1 in human B-cell chronic lymphocytic leukemia. Clin Cancer Res. 2008;14(2):396–404.

[45] Daneshmanesh AH, Mikaelsson E, Jeddi-Tehrani M, Bayat AA, Ghods R, Ostadkarampour M, et al. Ror1, a cell surface receptor tyrosine kinase is expressed in chronic lymphocytic leukemia and may serve as a putative target for therapy. Int J Cancer. 2008;123(5):1190–5.

[46] Gentile A, Lazzari L, Benvenuti S, Trusolino L, Comoglio PM. Ror1 is a pseudokinase that is crucial for Met-driven tumorigenesis. Cancer Res. 2011;71(8):3132–41.

[47] Hudecek M, Schmitt TM, Baskar S, Lupo-Stanghellini MT, Nishida T, Yamamoto TN, et al. The B-cell tumor-associated antigen ROR1 can be targeted with T cells modified to express a ROR1-specific chimeric antigen receptor. Blood. 2010;116(22):4532–41.

[48] Grada Z, Hegde M, Byrd T, Shaffer DR, Ghazi A, Brawley VS, et al. TanCAR: a novel bispecific chimeric antigen receptor for cancer immunotherapy. Mol Ther Nucleic Acids. 2013;2:e105.

[49] Zah E, Lin M-Y, Silva-Benedict A, Jensen MC, Chen YY. T cells expressing CD19/CD20 bispecific chimeric antigen receptors prevent antigen escape by malignant B cells. Cancer Immunol Res. 2016;4(6):498–508.

[50] Wilkie S, van Schalkwyk MCI, Hobbs S, Davies DM, van der Stegen SJC, Pereira ACP, et al. Dual targeting of ErbB2 and MUC1 in breast cancer using chimeric antigen receptors engineered to provide complementary signaling. J Clin Immunol. 2012;32(5):1059–70.

[51] Lanitis E, Poussin M, Klattenhoff AW, Song D, Sandaltzopoulos R, June CH, et al. Chimeric antigen receptor T Cells with dissociated signaling domains exhibit focused antitumor activity with reduced potential for toxicity in vivo. Cancer Immunol Res. 2013;1(1):43–53.

[52] Kloss CC, Condomines M, Cartellieri M, Bachmann M, Sadelain M. Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells. Nat Biotechnol. 2012;31(1):71–5.

[53] Kudo K, Imai C, Lorenzini P, Kamiya T, Kono K, Davidoff AM, et al. T lymphocytes expressing a CD16 signaling receptor exert antibody-dependent cancer cell killing. Cancer Res. 2014;74(1):93–103.

[54] Cartellieri M, Feldmann A, Koristka S, Arndt C, Loff S, Ehninger A, et al. Switching CAR T cells on and off: a novel modular platform for retargeting of T cells to AML blasts. Blood Cancer J. 2016;6(8):e458.

[55] Kim MS, Ma JSY, Yun H, Cao Y, Kim JY, Chi V, et al. Redirection of genetically engineered CAR-T cells using bifunctional small molecules. J Am Chem Soc. 2015;137(8):2832–5.

[56] Rodgers DT, Mazagova M, Hampton EN, Cao Y, Ramadoss NS, Hardy IR, et al. Switch-mediated activation and retargeting of CAR-T cells for B-cell malignancies. Proc Natl Acad Sci USA. 2016;113(4):E459–468.

[57] Wu C-Y, Roybal KT, Puchner EM, Onuffer J, Lim WA. Remote control of therapeutic T cells through a small molecule-gated chimeric receptor. Science. 2015;350(6258):aab4077.

[58] Morsut L, Roybal KT, Xiong X, Gordley RM, Coyle SM, Thomson M, et al. Engineering customized cell sensing and response behaviors using synthetic notch receptors. Cell. 2016;164(4):780–91.

[59] Roybal KT, Rupp LJ, Morsut L, Walker WJ, McNally KA, Park JS, et al. Precision tumor recognition by T cells with combinatorial antigen-sensing circuits. Cell. 2016;164(4):770–9.

[60] Kobold S, Grassmann S, Chaloupka M, Lampert C, Wenk S, Kraus F, et al. Impact of a new fusion receptor on PD-1-mediated immunosuppression in adoptive T cell therapy. J Natl Cancer Inst. 2015;107(8).

[61] Liu X, Ranganathan R, Jiang S, Fang C, Sun J, Kim S, et al. A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR T cells in advanced solid tumors. Cancer Res. 2016;76(6):1578–90.

[62] Prosser ME, Brown CE, Shami AF, Forman SJ, Jensen MC. Tumor PD-L1 co-stimulates primary human CD8(+) cytotoxic T cells modified to express a PD1:CD28 chimeric receptor. Mol Immunol. 2012;51(3–4):263–72.

[63] Fedorov VD, Themeli M, Sadelain M. PD-1- and CTLA-4-based inhibitory chimeric antigen receptors (iCARs) divert off-target immunotherapy responses. Sci Transl Med. 2013;5(215):215ra172.

[64] Torikai H, Reik A, Liu P-Q, Zhou Y, Zhang L, Maiti S, et al. A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. Blood. 2012;119(24):5697–705.

[65] Poirot L, Philip B, Schiffer-Mannioui C, Le Clerre D, Chion-Sotinel I, Derniame S, et al. Multiplex genome-edited T-cell manufacturing platform for "Off-the-Shelf" adoptive T-cell immunotherapies. Cancer Res. 2015;75(18):3853–64.

[66] Holzinger A, Barden M, Abken H. The growing world of CAR T cell trials: a systematic review. Cancer Immunol Immunother. 2016; 65(12):1433-50.

[67] Kay MA. State-of-the-art gene-based therapies: the road ahead. Nat Rev Genet. 2011; 12(5):316–28.

[68] Gueguen E, Rousseau P, Duval-Valentin G, Chandler M. The transpososome: control of transposition at the level of catalysis. Trends Microbiol. 2005;13(11):543–9.

[69] Liu H, Visner GA. Applications of Sleeping Beauty transposons for nonviral gene therapy. IUBMB Life. 2007;59(6):374–9.

http://dx.doi.org/10.5772/66496

[70] Singh H, Figliola MJ, Dawson MJ, Olivares S, Zhang L, Yang G, et al. Manufacture of clinical-grade CD19-specific T cells stably expressing chimeric antigen receptor using Sleeping Beauty system and artificial antigen presenting cells. PloS One. 2013;8(5):e64138.

[71] Singh H, Moyes JSE, Huls MH, Cooper LJN. Manufacture of T cells using the Sleeping Beauty system to enforce expression of a CD19-specific chimeric antigen receptor. Cancer Gene Ther. 2015;22(2):95–100.

[72] Manuri PVR, Wilson MH, Maiti SN, Mi T, Singh H, Olivares S, et al. piggyBac transposon/transposase system to generate CD19-specific T cells for the treatment of B-lineage malignancies. Hum Gene Ther. 2010;21(4):427–37.

[73] Kaneko S, Mastaglio S, Bondanza A, Ponzoni M, Sanvito F, Aldrighetti L, et al. IL-7 and IL-15 allow the generation of suicide gene-modified alloreactive self-renewing central memory human T lymphocytes. Blood. 2009;113(5):1006–15.

[74] Vera JF, Brenner LJ, Gerdemann U, Ngo MC, Sili U, Liu H, et al. Accelerated production of antigen-specific T cells for preclinical and clinical applications using gas-permeable rapid expansion cultureware (G-Rex). J Immunother. 2010;33(3):305–15.

[75] Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014; 371(16):1507–17.

[76] Singh N, Frey NV, Grupp SA, Maude SL. CAR T cell therapy in acute lymphoblastic leukemia and potential for chronic lymphocytic leukemia. Curr Treat Options Oncol. 2016;17(6):28.

[77] Hombach AA, Abken H. Costimulation by chimeric antigen receptors revisited the T cell antitumor response benefits from combined CD28-OX40 signalling. Int J Cancer. 2011;129(12):2935–44.

[78] Shen C-J, Yang Y-X, Han EQ, Cao N, Wang Y-F, Wang Y, et al. Chimeric antigen receptor containing ICOS signaling domain mediates specific and efficient antitumor effect of T cells against EGFRvIII expressing glioma. J Hematol Oncol. 2013;6:33.

[79] Guedan S, Chen X, Madar A, Carpenito C, McGettigan SE, Frigault MJ, et al. ICOS-based chimeric antigen receptors program bipolar TH17/TH1 cells. Blood. 2014;124(7):1070–80.

[80] Song D-G, Ye Q, Poussin M, Harms GM, Figini M, Powell DJ. CD27 costimulation augments the survival and antitumor activity of redirected human T cells in vivo. Blood. 2012;119(3):696–706.

[81] Foster AE, Chang P, Lin P-Y, Crisostomo J, Mahendravada A, Lu A, et al. MyD88/CD40-based costimulation to enhance survival and proliferation of chimeric antigen receptor (CAR)-modified T cells. ASCO Meet Abstracts. 2015;33(15_suppl):3064.

[82] Cheadle EJ, Rothwell DG, Bridgeman JS, Sheard VE, Hawkins RE, Gilham DE. Ligation of the CD2 co-stimulatory receptor enhances IL-2 production from first-generation chimeric antigen receptor T cells. Gene Ther. 2012;19(11):1114–20.

[83] Altvater B, Landmeier S, Pscherer S, Temme J, Juergens H, Pule M, et al. 2B4 (CD244) signaling via chimeric receptors costimulates tumor-antigen specific proliferation and in vitro expansion of human T cells. Cancer Immunol Immunother. 2009;58(12):1991–2001.

[84] Louis CU, Savoldo B, Dotti G, Pule M, Yvon E, Myers GD, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood. 2011;118(23):6050–6.

[85] Klebanoff CA, Gattinoni L, Restifo NP. Sorting through subsets: which T-cell populations mediate highly effective adoptive immunotherapy? J Immunother. 2012;35(9):651–60.

[86] Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF, et al. A human memory T cell subset with stem cell-like properties. Nat Med. 2011;17(10):1290–7.

[87] Singh N, Perazzelli J, Grupp SA, Barrett DM. Early memory phenotypes drive T cell proliferation in patients with pediatric malignancies. Sci Transl Med. 2016;8(320):320ra3.

[88] Faitschuk E, Hombach AA, Frenzel LP, Wendtner C-M, Abken H. Chimeric antigen receptor T cells targeting Fc μ receptor selectively eliminate CLL cells while sparing healthy B cells. Blood. 2016;128(13):1711–22.

[89] Kershaw MH, Wang G, Westwood JA, Pachynski RK, Tiffany HL, Marincola FM, et al. Redirecting migration of T cells to chemokine secreted from tumors by genetic modification with CXCR2. Hum Gene Ther. 2002;13(16):1971–80.

[90] Craddock JA, Lu A, Bear A, Pule M, Brenner MK, Rooney CM, et al. Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b. J Immunother. 2010;33(8):780–8.

[91] Kandalaft LE, Facciabene A, Buckanovich RJ, Coukos G. Endothelin B receptor, a new target in cancer immune therapy. Clin Cancer Res. 2009;15(14):4521–8.

[92] Chinnasamy D, Yu Z, Theoret MR, Zhao Y, Shrimali RK, Morgan RA, et al. Gene therapy using genetically modified lymphocytes targeting VEGFR-2 inhibits the growth of vascularized syngenic tumors in mice. J Clin Invest. 2010;120(11):3953–68.

[93] Huang Y, Yuan J, Righi E, Kamoun WS, Ancukiewicz M, Nezivar J, et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. Proc Natl Acad Sci USA. 2012;109(43):17561–6.

[94] Pule MA, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med. 2008;14(11):1264–70.

[95] Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood. 2016;127(26):3321–30.

[96] Engelhardt B, Ransohoff RM. Capture, crawl, cross: the T cell code to breach the blood–brain barriers. Trends Immunol. 2012;33(12):579–89.

http://dx.doi.org/10.5772/66496

[97] Motz GT, Coukos G. Deciphering and reversing tumor immune suppression. Immunity. 2013;39(1):61–73.

[98] Bouzin C, Brouet A, De Vriese J, Dewever J, Feron O. Effects of vascular endothelial growth factor on the lymphocyte-endothelium interactions: identification of caveolin-1 and nitric oxide as control points of endothelial cell anergy. J Immunol. 2007;178(3):1505–11.

[99] Franciszkiewicz K, Boissonnas A, Boutet M, Combadière C, Mami-Chouaib F. Role of chemokines and chemokine receptors in shaping the effector phase of the antitumor immune response. Cancer Res. 2012;72(24):6325–32.

[100] Oelkrug C, Ramage JM. Enhancement of T cell recruitment and infiltration into tumours. Clin Exp Immunol. 2014;178(1):1–8.

[101] Katz SC, Burga RA, McCormack E, Wang LJ, Mooring W, Point GR, et al. Phase I hepatic immunotherapy for metastases study of intra-arterial chimeric antigen receptor-modified T-cell therapy for CEA+ liver metastases. Clin Cancer Res. 2015;21(14):3149–59.

[102] Kofler DM, Chmielewski M, Rappl G, Hombach A, Riet T, Schmidt A, et al. CD28 costimulation Impairs the efficacy of a redirected t-cell antitumor attack in the presence of regulatory t cells which can be overcome by preventing Lck activation. Mol Ther. 2011;19(4):760–7.

[103] Koehler H, Kofler D, Hombach A, Abken H. CD28 costimulation overcomes transforming growth factor-beta-mediated repression of proliferation of redirected human CD4+ and CD8+ T cells in an antitumor cell attack. Cancer Res. 2007;67(5):2265–73.

[104] Foster AE, Dotti G, Lu A, Khalil M, Brenner MK, Heslop HE, et al. Antitumor activity of EBV-specific T lymphocytes transduced with a dominant negative TGF-beta receptor. J Immunother. 2008;31(5):500–5.

[105] Bendle GM, Linnemann C, Bies L, Song J-Y, Schumacher TNM. Blockade of TGF-β signaling greatly enhances the efficacy of TCR gene therapy of cancer. J Immunol. 2013;191(6):3232–9.

[106] Textor A, Listopad JJ, Wührmann LL, Perez C, Kruschinski A, Chmielewski M, et al. Efficacy of CAR T-cell therapy in large tumors relies upon stromal targeting by IFNγ. Cancer Res. 2014;74(23):6796–805.

[107] Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther. 2010;18(4):843–51.

[108] Ahmed N, Brawley VS, Hegde M, Robertson C, Ghazi A, Gerken C, et al. Human epidermal growth factor receptor 2 (HER2)-specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma. J Clin Oncol. 2015;33(15):1688–96.

[109] Chen F, Teachey DT, Pequignot E, Frey N, Porter D, Maude SL, et al. Measuring IL-6 and sIL-6R in serum from patients treated with tocilizumab and/or siltuximab following CAR T cell therapy. J Immunol Methods. 2016;434:1–8.

[110] Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and toxicity management of 19–28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med. 2014;6(224):224ra25.

[111] Maude SL, Barrett D, Teachey DT, Grupp SA. Managing cytokine release syndrome associated with novel T cell-engaging therapies. Cancer J. 2014;20(2):119–22.

[112] Teachey DT, Lacey SF, Shaw PA, Melenhorst JJ, Maude SL, Frey N, et al. Identification of predictive biomarkers for cytokine release syndrome after chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. Cancer Discov. 2016;6(6):664–79.

[113] Beatty GL, Haas AR, Maus MV, Torigian DA, Soulen MC, Plesa G, et al. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. Cancer Immunol Res. 2014;2(2):112–20.

[114] Maus MV, Haas AR, Beatty GL, Albelda SM, Levine BL, Liu X, et al. T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. Cancer Immunol Res. 2013;1(1):26–31.

[115] Birkholz K, Hombach A, Krug C, Reuter S, Kershaw M, Kämpgen E, et al. Transfer of mRNA encoding recombinant immunoreceptors reprograms CD4+ and CD8+ T cells for use in the adoptive immunotherapy of cancer. Gene Ther. 2009;16(5):596–604.

[116] Lamers CHJ, Sleijfer S, Vulto AG, Kruit WHJ, Kliffen M, Debets R, et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. J Clin Oncol. 2006;24(13):e20–22.

[117] Wang X, Chang W-C, Wong CW, Colcher D, Sherman M, Ostberg JR, et al. A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. Blood. 2011;118(5):1255–63.

[118] Philip B, Kokalaki E, Mekkaoui L, Thomas S, Straathof K, Flutter B, et al. A highly compact epitope-based marker/suicide gene for easier and safer T-cell therapy. Blood. 2014;124(8):1277–87.

[119] Jena B, Maiti S, Huls H, Singh H, Lee DA, Champlin RE, et al. Chimeric antigen receptor (CAR)-specific monoclonal antibody to detect CD19-specific T cells in clinical trials. PloS One. 2013;8(3):e57838.

[120] Straathof KC, Pulè MA, Yotnda P, Dotti G, Vanin EF, Brenner MK, et al. An inducible caspase 9 safety switch for T-cell therapy. Blood. 2005;105(11):4247–54.

[121] Kershaw MH, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res. 2006;12(20 Pt 1):6106–15.

http://dx.doi.org/10.5772/66496

[122] Ritchie DS, Neeson PJ, Khot A, Peinert S, Tai T, Tainton K, et al. Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. Mol Ther. 2013;21(11):2122–9.

[123] Parente-Pereira AC, Burnet J, Ellison D, Foster J, Davies DM, van der Stegen S, et al. Trafficking of CAR-engineered human T cells following regional or systemic adoptive transfer in SCID beige mice. J Clin Immunol. 2011;31(4):710–8.

[124] Katz SC, Point GR, Cunetta M, Thorn M, Guha P, Espat NJ, et al. Regional CAR-T cell infusions for peritoneal carcinomatosis are superior to systemic delivery. Cancer Gene Ther. 2016;23(5):142–8.

[125] Gordon WR, Zimmerman B, He L, Miles LJ, Huang J, Tiyanont K, et al. Mechanical allostery: evidence for a force requirement in the proteolytic activation of notch. Dev Cell. 2015;33(6):729–36.

[126] Daringer NM, Dudek RM, Schwarz KA, Leonard JN. Modular extracellular sensor architecture for engineering mammalian cell-based devices. ACS Synth Biol. 2014;3(12):892–902.

[127] Sampson JH, Choi BD, Sanchez-Perez L, Suryadevara CM, Snyder DJ, Flores CT, et al. EGFRvIII mCAR-modified T-cell therapy cures mice with established intracerebral glioma and generates host immunity against tumor-antigen loss. Clin Cancer Res. 2014;20(4):972–84.

Chapter 10

The Memory Activation of NK Cells: New Methods in Cancer Immunotherapy

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Additional information is available at the end of the chapter

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Abstract

Cancer remains a main cause of mortality, despite the research efforts to unravel molecular mechanisms and for developing personalized targeted therapies with acceptable side effects. In cancer, both players, the aggressor (tumor cells) and the endogenous defenders (immune cells), are key therapeutic targets. Immunotherapy is nowadays considered the fourth therapeutical approach in cancer, complementing and sometimes replacing surgery and chemo- and radiotherapy. Natural killer (NK) cells, generally considered part of the innate immune system, play a critical role in defense against pathogens and tumors. Immunological memory is a hallmark of the adaptive immune system. However, NK cells have been shown to mediate Ag-specific recall responses and acquire immunological memory in a manner similar to that of T and B cells. This chapter summarizes evidence for NK cell immunotherapy, evidence and characteristics of NK cell memory and mechanisms involved in the generation and survival of these cells. There is no doubt that NK cells have major role in cancer treatments and viral infections, and in the future, NK cell immunotherapy from "a new hope" may become "a reality" for malignant diseases.

Keywords: immunotherapy, natural killer (NK) cells, innate memory, adoptive cell transfer, cancer

1. Introduction

Cancer represents one of the major causes of mortality, despite huge research efforts for deciphering the molecular mechanisms of disease and for developing new targeted and personalized therapeutic approaches with acceptable side effects. There are still problems to overcome, which are linked to the following: (a) heterogeneity of cancer cells within tumors, which is mirrored in their response to a specific therapy; for example, cancer stem cells possess inherent mechanisms for self-repair and renewal and are therefore often responsible of tumor

recurrence and metastasis; (b) complex mechanisms by which tumor cells avoid the innate and adaptive immune responses (adaptability, versatility, mimicry), including by immune suppression induction [1].

In addition to conventional therapeutic approaches (chemo- and radiotherapy), we face the shifting of the attention to the immune antitumor defense mechanisms which might offer a steady improvement to conventional therapies for tumor cells eradication. Cancer defiantly induces immune suppression and this is further deepened by anticancer therapies, which weaken the immune response, apart from killing tumor cells [2, 3].

In the last decade, immunotherapy gained a leading position in cancer research and management, due to promising results recently reported in clinical studies for (a) vaccines (sipuleucel-T) and monoclonal antibodies (ipilimumab), (b) recombinant cytokines and hematopoietic growth factors, and (c) cellular and gene therapies.

The adoptive transfer of lymphocytes with high antitumor reactivity can trigger tumor regression in metastatic melanoma [4–6]. Nowadays, autologous T lymphocytes are clinically used. *Ex vivo* multiplied tumor infiltrating lymphocytes (TILs) are adoptively transferred at the very patient from which they have been harvested and IL-2 is concomitantly administered in order to enhance T lymphocyte activation. This kind of immunotherapy is not fitted for all cancer patients, as TILs with antitumor reactivity are only seldom found in patients with other types of cancer than melanoma. Even in melanoma, TILs harvested from certain patients cannot be sufficiently expanded *ex vivo*, and this undermines the odds for their adoptive transfer. Moreover, there are some cases when tumor cells do not express class I major histocompatibility complex (MHC) molecules and therefore cannot be recognized by T lymphocytes. Natural killer (NK) cells might be an alternative to T lymphocytes, as they are able to kill tumor cells independently of MHC.

Natural killer cells are a component of the innate immune response against viruses and malignant cells. Individuals having low NK cells activity are at risk to develop cancer [7]. The presence of high NK cells number within the tumor confers a good prognostic for cancer patients [8]. NK cells represent a relatively poor population, about 1–32% from the peripheral lymphocytes in healthy individuals [9]. As opposed to T cells, which hold MHC-restricted antigen specificity, NK cells are able to directly and quickly lyse target cells without the need of an initial sensitization. It was proven that many types of tumor cells express high levels of ligands for NK cells receptors [10], which leads to their recognition and killing by NK cells [11]. The role of NK cells is regulated by (a) cytokines and chemokines which interact with inhibiting or activating receptors on NK cells [12, 13]; (b) communication with other immune cells, such as dendritic cells [14], effector TCD4+ lymphocytes [15] and regulatory T lymphocytes [16].

Until now, therapies with NK cells have been successful mainly for patients with leukemia [17–22], but adoptive transfer of interleukin (IL)-2-activated NK cells in patients with solid tumors (melanoma or renal carcinoma) did not show clear clinical benefits [23]. Based on the fact that NK cells possess the memory of being previously activated [23–29], new strategies can be developed for enhancing *ex vivo* the antitumor activity of NK cells intended to be transferred in patients with solid tumors. The use and clinical efficiency of immune therapy with

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NK cells have been limited by the difficulty to obtain sufficient cells for adoptive transfer. NK cells represent only a small fraction of blood leukocytes, have a low *ex vivo* proliferation rate and have a limited lifetime in vivo. Identifying the optimal activator for expanding NK cells in vitro is difficult, due to the high number of activating and inhibiting receptors, pairs of cooperative receptors, overlapping of the signaling transduction pathways involved in their maturation, activation and proliferation.

It was shown that the multiplication of NK cells can be achieved by modulation with cytokines [30–38]. The development of the methods for growing human NK cells in vitro has incited a special interest for immunotherapy [32, 36, 39–41]. With the development of methods for multiplication of human NK cells in vitro, these cells have incited a special interest for immunotherapy.

2. NK cells biology

NK cells are large granular cells that play a major role in the innate immune response against viruses, bacteria, as well as malignant cells [12, 24, 42]. They were first identified in 1975 by their ability to kill tumor cells without MHC restriction or prior sensitization to tumor antigens [43–45]. The name "natural killer" refers to their natural occurrence and spontaneous ability to kill malignant cells in non-immunized animals. NK cells are found in a variety of lymphoid and nonlymphoid tissues, including bone marrow, lymph nodes, spleen, peripheral blood, liver and lung. NK cells develop in the bone marrow from a common lymphoid progenitor cells.

2.1. Phenotypes

NK cells are characterized by the expression of CD16 and CD56 surface antigens and the lack of CD3/T-cell receptor molecules. In humans, there are two subsets of NK cells based on CD56 expression levels: $CD56^{dim}$ and $CD56^{bright}$. Morphologically, the $CD56^{dim}$ NK cells are large granular lymphocytes, while $CD56^{bright}$ NK cells are small lymphocytes. The $CD56^{dim}$ subset represents the majority of NK cell population in the peripheral blood and spleen (90–95%), exhibit a high cytotoxic potential after interaction with target cells [46, 47]. They produce negligible amounts of cytokines and bear the Fc receptors (CD16) to mediate antibody-dependent cell-mediated cytotoxicity (ADCC). In contrast, the $CD56^{bright}$ subset predominates (approximately 90%) in lymph nodes and tonsils, have poor cytotoxic activity and produce very significant amounts of cytokines and chemokines [48]. The $CD56^{bright}$ NK cells produce chemokines and cytokines in response to cytokine stimulation, while the $CD56^{dim}$ population chemokines and cytokines production is stimulated by target cell recognition.

These subsets also differ in the expression of interleukin (IL)-2 receptor α chain (IL-2Rα/CD25). $CD56^{bright}$ subset exclusively expresses CD25, while the $CD56^{dim}$ subset lacks CD25 expression. There are differences in receptor expression between the two subsets of NK cells. As opposed to the $CD56^{bright}$ NK cells, $CD56^{dim}$ NK cells express high levels of killer-cell immunoglobulin-like receptors (KIRs) and low levels of CD94/natural killer group 2 (NKG2) receptors. These differences have been correlated with the specific alloreactive properties of $CD56^{dim}$ NK cells due to their high KIR levels and their ability to kill various tumor cells [49].

The murine NK cells do not express CD56 marker, but they can be divided in four subsets according to the expression of CD11b and CD27 markers: $CD11b^{low}CD27^{low}$, $CD11b^{low}CD27^{high}$, $CD11b^{high}CD27^{high}$, $CD11b^{high}CD27^{low}$ [50]. Mouse $CD27^{high}$ NK cells predominate in lymph nodes and produce large amounts of cytokines, but in contrast with $CD56^{bright}$ NK cells, they have cytotoxic potential. In humans, expression of CD11b and CD27 markers have also revealed four subsets of NK cells with distinct maturation stages, tissue distribution patterns and functional properties [51, 52].

The NK cell activating receptor NKp46 (Ncr1), in contrast to other human NK cell markers (CD56, CD16) or murine NK cell markers (NK1.1, DX5), is almost exclusively expressed by NK cells, and it can be used as an additional marker for identify NK cells [53, 54].

2.2. Receptors

NK cells do not express rearranged, antigen-specific receptors, but they express a variety of germ-line encoded receptors that can recognize ligands on their cellular targets.

NK cell function, including proliferation, production of cytokines and chemokines, natural killing, lymphokine-activated killing and ADCC, depends on an intricate balance between signals from inhibitory and activating receptors. The activating receptors interact with ligands expressed on stressed, infected, or transformed cells, or antibody-opsonized targets (CD16/FcγRIIIa), while inhibitory receptors recognize MHC class I or class I-like molecules [11].

Inhibitory receptors include killer immunoglobulin-like receptors (KIRs), the c-type lectin, NKG2A/CD94 and leukocyte immunoglobulin-like receptors (LILRs) [55]. The ligands for these inhibitory receptors are mostly the major histocompatibility complex class-I (MHC-I) molecules. Inhibitory signals prevent NK cells from becoming activated, blocking degranulation and cytokine production. Ligation of MHC-I molecules to the inhibitory receptors acts as a form a NK cell tolerance. By this mechanism, NK cells save healthy cells from killing as long as they express normal levels of MHC class I molecules and low amounts of stress-induced self-molecules. During the NK cell development, signals coming from inhibitory receptors help NK cells to be "educated" to respond to MHC-I deficient cells [56].

Activating receptors include the natural cytotoxicity receptors or NCRs, the c-type lectins, NKG2D and NKG2C/CD94, the SLAM family receptors and others. NK cells express the low-affinity Fc receptor or CD16 and the death ligands FasL and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) that after interaction with death receptors induce apoptosis of the target cell. Although some of the ligands to activation receptors are already present on healthy cells, the expressions of many of them are induced upon cell stress [57].

In case of cellular transformation or viral infection, surface MHC class I expression on the cell surface is downregulated or lost to escape from recognition by T cells. When mature NK cells encounter that cell, their inhibitory receptors are not engaged, and the unsuppressed activating signals can trigger cytokine secretion and attack of the targeted cell. In parallel, cellular stress and DNA damage upregulate "stress ligands" that can be recognized by activating NK receptors.

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NK cells kill tumor targets through a variety of mechanisms, including release of cytoplasmic granules containing perforin and granzyme, expression of tumor necrosis factor (TNF) family members FasL or TNF-related apoptosis-inducing ligand (TRAIL), that induce tumor cell apoptosis by interacting with their respective receptors Fas and TRAIL receptor (TRAILR) as well as ADCC [58].

During tumor progression, tumor cells develop the ability through which they escape from NK cell response. These mechanisms include downregulation of adhesion molecules, costimulatory ligands or ligands for activating receptors, upregulation of MHC-I molecules, soluble MIC, FasL or NO expression [59, 60], secretion of immunosuppressive factors such as IL-10, transforming growth factor (TGF)-β or indoleamine 2,3-dioxygenase (IDO) and resisting Fas- or perforin-mediated apoptosis [61]. In cancer patients, there are observed some NK cell defects, including decreased numbers in peripheral blood and in tumor infiltrate, reduced expression of activation receptor or intracellular signaling molecules and overexpression of inhibitory receptors, decreased cytotoxicity, defective proliferation and cytokine production.

2.3. Cytokines

Cytokines are the main regulators of growth, proliferation, survival and differentiation for various cell types involved in the innate and adaptive immunity. The exhibition of NK cells to cytokines induces enhanced proliferation, augmented cytokine production, higher cytotoxicity against target cells and upregulation of cytotoxic and adhesion molecules. The common γ-chain interleukins (ILs) such as IL-2, IL-15, IL-21 can activate NK cells, and the combination of IL-12 and IL-18 is especially potent to trigger interferon (IFN)-γ.

IL-2 and IL-15 are the best studied cytokine activators of NK cells; they have central role for NK cell development and homeostasis, induce proliferation, costimulate cytokine production and enhance cytotoxic effector mechanisms. The both cytokine share the IL-2/15Rβ and γ_c as the primary signaling subunits, but these cytokine require α receptor subunit for efficient ligation of the IL-2/15R$\beta\gamma_c$. IL-15 plays an important role in the maturation, survival and homeostatic expansion of NK cells. For generating in vivo functional NK cells, IL-15R signaling is required [62]. The first surface marker exhibited by NK cell progenitors is IL-2/15R β (CD122), which is expressed even before the cell lineage marker, NK1.1 (CD161c or NKR-P1C) [63]. The factors which further regulate differentiation and homeostasis of mature NK cells are largely unknown. Mature NK1.1+ cells continuously need IL-15 for survival [64–66] and NK cells can be stimulated in vitro by IL-15 or IL-2. Once NK cells are activated, their differentiation can be induced/increased by cytokines. It has been shown that IL-15 is a powerful *ex vivo* stimulator for immune cells. Preclinical studies have shown that NK cells, naïve/memory T CD8+ lymphocytes and dendritic cells cultivated with IL-15 develop enhanced functions when being adoptively transferred in animals [67–69]. Several studies shown that coadministration of IL-15/IL-15Ra complexes on NK cells enhanced in vivo activity, and the use of IL-15/IL-15Rα complexes remains highly promising as an IL-15 immunotherapy [70–72]. IL-2 and IL-15 are also used in *ex vivo* activation and/or expansion of NK cells for adoptive therapy and to support the expansion and function of NK cells after infusion.

IL-21 is a cytokine structurally-related to IL-2, IL-4, IL-15. IL-21Rα is expressed by lymphoid tissues, shows similarities with IL-2/IL-15R β chain and forms a complex with the common γ chain [73]. IL-21 exerts antitumor effects through its ability to induce activation and proliferation of cytotoxic cells (T CD8+ cytotoxic lymphocytes, NK cells, NKT cells). Meanwhile, IL-21 suppresses Foxp3 expression and the expansion of immunosuppressive Treg lymphocytes. Accordingly, IL-21 was associated with antitumor activity in clinical practice [74]. The discovery of IL-21 was linked to its role in NK cells proliferation and maturation. Further studies have provided however contradictory data, highlighting both the activator and the suppressor role of IL-21. Soluble IL-21 alone does not induce significant proliferation in mature mouse NK cells, and IL-21R knockout mice possess normal NK cells number. Meanwhile, IL-21 synergizes with IL-2, IL-15 and Flt-3L for NK cells generation in the bone marrow and in umbilical cord blood. IL-21 can activate the cytotoxic activity of NK cells through over expression of costimulating receptors and cytolytic molecules (perforin, granzymes). IL-21 enhances multipotent progenitor maturation from bone marrow and activates peripheral NK cells in the absence of other stimuli.

IL-12, originally identified as "NK cell stimulatory factor (NKSF)" based on its ability to enhance NK cell cytotoxicity. The primary effects of IL-12 on NK cells are IFN-γ and TNF-α production. In vitro and in vivo studies have shown that IL-12 acts on NK cells in concert with other activating cytokines, such as IL-2 and IL-18, or with receptor-based interactions from pathogenic cells.

Although the IL-18Rα is constitutively expressed on unstimulated NK cells and can induce NK cell proliferation alone, IL-18 has been described as a costimulatory cytokine that functions synergistically with IL-12 and IL-15. NK cells from IL-18 deficient mice have impaired cytotoxicity and IFN-γ production. These findings indicate the importance of IL-18 to NK-mediated host defense.

Successful adoptive cell transfer (ACT) and *ex vivo* modulation of cellular functions with cytokines has aroused the interest for immunotherapy in cancer. Currently, immunotherapy is known as the forth treatment alternative in cancer, after surgery, chemo- and radiotherapy.

3. NK cells for adoptive transfer

3.1. Autologous NK cells

Based on results from experimental animal models, adoptive transfer of autologous NK cells seems to be safe and promising for cancer therapy. Initial trials of adoptive NK cells involved infusion of CD56 bead-selected autologous NK cells from a leukapheresis product followed by administration of systemic cytokines [75]. Upon cytokine stimulation, NK cells become lymphokine-activated killer (LAK) cells and exhibit greater cytotoxicity against tumor cells. Although administration of cytokines improve the antitumor activity of NK cells in vitro, only limited antitumor activity of LAK cells was observed in cancer patients [23]. Similar results were also obtained when autologous NK cells and systemic IL-2 were administrated to patients with lymphoma. Partially effective clinical outcomes were observed in metastatic renal cell carcinoma (RCC) patients that received a combination of high-dose IL-2 and LAK cell infusions, while

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treatment of non-Hodgkin's lymphoma and RCC patients with *ex vivo* IL-2-activated autologous NK cells followed by daily subcutaneous IL-2 injection shown no improvement in the disease status. In patients with recurrent malignant glioma infusion of NK cells combined with IFN-α was safe and partially effective. It is known that high IL-2 doses induced severe toxic side effects such as vascular leak syndrome and also promote expansion of regulatory T cells that directly inhibit NK-cell functions and induce activation-induced cell death of NK cells. The adoptive transfer of IL-2-activated LAK cells was more successful rather than administering IL-2 systemically. Other cytokines, such as IL-12, IL-15, IL-18 and IL-21, have been successfully tested in preclinical cancer models [76]. Adoptively transferring autologous NK cells has been evaluated clinically for cancer immunotherapy and was found to greatly improve clinical responses without any obvious adverse side effects in metastatic renal cell carcinoma (RCC), malignant glioma and breast cancer patients. However, these autologous NK cells could not yet exhibit their full cytotoxic capacity in vivo and were not consistently effective in cancer patients; this may be due to MHC class I expression in cancer patients that suppress autologous NK cells in vivo.

As was shown, adoptively transferring autologous NK cells was found to greatly improve clinical responses without any obvious adverse side effects in some cancers. However, these NK cells could not yet exhibit their full cytotoxic capacity in vivo, this may be due to many tumors expressing high levels of human leukocyte antigen (HLA) class I receptor and/or low levels of ligands for activating receptors that suppress autologous NK cells in vivo. For these reasons, they are focused on therapies using allogenic NK cells from related donors or other strategies to prevent such NK cell resistance.

3.2. Allogeneic NK cells

Using the allogeneic NK cells has the advantage of the inherent alloreactivity afforded by the "missing self" concept. When donor NK cells encounter an altered MHC environment, the NK cells can be "re-educated" by host HLA and can acquire cytotoxicity against host tumor cells without causing graft versus host disease (GVHD). Allogenic NK cells with KIR mismatch have greater tumor-killing activity and the ability to control acute myeloid leukemia (AML) relapse [77, 78]. Adoptively transferred human-mismatched (haploidentical) allogeneic NK cells have been shown to be a safe therapy with minimal toxicity and have been more successful for cancer immunotherapy, including against leukemia and solid cancers. In some clinical trials using adoptively transferred haploidentical allogeneic NK cells to treat AML patients, including pediatric AML patients and older AML patients no graft-versus-host disease (GvHD) response was observed and NK cell therapy was well tolerated. Besides hematopoietic-derived tumors, strategies using adoptively transferred haploidentical allogeneic NK cells can also expand in patients with various malignancies, including metastatic melanoma, renal cell carcinoma, Hodgkin's disease and poor-prognosis AML

3.3. Memory-like NK cells

NK cells are traditionally considered members or the innate branch of the immune system that responds rapidly but lack immunologic specificity in the form of a clonal antigen receptor and memory of prior activation. Recently several groups have challenged this paradigm of NK cells as pure innate lymphocytes and demonstrated memory-like functions in NK cells.

The immune system capacity to learn from previous encounters with pathogens, and respond more rapidly and effectively upon secondary infection has been termed adaptive immunity or immunological memory. After primary encounter with antigen, naïve antigen-specific T or B cells proliferate vigorously and some of them differentiate into memory cells [79]. This stage represents the expansion phase, when the naïve cells clonally expand. Following the primary response, in the contraction phase, the majority of effector cells die and surviving cells enter the memory phase. Upon reencounter with their cognate antigen, memory cells exert their functional responses more rapidly than do naïve cells. This response to a second antigen exposure called the "recall response."

Antigen-specific responses and memory responses, both are hallmarks of adaptive immunity. Innate responses do not require pre-sensitization and rely on germline encoded receptors and do not require clonal expansion. NK cells have long been categorized as a component of innate immunity. Although NK cells lack the ability to undergo somatic rearrangements of their receptors, these cells are developmentally and functionally more related to adaptive immune lymphocytes than innate immune cells.

Initially it was believed that NK cells act in the first days of infection, but now we know that NK cells function in parallel and complementary to the adaptive immune response over extended periods of time. Moreover, there are many evidences for adoptive-like features of NK cells. Evidence for adoptive-like features of NK cells has come from a variety of studies, and NK cell-mediated memory can be generated in response to haptens, viruses and following combined cytokine activation.

3.3.1. NK cell response to haptens

NK cell memory was first described in a mouse model of hapten-induced contact hypersensitivity (CHS), induced by chemical haptens such as 2,4-dinitro-1-fluorobenzene (DNFB) and 4-ethoxymethylene-2-phenyloxazol-5-one (oxazolone) and picryl chloride [25]. T- and B-deficient mice developed vigorous specific contact hypersensitivity responses to haptens and response persisted for at least four weeks. The mice exhibited enhanced recall responses to the same chemical, but not to a different one, demonstrating antigen specificity. Furthermore, contact hypersensitivity responses could be conferred to naive mice by adoptive transfer of natural killer cells from sensitized mice.

These responses possessed the hallmarks of adaptive immunity: they were sensitization dependent, persisted for at least four weeks and were only elicited by haptens to which mice had previously been sensitized. These observations indicate that natural killer cells can mediate long-lived, antigen-specific adaptive recall responses independent of B cells and T cells. It was shown that transfer of a subset of liver-derived NK cells, not splenic or naïve NK cells, could confer the recall response. NK cell memory to haptens depended on expression of CXCR6, a chemokine receptor on hepatic NK cells critical for intrahepatic survival and homeostasis [80]. CXCR6 is a chemo-attractant receptor which is expressed on roughly 50% of liver NK cells. The molecular mechanism leading to the generation of antigen-specific memory NK cells remained elusive.

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3.3.2. NK cell response to viruses

Also, NK cells could mount recall responses to diverse viral antigens such as vesicular stomatitis virus (VSV), virus-like particles (VLP) containing influenza A-derived hemaglutinin and/or matrix protein 1, or VLP containing the HIV-1-derived Gag protein and/or Env protein [80]. In order to confer the recall response, liver NK cells had to express the CXCR6. Blocking CXCR6 with antibodies can impair the recall response. NK cells can respond more vigorously upon secondary stimulation against challenges with additional irritants (such as fluorescein isothiocyanate [FITC]), as well as the viruses vaccinia, and herpes simplex virus (HSV)-2 [81–83].

A central issue that remains unresolved is how NK cells can recognize and differentiate between all these different antigens, since there are no known VSV, vaccinia, HSV-2, or HIV-specific NK receptors. The ability of NK cells to respond to such a wide diversity of distinct antigens, including pathogens that are not endemic to mice, such as HIV1, is puzzling and suggests that a hitherto unknown recombination-activating genes (RAG)-independent receptor diversification mechanism may exist in NK cells [84].

Most of the evidence that NK cells exhibit a memory-like adaptive response has come from studies involving mouse cytomegalovirus (MCMV) infection. NK cells play a crucial role in the protective immune response to herpesvirus family members, especially to CMV infection. It have been shown that a subset of NK cells in the C57BL/6 strain of mice bearing the activating receptor Ly49H, specifically recognize the MCMV-expressed ligand m157 and proliferate in response to viral infection [85–87]. Ly49H, a germ-line encoded receptor, is expressed by a very large fraction of NK cells in naïve mice (~50% of NK cells). During viral infection, a Ly49H-expressing subset of NK cells is extended and rapidly responds upon reinfection with MCMV, similar to classic lymphocytes memory. The germ-line encoded MCMV-specific receptors exist too in mouse strains, such as Bagg Albino (BALB/c), non-obese diabetic (NOD) and others, and that clonal expansions of MCMV-specific NK cells could also be observed in those strains [88]. Similar to CD8+ T cell response, the proliferation was Ag-specific because infection of mice with a mutant MCMV lacking m157 did not cause expansion of Ly49H+ NK cells [25]. Ly49H+ NK cells adoptively transferred in mice lacking functional Ly49H receptor proliferate 100-1000-fold after MCMV infection, and after a contraction phase, persist for months. When these cells are restimulated *ex vivo* with agonistic antibodies against NK1.1 or Ly49H, they respond more robustly and offer increased protection against MCMV infection then naïve NK cells. MCMV-induced NK cell memory is critically dependent on the IL-12; NK cells that lack the IL-12 receptor do not proliferate in response to MCMV [89].

In human, studies have focused on the memory NK cell response in infection with cytomegalovirus (CMV), Hanta virus or Chikungunya virus [90–92]. The results shown that NK cells that express the germ-line encoded NKG2C receptor appeared in increased frequency in response to infections with these viruses. During acute HCMV infection, $NKG2C^+$ NK cells expanded in number, and diminished partially in numbers after the resolution of the acute phase. These memory NK cells persisted for up to a year. After expansion, the $NKG2C^+$ NK cells produced significantly more IFN-γ in response than $NKG2C^-$ NK cells. A follow-up study demonstrated that after adoptive transfer of $NKG2C^+$ NK cells from CMV-seropositive donors exhibited

enhanced effector function against a secondary CMV challenge [93]. In the Hanta virus infection or Chikungunya virus infection, NKG2C^{+} NK cells expanded three- to fourfold compared to uninfected controls. In both cases, these increases were only seen in patients who were HCMV seropositive, raising the possibility that the increase in NKG2C+ NK cells reflected reactivation of latent CMV. It has been noted that in HCMV-infected patients, after the acute infection was cleared, NKG2C+ NK cell numbers remained elevated in contrast with uninfected individuals. It is possible that these NKG2C^{+} NK cells present in HCMV-seropositive individuals respond to other infections. Another study has demonstrated that the combination of CMV-infected fibroblasts plus IL-12-producing monocytes induced the expansion of NKG2C^{+} NK cell in vitro [94].

3.3.3. Cytokine-induced NK cell responses

Memory-like NK cells could be induced in vitro by cytokines activation in both mice and humans. NK cells from Rag 1-deficent mice were pre-activated overnight in vitro with IL-12, IL-18 and IL-15, and then adoptively transferred into syngeneic Rag-1$^{-/-}$ recipients. After resting in vivo, when the NK cells had reverted to a quiescent state, the cytokine pre-activated NK cells were phenotypically similar to control NK cells. They expressed similar levels of CD69, CD11b, CD27, B220, as well as the cytokine receptors CD122, IL12Rβ1, IL-15Rα and CD127. Also, they expressed comparable levels of granzyme B and lysed target cells similar to control NK cells in vitro [27, 95]. However, up to 3 weeks following adoptive transfer, the cytokine pre-activated NK cells were found to respond more robustly compared to resting NK cells. These pre-activated NK-cells displayed enhanced IFN-γ production upon either activating receptor ligation (Ly49H or NK1.1 receptors) or cytokines (IL-12 and IL-15) restimulation. This enhanced ability to produce IFN-γ occurs in cells that have not undergone division and those that have replicated. It has been demonstrated that the enhanced functionality of memory-like NK cells is not due to alteration in IFN-γ transcription or mRNA stability.

Adoptively transferred cytokine pre-activated NK cells proliferated rapidly in an IL-2-dependent manner into recipient mice bearing MHC class I-deficient RMA-S lymphoma or B16-Rae1ε melanoma cell lines [96]. Exposure of NK cells to cytokines upregulated the IL-2Rα chain, making these cells more responsive to IL-2. It was observed a significantly reduced tumor growth and prolonged survival in recipient mice, pre-activated NK cells exhibited enhanced functionality months following adoptive transfer, and were able to mediate more effective in vivo antitumor responses.

Human NK cells also exhibit enhanced IFN-γ production after short-term pre-activation with various combinations of IL-12, IL-15 and IL-18 [97]. Both NK cell subsets, CD56^{bright} and CD56^{dim}, exhibited cytokine-induced memory-like NK cell. Cytokine pre-activation led to extensive proliferation, and memory-like NK cells maintained their capacity for enhanced recall responses. Similar to mice, IFN-γ mRNA transcript levels did not differ between control and memory-like NK cells. In contrast to murine memory-like NK cells, phenotypic differences were identified between pre-activated NK cells and controls. Human memory-like NK cells had increased CD94, NKG2A, NKp46 and CD69 surface expression and reduced KIR and CD57 expression. Human memory-like NK cells were also shown to be responsive to low concentrations of IL-2.

A pre-clinical study has shown that human memory-like NK cells also have potential as anti-leukemia cellular therapy [98]. They exhibited enhanced IFN-γ production and increased cytotoxicity when restimulated with leukemia cell lines or acute myeloid leukemia (AML) blasts in vitro. Following adoptive transfer into immunodeficient NOD-severe combined immunodeficiency (SCID)-$\gamma_c^{-/-}$ mice, human cytokine-induced memory-like NK cells exhibited increased IFN-γ production following restimulation [99].

Therefore, human NK cells can acquire memory-like properties after a brief cytokine pre-activation.

4. Conclusion

Although, the concept of NK cell memory is rather new, many studies in recent years have provided substantial evidence for adaptive features of the NK cell response. The enhanced function of memory NK cells makes them an area of interest for future use in preventing or treating inflammatory diseases, infectious diseases and cancer.

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References

[1] Harris BN, Sinha UK. Cancer stem cells: a review of the literature and the implications. J Cancer Res Updat. 2013;**2**:186–193. DOI: 10.6000/1929-2279.2013.02.03.4

[2] Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol. 2013;**14**:1014–1022. DOI: 10.1038/ni.2703

[3] Kawakami Y, Yaguchi T, Sumimoto H, Kudo-Saito C, Tsukamoto N, Iwata-Kajihara T, Nakamura S, Nishio H, Satomi R, Kobayashi A, Tanaka M, Park JH, Kamijuku H, Tsujikawa T, Kawamura N. Cancer-induced immunosuppressive cascades and their reversal by molecular-targeted therapy. Ann N Y Acad Sci. 2013;**1284**:80–86. DOI: 10.1111/nyas.12094.

[4] Rosenberg SA, Dudley ME. Adoptive cell therapy for the treatment of patients with metastatic melanoma. Curr Opin Immunol. 2009;**21**:233–240. DOI: 10.1016/j.coi.2009.03.002

[5] Dudley ME, Yang JC, Sherry R, Hughes MS, Royal R, Kammula U, Robbins PF, Huang J, Citrin DE, Leitman SF, Wunderlich J, Restifo NP, Thomasian A, Downey SG, Smith FO, Klapper J, Morton K, Laurencot C, White DE, Rosenberg SA. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol. 2008;**26**:5233–5239. DOI: 10.1200/JCO.2008.16.5449

[6] Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, Royal RE, Topalian SL, Kammula US, Restifo NP, Zheng Z, Nahvi A, de Vries CR, Rogers-Freezer LJ, Mavroukakis SA, Rosenberg SA. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science. 2006;**314**:126–129. DOI: 10.1126/science.1129003

[7] Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet. 2000;**356**:1795–1799. DOI: 10.1016/S0140-6736(00)03231-1

[8] Villegas FR, Coca S, Villarrubia VG, Jiménez R, Chillón MJ, Jareño J, Zuil M, Callol L. Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer. Lung Cancer. 2002;**35**:23–28. DOI: 10.1016/S0169-5002(01)00292-6

[9] Pittari G, Fregni G, Roguet L, Garcia A, Vataire AL, Wittnebel S, Amsellem S, Chouaib S, Bourhis JH, Caignard A. Early evaluation of natural killer activity in post-transplant acute myeloid leukemia patients. Bone Marrow Transpl. 2010;**45**:862–871. DOI: 10.1038/bmt.2009.265

[10] Raulet DH, Guerra N. Oncogenic stress sensed by the immune system: role of natural killer cell receptors. Nat Rev Immunol. 2009;**9**:568–580. DOI: 10.1038/nri2604

[11] Pegram HJ, Andrews DM, Smyth MJ, Darcy PK, Kershaw MH. Activating and inhibitory receptors of natural killer cells. Immunol Cell Biol. 2011;**89**:216–224. DOI: 10.1038/icb.2010.78

[12] Farag SS, Caligiuri MA. Human natural killer cell development and biology. Blood Rev. 2006;**20**:123–137. DOI: 10.1016/j.blre.2005.10.001

[13] Ljunggren HG, Malmberg KJ. Prospects for the use of NK cells in immunotherapy of human cancer. Nat Rev Immunol. 2007;**7**:329–339. DOI: 10.1038/nri2073

[14] Moretta L, Ferlazzo G, Bottino C, Vitale M, Pende D, Mingari MC, Moretta A. Effector and regulatory events during natural killer-dendritic cell interactions. Immunol Rev. 2006;**214**:219–228. DOI: 10.1111/j.1600-065X.2006.00450.x

[15] Zingoni A, Sornasse T, Cocks BG, Tanaka Y, Santoni A, Lanier LL. Cross-talk between activated human NK cells and CD4+ T cells via OX40-OX40 ligand interactions. J Immunol. 2004;173:3716–3724. DOI: 10.4049/jimmunol.173.6.3716

[16] Ghiringhelli F, Ménard C, Martin F, Zitvogel L. The role of regulatory T cells in the control of natural killer cells: relevance during tumor progression. Immunol Rev. 2006;**214**:229–238. DOI: 10.1111/j.1600-065X.2006.00445.x

[17] Moretta L, Locatelli F, Pende D, Marcenaro E, Mingari MC, Moretta A. Killer Ig-like receptor-mediated control of natural killer cell alloreactivity in haploidentical hematopoietic stem cell transplantation. Blood. 2001;**117**:764–771. DOI: 10.1182/blood-2010-08-264085

[18] Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, Posati S, Rogaia D, Frassoni F, Aversa F, Martelli MF, Velardi A. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;**295**:2097–2100. DOI: 10.1126/science.1068440

[19] Passweg JR, Tichelli A, Meyer-Monard S, Heim D, Stern M, Kühne T, Favre G, Gratwohl A. Purified donor NK-lymphocyte infusion to consolidate engraftment after haploidentical stem cell transplantation. Leukemia. 2004;**18**:1835–1838. DOI: 10.1038/sj.leu.2403524

[20] Geller MA, Miller JS. Use of allogeneic NK cells for cancer immunotherapy. Immunotherapy. 2011;**3**:1445–1459. DOI: 10.2217/imt.11.131

[21] Geller MA, Cooley S, Judson PL, Ghebre R, Carson LF, Argenta PA, Jonson AL, Panoskaltsis-Mortari A, Curtsinger J, McKenna D, Dusenbery K, Bliss R, Downs LS, Miller JS. A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer. Cytotherapy. 2011;**13**:98–107. DOI: 10.3109/14653249.2010.515582

[22] Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, McKenna D, Le C, Defor TE, Burns LJ, Orchard PJ, Blazar BR, Wagner JE, Slungaard A, Weisdorf DJ, Okazaki IJ, McGlave PB. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood. 2005;**105**:3051–3057. DOI: 10.1182/blood-2004-07-2974

[23] Parkhurst MR, Riley JP, Dudley ME, Rosenberg SA. Adoptive transfer of autologous natural killer cells leads to high levels of circulating natural killer cells but does not mediate tumor regression. Clin Cancer Res. 2011;**17**:6287–6297. DOI: 10.1158/1078-0432.CCR-11-1347

[24] Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, Yokoyama WM, Ugolini S. Innate or adaptive immunity? The example of natural killer cells. Science. 2011;**331**:44–49. DOI: 10.1126/science.1198687

[25] O'Leary JG, Goodarzi M, Drayton DL, von Andrian UH. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. Nat Immunol. 2006;**7**:507–516. DOI: 10.1038/ni1332

[26] Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. Nature. 2009;**457**:557–561. DOI: 10.1038/nature07665

[27] Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. Proc Natl Acad Sci U S A. 2009;**106**:1915–1919. DOI: 10.1073/pnas.0813192106

[28] Paust S, von Andrian UH. Natural killer cell memory. Nat Immuno. 2011;**12**:500–508. DOI: doi:10.1038/ni.2032

[29] Sun JC, Lopez-Verges S, Kim CC, DeRisi JL, Lanier LL. NK cells and immune "memory". J Immunol. 2011;**186**:1891–1897. DOI: 10.4049/jimmunol.1003035

[30] Decot V, Voillard L, Latger-Cannard V, Aissi-Rothé L, Perrier P, Stoltz JF, Bensoussan D. Natural-killer cell amplification for adoptive leukemia relapse immunotherapy: comparison of three cytokines, IL-2, IL-15, or IL-7 and impact on NKG2D, KIR2DL1, and KIR2DL2 expression. Exp Hematol. 2010;**38**:351–362. DOI: 10.1016/j.exphem.2010.02.006

[31] Koehl U, Sörensen J, Esser R, Zimmermann S, Grüttner HP, Tonn T, Seidl C, Seifried E, Klingebiel T, Schwabe D. IL-2 activated NK cell immunotherapy of three children after haploidentical stem cell transplantation. Blood Cells Mol Dis. 2004;**33**:261–266. DOI: 10.1016/j.bcmd.2004.08.013

[32] Koehl U, Esser R, Zimmermann S, Tonn T, Kotchetkov R, Bartling T, Sörensen J, Grüttner HP, Bader P, Seifried E, Martin H, Lang P, Passweg JR, Klingebiel T, Schwabe D. Ex vivo expansion of highly purified NK cells for immunotherapy after haploidentical stem cell transplantation in children. Klin. Padiatr. 2005;**217**:345–350. DOI: 10.1055/s-2005-872520

[33] Clausen J, Petzer AL, Vergeiner B, Enk M, Stauder R, Gastl G, Gunsilius E. Optimal timing for the collection and in vitro expansion of cytotoxic CD56(+) lymphocytes from patients undergoing autologous peripheral blood stem cell transplantation. J Hematother Stem Cell Res. 2001;**10**:513–521. DOI: 10.1089/15258160152509127

[34] Clausen J, Vergeiner B, Enk M, Petzer AL, Gastl G, Gunsilius E. Functional significance of the activation-associated receptors CD25 and CD69 on human NK-cells and NK-like T-cells. Immunobiology. 2003;**207**:85–93. DOI: 10.1078/0171-2985-00219

[35] Clausen J, Enk M, Vergeiner B, Eisendle K, Petzer AL, Gastl G, Gunsilius E. Suppression of natural killer cells in the presence of CD34+ blood progenitor cells and peripheral blood lymphocytes. Biol Blood Marrow Transpl. 2004;**10**:691–697. DOI: 10.1016/j.bbmt.2004.06.009

[36] Klingemann HG, Martinson J. Ex vivo expansion of natural killer cells for clinical applications. Cytotherapy. 2004;**6**:15–22. DOI: 10.1080/14653240310004548

[37] de Rham C, Ferrari-Lacraz S, Jendly S, Schneiter G, Dayer JM, Villard J. The proinflammatory cytokines IL-2, IL-15 and IL-21 modulate the repertoire of mature human natural killer cell receptors. Arthritis Res Ther. 2007;**9**:R125. DOI: 10.1186/ar2336

[38] Spanholtz J, Tordoir M, Eissens D, Preijers F, van der Meer A, Joosten I, Schaap N, de Witte TM, Dolstra H. High log-scale expansion of functional human natural killer cells from umbilical cord blood CD34-positive cells for adoptive cancer immunotherapy. PLoS One. 2010;**5**:e9221. DOI: 10.1371/journal.pone.0009221.

[39] Iliopoulou EG, Kountourakis P, Karamouzis MV, Doufexis D, Ardavanis A, Baxevanis CN, Rigatos G, Papamichail M, Perez SA. A phase I trial of adoptive transfer of allogeneic natural killer cells in patients with advanced non-small cell lung cancer. Cancer Immunol Immunother. 2010;**59**:1781–1789. DOI: 10.1007/s00262-010-0904-3

[40] Berg M, Lundqvist A, McCoy P Jr, Samsel L, Fan Y, Tawab A, Childs R. Clinicalgrade ex vivo-expanded human natural killer cells up-regulate activating receptors and death receptor ligands and have enhanced cytolytic activity against tumor cells. Cytotherapy. 2009;**11**:341–355. DOI: 10.1080/14653240902807034

[41] Fujisaki H, Kakuda H, Shimasaki N, Imai C, Ma J, Lockey T, Eldridge P, Leung WH, Campana D. Expansion of highly cytotoxic human natural killer cells for cancer cell therapy. Cancer Res. 2009;**69**:4010–4017. DOI: 10.1158/0008-5472.CAN-08-3712

[42] Parham P. Immunology. NK cells lose their inhibition. Science. 2004;**305**:786–787. DOI: 10.1126/science.1102025

[43] Kiessling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. Eur J Immunol. 1975;**5**:112–117. DOI: 10.1002/eji.1830050208

[44] Kiessling R, Klein E, Pross H, Wigzell H. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. Eur J Immunol. 1975;**5**:117–121. DOI: 10.1002/eji.1830050209

[45] Herberman RB, Nunn ME, Holden HT, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. Int J Cancer. 1975;**16**:230–239.

[46] De Maria A, Bozzano F, Cantoni C, Moretta L. Revisiting human natural killer cell subset function revealed cytolytic CD56(dim)CD16+ NK cells as rapid producers of abundant IFN-gamma on activation. Proc Natl Acad Sci U S A. 2011;**108**:728–732. DOI: 10.1073/pnas.1012356108

[47] Fauriat C, Long EO, Ljunggren HG, Bryceson YT. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. Blood. 2010;**115**:2167–2176. DOI: 10.1182/blood-2009-08-238469

[48] Romee R, Leong JW, Fehniger TA. Utilizing cytokines to function-enable human NK cells for the immunotherapy of cancer. Scientifica. 2014;**2014**:205796. DOI: 10.1155/2014/205796

[49] Bodduluru LN, Kasala ER, Madhana RM, Sriram CS. Natural killer cells: The journey from puzzles in biology to treatment of cancer. Cancer Lett. 2015;**357**:454–467. DOI: 10.1016/j.canlet.2014.12.020

[50] Chiossone L, Chaix J, Fuseri N, Roth C, Vivier E, Walzer T. Maturation of mouse NK cells is a 4-stage developmental program. Blood. 2009;**113**:5488–5496. DOI: 10.1182/blood-2008-10-187179

[51] Fu B, Wang F, Sun R, Ling B, Tian Z, Wei H. CD11b and CD27 reflect distinct population and functional specialization in human natural killer cells. Immunology. 2011;**133**:350–359. DOI: 10.1111/j.1365-2567.2011.03446.x

[52] Fu B, Tian Z, Wei H. Subsets of human natural killer cells and their regulatory effects. Immunology. 2014;**141**:483–489. DOI: 10.1111/imm.12224

[53] Caligiuri MA. Human natural killer cells. Blood. 2008;**112**:461–469. DOI: 10.1182/blood-2007-09-077438

[54] Narni-Mancinelli E, Chaix J, Fenis A, Kerdiles YM, Yessaad N, Reynders A, Gregoire C, Luche H, Ugolini S, Tomasello E, Walzer T, Vivier E. Fate mapping analysis of lymphoid cells expressing the NKp46 cell surface receptor. Proc Natl Acad Sci U S A. 2011;**108**:18324–18329. DOI: 10.1073/pnas.1112064108

[55] Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol. 2008;**9**:495–502. DOI: 10.1038/ni1581

[56] Anfossi N, André P, Guia S, Falk CS, Roetynck S, Stewart CA, Breso V, Frassati C, Reviron D, Middleton D, Romagné F, Ugolini S, Vivier E. Human NK cell education by inhibitory receptors for MHC class I. Immunity. 2006;**25**:331–342. DOI: 10.1016/j.immuni.2006.06.013

[57] Davis ZB, Felices M, Verneris MR, Miller JS. natural killer cell adoptive transfer therapy: exploiting the first line of defense against cancer. Cancer J. 2015;**21**:486–491. DOI: 10.1097/PPO.0000000000000156

[58] Campbell KS, Hasegawa J. Natural killer cell biology: an update and future directions. J Allergy Clin Immunol. 2013;**132**:536–544. DOI: 10.1016/j.jaci.2013.07.006

[59] Pietra G, Manzini C, Rivara S, Vitale M, Cantoni C, Petretto A, Balsamo M, Conte R, Benelli R, Minghelli S, Solari N, Gualco M, Queirolo P, Moretta L, Mingari MC. Melanoma cells inhibit natural killer cell function by modulating the expression of activating receptors and cytolytic activity. Cancer Res. 2012;**72**:1407–1415. DOI: 10.1158/0008-5472.CAN-11-2544

[60] Vitale M, Cantoni C, Pietra G, Mingari MC, Moretta L. Effect of tumor cells and tumor microenvironment on NK-cell function. Eur J Immunol. 2014;**44**:1582–1592. DOI: 10.1002/eji.201344272

[61] Godal R, Bachanova V, Gleason M, McCullar V, Yun GH, Cooley S, Verneris MR, McGlave PB, Miller JS. Natural killer cell killing of acute myelogenous leukemia and acute lymphoblastic leukemia blasts by killer cell immunoglobulin-like receptor-negative natural killer cells after NKG2A and LIR-1 blockade. Biol Blood Marrow Transplant. 2010;**16**:612–621. DOI: 10.1016/j.bbmt.2010.01.019

[62] Colucci F, Caligiuri MA, Di Santo JP. What does it take to make a natural killer? Nat Rev Immunol. 2003;**3**:413–425. DOI: 10.1038/nri1088

[63] Rosmaraki EE, Douagi I, Roth C, Colucci F, Cumano A, Di Santo JP. Identification of committed NK cell progenitors in adult murine bone marrow. Eur J Immunol. 2001;**31**:1900–1909. DOI: 10.1002/1521-4141(200106)31:6<1900::AID-IMMU1900>3.0.CO;2-M

[64] Cooper MA, Bush JE, Fehniger TA, VanDeusen JB, Waite RE, Liu Y, Aguila HL, Caligiuri MA. In vivo evidence for a dependence on interleukin 15 for survival of natural killer cells. Blood. 2002;**100**:3633–3638. DOI: 10.1182/blood-2001-12-0293

[65] Koka R, Burkett PR, Chien M, Chai S, Chan F, Lodolce JP, Boone DL, Ma A. Interleukin (IL)-15Rα-deficient natural killer cells survive in normal but not IL-15Rα-deficient mice. J Exp Med. 2003;**197**:977–984. DOI: 10.1084/jem.20021836

[66] Ranson T, Vosshenrich CA, Corcuff E, Richard O, Laloux V, Lehuen A, Di Santo JP. IL-15 availability conditions homeostasis of peripheral natural killer T cells. Proc Natl Acad Sci U S A. 2003;**100**:2663–2668. DOI: 10.1073/pnas.0535482100

[67] Salagianni M, Lekka E, Moustaki A, Iliopoulou EG, Baxevanis CN, Papamichail M, Perez SA. NK cell adoptive transfer combined with Ontak-mediated regulatory T cell elimination induces effective adaptive antitumor immune responses. J Immunol. 2011;**186**:3327–3335. DOI: 10.4049/jimmunol.1000652

[68] Klebanoff CA, Finkelstein SE, Surman DR, Lichtman MK, Gattinoni L, Theoret MR, Grewal N, Spiess PJ, Antony PA, Palmer DC, Tagaya Y, Rosenberg SA, Waldmann TA, Restifo NP. IL-15 enhances the in vivo antitumor activity of tumor-reactive CD8+ T cells. Proc Natl Acad Sci U S A. 2004;**101**:1969–1974. DOI: 10.1073/pnas.0307298101

[69] Anguille S, Smits EL, Cools N, Goossens H, Berneman ZN, Van Tendeloo VF. Short-term cultured, interleukin-15 differentiated dendritic cells have potent immunostimulatory properties. J Transl Med. 2009;**18**:109. DOI: 10.1186/1479-5876-7-109

[70] Rubinstein MP, Kovar M, Purton JF, Cho JH, Boyman O, Surh CD, Sprent J. Converting IL-15 to a superagonist by binding to soluble IL-15R{alpha}. Proc Natl Acad Sci U S A. 2006 Jun 13;103(24):9166-71. 2006;**103**:9166–9171. DOI: 10.1073/pnas.0600240103

[71] Stoklasek TA, Schluns KS, Lefrançois L. Combined IL-15/IL-15Rα immunotherapy maximizes IL-15 activity in vivo. J Immunol. 2006;**177**:6072–6080. DOI: 10.4049/jimmunol.177.9.6072

[72] Isvoranu G, Marinescu B, Surcel M, Ursaciuc C, Manda G. Immunotherapy in cancer—in vivo study of the antitumor activity of the IL-15/IL-15R alfa combination in an experimental model of melanoma. Farmacia. 2015;**63**:631–636.

[73] Asao H, Okuyama C, Kumaki S, Ishii N, Tsuchiya S, Foster D, Sugamura K. Cutting edge: the common γ-chain is an indispensable subunit of the IL-21 receptor complex. J Immunol. 2001;**167**:1–5. DOI: 10.4049/jimmunol.167.1.1

[74] Petrella TM, Tozer R, Belanger K, Savage KJ, Wong R, Smylie M, Kamel-Reid S, Tron V, Chen BE, Hunder NN, Hagerman L, Walsh W, Eisenhauer EA. Interleukin-21 has activity in patients with metastatic melanoma: a phase II study. J Clin Oncol. 2012;**30**:3396–3401. DOI: 10.1200/JCO.2011.40.0655

[75] Burns LJ, Weisdorf DJ, DeFor TE, Vesole DH, Repka TL, Blazar BR, Burger SR, Panoskaltsis-Mortari A, Keever-Taylor CA, Zhang MJ, Miller JS. IL-2-based immunotherapy after autologous transplantation for lymphoma and breast cancer induces immune activation and cytokine release: a phase I/II trial. Bone Marrow Transplant. 2003;**32**:177–1786. DOI: 10.1038/sj.bmt.1704086

[76] Farag SS, Caligiuri MA. Cytokine modulation of the innate immune system in the treatment of leukemia and lymphoma. Adv Pharmacol. 2004;**51**:295–318. DOI: 10.1016/S1054-3589(04)51013-X

[77] Rubnitz JE, Inaba H, Ribeiro RC, Pounds S, Rooney B, Bell T, Pui CH, Leung W. NKAML: a pilot study to determine the safety and feasibility of haploidentical natural killer cell

transplantation in childhood acute myeloid leukemia. J Clin Oncol. 2010;**28**:955–959. DOI: 10.1200/JCO.2009.24.4590

[78] Curti A, Ruggeri L, D'Addio A, Bontadini A, Dan E, Motta MR, Trabanelli S, Giudice V, Urbani E, Martinelli G, Paolini S, Fruet F, Isidori A, Parisi S, Bandini G, Baccarani M, Velardi A, Lemoli RM. Successful transfer of alloreactive haploidentical KIR ligand-mismatched natural killer cells after infusion in elderly high risk acute myeloid leukemia patients. Blood. 2011;**118**:3273–3279. DOI: 10.1182/blood-2011-01-329508

[79] Williams MA, Bevan MJ. Effector and memory CTL differentiation. Annu Rev Immunol. 2007;**25**:171–192. DOI: 10.1146/annurev.immunol.25.022106.141548

[80] Paust S, Gill HS, Wang BZ, Flynn MP, Moseman EA, Senman B, Szczepanik M, Telenti A, Askenase PW, Compans RW, von Andrian UH. Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigenspecific memory of haptens and viruses. Nat Immunol. 2010;**11**:1127–1135. DOI: 10.1038/ni.1953

[81] Peng H, Jiang X, Chen Y, Sojka DK, Wei H, Gao X, Sun R, Yokoyama WM, Tian Z. Liver-resident NK cells confer adaptive immunity in skin-contact inflammation. J Clin Invest. 2013;**123**:1444–1456. DOI: 10.1172/JCI66381

[82] Gillard GO, Bivas-Benita M, Hovav AH, Grandpre LE, Panas MW, Seaman MS, Haynes BF, Letvin NL. Thy1+ NK cells from vaccinia virus-primed mice confer protection against vaccinia virus challenge in the absence of adaptive lymphocytes. PLoS Pathog. 2011;**7**:e1002141. DOI: 10.1371/journal.ppat.1002141

[83] Abdul-Careem MF, Lee AJ, Pek EA, Gill N, Gillgrass AE, Chew MV, Reid S, Ashkar AA. Genital HSV-2 infection induces short-term NK cell memory. PLoS One. 2012;**7**:e32821. DOI: 10.1371/journal.pone.0032821

[84] Min-Oo G, Kamimura Y, Hendricks DW, Nabekura T, Lanier LL. Natural killer cells: walking three paths down memory lane. Trends Immunol. 2013;**34**:251–258. DOI: 10.1016/j.it.2013.02.005

[85] Arase H, Mocarski ES, Campbell AE, Hill AB, Lanier LL. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. Science. 2002;**296**:1323–1326. DOI: 10.1126/science.1070884

[86] Dokun AO, Kim S, Smith HR, Kang HS, Chu DT, Yokoyama WM. Specific and nonspecific NK cell activation during virus infection. Nat Immunol. 2001;**2**:951–956. DOI: 10.1038/ni714

[87] Smith HR, Heusel JW, Mehta IK, Kim S, Dorner BG, Naidenko OV, Iizuka K, Furukawa H, Beckman DL, Pingel JT, Scalzo AA, Fremont DH, Yokoyama WM. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. Proc Natl Acad Sci U S A. 2002;**99**:8826–8831. DOI: 10.1073/pnas.092258599

[88] Pyzik M, Charbonneau B, Gendron-Pontbriand EM, Babić M, Krmpotić A, Jonjić S, Vidal SM. Distinct MHC class I-dependent NK cell-activating receptors control cytomegalovirus infection in different mouse strains. J Exp Med. 2011;(208):1105–1117. DOI: 10.1084/jem.20101831

[89] Sun JC, Madera S, Bezman NA, Beilke JN, Kaplan MH, Lanier LL. Proinflammtory cytokine signaling required for the generation of natural killer cell memory. J Exp Med. 2012;(209):947–954. DOI: 10.1084/jem.20111760

[90] Foley B, Cooley S, Verneris MR, Pitt M, Curtsinger J, Luo X, Lopez-Vergès S, Lanier LL, Weisdorf D, Miller JS. Cytomegalovirus reactivation after allogeneic transplantation promotes a lasting increase in educated NKG2C+ natural killer cells with potent function. Blood. 2012;**119**:2665–2674. DOI: 10.1182/blood-2011-10-386995

[91] Björkström NK, Lindgren T, Stoltz M, Fauriat C, Braun M, Evander M, Michaëlsson J, Malmberg KJ, Klingström J, Ahlm C, Ljunggren HG. Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. J Exp Med. 2011;**208**:13–21. DOI: 10.1084/jem.20100762

[92] Petitdemange C, Becquart P, Wauquier N, Béziat V, Debré P, Leroy EM, Vieillard V. Unconventional repertoire profile is imprinted during acute chikungunya infection for natural killer cells polarization toward cytotoxicity. PLoS Pathog. 2011;**7**:e1002268. DOI: 10.1371/journal.ppat.1002268

[93] Foley B, Cooley S, Verneris MR, Curtsinger J, Luo X, Waller EK, Anasetti C, Weisdorf D, Miller JS. Human cytomegalovirus (CMV)-induced memory-like NKG2C(+) NK cells are transplantable and expand in vivo in response to recipient CMV antigen. J Immunol. 2012;**189**:5052–5088. DOI: 10.4049/jimmunol.1201964

[94] Rölle A, Pollmann J, Ewen EM, Le VT, Halenius A, Hengel H, Cerwenka A. IL-12-producing monocytes and HLA-E control HCMV-driven NKG2C+ NK cell expansion. J Clin Invest. 2014;**124**:5305–5316. DOI: 10.1172/JCI77440

[95] Keppel MP, Yang L, Cooper MA. Murine NK cell intrinsic cytokine-induced memory-like responses are maintained following homeostatic proliferation. J Immunol. 2013;**190**:4754–4762. DOI: 10.4049/jimmunol.1201742

[96] Ni J, Miller M, Stojanovic A, Garbi N, Cerwenka A. Sustained effector function of IL-12/15/18-preactivated NK cells against established tumors. J Exp Med. 2012;**209**:2351–2365. DOI: 10.1084/jem.20120944

[97] Romee R, Schneider SE, Leong JW, Chase JM, Keppel CR, Sullivan RP, Cooper MA, Fehniger TA. Cytokine activation induces human memory-like NK cells. Blood. 2012;**120**:4751–4760. DOI: 10.1182/blood-2012-04-419283

[98] Rosario M, Romee R, Schneider S, Wagner J, Berrien-Elliott M, Leong J, Sullivan RP, Fehniger TA. Human cytokine-induced memory-like NK cells are active against myeloid leukemia in vitro and in vivo. Blood. 2014;**124**:1117. DOI: dx.doi.org/

[99] Leong JW, Chase JM, Romee R, Schneider SE, Sullivan RP, Cooper MA, Fehniger TA. Preactivation with IL-12, IL-15, and IL-18 induces CD25 and a functional high-affinity IL-2 receptor on human cytokine-induced memory-like natural killer cells. Biol Blood Marrow Transplant. 2014;**20**:463–473. DOI: 10.1016/j.bbmt.2014.01.006

Section 3

Infection and Inflammation

Chapter 11

Therapeutic Antibody-Based Drugs in the Treatment of Human Inflammatory Disorders

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Additional information is available at the end of the chapter

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Abstract

Inflammation causes debilitating human conditions and older treatments rely on global immunosuppression that non-specifically alleviates symptoms. Currently, several monoclonal antibodies (mAbs) are available that specifically block pro-inflammatory cytokines. These include mAbs specific to tumour necrosis factor (TNF), interleukin (IL)-1β, IL-6, IL-17 and IL-12/IL-23. The chapter summarises the key elements in human inflammatory disease conditions, including various forms of arthritis, psoriasis, Crohn's disease and ulcerative colitis, plus pyrin-associated inflammatory syndromes and periodic fevers, to explain the benefit of cytokine neutralisation through mAb-type reagents. The chapter reviews the efficacy and safety of the current repertoire of anti-cytokine/cytokine receptor mAbs. It also discusses the known side effects and adverse events that are sometimes associated with systemic blockade of cytokines *in vivo*, and concludes that the accumulating knowledge of treatment failures can reveal unappreciated aspects of cytokine biology and even new treatment opportunities. The chapter includes mention of the rapidly expanding cohort of biosimilar mAbs and the mAbs to IL-4, IL-5 and IL-13 that are now emerging, in addition to the need for treatments for disorders that remain refractory to the current repertoire of anti-cytokine mAbs and conventional treatments. Thus, here we summarise the current status of anti-cytokine mAbs for human inflammatory diseases.

Keywords: arthritis, asthma, crohn's disease, cytokines, biosimilar, inflammation, interleukin (IL), IL-1β, IL-4, IL-5, IL-6, IL-13, IL-12, IL-17, IL-23, monoclonal antibodies (mAbs), periodic fevers, psoriasis, pyrin, tumour necrosis factor (TNF), and ulcerative colitis

1. Introduction

Human inflammatory diseases are among some of the most debilitating conditions described and they inflict varying degrees of functional impairment and may be long-lasting, causing chronic pain. Some examples of inflammatory conditions include rheumatoid arthritis (RA) and other related or non-related arthritides, skin diseases such as psoriasis, or intestinal conditions such as Crohn's disease (CD) and ulcerative colitis (UC), as well as pyrin-associated inflammatory syndromes and periodic fevers. These conditions generally present as acute bouts of inflammation, but are in most cases active as chronic conditions with periods of worsening or 'flares'. Intense research, over many decades, has revealed important details regarding the mechanisms that contribute to the pathology of these conditions, although in many situations the initial trigger continues to remain undefined. This knowledge led to the use of broad-acting anti-inflammatory agents that exert benefit due to global immune suppression. Thus, drugs such as corticosteroids became the mainstream treatment option. Over time, however, as knowledge of the underlying pathobiology deepened, so the role of individual cytokines emerged as critical drivers of the *in vivo* inflammatory processes. Eventually, as it became known that microbes, especially viruses, encode and express cytokine-receptor mimics that block the biological effects of specific cytokines, and inhibit cytokine-mediated inflammation [1, 2], thus it became obvious to trial soluble receptor proteins as inhibitors of pro-inflammatory cytokines to treat human inflammatory diseases. Although the microbial products themselves are potent neutralising reagents, they were viral in origin—not human—and therefore immunogenic (not suitable for long-term human use). The use of neutralising cytokine-specific monoclonal antibodies (mAbs), and/or recombinant forms of soluble cytokine receptors, however, efficiently solved this problem, because these recombinant Ig-based molecules are essentially identical copies of endogenous human protein—purified monoclonal Ig. Thus was born the era of cytokine-neutralising mAb-based therapeutic reagents for the treatment of human inflammatory diseases.

2. Clinical presentation and processes of inflammatory diseases amenable for treatment with cytokines targeted neutralising mAbs

Inflammation is a natural and spontaneous process that occurs in response to an insult causing tissue damage. It involves the activation of innate and adaptive immune system components, including both vascular and cellular responses. Essentially, there are four signs that represent the clinical manifestations of inflammation: redness (Latin: *rubor*), warmth/heat (*calor*), swelling (*tumour*) and pain (*dolor*), and when unresolved, inflammation frequently results in the loss of physiological function (*function laesa*). Systemic symptoms such as fever also frequently occur. Together, these are the universal or classical hallmarks of inflammation in mammals.

The magnitude of the response is initially directly proportional to the severity of the insult, but reactivation of inflammation can be triggered, either by a reoccurrence of the same or similar event, or sometimes via an unrelated event. During times of exacerbation, the severity of symptoms escalates dramatically, and this is often referred to as an inflammation 'flare'. In either instance, the physiological events that follow are becoming increasingly well understood at a molecular level and this detailed mechanistic understanding has revealed a number of

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opportunities for therapeutic blockage of specific mediators of inflammation. In many cases, the results of these specific interventions have been truly remarkable, such that previously debilitating disease conditions are now entirely manageable or, in some cases, almost unnoticeable. The following sections provide a summary of current knowledge of the molecular basis of the events that occur in several human inflammatory disorders together with a description of the mAbs and recombinant protein-based reagents that can be applied to successfully ameliorate inflammation.

2.1. Inflammatory cytokines in the pathology of arthritides: rheumatoid arthritis (RA), idiopathic juvenile arthritis (IJA) and ankylosing spondylitis (AS)

Rheumatoid arthritis (RA) is an autoimmune disease that comprises both systemic and tissues-specific inflammation, primarily inflammation of joint synovium, leading ultimately to erosion of the joint tissue. The initial trigger of the inflammation is usually unknown. Once present, however, it usually progresses and is characterised by episodes of greater intensity or flares. The systemic nature of the condition is exemplified by the fact that diverse tissues may be involved, including skin and kidneys. It is generally believed that there are three main phases of pathology in RA: (i) an initial induction phase of non-specific tissue inflammation, (ii) an expansion phase involving T lymphocyte (T cell) responses and (iii) a chronic systemic inflammation phase mediated by the production of cytokines such as interleukin (IL)-1β, tumour necrosis factor (TNF) and IL-6 [3] and the production of citrullinated fibrinogen among other substrates. The 'unnatural' citrullinated proteins are frequently the targets of rheumatoid factor IgM and IgG autoantibodies [4]. Although the systemic phase is debilitating in its own right, the inflammatory destruction of joint synovium results in immobile and dysfunctional joints, and this is often amplified by the involvement of multiple affected joints, that is, polyarthritis (**Figure 1**); for most patients, the painful chronic synovitis ultimately results in irreparable joint destruction.

RA occurs not only in adults but also in children [5]. There are many presentations of juvenile arthritis and most are idiopathic in nature, and include polyarticular and/or systemic arthritis, as well as fever, skin rash, anaemia, spleen, liver and sometimes even cardiac tissue inflammation [6, 7]. The inflammation is thought to be due to activation of macrophages and other immune cells (monocytes, dendritic cells, T cells, etc.), which may explain the different subtypes of juvenile idiopathic arthritis (JIA) [8], and in all cases there is inflammation mediated primarily

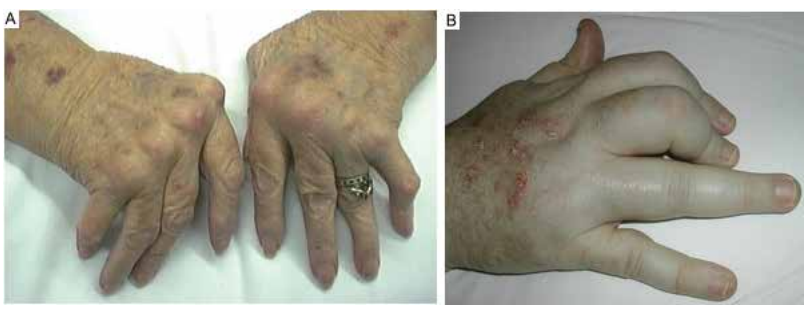

Figure 1. Clinical presentation of rheumatoid and psoriatic arthritis. (A) Long-standing RA characterised by ulnar deviation, metacarpal phalangeal joint subluxation and boutonniere deformity, and (B) Psoriatic arthritis with boutonniere deformity. (Images generously provided by Prof. Manolios, Westmead Hospital, Sydney, Australia).

by the production of soluble mediators—especially pro-inflammatory cytokines such as TNF [9, 10]. Systemic JIA (SJIA) is thus considered a multifactorial auto-inflammatory disease [6].

Previous treatments for SJIA have traditionally comprised non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids in more severe cases, but these drugs can have significant side effects and are often poorly tolerated in children, sometimes causing lifelong sequelae such as sterility. Fortunately, most anti-TNF-based mAbs have yielded promising results, although efficacy rates and treatment retention rates decrease with treatment time [11]. With the emergence of IL-6 neutralising mAbs, such as tocilizumab, other effective treatment options also now exist [12]. However, IL-1β-blocking agents such as anakinra or canakinumab also show significant efficacy in JIA [13, 14]. Taken together, these findings support the current dogma that the pathobiology of JIA is not entirely identical to adult RA even when joint arthropathy is the primary common lesion. Secondly, these results suggest that there exists an inflammation hierarchy among the contributing cytokines—some being critical to the production of other cytokines or inflammatory mediator substances, and others less significant, that is, some are 'non-drivers' of the pathology but elevated nonetheless [9]. Furthermore, the presence of TNF, IL-6 and IL-1β strongly points to the involvement of macrophages—particularly type-I macrophages (M1 MØ) which, when activated, produce this combination of cytokines. Interestingly, a polymorphism in macrophage migration-inhibitory factor (MIF) has been found to be associated with SJIA [15, 16]. Indeed, the diverse presentation of juvenile arthritis suggests that there is still much more to learn about the aetiology of arthritis in children.

Ankylosing spondylitis (AS) is another type of inflammatory arthritis that usually involves the sacroiliac joint and spine [17]. As this disease worsens, shoulders can also be affected. The predominant symptoms are joint stiffness and pain caused by a chronic low-grade inflammation [17]. In advanced cases, vertebra can actually fuse and remain in a fixed and immobile position, explaining why many AS patients frequently present with a classical 'forward-leaning' posture or limited flexion in the lumbar spine and inter-vertebral calcification (**Figure 2A** and **B**). Despite a long-known association to HLA-B27, and other immune gene loci [18], and an increased prevalence in males, the trigger for this condition remains unknown [17]. The disease can be either undifferentiated or more specific in its presentation, for example, presenting in a more defined manner such as with reactive arthritis, psoriatic arthritis (see **Figure 1**) or more dispersed symptoms such as arthritis with an associated inflammatory

Figure 2. Clinical presentation of ankylosing spondylitis (AS) and psoriasis. (A) AS in a 30-year-old male with limited flexion of lumbar spine, (B) AS involving cervical spine; X-ray features show calcification of anterior longitudinal ligament, (C) psoriatic erosions involving proximal interphalangeal joints and second distal interphalangeal joint and (D) psoriatic skin lesion characterised by flacking and silver scales. (Images generously provided by Prof. Manolios, Westmead Hospital, Sydney, Australia).

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bowel disease (IBD) condition. The link with IBD is intriguing, and although this has long been a rather poorly understood AS disease association (or presentation), recent evidence suggests a potential role of IL-17-family cytokines.

Early treatments for AS have been focused primarily on relieving pain, for example, through non-steroidal anti-inflammatory drugs such as aspirin, ibruprofen or voltaren and so on. Cox-2 inhibitors have also been used. As these are broad inhibitors of inflammation and pain-reducing mimetics, they do not specifically target the specific factors that are critical to the underlying aetiology of the condition. Similarly, drugs, such as sulfasalazine, methotrexate or corticosteroids, while offering some degree of efficacy in the treatment of AS, are, again, broad-acting immune suppressants. As knowledge of the molecular aetiology of this disease has increased, it was found that TNF-neutralising drugs etanercept, adalimumab, certolisumab pegol, infliximab or golimumab can be effective [19]. Yet, precisely how anti-TNF mAbs provide benefit in AS patients, however, is still not entirely clear, due essentially to the gaps in knowledge surrounding this disease; the ability of the anti-TNF agents to prevent new bone formation, for example, is still controversial and poorly explained through existing knowledge. Moreover, anti-TNF mAbs are not beneficial in all AS patients. Thus, most clinicians conclude that while TNF may be produced in certain circumstances in AS pathology, it may or may not be the driving factor in AS disease pathology [20].

There is currently much excitement surrounding the role for cytokines IL-17 and IL-23 in AS. Indeed, the demonstrated efficacy of IL-17 and IL-23 neutralising mAbs in clinical trials has recently cemented these cytokines as central mediators of AS inflammation. Several previously unexpected immune cells are now therefore strongly implicated as being critical components of the pathobiology of AS, specifically Th-17 cells and lineage-negative innate-like immune cells (ILC) type 3 [21]. The different subsets of ILC3 cells typically produce not only IL-17-type cytokines but also other cytokines such as IL-6, TNF and IFNγ (thus explaining the partial benefits of treatment with anti-TNF mAbs, and global immune-suppressive treatments). These ILCs are interesting in AS because they are exposed to bacteria and microbial products as they are found in skin and in gut and recognised for their role in preserving barrier function. Moreover, the detection of these innate cell types in the blood of AS patients [22] thus provides a mechanistic link with the AS arthritis and the inflammatory bowel disease-type symptoms that occurs in many AS patients. Moreover, both TNF and IL-17 have long been implicated in the structural bone damage and remodelling that is evident in AS [23, 24]. More research will be required to define the precise pathogenic mechanisms of IL-17-producing innate immune cells in AS.

2.2. Inflammatory cytokines in psoriasis (and psoriatic-type arthritis)

Psoriasis is an autoimmune skin condition where patches of scaly skin accumulate. The locations of these patches are usually elbows, knees or scalp, although the location is not a diagnostic feature per se, and the psoriatic skin lesions can occur almost anywhere on the body. A proportion of people with chronic skin psoriasis will also develop a type of psoriatic arthritis of joints (**Figure 1**). Like RA, this can result in significant joint erosion (**Figure 2**) but this type of arthritis is rheumatoid factor negative, and thus distinct from RA [25]. Psoriasis is also different from eczema, in that there is a thickening of the epidermis and the condition almost always persists, whereas eczema often fades spontaneously, for example, as children grow older. In fact, there are

various forms of psoriasis, including the most common form—plaque psoriasis, comprising an accumulation of dead skin cells building up, forming a cracked 'plague' skin lesion (**Figure 2**). Some patients, however, develop smooth, shiny skin lesions; these usually being on the knee or under the arm. In addition, guttate psoriasis is a form of the psoriatic disease that sometimes form after *Streptococcal* sp. bacterial infections. Erythrodermic psoriasis is the most severe form of the disease, and in this condition large areas of skin eventually sloth off.

In psoriasis treatment, a number of systemic immunosuppressive agents have been used, for example, cyclosporine or methotrexate. Nevertheless, the dysfunction of cytokines, especially IL-13, IL-17 and IL-23, appears to be integral to the pathology of all forms of psoriasis—consistent with the broad benefits of cyclosporin in psoriatic pathologies [26]. Benefit has long been established with mAb-based reagents that neutralise TNF [27], and more recently, new IL-23-neutralising mAbs are demonstrating considerable efficacy [28, 29]. The IL-17A-neutralising mAbs secukinumab and ixekizumab, and IL-17R-blocking mAbs brodalumab are also showing significant efficacy in ameliorating psoriatic-based skin conditions [30–32]. This is consistent with the observations of elevated IL-17A within psoriatic plaques (skin lesions), being produced from many immune cell types [33], as well as IL-13 [34]. In fact, it has also been recently demonstrated that IL-17 is intimately linked to IL-13 biology, whereby IL-13 regulates IL-17A production in Th17 cells [35, 36]. These findings are also consistent with the observation that transgenic IL-17A expression mice develop psoriatic-type skin lesions that resembles human psoriasis [37]. The striking efficacy of anti-IL-17 mAbs indicates that IL-17-producing cells, such as Th-17 cells, are integral to the pathobiology of many forms of psoriasis. Recently, however, IL-17-producing ILC3s have been shown to be present in psoriatic tissues [38, 39]. It has also been shown that CD1a-restricted IL-17-producing lipid antigen recognising T cells are present both in skin and in blood of psoriasis patients [40]. Hence, the dramatic success of these new mAbs not only brings psoriasis patients the promise of relief of their symptoms but also simultaneously reveals new and otherwise unappreciated knowledge of the critical aspects of the disease mechanisms at play in psoriasis.

Other interesting recent developments are new oral treatments for psoriasis [41]. For example, a small molecule phosphodiesterase-4 inhibitor (apremilast) works by preventing cAMP activation in immune cells, thereby limiting pro-inflammatory cytokine production [42–45]. It should be noted, however, that initial clinical trials were discontinued due to unexpected side effects such as diarrhoea, headache and nausea, although careful re-examination of dosing regiments and/or new molecular modifications may still be possible. Nonetheless, phosphodiesterase-4 has itself been found to be elevated in psoriatic lesion inflammatory cells [44], and thus the amelioration of symptoms correlates perfectly with its potent inhibition *in vivo*. In summary, these findings again strongly substantiate the involvement of inflammatory cytokines, especially IL-17 and IL-23, in the aetiopathology of human psoriasis. It is no exaggeration to conclude that newly developed mAbs blocking IL-17 and IL-23 pathways have completely revolutionised the treatment of chronic psoriasis— they now already comprise the 'standard of care' in plaque psoriasis treatments [46]. Even so, there is much more to learn about this complex condition, such as the roles of IL-12 versus IL-23, for example, in limiting IL-17 production, and the role of IL-17-producing skin γδ T cells [47].

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2.3. Cytokines in the pathology of inflammatory bowel disease (IBD): Crohn's disease (CD) and ulcerative colitis (UC)

There are several autoimmune-based chronic inflammatory bowel diseases (IBD) involving the gastrointestinal tract (GIT) and these most frequently include Crohn's disease (CD) and ulcerative colitis (UC). Generically speaking, CD is considered to involve the distal junction of the small intestine and thus primarily involves inflammation in the large intestine, whereas UC inflammation can occur anywhere within the entire GIT. These conditions are both progressive and characterised by relapsing inflammation [48]. CD lesions usually involve only the superficial mucosal tissue layers, whereas UC inflammation is often more extensive, even presenting through the full-tissue thickness of the intestine. A less-well-known feature of CD is that the inflammation may involve non-GIT mucosa, for example, skin, eyes or joints, and even liver can be affected. The pathological processes in CD and UC are complex, with a deep and interconnecting interplay between inflammation and fibrosis as there is often a constant need for tissue healing [49]. For both CD and UC, the differential diagnosis is usually confirmed through endoscopy, as this procedure permits the delineation of the anatomical location that is affected (site of the inflammation). Importantly, the endoscopy also provides the opportunity for the grading of lesion severity.

In both CD and UC the immune system is highly activated, explaining the clinical benefits experienced from treatments that induce global immune suppression. Cytokine-specific mAb-based treatments are also effective at blocking and preventing IBD inflammation. It has become increasingly evident that environmental triggers are both constitutive and exacerbating during times of inflammatory flares, and hence the systemic presence of therapeutic mAbs provides a long-lasting inhibition towards the chronic inflammation. There is also a growing appreciation of the role of the gut microbiota in IBD [50]. Although the intestinal (mucosal) immune system is meant to remain unresponsive to commensal microorganisms, just as it is to food-based antigens, it retains a capacity to respond to intestinal pathogens. The current theory, however, is that there is an inappropriate, and potentially constitutive, activation of innate immune cells within the bowel and these activated cells constitute the basis of chronic IBD inflammation [48]. Theoretically, IBD inflammation may involve almost any innate immune cell residing within the GIT mucosa, but Th1- and/or Th-17-type pro-inflammatory cytokines appear to be involved—and these cells produce both TNF and/or IL-17 plus IFNγ [48]. Also, there is currently a high level of interest in the ILC3 cells in acting as the initial triggers of IBD inflammation [51, 52]. However, changes in commensal gut microflora are also now in focus, and especially the ability of bacteriophage viruses, due to their capacity to lyse bacteria and thereby alter the GIT microbiome diversity [53]. Thus, both a dysbiosis and inflammation-mediated disruption of the GIT epithelial barriers are currently thought to be integral to both UC and CD conditions. Fortunately, there are already several neutralising mAb-based therapies for IBD patients, especially for those who are refractory to traditional treatment of aminosalicylates and corticosteroides. These include the anti-TNF mAbs (infliximab, adalimumab, golimumab and certolizumab pegol) and two anti-integrin-blocking mAbs (natalizumab and vedolizumab). In contrast to the benefit evident in neutralising TNF, a contributing role for IL-17 in IBD is still uncertain, and IL-12/IL-23 are likely not the driver cytokines as there is only marginal efficacy from

ustekinumab (anti-12/IL-23) in CD patients, and no benefit was evident in initial trials with briakinumab (anti-IL-12/IL-23 p40-neutralising mAbs) [54]. Indeed, brodalumab (anti-IL-17RA-neutralising mAb) caused worsening symptoms in CD [55]. Clearly, further investigation into the complex interactions between the normal and altered microbiome, and the endogenous intestinal cells, including resident innate and adaptive immune cells, is required to better understand these IBD pathologies.

2.4. Autoinflammatory diseases: TNF-receptor-associated periodic syndrome (TRAPS), cryopyrin-associated periodic syndrome (CAPS) and Muckle-Wells syndrome

One of the clearest cases of the mechanistic role of cytokines in the aetiology of human inflammation concerns the hereditary periodic fever conditions. Here, an autoinflammatory trigger (or triggers) involves genes that are embedded within the innate immune system, but the response occurs in the absence of demonstrable infection—although there still remains the possibility that a subclinical and undetectable infection is present [56]. For example, patients with TNFR1 mutations are usually classified as TNF-Receptor-Associated Periodic Syndrome (TRAPS) [57]. TRAPS fevers typically last more than a week and exhibit a range of symptoms, such as myalgia, arthritis, fasciitis, abdominal pain, skin rashes and patches (**Figure 3**), or periorbital oedema, and even amyloidosis in severe cases [58, 59]. The precise mechanism(s) of pathology resulting in TRAPS has continued to mature over time, as TRAPS mutant TNFRs have been successively thought to result in altered activation of a key transcription factor within the immune system (NF-κB), an inability to bind to TNF, reduced surface expression of TRAPS TNFRs, the incorrect folding of the receptors leading to an 'unfolded protein response' which appears to activate the inflammasome and lead to mitochondrial reactive oxygen species, and ultimately to inflammation [56, 60]. Despite the varied presentations, a unifying presentation in TRAPS patients is the elevated levels of serum TNF, IL-1β and IL-6 cytokines. TRAPS treatment options vary but broad immunosuppression, such as with colchicine, is no longer generally recommended, as it is accepted that there is significant benefit in treating patients only at the times of inflammation, that is, during disease flares, and potentially monitored via levels of serum S100 proteins, IL-18, serum amyloid A, and even miRNA molecules [61, 62]. With the number of inflammatory cytokines that are elevated, the treatment options range from generic immune suppressants (e.g. colchicine) to the use of specific cytokine-neutralising mAbs. Unexpectedly perhaps, anti-TNF mAbs have largely proven ineffective in TRAPS, and they may even unexpectedly sometimes provoke a cytokine storm via the activation of the cRel (a component of the NF-κB system), and thereby escalating the inflammation [63]. Interestingly, the current standard treatment for TRAPs and the majority of hereditary autoinflammatory diseases is the neutralisation of IL-1β, and either recombinant IL-1 receptor antagonist (anakinra) or human IgG1 anti-IL-1β mAb (canakinumab) alleviates inflammation in TRAPS [64, 65]. Hence, it appears that targeting only IL-1β is beneficial in TRAPS. This, again, implies that there exists a hierarchy of inflammatory cytokines, such that blocking one cytokine has a broader effect of reducing the production of others. In fact, the administration of recombinant human TNF in human clinical trials for cancer and sepsis clearly demonstrated this principle: the administration of TNF induced elevated IL-1β and IL-6 [66, 67] (recently reviewed in Ref. [68]).

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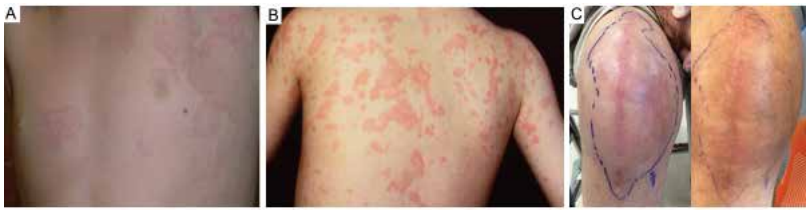

Figure 3. Clinical presentation of auto-inflammatory syndrome skin rashes and pseudo-gout inflammation. (A) TRAPS skin rash (from [69]), (B) Muckle-Wells syndrome / CAPS rash (image from autoinflammatory.org) and (C) joint and tissue inflammation due to pseudo-gout flair after total knee arthroplasty of right knee, both before (left) and after (right) antibiotics for potential culture-negative post-operative infection (Images used with permission).

Other autoinflammatory syndromes include Muckle-Wells syndrome (MWS), which presents with periodic episodes of skin rashes (**Figure 3**), sensorineural deafness, hives, episodal fever, joint pain and/or amyloidosis and other symptoms. These conditions are collectively known as cryopyrin-associated periodic syndrome (CAPS) and they are all universally associated with activation of pro-caspase-1 [70, 71] and thus also with mutations in NLRP3/CIAS1 and LNRC4 genes [72, 73] (see www.autoinflammatory-search/diseases). The central mechanism of pathogenesis of CAPS-type diseases is the elevated production of IL-1β, usually from activated monocytes/macrophages, and because of the involvement of caspase-1, there is usually a concomitant elevated production of IL-18. Thus, the neutralisation of IL-1β as the fundamental driver of the inflammation is proving to be beneficial in these conditions, that is, either with mAb canakinumab or with recombinant IL-1Ra protein (anakinra). Even deafness in Muckle-Wells syndrome patient was alleviated by neutralising IL-1β [74]. Finally, NLRP3 activation also results in elevated IL-1β in other unrelated sterile inflammatory conditions such as those involving monosodium urate (gout) and calcium pyrophosphate dihydrate (CPPD) (pseudo-gout) crystalline-induced arthritis (**Figure 3**) [75, 76]. Thus, neutralising IL-1β is effective in nearly all CAPS-type autoinflammatory conditions [60].

3. Biological therapeutics for inflammation

There are currently more than 20 recombinant cytokine receptor- and mAb- based protein drugs that have been developed and widely approved for the treatment of human inflammation (see **Boxes 1–5**). These can be classified as recombinant cytokine receptor-based proteins, or cytokine- or cytokine receptor-specific-neutralising mAbs (**Figure 4**).

3.1. Recombinant cytokine receptors and receptor-Ig fusion proteins

Etanercept (trade name Enbrel; www.enbrel.com) was the first human cytokine-receptor immunoglobulin chimeric fusion protein approved for the treatment of human diseases. Etanercept comprises the extracellular region of human TNFR2 and the Fc region of human IgG1, and is produced in Chinese hamster ovary (CHO) cells. As a TNFR2-based-Ig protein, it has properties of both a human cytokine receptor and human Ig protein: the TNFR2 component binds to TNF and lymphotoxin-α, whereas the human IgG1 portion confers serum longevity and Ig Fc receptor (FcR)-binding capacity. Etanercept is thus a TNF inhibitor capable of neutralising soluble

Box 1. Current therapeutic TNF and TNF-receptor-specific inhibitory agents.

TNF-receptor Ig fusion proteins and anti-tumour necrosis factor (TNF)				
Drug name and structure	**Brand name**	**Usual delivery**	**Approved disease indications**	**Additional potential uses**
Etanercept Recombinant fusion protein: Human TNFR2:IgG1-Fc	Enbrel®	s.c. injection	Rheumatoid arthritis Polyarticular juvenile idiopathic arthritis (PJIA) Psoriatic arthritis Ankylosing spondylitis Plaque psoriasis	Cognitive impairment (peri-spinal delivery)?
Infliximab Humanised (chimeric) IgG1κ	Remicade®	i.v. infusion	Rheumatoid arthritis* Psoriatic arthritis* Ankylosing spondylitis Plaque psoriasis Crohn's disease Paediatric RA Paediatric Crohn's disease	
Adalimumab Human IgG1κ	Humira®	s.c. injection	Rheumatoid arthritis* Psoriatic arthritis* Plaque psoriasis Active ankylosing spondylitis Crohn's disease Juvenile idiopathic arthritis Ulcerative colitis	
Golimumab Human IgG1κ	Simponi®	s.c. injection	Rheumatoid arthritis* Psoriatic arthritis* Plaque psoriasis Ulcerative colitis	
Certolizumab Pegol Pegylated-Fab' of humanised IgG1κ	Cimzia®	s.c. injection	Rheumatoid arthritis* Psoriatic arthritis* Ankylosing spondylitis Crohn's disease	
Biosimilars: (Among others)				
Erelzi TNFR2-IgG1 Etanercept biosimilar	etanercept-szzs® (Sandoz)	i.v. infusion	Same indications as per etanercept	
Brenzys (SB4) TNFR2-IgG1 Etanercept biosimilar	(Samsung Bioepis; Merck and Biogen)	i.v. infusion	Same indications as per etanercept	
CTP-13 Humanised IgG1κ Infliximab biosimilar	Remsima® (Infliximab) Inflectra® (Hospira)	i.v. infusion	Same as per infliximab	
BOW015 Human IgG1κ Infliximab biosimilar	Infimab® (Reliance Life Sciences)	i.v. infusion	Same as per infliximab	
SB2 Human IgG1κ Infliximab biosimilar	(Samsung Bioepis; Merck and Biogen)	i.v. infusion	Same as per infliximab	

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TNF-receptor Ig fusion proteins and anti-tumour necrosis factor (TNF)				
Drug name and structure	**Brand name**	**Usual delivery**	**Approved disease indications**	**Additional potential uses**
Adalimumab-atto Human IgG1κ Adalimumab biosimilar	Amjevita® (AMGEN)	s.c. injection	Same as per adalimumab	
Adalimumab (India) Human IgG1κ Adalimumab biosimilar	Adfrar® (Torrent Pharma)	s.c. injection	Same as per adalimumab	
SB5 Human IgG1κ Adalimumab biosimilar	(Samsung Bioepis; Merck and Biogen)	s.c. injection	Same as per adalimumab	

Note: *These agents can be used alone or in combination with methotrexate or other non-biologic disease-modifying anti-rheumatic drugs.

Box 2. Current therapeutic IL-1β-specific mAb, or IL-1-receptor antagonist, inhibitory agents.

Anti-interleukin-1β or IL-1-receptor-antagonist				
Drug name and structure	**Brand name**	**Usual delivery**	**Approved disease indications**	**Additional potential uses**
Anakinra Recombinant human IL-1Rα (*E. coli*-derived protein; non-mAb)	Kineret® (AMGEN/ Biovitrum)	s.c. injection	Adult rheumatoid arthritis (moderate-to-severe, monotherapy or with DMARDS)	Lupus nephritis Inflammatory joint diseases: psoriatic arthritis, spondyloarthritis, osteoarthritis, etc. Periodic fevers Gout Asbestosis Epilepsy Stroke
Rilonacept Recombinant IL-1R accessory protein (*E. coli*-derived)	Arcalyst® (Regenron Pharmaceuticals)	s.c. injection	Cryopyrin-associated periodic syndromes (CAPS), including familial cold auto-inflammatory syndrome (FCAS) and Muckle-Wells syndrome (MWS)	
Canakinumab Humanised anti-IL-1β IgG1κ	Ilaris™ (ACZ885) (Novatis)	s.c. injection	Cryopyrin-associated periodic syndrome (CAPS) Familial cold auto-inflammatory syndrome (FCAS) and Muckle-Wells syndrome (MWS) Systemic juvenile idiopathic arthritis (SJIA)	Rheumatoid arthritis Chronic obstructive pulmonary disease Coronary artery disease Gout Schizophrenia
Gerokizumab Humanised mouse anti-human IL-1β IgG2κ (Fab)	Eyeguard™ (XOMA Corp.)		No approved medical indications at present	Behçets Uveitis Non-infectious uveitis Pyoderma gangrenosum

Box 3. Current therapeutic IL-6 and IL-6-receptor-specific inhibitory agents.

Anti-interleukin-6 and IL-6Rα				
Drug name and structure	**Brand name**	**Usual delivery**	**Approved disease indications**	**Additional potential uses**
Tocilizumab Humanised mouse anti-IL-6R IgG1κ	Actemra® (Hoffmann–La Roche)	i.v. infusion (monthly) or more usually s.c. injection	Rheumatoid arthritis Systemic juvenile idiopathic arthritis (SJIA) Crohn's disease (moderate/severe) Castleman's disease	Neuromyelitis Optica (Devic's disease) GVHD? TRAPS?
Sarilumab Human anti-IL-6R IgG1κ	VelocImmune® (Sanofi & Regeneron)	s.c injection	Rheumatoid arthritis (with methotrexate) Plaque psoriasis (moderate/severe)	AS?* (**failed trials)
Sirukumab Human mAb IgG1κ	(GlaxoSmithKline)	s.c. injection	Rheumatoid arthritis (with or without methotrexate)	Giant cell arteritis (vasculitis) Non-eosinophilic asthma

Box 4. Current therapeutic IL-17 and IL-17-receptor-specific inhibitory agents.

Anti-interleukin-17 and IL-17R				
Drug name and structure	**Brand name**	**Usual delivery**	**Approved disease indications**	**Additional potential uses**
Brodalumab Human anti-IL-17R IgG2κ	(KHK4827, AMG827) (Valeant Pharmaceutical & Kyowa Hakko Kirin)	s.c. injection	Psoriasis (severe) Psoriatic arthritis Rheumatoid arthritis Asthma Crohn's disease (moderate/severe)	None yet known
Ixekizumab Humanised anti-IL-17A and anti-IL-17A/F IgG4	Taltz® (LY2439821 Eli Lily & Co).	s.c. injection	Plaque psoriasis (moderate/severe)	None yet known
Secukinumab Human anti-I7A IgG1κ	Cosentyx® (Novartis Pharma AG)	s.c. injection	Plaque psoriasis (moderate/severe) Psoriatic arthritis Ankylosing spondylitis	None yet known

serum TNF and LTα, engaging with cytokine-expressing cells (i.e. membrane-bound TNF), and simultaneously also in engaging with FcR-expressing cells and henceforth of triggering FcR-mediated cell signalling (for a recent review, see [68]). An analogous TNFR1 p55-IgG1 Fc fusion protein (Lenercept) was similarly produced and tested in a double-blind placebo-controlled clinical trial for multiple sclerosis (MS). This disease choice was based on the fact that TNF is produced in MS and has demonstrable cytotoxic activity against oligodendrocytes—the cells that are destroyed by the immune system in MS—and because TNF neutralisation had been shown to be beneficial in mice with experimental autoimmune encephalitis (a murine model for MS-like disease). However, MS patients reported no benefits from the Lenercept treatment and

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Box 5. Current therapeutic IL-12/IL-23 and common receptor-specific inhibitory agents.

Anti-interleukin-12 and interleukin-23 (IL-12 and IL-23)				
Drug name and structure	**Brand name**	**Usual delivery**	**Approved disease indications**	**Additional potential uses**
Ustekinumab Humanised mAb anti-IL-12/IL-23 p40 IgG1κ	Stelara® (CNTO 1275) (Centocor & Jassen-Cilag)	s.c. injection	Plaque psoriasis (moderate/severe)	RA AS CD Systemic lupus erythematosis Ankylosing spondylitis
Briakinuman Human mAb anti-IL-12/IL-23 p40 IgG1κ	ABT-874 (Abbott)	s.c. injection	Plaque psoriasis Psoriatic arthritis	RA, CD? MS?
Tildrakizumab Humanised mAb Anti-IL-23 p19 IgG1κ	(Merck; and now Sun Pharma)	s.c. injection	Plaque psoriasis (moderate/severe)	CD?
Guselkumab Humanised mAb Anti-IL-23 p19 IgG1κ	(Janssen Research & Development)	s.c. injection	Plaque psoriasis (moderate/severe)	CD?
AMG139 Human mAb anti-IL-12/IL-23 p40 IgG1κ	(Amgen)		In Phase II trial for CD	
BI655066 Human mAb anti-IL-12/IL-23 p40 IgG1	(Boehringer Ingelheim Pharmaceuticals)		In Phase II trial for psoriasis	

unfortunately many trial patients experienced an unexpected worsening of their disease [77]. Lenercept also failed clinical trials for sepsis [78]. The reasons for this failure, especially in the face of the success of etanercept, were enigmatic at the time and remain incompletely explained even today; it is not clear whether ligand-binding differences, or even minor differences in the Ig component, explain the divergence in *in vivo* behaviour and therapeutic efficacy. Onercept, a TNFR1-extracellular region without an FcR component was also created by molecular biology engineering. Onercept neutralised TNF *in vitro*, but it failed in clinical trials for psoriasis [79]. In fact, several other human TNF-inhibitory TNFR-based reagents have also been developed, such as pegsunercept (a pegylated recombinant soluble TNFR1 protein), but these were not licensed for various reasons, primarily a lack of efficacy for the disease situations in which they were tested (reviewed in Ref. [68]).

In an analogous manner, a recombinant bio-therapeutic IL-1β inhibitor comprising a purified recombinant IL-1 receptor antagonist protein, anakinra (trade name Kineret; www.kineretrx.com), has been developed and approved for the treatment of adult RA, usually administered as a weekly subcutaneous (s.c.) injection. Moreover, another IL-1RA (accessory) protein, rilonacept (trade name Arcalyst; www.arcalyst.com), is a dimeric fusion protein comprising

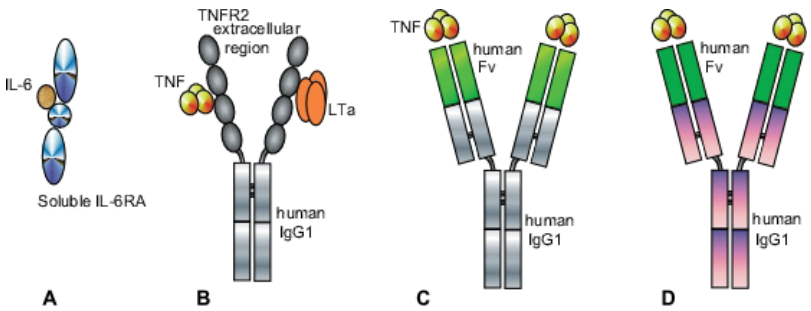

Figure 4. Examples of recombinant protein mAb-based drugs. (A) Soluble (extracellular region) cytokine receptor, (B) soluble (extracellular region) cytokine receptor—Ig Fc fusion protein, (C) humanised or fully human mAb and (D) biosimilar human or humanised mAb.

IL-1R1, and an IL-1RA linked to IgG1-Fc. It is approved for the treatment of Cryopyrin-Associated Periodic Syndromes, including Muckle-Wells syndrome in adults and in children of 12 and older. It should be noted that these IL-1β receptor-based inhibitors are specifically contraindicated for simultaneous use with anti-TNF agents due to a dramatically increased risk of infection (see below for a full list of contraindications).

3.2. Cytokine-neutralising mAbs

Infliximab (trade name Remicade; www.remicade.com) was the first anti-human cytokine mAb to be approved for therapeutic use. Infliximab binds to both soluble and membrane-bound human TNF, and this interaction prevents TNF from binding to either of its receptors TNFR1 or TNFR2. Since antibodies are high-affinity reagents, infliximab is thus a potent inhibitor of TNF's biological activities. Infliximab is administered by intravenous (i.v.) infusion, usually 5 mg/kg, every 8 weeks (see **Box 1**). Other human TNF-specific therapeutic mAbs now also exist. Adalimumab (trade name Humira; www.humira.com) and golimumab (trade name Simponi; www.simponi.com) are both human and humanised anti-human TNF IgG1 mAbs. These mAbs are generally administered by s.c. injection, every 1–2 weeks (see **Box 1**). Certolizumab pegol (trade name Cimzia; www.cimzia.com) is a pegylated human immunoglobulin Fab' fragment of an anti-TNF IgG1 mAb. It is also administered by s.c. injection, usually monthly. These agents are all approved for use in a broad array of arthritic- and psoriatic-related human inflammatory conditions (see **Box 1**). In the USA, Adalimumab has also recently been approved for hidradenitis suppurativa (apocrine acne). This is a chronic inflammatory condition that affects apocrine gland-bearing skin, such as that found in the axillae and groin, where recurrent boil-like nodules develop and fail to heal.

More recently, neutralising IL-1β-specific mAbs have also emerged, canakinumab (trade name Ilaris; www.ilaris.com) and gerokizumab (trade name Eyeguard). These are approved for CAPS-type auto-inflammatory conditions, including MWS, as well as systemic JIA (see **Box 2**). Similarly, blocking mAbs specific to IL-6R, tocilizumab (trade name Actemra; www.actemra.com), sarilumab and sirukumab, have also been developed (**Box 3**). Sarilumab has recently successfully completed a phase III clinical trials in combination with methotrexate for RA, and

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its approval appears to be imminent in the USA. These anti-IL-6 mAbs are being used in combination with methotrexate to slow RA and JIA progression in patients who do not benefit from anti-TNF agents, or especially when methotrexate monotherapy is less efficacious than expected. Tocilizumab is additionally approved for the B cell tumour Castleman's disease [80], and there is preliminary evidence that it might be effective against treating the refractory neuromyelitis known as Devic's disease [81].

Other recent additions to the repertoire of human cytokine-neutralising mAbs are those that inhibit IL-17 and IL-23 which are showing efficacy in the treatment of psoriasis and psoriatic-related conditions (see **Box 4**). Brodalumab, an IL-17RA-specific mAb, is one such reagent that acts by preventing IL-17-family cytokines from binding to the IL-17 receptor (**Box 4**). Recent Brodalumab data, derived from phase II and III clinical trials, have demonstrated effectiveness in the treatment of psoriasis [32], and reportedly with superior skin clearance than the anti-IL-12/IL-23 mAb ustekinumab [55, 82]. These are long-awaited treatment for a skin condition that has previously proven to be difficult to treat. However, the clinical trials with Brodalumab were unpredictable, in that trial-related adverse events apparently included suicidal ideation with trial-related harmful behaviours in some patients even suicide [83]. This unexpected outcome may translate to limitations with its use and has necessitated restrictive labelling and specific cautions in its use. On the other hand, ixekizumab (trade name Taltz; www.taltz.com), an IL-17A cytokine-neutralising mAb, is already approved for plaque psoriasis without any noted psychological symptoms or unfavourable behavioural side effects [30]. These IL-17-family cytokine-neutralising drugs represent a major breakthrough in psoriasis treatment.

Finally, the most recent addition to cytokine-neutralising mAb-based reagents are those that neutralise IL-12 and IL-23 (**Box 5**), which act, for example, by binding to the shared p40 subunit of these cytokines. Ustekinumab (trade name Stelera; www.stelerainfo.com) is an IL-12- and IL-23-neutralising mAb, and as mentioned, it is now approved for the treatment of moderate-to-severe plaque psoriasis, psoriatic arthritis and moderately active CD [46]. Ustekinumab offers improved efficacy over anti-TNFs agents in CD patients, and, moreover, requires only tri-monthly administration (after an initial monthly dosing induction). Briakinuman, guselkumab and tildrakizumab also all block IL-23; briakinuman is a human IgG anti-IL-23p40 mAb, and tildrakizumab is a humanised IgG1κ anti-IL-23p19 mAb and both are effective and approved for psoriasis [46, 84]. Finally, guselkumab, an IgG1λ anti-IL-23p19 mAb, is reported to be safe in early-stage trials, and is also intended for use in psoriasis [85], where it outcompeted the anti-TNF mAb adalimumab in phase II trials [86]. As these are recently developed mAbs, their safety profiles will require ongoing monitoring, although early data suggest that they do not represent an increased risk of infection [87].

4. New 'biosimilars' antibody reagents—biosimilars and interchangeables

It is now well over a decade since the first anti-cytokine mAbs have been used internally to treat human inflammatory conditions and already the next generation of reagents are emerging. These are the 'copy' reagents and they are generally known as 'biosimilar' reagents [88]. As the initial cohorts of biologics are all now nearing the end of their patent protection, many

pharmaceutical companies currently dedicate a large effort towards producing their new generation of mAbs. This is not just of benefit to the pharmaceutical companies that produce these drugs, but potentially hugely advantageous for mankind. The greater the competition in the marketplace the more downward pressure on the current high costs of cytokine-neutralising mAbs and protein biologics [89]; in other words, the development of biosimilar mAbs should ultimately translate into significant savings for the patient/consumer. The production of biosimilar reagents should therefore quickly provide access to these drugs for a much larger proportion of patients who might not otherwise be able to afford them. Already, the estimates of the monetary savings are being generated and they are in the order of over Euro 20M within the first 3 years, which equates to at least an additional estimated 1200–1800 patients [90].

A *'biosimilar'* reagent is defined by the US Federal Drug Administration (FDA) as a biological product that is approved on the basis that it has highly similar physical and functional properties to an existing FDA-approved biological product—known as the *'reference'* product. The US FDA guidelines for biosimilars and other drugs are available online (http://www.fda.gov/) and a review of the current guidelines for the production of biosimilar regents has recently been published [91]. Theoretically, there are no clinically meaningful differences between a biosimilar reagent and its reference product in terms of either safety or efficacy. While this is essentially true in reality, it is important to note, however, that a biosimilar and reference product may not be entirely identical; minor differences in clinically inactive components are allowable in a biosimilar product [88]. Another term that is used in this field is that of an *interchangeable* product. This is a biosimilar that meets additional standards, that is, that it produces essentially the same clinical results as the referenced product within an identical patient cohort. This was achieved, for example, with Remsima (infliximab biosimilar), both in RA and in SA patient cohorts [92, 93]. An interchangeable biological can therefore be substituted for the reference product by a clinician or a pharmacist with essentially no discernable impact.

The establishment of the degree of similarity of a given candidate biosimilar is determined through extensive physical, chemical and functional characterisation—directly comparing the biosimilar product against the original reference product [94–96]. This includes a formal demonstration of the similarity of the primary, secondary and tertiary structure of the biosimilar, and examination of the similarity of the structural motifs that determine its mechanism of action. Firstly, the affinity of a given mAb for its cognate antigen needs to be identical, or closely similar, to that of the reference product, and analytical techniques such as surface plasmon resonance (SPR) are used to provide real-time-binding kinetic assessments (on- and off-rates) of the biosimilar and reference mAbs. Secondly, the biosimilar mAb must possess inherent properties integral on the reagent as a whole, for example, the capacity of the mAb to induce immune effector functions such as antibody-dependent cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) [97], and the class of the mAb Ig is therefore an essential aspect that much be matched in the biosimilar; if the original reference mAb is an IgG1, then the biosimilar must also be an IgG1. Thirdly, glycosylation patterns are being increasingly recognised as critically important, as differences in sugars can interfere with an Ig's biological activity [98]. Taken together, the similarity of the biosimilar mAb is essential as it ensures identical interactions with (i) antigen, (ii) FcRs and (iii) its *in vivo* half-life. Often, more than 30 analytical methods may be required to establish a new product as a bone fide biosimilar [99].

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4.1. Approval processes for biosimilar mAbs

The US FDA recommends a step-wise approach for approving a biosimilar [100]. (Note: biosimilars are not generic drugs, and their development and licensing do not fall under the same regulatory pathways as generics.) The first step is the assessment of the critical quality attributes (CQAs) of the molecule, that is, those that are relevant to the clinical outcomes. Factors thought to be affecting the identity, purity and potency of a biosimilar molecule constitute its CQA. The FDA also suggests that CGQs should be classified into three tiers and there is a statistical approach for assessing CQAs, namely an equivalence testing for Tier 1, a quality-range approach for Tier 2 and descriptive testing (raw data and graphical comparison) for Tier 3. The processes are relatively similar worldwide, although there are differences in how biosimilars are assessed in different countries or regions throughout the world with respect to the need for *in vivo* toxicity testing [101]. There is also a need to provide evidence that all batches of the biosimilar will fall within the established range. This challenge occurs because recombinant mAbs are usually produced using a variety of host cell types and the newly generated recombinant biosimilar protein may be associated with production impurities including host cell proteins that co-purify with the biosimilar [102]—these are best identified by mass spectrometry-type approaches [103]. Additionally, a number of post-translational modifications, including glycosylation, oxidation, deamidation, pyroglutamation and formylation, can be introduced into a mAb during its production. Thus, the biochemical and biophysical profiles of a biosimilar molecule must closely match the reference product and any differences need to be investigated to understand the nature of the divergence between the biosimilar and the reference product and the potential effect(s) on safety, toxicity and biological function [88].

There are generally four phases of clinical research that are required for a new drug to be developed and approved for human use: A phase I study to establish an initial safe dose range and identify potential side effects, a phase II further assessing the efficacy and safety, followed by a phase III study that confirms the drugs' efficacy in comparison to a current treatment and further establishes its safety versus the severity of any detectable side effects. Sometimes, a phase IV study is additionally performed for further assessment of the drugs' efficacy in different populations and/or a better assessment the extent of side effects, for example, issues associated with long-term drug use, or its use within a different population. By contrast, the benefit of the biosimilar agent is its abbreviated assessment process. This is justified because of the existing breadth of understanding of the reference product and its mechanisms of efficacy, which have already been extensively demonstrated via the original, the reference product assessment [104]. This permits the approval process for biosimilars to be focused mostly on the analytical demonstration of similarity to the original reference product, and only two phases of clinical studies are required for a full approval of a biosimilar. First, a phase I study to demonstrate a similar pharmacokinetic and pharmacodynamic profile. This is generally followed by a pivotal phase III-type study that demonstrates similar efficacy, safety and immunogenicity—usually comparing against the reference product. The first biosimilar (Resima) for infliximab was assessed in RA and AS patients in exactly this manner [92, 93]. Importantly, it is assumed that the biosimilar product will be delivered by the same route and at the same dosage as the reference product. This process assumes that any newly produced biosimilar mAb reagent is therefore unlikely to reveal any new adverse drug responses that

have not already been documented in the original reagent. This process permits attention to be focused on testing the immunogenic potential of the biosimilar, for example, via close attention to the production processes, the mAb's physical similarity (glycosylation, etc.), and the presence (and quantity) of any co-purifying entities. Only time will determine if there are any subtle differences in the new-generation biosimilar mAbs, that is, compared to original product, and, henceforth, whether specific prescription guidelines need to be developed.

4.2. New cytokine-neutralising biosimilar reagents and mAbs

Two etanercept (Enbrel®) TNFR-IgFc biosimilar reagents have been approved to date: Erelzi (*etanercept*-szzs) was approved in the US in mid-2016 and Brenzys (also known as SB4) was approved in Korea in 2015. SB4 is also now approved in Europe, Australia and Canada (See **Box 1**). Furthermore, there are several biosimilar anti-TNF mAbs that are either approved or in development (see **Box 1**). For example, CTP-13 (trade name Remsima) was the worlds' first registered biosimilar anti-TNF infliximab (Remicade®) mAb therapeutic, first registered in Europe and Korea in 2013. Additionally, inflectra (infliximab-dyyb) was approved in the US in early 2016 and Infimab is produced in India. Similarly, Adalimumab-atto (trade name Amjevita) and SB5 are other adalimumab (Humira®) biosimilars. For approval, Remsima was extensively evaluated in comparison to infliximab. It was found to have (i) virtually identical primary and higher-order structures, (ii) similar monomer and aggregate content, (iii) some less basic variants due to C-terminal lysine amino acid residues (but these appear to be rapidly removed in serum) and (iv) highly similar glycosylation patterns, to infliximab [105]. Nevertheless, the situation at present is that these new biosimilar drugs exist, but they are not commercially available because the original US patent for anti-human TNF mAb does not expire until late 2018. In fact, it has been estimated that there may already be as many as 20 anti-TNF biosimilar mAbs and mAb-based reagents in development, or under clinical assessment. It is expected that these drugs will be marketed for the treatment of RA, JIA, AS and psoriatic arthritis, that is, the indications as their reference drug(s) [106]. It is expected that eventually biosimilars will be produced for all of the anti-IL-1β-, IL-6-, IL-17- and IL-12/23-therapeutic mAbs (see **Box 1**–**5**).

Arguably, the most pressing issue with respect to the use of biosimilars and interchangeables is *when* and *how* to use them. Since there appears to be equivalent efficacy between these first- and second-generation drugs, then it can be assumed that either the original or the new-generation reagent can provide immediate benefit to treatment-naïve patients. Furthermore, initial studies also suggest that it is safe to switch to a biosimilar drug in anti-drug antibody-naïve patient [107]. However, a recent study has demonstrated that virtually all patients who developed anti-infliximab antibodies react to both inflectra and remsima—the infliximab biosimilar mAbs [108]. This suggests that epitopes that are present in infliximab that elicit the drug-specific antibodies are also present in the biosimilar mAbs [108]. It is also possible that new epitopes are present in the biosimilar, and, similarly, that unique drug epitopes can be present in the reference product. Data also exist showing that adalimumab-treated patient serum does not show cross-reactivity with either infliximab or its biosimilar remsima [109]. Thus, the cross-reactivity appears to be drug specific.

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5. mAb and biosimilar Ig effector functions

Igs are complex tetrameric molecules comprising two glycosylated heavy chains and two light-chain polypeptide molecules, bound together by disulphide bonds. The structure has different domains, termed 'constant' (C) and 'variable' (V) domains (**Figure 5**). The domains are encoded by different gene segments: C gene segments, plus a unique combination of V, plus 'diversity' (D) and 'joining' (J) gene segments conferring the antigen-binding site specificity.

5.1. mAb-antigen specificity and neutralisation

mAbs are highly specific reagents due to their extremely high affinity to their cognate Ag. Biochemically, the reactivity is generally nanomolar to picomolar (10^8–10^{11} K_D). When antibodies bind to epitopes that block the antigen's normal Ag reactivity, that is, to their naturally occurring ligand, their on- and off-rates define them as blocking reagents. Thus, mAb reagents that are specific to cytokines or cytokine receptors can be strong inhibitors of cytokine biology *in vivo*. Therapeutic mAbs are long lasting (approximately 15 days) due primarily to the normal longevity of Ig in human plasma. Thus, the high affinity, neutralising capacity and longevity of mAbs make them ideal therapeutic reagents.

5.2. mAb-FcR binding

Ig molecules bind to their antigens, and also to Fc receptor (FcR) proteins that are typically expressed on many cells in the hematopoietic system, especially myeloid-lineage cells. FcγR1 is a high-affinity receptor (typically $K_A > 10^7$M), whereas FcγRIIA/B/C (CD32) and FcγRIIIA/B (CD16) are low-affinity receptors (typically $K_A < 10^7$M) for human IgG1 [110] (See **Table 1**) and this difference means that low-affinity FcRs generally exist unbound by high-plasma circulating Ig [111]. The Ig affinity difference of FcRs also explains why FcγR1 can bind to monomeric IgG, whereas FcγRII and III tend to bind to IgG complexes.

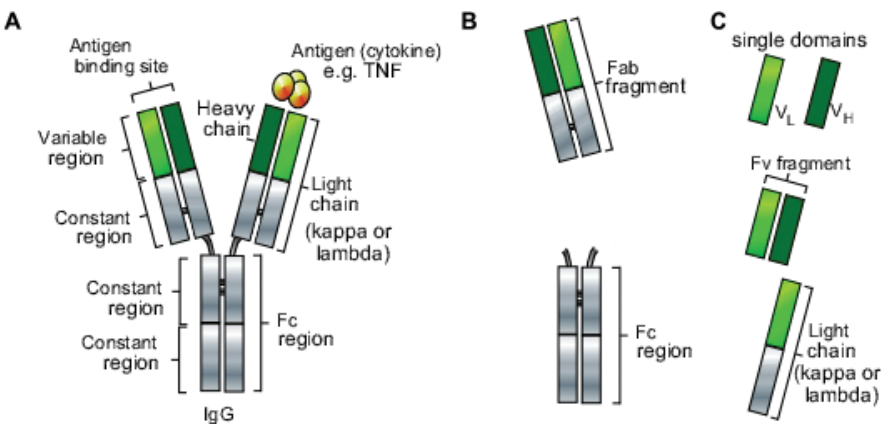

Figure 5. Immunoglobulin (Ig) and antibody fragments. (A) Soluble intact mAb, (B) Fab and Fc fragments and (C) single (light-chain) domain antibodies (Dabs), mAB Fv antigen-binding fragment and intact whole light-chain (kappa, κ or lambda, λ).

	Ag presentation	Ig type	Fc receptor type and function	LIR type	Refs.
1	T-independent	IgM	Polymeric IgR Fc**α**/μR FcμR	?	[118] [111, 119]
2	T-dependent	IgG1	All FcγRs	LIR-1/2	[120]
3	T-independent and carbohydrate Ag's	IgG2	FcγRIIA H131 (high affinity) FcγRIIA R131 + V158 (low affinity)		
4	T-dependent	IgG3	All FcγRs	?	[121]
5	Chronic Ag and allergic responses	IgG4	*FcγRI (CD64)—high affinity FcγRIIA (CD32)—low affinity FcγRIIB FcγRIIC *FcγRIIIA V158 (CD16)	LIR-1/2	
6		IgG (all isotypes)	FcγRIIIB (CD16) low affinity inhibitory receptor; GPI-linked	?	
7		IgA	Fc**α**R1 (inhibitory and activating) Fc**α**/μR	?	[121–124]

Notes: (1) High affinity Ig receptor (*) [120].
(2) FcγRIIA and FcγRIIC are single-domain-activating receptors [120].
(3) FcγRIIB is a single-chain inhibitory receptor [120].
(4) Other human variants:
FcγRIIA: two alleles H131 (low responder) and R131 (high responder).
FcγRIIIA: two variants—V158 and F155.
FcγRIIIB: two variants at four positions—R36, N65, D82 and V106; S36, S65, N82 and I106.
Plus point mutant A78D (SH) [120].

Table 1. Human immunoglobulin interactions with FcR and LIRs.

FcR binding to Igs can be activating to the cells that express them (e.g. typically FcγRIIA or FcγRIIIA) or, alternatively, Ig binding of FcRs can trigger inhibitory signals (e.g. FcγRIIB and FcγRIIIB). This is due to FcR-activating receptors containing intracellular immunoreceptor tyrosine-based activation motifs (ITAM) defined as YXXL/I(X6-8)YXXL/I amino acids, while inhibitory FcRs contain cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs) defined as (S/I/V/LxYxxI/V/L). The capacity to trigger activation or inhibitory FcR signalling also explains why circulating monomeric IgGs are generally not as stimulatory to immune cells as compared to Igs when they exist as immune complexes. Although therapeutic mAbs were initially designed to bind and neutralise cytokines, it is clear that these mAbs also bind to FcRs, and that this property is required for ADCC or CDC. However, FcRs are themselves associated with and regulated by additional proteins such as the immunoglobulin-like receptor (LIRs) [112]. LIRs fall into two basic categories: those that contain ITAMs (defined above), for example, LIR-6 and LIR-7, and those that contain inhibitory ITIMs, for example, LIR-1, -2, -3, -5 and -8. Some LIRS also contain asparagine (NxYxxL/V) or a serine residue (SxYxxL/V) [113, 114]. LIR-1, LIR-6 (a and b) and LIR-7 associate with the γ-chain of FcRs for human IgG, IgA and IgE (see **Table 1**) [112, 115]. The co-association of these molecules results in the LIR's intracellular region being physically close to the FcR, and

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this permits the LIRs ITIM to dampen the FcR-signalling capacity [112]. Thus, interactions between Igs with an FcR are influenced by FcR-adjacent LIRs.

Since the LIRs themselves have not been extensively studied, the potential function(s) of LIRs with respect to mAb therapeutics is only now emerging. Nevertheless, the interactions of mAbs with FcRs, as well as the FcR-associated LIR molecules, are becoming increasingly appreciated as vitally important in understanding and predicting mAb effector function [116]. It is therefore equally important to consider the expression of both FcRs and LIRS in various disease settings. It is known, for example, that LIRs are expressed in the synovium of RA or AO patients [117]; however, how they modulate autoantibody-dominated diseases is only now emerging.

6. Adverse events related to cytokine-neutralising and biosimilar mAbs

6.1. Antigenicity of anti-cytokine mAbs and development of drug-immune complexes

mAb-based therapeutics and related agents represent some of the most biologically complex drugs currently available. The most common bio-manufacturing process involves the production of cell culture-expressed Ig proteins, most frequently a Chinese hamster ovary cell lines engineered to express the human, humanised or chimeric Ig-type mAbs. Unlike classic small-molecule drugs, these intact Ig-type drugs are large multicomponent proteins that are essentially similar to natural molecules: mAb, or unique molecules generated by recombinant technology, for example, fusion proteins comprising two (or more) naturally encoded proteins such as cytokine receptor proteins with or without an Ig Fc. Nevertheless, these mAb-based agents and their biosimilar counterparts can vary in numerous ways from the naturally existing component (see Section 4.1). This includes alterations in post-translational modifications of proteins as well as contamination by host cell proteins [103]. This, in part, explains why factors intrinsic to the drug production can contribute to the immunogenicity of the drug, even though mAbs (and biosimilar mAbs) are highly similar to human endogenously produced Ig proteins.

The formation of therapeutic mAb-type drug reagent-immune complexes can be significant to the patient for a variety of reasons. Drug-immune complexes can alter the activation threshold for FcRs; note that high-affinity FcγRII and FcγRIII preferentially bind to immune-complexed Ig [120] and the activation threshold for FcR signalling is lowered when engaging with higher-ordered complexes—meaning that smaller or mid-sized immune-complexed mAb drugs can have extended *in vivo* half-lives and engage with what would otherwise normally be low-affinity FcRs. This explains, at least in theory, the potential for mAb-based reagents to sometimes induce inflammatory reactions despite the fact that they are otherwise virtually identical to naturally produced endogenous Igs. (There is decreasing identity to endogenous host Ig for whole mAb, then Mab fragments, recombinant soluble receptor proteins, and finally receptor-IgG Fc proteins.) Moreover, tissue deposition of immune-complexed mAbs can lead to vascular thrombosis, neutrophil recruitment or tissue monocyte/macrophage cell activation resulting in the release of inflammatory and chemotactic molecules, cytokines and chemokines—in this case exactly the opposite to what the

anti-cytokine or cytokine-receptor mAb is designed to achieve. Ultimately, the immune-complexed-mAb drug can eventually elicit the anti-drug antibodies (ADAs) and cross-link B cell receptors, amplifying immune activation.

The production of anti-drug antibodies has long been debated as being either harmful or irrelevant. For example, the presence of anti-drug Igs might decrease the half-life of the mAb drug (when bound to the mAb drug), or the anti-drug Ig could bind to an epitope located within the Fv region of the mAb such that it naturally competes with its antigen specificity of the mAb drug, thereby rendering the drug incapable of neutralising (blocking) antigen binding. It is generally considered that there are two types of antibodies-drug mAb reactions: (i) mAb interactions with natural antibodies (usually IgM isotypes) and (ii) mAb interactions with matured, isotype class-switched IgG effector Igs. Natural antibodies exist in most individuals and are usually low-affinity IgM antibodies with broad specificity, secreted by $CD5^+$ B1 lymphocytes. Because they are IgM, they have an innate propensity to form immune complexes. On the other hand, immune-complexed mAb drugs can be taken up by antigen-processing cells, such as marginal zone macrophages, and presented to naïve B cells, eventually resulting in the production of high-affinity IgG. This type of 'mature' anti-drug Ig can ultimately involve the activation of T cells and thus also to drug-based T cell-mediated inflammation. In clinical practice, there is little evidence of Ig-based adverse drug reactions to therapeutic mAbs, thus the anti-mAb-based drug Abs, even when present, are often not pathological per se—although they may block the mAbs capacity to bind and neutralise cytokines thus rendering the mAb drug ineffective.

The evidence of mAb immune complexes, and B- and T-cell reactivity, is arguably best considered with respect to the anti-TNF-neutralising antibodies, as these agents have now been used for well over a decade and in various disease populations. Thus with time it has become clear that mAb-specific Igs are (i) not infrequent (they occur in as many as 14% of patients taking anti-TNF mAb-type drugs) [125], (ii) capable of immune clearance of the mAb drug, (iii) can alter the pharmacokinetic profile of the mAb (e.g. drug half-life), (iv) capable of inducing immune cross-recognition to the endogenously arising protein—particularly, a cytokine-receptor component of the drug and (v) capable of inducing an array of adverse events spanning less significant infusion-type reactions to severe hypersensitivity reactions. It is evident, therefore, that any patient with a history of prior sensitisation to mAb-type reagents should carefully consider the safety of using another mAb-type drug.

6.2. Adverse events related to cytokine neutralisation

The vast majority of conditions requiring cytokine blockade by neutralising mAbs are chronic conditions. This raises the important issue of what happens when the normal function of the cytokine is being blocked *in vivo*. Indeed, most of the cytokines highlighted here are central to inflammation that is beneficial to the host, especially that which is central to an efficient antiviral and/or antibacterial immune response, such as IL-1β, IL-6 and TNF—all of which are produced during the normal response to infection. This is because IL-1β helps initiate immune responses during infected-related inflammation (since RIG-I activates NF-κB and the inflammasome, and thus contributes to the aetiopathology of viral arthritis

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[126, 127]), IL-6 and TNF are produced by activated macrophages *in vivo* [128], and TNF and IFNγ are produced at virtually all stages of infection where they have potent antiviral effector functions [129]. IL-6 also aids Ig development, especially IgA at mucosal sites [130]. Neutralising these cytokines therefore necessarily significantly compromises the host's natural ability to effectively combat infections. This explains why anti-TNF therapy recipients are at serious risk of more severe acute virus infections [131], and reactivation of chronic viral or bacterial infection, especially tuberculosis [132]. This explains why there are numerous reports of reactivation of chronic virus infections such as varicella zoster virus ('shingles') in patients using anti-TNF mAbs [133]. It also explains why all therapeutic mAbs that neutralise IL-1β, IL-6 and TNF are naturally contraindicated for the use during times of active acute infection (**Boxes 6**, **7** & **8**).

There is also evidence, albeit less convincing, that long-term use of anti-TNF therapeutics might be associated with an increased risk of certain cancers, especially lymphomas [134]. However, many of the chronic inflammatory conditions that triggered the use of anti-cytokine mAbs occur in older patients, and these are people who might also naturally be at risk of certain cancers. Thus, without this type of clinical trial data health professionals

Box 6. Contraindications and adverse events associated with anti-cytokine/cytokine receptor mAbs.

Therapeutic anti-cytokine and cytokine receptor reagents: Anti-tumour necrosis factor (TNF)		
Drug name and reagent	**Known adverse event**	**Specific contraindication**
Etanercept (Human TNFR2:IgG1-Fc) **Infliximab** (Humanised mouse IgG1κ) **Adalimumab** (Human IgG1κ) **Golimumab** (Human IgG1κ) **Certolizumab Pegol** (Pegylated-Fab' IgG1κ)	• **Common side effects and cautions:** Injection-site reactions (redness) Upper respiratory infections (sinus) Headache. • **Serious side effects:** Infection (new) infections, especially Tuberculosis, histoplasmosis, influenza and other viral infections, e.g. chickenpox Hepatitis B (reactivation) Nervous system demyelination Blood pressure Heart failure Psoriasis Lupus-like syndrome Lymphoma and other cancers Autoimmune hepatitis	• Existing (chronic) infections, especially Tuberculosis, HIV, Hepatitis B but also varicella (chickenpox) and influenza or other respiratory infections • Vaccination with live microorganisms • Co-use of certain other immunosuppressant agents, e.g. anti-IL1β agents, e.g. anakinra (Kineret®), anti-CLTA4 mAbs, e.g. abatacept (Orencia®), or Cytoxan (cyclophosphamide) • Multiple sclerosis • Guillain-Barré syndrome • Pregnancy • Confirmed drug hypersensitivity
BIOSIMILARS: **CTP-13** (humanised mouse IgG1κ) **Adalimumab biosimilar** (human IgG1κ) **Infimab** (human IgG1κ)	Expected to be similar to those listed above	Expected to be similar to those listed above

Box 7. Contraindications and adverse events associated with anti-cytokine/cytokine receptor mAbs.

Therapeutic anti-cytokine and cytokine receptor reagents: Anti-interleukin-1β or IL-1-receptor-α		
Drug name and reagent type	**Known adverse event**	**Specific contraindication**
Anakinra (Recombinant IL-1R) **Rilonacept** (Recombinant IL-1R) **Canakinumab** (Humanised mouse IgG1κ) **Gerokizumab** (Humanised mouse IgG2κ)	• **Common side effects and cautions:** Injection-site reactions (redness) Upper respiratory infections (sinus) Headache Latex allergy (needle cover contains latex) • **Serious side effects:** Infection (new) infections Vertigo Nasopharyngitis/respiratory tract infection, especially Tuberculosis	• Existing infections, especially Tuberculosis, HIV, Hepatitis B but also varicella, influenza or other respiratory infections • Vaccination with live microorganisms • Co-use of TNF- inhibitory agents: e.g. anakinra (Kineret®) • Pregnancy and breastfeeding • Confirmed drug hypersensitivity

Box 8. Contraindications and adverse events associated with anti-cytokine/cytokine receptor mAbs.

Therapeutic anti-cytokine and cytokine receptor reagents: Anti-interleukin-6 or IL-6-receptor		
Drug name and reagent type	**Drug name and reagent type**	**Drug name and reagent type**
Tocilizumab (Human IgG1κ) **Sarilumab** (Human IgG1κ) **Sirukumab** (Human IgG1κ)	• **Common side effects and cautions**: Injection-site reactions (redness) Upper respiratory infections (sinus) perforations of stomach or intestines/prior diverticulitis, especially if taking other NSAID, corticosteroids or methotrexate Changes in blood tests (platelet and neutrophil count, LFTs, increased cholesterol) • **Serious side effects**: Infection (new) infections Nasopharyngitis/respiratory tract infection especially Tuberculosis	• Existing infections, especially Tuberculosis, HIV, Hepatitis B but also varicella and influenza or other respiratory infections • Vaccination with live microorganisms • Co-use of TNF- inhibitory agents, for example: Etanercept (Enbrel®), Adalimumab (Humira®), Infliximab (Remicade®), Golimumab (Simponi®) or Certolizumab (Cimzia®) • Co-use of B cell suppressive agents, e.g. rituximab (Rituxan®) • Co-use of T cell suppressive agents, e.g. anti-CTLA4 abatacept (Orencia®) • Pregnancy and breastfeeding • Confirmed hypersensitivity

and epidemiologists only have access to patient data that are predominantly anecdotal in nature. Arguably, the lack of overwhelming evidence of increased tumour incidence in patients using anti-TNF mAbs-type drugs is consistent with the fact that clinical trials with

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TNF as an anticancer agent induced systemic inflammation rather than controlling the tumour [135]. Yet, this is countered by clear *in vivo* evidence that TNF is tumouricidal [136]. It would seem wise, therefore, for patients to remain vigilant to the potential risks where practicable.

6.3. Unexpected anti-cytokine mAb adverse events—negative neurological events

Evidence comprising over a decade of use of anti-TNF-blocking reagents (TNFR-IgFc fusion proteins and anti-TNF mAbs) has substantiated that in some patients there is the unpredictable adverse event of developing demyelinating lesions in brain white matter (**Figure 6**). The spectrum of clinical presentation of demyelinating events includes optic neuritis, MS-like symptoms of paralysis, demyelinating neuropathies, or Guillain-Barre syndrome (for a recent review, see [137]). The incidence of these conditions in the general populations is normally quite low, but it is accepted that some patients develop these conditions within a few months of starting anti-TNF therapies [138, 139]. In fact, MS as an existing condition is strongly contraindicated for the use of anti-TNF therapeutics, and, as expected, cessation of anti-TNF drugs is mandated if demyelinating symptoms occur [140]. Alternative MS treatments such as glatiramir acetate (an undefined mixture of decoy CNS substrates) or interferon-β are recommended in these patients. For the most part, demyelination events are transitory, however, in a small subset of patients the neurological symptoms persist.

Another unexpected concern is that with the use of the IL-17-inhibiting reagent, soluble IL-17RA (Brodalumab), there have been unexpected reports of an increased incidence of depression and suicidal ideation-type behaviours in some trial patients (https://www. aad.org/eposters/Submissions/getFile.aspx?id=1146&type=sub) (**Box 9**). These unfortunate adverse events resulted in a decision by Amgen and AstraZeneca to offload the drug to another pharma company, the Canadian-based multinational Valeant Pharmaceuticals and Kyowa Hakko Kirin Company in Japan [83]. Nevertheless, the lack of any negative psychological symptoms when using Ixekizumab (an IL-17A-neutralising mAb) indicates that IL-17A itself

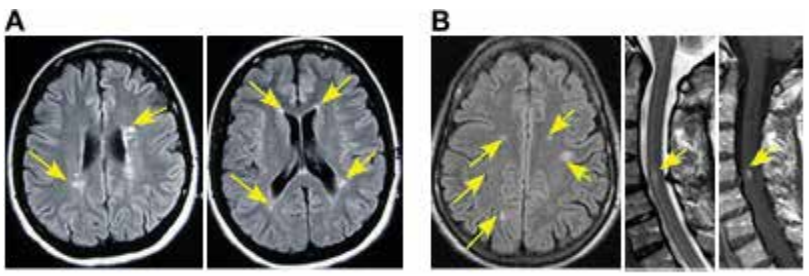

Figure 6. Two patients showing MRIs of demyelinating CNS lesions associated with anti-TNF agents. (A) A 46-year-old Caucasian female taking etanercept for 4 years for psoriatic arthritis developed multiple periventricular and subcortical lesions (arrows), and (B) a 57-year-old Caucasian female with AS treated with etanercept for 6 years developed multiple periventricular and subcortical frontal, parietal and temporal lobe lesions and level a C4–C5 cervical spine lesion (arrow). (Images adapted from [138] in compliance with copyright).

Box 9. Contraindications and adverse events associated with anti-cytokine/cytokine receptor mAbs.

Therapeutic anti-cytokine and cytokine receptor reagents: Anti-interleukin-17 and IL-17Rα		
Drug name and structure	**Known adverse events**	**Specific contraindications**
Brodalumab** (Human IgG2κ) **Ixekizumab** (Human IgG4) **Secukinumab** (Human IgG1κ)	**Common side effects and cautions:** Injection-site reactions (redness) Upper respiratory infections and/or nasopharyngitis Headache Arthralgia **Serious side effects:** Major cardiovascular events (including myocardial infarction) Cholelithiasis Suicidal ideation and behaviour**	• Patients suffering from psoriasis are sometimes afflicted with co-morbidities including psychiatric conditions (depression, anxiety, suicidality**). Patients with these conditions are not excluded; however, depression (PHQ-8) and suicidality (eC-SSRS) test monitoring are recommended • Drug hypersensitivity • Infection

is not the culprit per se. Thus, other IL-17-related cytokines, or other IL-17R-binding partners (but not IL-17A), may be necessarily required for the development of negative emotions, especially those related to depression and suicide. This unexpected trial outcome, although highly unfortunate, may have simultaneously inadvertently illustrated a previously unappreciated role for the IL-17/IL-17R axis in depression and suicidality. Although the mechanism is currently unknown, it has been reported that inflammatory cytokines IL-1β and IL-6 are elevated in blood of suicide victims [141], and recombinant interferon-α therapy has been associated with depression in chronic hepatitis patients [142, 143]. There is growing evidence that cytokines such as IFN-α drive neuroinflammation via triggering the tryptophan pathway [144], and high levels of the downstream tryptophan metabolite, quinolinic acid, has been linked to microglia expression in suicide victims [145, 146]. Furthermore, one might hypothesise that blocking IL-17, but not IFNs, might still leave type-I IFN levels high, and promoting depression and suicidality by mechanisms described above. However, another possibility could be that IL-17R agonistic mAbs, or IL-17 small-molecule agonists, might have value in potentially preventing depression, suicide and other negative emotions. It is currently unknown, for example, whether the brodalumab-IL-17RA interactions completely block all IL-17-related cytokines, prevent IL-17RA from interactions with one or more of its potential hetero-complexed IL-17 receptors, for example, IL-17 receptor RB, IL-17RC or IL-17R. Nevertheless, it is clear that the clinical use of brodalumab must likely only occur with a clear 'suicide-risk' warning for those who choose to use it to ameliorate inflammatory conditions such as psoriasis. Additionally, it remains a plausible possibility that altering the IL-17R mAb epitope may generate a non-'suicide-risk' next-generation reagent, that retains its anti-inflammatory properties. Even more intriguing, the current FDA submissions claim that the latest clinical data do not replicate the initial finding of an increased risk of suicidal ideation. Further investigation will be needed to determine the broader and

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usual spectrum of adverse events of brodalumab. No adverse events are known yet for IL-12/IL-23 neutralising mAbs.

7. Expanding treatment indications for existing cytokine-neutralising and biosimilar mAbs—current realities and exciting futures

mAb-type drug development procedures in the US and Europe typically involve small-scale clinical trials demonstrating safety followed by trials showing efficacy relative to a specific disease(s) indication. These so-called landing indications are often followed by fast-tracked priority review. The expanded use may include a different disease indication or a different use of the mAb, such as the delivery of a radio-isotype conjugated to the mAb drug, such as was the case for the anti-CD20 mAb rituximab. The fast track and priority review is justified primarily because of the availability of existing safety and toxicity data.

With existing safety data in place, there is the ability to file for expanded use of mAb-based drugs. This is particularly the case for cytokine- and cytokine-receptor-specific mAbs, as the target cytokine/cytokine receptor may be elevated and involved in additional pathologies, apart from the disease indication directly assessed in the original clinical trials. (In some cases, a mAb drug has even failed in the original trial, but has been successful in subsequent trials, e.g., the TNF-neutralising mAb infliximab failed in clinical trials of sepsis, but is successful when used in RA and Crohn's disease patients, etc.; see **Box 1**). Most often, the expanded use label is related to diseases or conditions that are similar in terms of aetiopathology. For example, anti-TNF mAb-based reagents Enbrel, infliximab and adalimumab are recommended for a spectrum of arthritis and tissue-related inflammatory diseases: RA, psoriatic arthritis, plaque psoriasis, AS, JIA, CD and UC.

7.1. Anti-TNF mAb-based reagents in neuroinflammation and cognition

Etanercept (a TNFR2-Ig Fc) has additionally been used in off-label situations, most notably, in the treatment of cognitive decline after brain injury or Alzheimer's disease, and also in stroke. These uses are consistent with evidence that activated microglia produce TNF and with the idea that TNF is important in modulating neuronal synaptic function and neuropathic pain. In fact, there is an extensive literature base demonstrating important roles for TNF in the development and homeostasis of neurological systems [147]. One unifying hypothesis is that TNF causes glutamate excitotoxicity in neurones in a number of neurodegenerative diseases, and it is sobering to consider that cerebral TNF is elevated in degenerative CNS conditions, traumatic brain injury and even situations of post-operative delirium with cognitive decline [148]. So too, the levels of neuronal and microglial glutamate are important in these diseases, but it is also known that either TNF or IL-1β induces high level of neuronal glutamate and neurotoxicity [149]. Despite the growing body of evidence implicating TNF in neuroinflammation, there is still debate about the effectiveness and strategy of neutralising TNF in neurodegenerative

disorders. One of the reasons for this likely surrounds the difficulties in delivering the TNF-neutralising mAb-based reagents to the brain, although it appears that this can be successfully achieved by peri-spinal administration [150]. Moreover, the recent discovery of the brain lymphatics [151] provides an avenue for drug removal away from brain tissue.

Another off-label use of mAbs that neutralise cytokines in inflammation is stroke and traumatic brain injury. The main focus of treatment in stroke is thrombolytic therapy with an emphasis to reduce stroke size and reverse localised ischaemia. Nevertheless, there is evidence that the stroke penumbra region evokes or experiences an inflammatory response that comprises microglial TNF production and subsequent neurotoxicity. Peri-spinal-delivered etanercept appears to ameliorate this inflammation, even years after the neurological injury [152, 153]. Moreover, even a single injection of etanercept has been reported to alleviate symptoms of aphasia, speech apraxia, a hemiparesis in a patient with non-recent traumatic acute brain injury [154]. In animal models, traumatic brain injury induces both microglial and astrocytic activation with increasing production of TNF that can be neutralised by etanercept [155]. In humans, there is also strong evidence of elevated pro-inflammatory cytokine IFNγ, TNF and IL-1β and IL-6 which is associated with poorer cognitive outcomes [156]. This is an area of increasing investigation and current models suggest a key role for reactive oxygen species, matrix metalloproteases, angiogenic factor, inflammatory cytokine and leukocyte adhesions such that in early stages neuroprotection may be mediated by neurotrophic factors such as brain-derived neurotrophic factor, nerve growth factor and vascular endothelial growth factor, plus cytokines TGFβ, IL-1Ra, IL-4 and IL-10, among others, with a switch to neurodegenerative changes in chronic inflammation involving cytokines TNF, IL-1β and IL-6 [157]. Hence, brain microglia are essential for both neurorestoration and neurorecovery, but prolonged activation is more likely to be disadvantageous, that is, to have pathological sequelae [158]. With the apparent efficacy of etanercept treatment to neutralise TNF, even years after the initial insult or injury, it remains plausible that the administration of IL-1Ra might also be beneficial in early stages, that is, to block inflammation by IL-1β, with subsequent administration of mAb-based neutralisation of TNF, IL-1β and IL-6 in later stages. This is consistent with documented TNF immune reactivity in brain tissues from early times, extending to 18 days or more after ischaemic stroke in humans [159]. A greater understanding of the processes that regulate microglial activation and function will critically inform the potential standardised use of anti-cytokine treatments to neutralise inflammation-mediated tissue injury after TBI and stroke.

One of the most intriguing uses of anti-TNF mAbs has been in the treatment of cognitive impairment, a concept already introduced above. In infectious situations, prolonged activation of the transcription factor NF-κB and the sustained expression of TNF have been linked to AIDS-related dementia complex [160]. In particular, the regional location of TNF-producing cells correlated with HIV gp41-reactive cells, and correlated with increasing cognitive impairment and dementia [161]. In animal models, increased TNF is associated with cognitive decline that is linked to non-enzymatic glycation of proteins, for example, modification by D-glucose [162]. Similarly, exposure to certain anaesthetics is associated with the potential for post-surgery delirium and with later cognitive dysfunction [163], and this is especially apparent in the elderly [164]. Surgery-associated cognitive dysfunction has suggested to be linked to the production of pro-inflammatory cytokines [165], the activation of caspases, and to the increased synthesis and accumulation of β-amyloid (Aβ) protein, and thus to the induction of

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hyperphosphorylation of tau [166, 167], although contradictory studies also exist [168]. Recent studies further suggest that TNF and IL-6 are components of the pro-inflammatory response [169]. Furthermore, another recent study has even suggested that high IL-6 prior to surgery is a risk factor for post-operative delirium onset in the elderly [170]. Therefore, there is a potential use for TNF- and/or IL-6-neutralising mAbs in these conditions, although they are not currently a component of the standard treatment. At present, one can only surmise that these drugs might be beneficial to elderly patients, especially long term, particularly because of the possibility that post-operative delirium is associated with subsequent cognitive impairment [171] or indeed, potentially even, as a possible trigger for subsequent neurodegenerative pathologies.

7.2. IL-17- and IL-17R-related mAbs and negative emotions: anxiety and suicidal ideation

A recent and unexpected complication of IL-17 cytokine blockade via IL-17R-specific mAbs was a report of self-harm ideation and suicidality, as noted above (**Box 9**). This appears specific to IL-17R blockade, rather than IL-17A neutralisation alone, although a recent re-evaluation of the phase II and II trial data, literature and expert opinion has refuted these findings [172], and others interpret the data to imply accidental findings, rather than being suggestive of a direct suicidal causation [173]. Nevertheless, further investigation will clearly be required, and close monitoring of its use, in a broader population, will be required to confirm a role for IL-17-related cytokines, or other IL-17R-interacting molecules, in the propagation of negative emotions, especially depression and anxiety. In this regard, it is nevertheless intriguing that anxiety has previously been negatively correlated with serum levels of TGF-β1 and IL-17 [174], whereas others have reported increased TNF and IL-17 in individuals with generalised anxiety disorder [175]. Moreover, increased levels of dopamine-induced glucocorticoid-resistant Th-17 cells are reported in multiple sclerosis—a condition where depression is a frequent co-morbidity [176]. Although these intriguing observations clearly warrant further investigation, it remains possible, although quite controversial, that this represents a new opportunity: to target IL-17R in individuals experiencing suicidal ideation.

8. Inflammatory conditions still requiring new treatments

8.1. Other inflammatory diseases amenable to mAb cytokine blockade: anti-IL-4, IL-5 and IL-13 in asthma, allergy and atopic dermatitis

Asthma is a chronic disease of airways where pre-exposure and complement result in cytokine- and allergen-triggered inflammation that is characterised by the dysregulation of IL-4, IL-5 and/or IL-13. Mepolizumab (Nucala) and reslizumab (Cinquair) are IL-5-specific-neutralising mAbs that have recently been demonstrated to be capable of preventing and controlling moderate to severe asthma [177, 178]. Since eosinophilia is a feature of this condition, mepolizumab is also indicated for other hyper-eosinophilic conditions, such as eosinophilic airway inflammation, allergic rhinitis, atopic dermatitis, and eosinophilic oesophagitis [179]. Similarly, benralizumab is an IL-5Rα-neutralising mAb currently in development [180]. However, it is clear that asthma is more accurately defined as a heterogeneous syndrome, which explains why many patients do not respond well to older, more conventional asthma therapies. Apart from targeting IL-5, mAbs that target and neutralise IL-13 (e.g. tralokinumab,

produced by LEO Pharma) are also emerging as effective reagents in clinical trials for atopic dermatitis, and are additionally being considered for conventionally unresponsive asthma patients. Lebrikizumab neutralises IL-4 and IL-13 and prevents airway inflammation, mucous secretion and airway remodelling that occurs in chronic asthma [181, 182]. As with the other inflammatory conditions discussed in this chapter, the challenge for clinicians is to determine which of these recently developed anti-IL-4, -IL-5 and -IL-13 cytokine and IL-5Rα cytokine-receptor-neutralising reagents are optimal for a given disease condition. Comorbidities may be highly informative in this regard, and already it has been suggested that the lebrikizumab is most effective in patients with serum periostin, a potential predictor of airway eosinophilia [183, 184] and a correlate for IL-13 bioactivity *in vivo* [185]. Moreover, recent studies indicate that in the context of asthma, allergy and atopic dermatitis, Th-2 cytokines producing ILC2 cells play an important role in modulating IL-3, IL-5 and IL-13 functions at the lung mucosa or skin [186, 187]. ILC2 subsets may vary considerably accordingly to the anatomical location. For example, lung-resident IL-33R+ ILC2s produce IL-5 and IL-13, whereas skin ILC2s express thymic stromal lymphopoietin (TLSP) and IL-4 [188]. Indeed, vaccine adjuvants such as IL-13Ra2 or IL-4R antagonist can significantly alter ILC2 function at vaccination sites, acting within the first 24 h after administration [189, 190]. Thus, designing drugs that target the different ILC2 subsets at the lung mucosa or skin has high potential to provide the next-generation therapeutics for asthma, allergy and atopic dermatitis. So, too, the therapeutic value of the current Th-2 cytokine-neutralising antibodies will become clearer with time, and a current challenge is the paucity of treatments available for asthma patients who present with little or no evidence of Th-2 cytokine-based inflammation.

8.2. Remaining challenges including neurological inflammation

Still, there are several conditions or situations where treatments remain suboptimal, or difficult, and where treatment failure is inexplicably common. For example, despite advances in the current understanding of SJIA, up to 50% of cases experience a chronic disease and many patients appear to be refractory to existing treatments—including cytokine-specific mAbs [6]. This reality may again highlight the possibility that there exists a spectrum of aetiologies, some of which are not sufficiently affected by existing treatments. Alternatively, it is possible that the mechanisms that regulate checkpoints and exert inhibition of the immune system require additional specific enhancement. Other classic autoimmune diseases such as Scleroderma, although uncommon, involves systemic immune attack of tissues, including vascular endothelium, that remains extremely challenging to treat and can even require in-limb amputation by end-stages, in extreme cases. Even today, there remains no durable effective treatment for scleroderma. Other rare immune-destructive conditions such as myasthenia gravis, involving autoantibody blockade of neuromuscular junctions, urgently require better treatments rather than global B-cell immune suppression.

Remaining high on the list of current clinical challenges in neuroinflammatory conditions is multiple sclerosis, especially the chronic progressive forms of multiple sclerosis where patients experience progressive worsening with each disease flare. Also, there are other neurodegenerative conditions, such as amyotrophic lateral sclerosis (also known as Lou Gehrig's disease, or motor neurone disease) that present with elements of neuroinflammation, even if inflammation

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is not necessarily the primary driver of neuronal loss. The recent intriguing success of anti-TNF therapies in stroke and brain injury [152–154] highlighted above suggests that the cytokine/cytokine-receptor blockage in the brain is possible. Innovation for easier brain-specific delivery methods, and a considerably deeper knowledge of immune cells with their extensive tissue- and cell-specific interactions in the brain, in both normal and disease settings, should accelerate the development of new treatment options and address this opportunity and serious clinical need.

Food intolerances and food-related atopy also remain a clinical challenge. Ranging from peanut allergies to raw meat intolerance that arises after tick-bite, the current treatments remain global in nature and need to better embrace the microbiome, including dysbiosis exerted by viruses (especially bacteriophages) rather than just the diversity in bacteria communities. Hence, there remains a critical need for more information, that is, a more detailed mechanistic understanding of these immunological diseases and food allergies. Despite the successes of mAb-based biotherapeutics for human inflammatory diseases, a challenge for the global pharmaceutical companies who have benefited from these biotechnology successes is thus to direct more funding into these research areas.

9. Summary

Nearly 20 years have passed since the first cytokine-specific biological reagent, Enbrel (etanercept), was FDA approved in 1997, and, as reviewed here, there are already now more than 20 cytokine- or cytokine-specific mAbs and recombinant soluble cytokine receptor proteins in clinical use, or on the verge of approval, for inflammatory diseases. Thus, the treatment of human inflammatory diseases has experienced a watershed era. Arguably, three challenges now remain. The first is to address the less common but nevertheless devastating conditions for which there are no cures or effective treatments—irrespective of the number of people affected by them. The second is to determine how to better stratify the existing treatments for optimum use in selected subconditions. Thirdly, the overwhelming concern is that these treatment breakthroughs remain out of reach for millions of people worldwide; there are still, undoubtedly, millions of patients who cannot afford them. With the era of biosimilars upon us, there is an opportunity to provide cheaper mAb-based therapeutics to affected people. Yes, the opportunity is there, but whether it will change the unsustainable appetite for large financial gains and reduce costs in developed countries remains to be seen.

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References

[1] Upton C, Macen JL, Schreiber M, McFadden G. Myxoma virus expresses a secreted protein with homology to the tumor necrosis factor receptor gene family that contributes to viral virulence. Virology. 1991;184(1):370–82.

[2] Upton C, Mossman K, McFadden G. Encoding of a homolog of the IFN-gamma receptor by myxoma virus. Science. 1992;258 (5086):1369–72.

[3] Thomas R, Cope AP. Chapter 109: Pathogenesis of rheumatoid arthritis. In: Edited by Richard A. Watts PGC, Christopher Denton, Helen Foster, John Isaacs, and Ulf Müller-Ladner editor. Oxford Textbook of Rheumatology: Oxford University Press; 2016.

[4] England BR, Thiele GM, Mikuls TR. Anticitrullinated protein antibodies: origin and role in the pathogenesis of rheumatoid arthritis. Curr Opin Rheumatol. 2017;29(1):57–64. DOI: 10.1097/BOR.0000000000000356.

[5] Baildam E. Chapter 116: Juvenile idiopathic arthritis. In: Edited by Richard A. Watts PGC, Christopher Denton, Helen Foster, John Isaacs, and Ulf Müller-Ladner., editor. Oxford Textbook of Rheumatology: Oxford University Press; 2016.

[6] Gurion R, Lehman TJA, L.N. M. Systemic arthritis in children: a review of clinical presentation and treatment. Int J Inflam. 2012;2012:271569. doi: 10.1155/2012/271569.

[7] Ramanan A.V., Akikusa JD. Chapter 14: The systemically unwell child. In: Edited by Richard A. Watts PGC, Christopher Denton, Helen Foster, John Isaacs, and Ulf Müller-Ladner editor. Oxford Textbook of Rheumatology. 4th Edition (2016) ed: Oxford University Press; 2016.

[8] Mellins ED, Macaubas C, Grom AA. Pathogenesis of systemic juvenile idiopathic arthritis: some answers, more questions. Nat Rev Rheumatol. 2011;7(7): 416–26. doi: 10.1038/nrrheum.2011.68.

[9] Macaubas C, Nguyen K, Milojevic D, Park JL, Mellins ED. Oligoarticular and polyarticular JIA: epidemiology and pathogenesis. Nat Rev Rheumatol. 2009;5(11):616–26. doi: 10.1038/nrrheum.2009.209.

[10] Schulert GS, Grom AA. Pathogenesis of macrophage activation syndrome and potential for cytokine-directed therapies. Annu Rev Med 2. 2015;66:145–59. doi: 10.1146/annurev-med-061813-012806.

[11] Mourão AF, Santos MJ, Melo Gomes JA, Martins FM, Mendonça SC, Oliveira Ramos F, Fernandes S, Salgado M, Guedes M, Carvalho S, Costa JA, Brito I, Duarte C, Furtado C, Lopes A, Rodrigues A, Sequeira G, Branco JC, Fonseca JE, Canhão H. Effectiveness and long-term retention of anti-tumour necrosis factor treatment in juvenile and adult patients with juvenile idiopathic arthritis: data from Reuma.pt. Rheumatology (Oxford). 2016;55(4):697–703. DOI: 10.1093/rheumatology/kev398.

[12] Turnier JL, Brunner HI. Tocilizumab for treating juvenile idiopathic arthritis. Expert Opin Biol Ther. 2016;16(4):559–66. DOI: 10.1517/14712598.2016.1150997.

[13] Church LD, McDermott MF. Canakinumab: a human anti-IL-1β monoclonal antibody for the treatment of cryopyrin-associated periodic syndromes. Expert Rev Clin Immunol. 2010;6(6):831–41. DOI: 10.1586/eci.10.66.

[14] Peitz J, Horneff G. Treatment of systemic-onset juvenile arthritis with canakinumab. Open Access Rheumatol. 2015;7:23–31. DOI: 10.2147/OARRR.S54215.

[15] Donn R, Alourfi Z, Zeggini E, Lamb R, Jury F, Lunt M, Meazza C, De Benedetti F, Thomson W, Ray D, Group. BPRS. A functional promoter haplotype of macrophage migration inhibitory factor is linked and associated with juvenile idiopathic arthritis. Arthritis Rheum. 2004;50(5):1604–10. DOI: 10.1002/art.20178.

[16] Hersh AO, Prahalad S. Immunogenetics of juvenile idiopathic arthritis: A comprehensive review. J Autoimmun 2. 2015;64:113–24. DOI: 10.1016/j.jaut.2015.08.002.

[17] Sieper A. Chapter 113: Axial spondyloarthropathies. In: Edited by Richard A. Watts PGC, Christopher Denton, Helen Foster, John Isaacs, and Ulf Müller-Ladner., editor. Oxford Textbook of Rheumatology: Oxford University Press; 2016.

[18] International Genetics of Ankylosing Spondylitis Consortium (IGAS), Cortes A, Hadler J, Pointon JP, Robinson PC, Karaderi T, Leo P, Cremin K, Pryce K, Harris J, lee S., Joo JB, Shim S-C, Weisman M, Ward M, Zhou X, Garchon H-J, Chiocchia G, Nossent J, Lie BA,

Førre Ø, Tuomilehto J, Laiho K, Jiang L, Liu Y, m, Wu X, Bradbury LA, Elewaut D, Burgos-Vargas R, Stebbings S, Appleton L, Farrah C, Lau J, Kenna TJ, Haroon N, Ferreira MA, Yang J, Mulero J, Fernandez-Sueiro JL, Gonzalez-Gay MA, lopez-Larrea C, Deloukas P, Donnelly P, Australo-Anglo-American Spondyloarthritis Consortium (tASC), Groupe Française d'Etude Génétique des Spondylarthrites (GFeGS), Nord-Trøndelag Health Study (HUNT), Spondyloarthritis Research Consortium of Canada (SPARCC), Wellcome Trust Case Control Consortium 2 (ETCCC2), Bowness P, Gafney K, Gaston H, Gladman DD, Rahman P, Maksymowych WP, Xu H, Crusius JBA, van der Horst-Bruinsma IE, Chou C-T, Valle-Oñate R, Romero-Sánchez C, Hansen IM, Pimentel-Santos FM, Inman RD, Videm V, Martin J, Breban M, Reveille JD, Evans DM, Kim T-H, Wordsworth BP, Brown MA. Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. Nat Genet. 2013;45(7):730–8. doi: 10.1038/ng.2667.

[19] Maxwell LJ, Zochling J, Boonen A, Singh JA, Veras MM, Tanjong Ghogomu E, Benkhalti Jandu M, Tugwell P, Wells GA. TNF-α inhibitors for ankylosing spondylitis. Cochrane Database Syst Rev 2015;4:CD005468. doi: 10.1002/14651858. CD005468.pub2.

[20] Barr A, Keat A. Spondyloarthritis: evolving therapies. Arthritis Res Ther. 2010;12 (6):221. doi: 10.1186/ar3178.

[21] Ciccia F, Guggino G, Rizzo A, Saieva L, Peralta S, Giardina A, Cannizzaro A, Sireci G, De Leo G, Alessandro R, Triolo G. Type 3 innate lymphoid cells producing IL-17 and IL-22 are expanded in the gut, in the peripheral blood, synovial fluid and bone marrow of patients with ankylosing spondylitis. Ann Rheum Dis. 2015;74(9):1739–47. doi: 10.1136/annrheumdis-2014-206323.

[22] Triggianese P, Conigliaro P, Chimenti MS, Biancone L, Monteleone G, Perricone R, Monteleone I. Evidence of IL-17 producing innate lymphoid cells in peripheral blood from patients with enteropathic spondyloarthritis. Clin Exp Rheumatol. 2016;34(6): 1085–93.

[23] Wendling D, Cedoz JP, Racadot E, Dumoulin G. Serum IL-17, BMP-7, and bone turnover markers in patients with ankylosing spondylitis. Joint Bone Spine. 207;74(3):304–5. DOI: 10.1016/j.jbspin.2006.11.005.

[24] Barnabe C, Hanley DA. Effect of tumor necrosis factor alpha inhibition on bone density and turnover markers in patients with rheumatoid arthritis and spondyloarthropathy. Semin Arthritis Rheum. 2007;39(2):116–22. DOI: 10.1016/j.semarthrit.2008.04.004.

[25] Coates LC, Helliwell PS. Chapter 114: Psoriatic arthritis. In: Edited by Richard A. Watts PGC, Christopher Denton, Helen Foster, John Isaacs, and Ulf Müller-Ladner., editor. Oxford Textbook of Rheumatology: Oxford University Press; 2016.

[26] Walling HW, Swick BL. Update on the management of chronic eczema: new approaches and emerging treatment options. Clin Cosmet Investig Dermatol. 2010; 3:99–117.

[27] Campa M, Ryan C, Menter A. An overview of developing TNF-α targeted therapy for the treatment of psoriasis. Expert Opin Investig Drugs. 2015;24(10):1343–54. doi: 10.1517/13543784.2015.1076793.

[28] Krueger JG, Ferris LK, Menter A, Wagner F, White A, Visvanathan S, Lalovic B, Aslanyan S, Wang EE, Hall D, Solinger A, Padula S, Scholl P. Anti-IL-23A mAb BI 655066 for treatment of moderate-to-severe psoriasis: Safety, efficacy, pharmacokinetics, and biomarker results of a single-rising-dose, randomized, double-blind, placebo-controlled trial. J Allergy Clin Immunol. 2015;136(1):116–24.e7. doi: 10. 1016/j.jaci.2015.01.018.

[29] Sofen H, Smith S, Matheson RT, Leonardi CL, Calderon C, Brodmerkel C, Li K, Campbell K, Marciniak SJJ, Wasfi Y, Wang Y, Szapary P, Krueger JG. Guselkumab (an IL-23-specific mAb) demonstrates clinical and molecular response in patients with moderate-to-severe psoriasis. J Allergy Clin Immunol. 2014;133(4):1032–40. doi: 10. 1016/j.jaci.2014.01.025.

[30] Blauvelt A. Ixekizumab: a new anti-IL-17A monoclonal antibody therapy for moderate-to severe plaque psoriasis. Expert Opin Biol Ther. 2016;16(2):255–63. doi: 10.1517/14712598.2016.1132695.

[31] Leonardi C, Matheson R, Zachariae C, Cameron G, Li L, Edson-Heredia E, Braun D, Banerjee S. Anti-interleukin-17 monoclonal antibody ixekizumab in chronic plaque psoriasis. N Engl J Med. 2012;366(13):1190–9. doi: 10.1056/NEJMoa1109997.

[32] Papp KA, Leonardi C, Menter A, Ortonne JP, Krueger JG, Kricorian G, Aras G, Li J, Russell CB, Thompson EH, Baumgartner S. Brodalumab, an anti-interleukin-17-receptor antibody for psoriasis. N Engl J Med. 2012;366(13):1181–9. doi: 10.1056/NEJMoa1109017.

[33] Girolomoni G, Mrowietz U, Paul C. Psoriasis: rationale for targeting interleukin-17. Br J Dermatol. 2012;167(4):717–24. doi: 10.1111/j.1365-2133.2012.11099.x.

[34] Wongpiyabovorn J, Suto H, Ushio H, Izuhara K, Mitsuishi K, Ikeda S, Nakao A, Okumura K, Ogawa H. Up-regulation of interleukin-13 receptor alpha1 on human keratinocytes in the skin of psoriasis and atopic dermatitis. J Dermatol Sci. 2003;33(1):31–40.

[35] Newcomb DC, Boswell MG, Zhou W, Huckabee MM, Goleniewska Sevin CM, Hershey GK, Kolls JK, Peebles RSJ. Human TH17 cells express a functional IL-13 receptor and IL-13 attenuates IL-17A production. J Allergy Clin Immunol. 2011;127(4): 1006-13.e1-4. doi: 10.1016/j.jaci.2010.11.043.

[36] Ravichandran J, Jackson RJ, Trivedi S, Ranasinghe C. IL-17A expression in HIV-specific CD8 T cells is regulated by IL-4/IL-13 following HIV-1 prime-boost immunization. J Interferon Cytokine Res. 2015;35(3):176–85. doi: 10.1089/jir.2014.0078.

[37] Wohn C, Brand A, van Ettinger K, Brouwers-Haspels I, Waisman A, Laman JD, Clausen BE. Gradual development of psoriatic skin lesions by constitutive low-level expression of IL-17A. Cell Immunol. 2016;308:57–65. doi: 10.1016/j.cellimm.2015.11.006.

[38] Teunissen MB, Munneke JM, Bernink JH, Spuls PI, Res PC, Te Velde A, Cheuk S, Brouwer MW, Menting SP, Eidsmo L, Spits H, Hazenberg MD, Mjösberg J. Composition of innate lymphoid cell subsets in the human skin: enrichment of NCR(+) ILC3 in lesional skin and blood of psoriasis patients. J Invest Dermatol. 2014;134(9): 2351–60. doi: 10.1038/jid.2014.146.

[39] Villanova F, Flutter B, Tosi I, Grys K, Sreeneebus H, Perera GK, Chapman A, Smith CH, Di Meglio P, Nestle FO. Characterization of innate lymphoid cells in human skin and

blood demonstrates increase of NKp44+ ILC3 in psoriasis. J Invest Dermatol. 2014;134 (4):984–91. doi: 10.1038/jid.2013.477.

[40] Cheung KL, Jarrett R, Subramaniam S, Salimi M, Gutowska-Owsiak D, Chen YL, Hardman C, Xue L, Cerundolo V, Ogg G. Psoriatic T cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a. J Exp Med. 2016; 213(11):2399–412. DOI: 10.1084/jem.20160258.

[41] Mahmood T, Zaghi D, Menter A. Emerging oral drugs for psoriasis. Expert Opin Emerg Drugs. 2015;20(2):209–20. doi: 10.1517/14728214.2015.1010509.

[42] Papp K, Cather JC, Rosoph L, Sofen H, Langley RG, Matheson RT, Hu C, Day RM. Efficacy of apremilast in the treatment of moderate to severe psoriasis: a randomised controlled trial. Lancet. 2012;380(9843):738–46. doi: 10.1016/S0140-6736(12)60642-4.

[43] Papp KA, Kaufmann R, Thaçi D, Hu C, Sutherland D, Rohane P. Efficacy and safety of apremilast in subjects with moderate to severe plaque psoriasis: results from a phase II, multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-comparison study. J Eur Acad Dermatol Venereol. 2013;27(3):e376–83. doi: 10.1111/ j.1468-3083.2012.04716.x.

[44] Schafer PH, Truzzi F, Parton A, Wu L, Kosek J, Zhang LH, Horan G, Saltari A, Quadri M, Lotti R, Marconi A, Pincelli C. Phosphodiesterase 4 in inflammatory diseases: Effects of apremilast in psoriatic blood and in dermal myofibroblasts through the PDE4/CD271 complex. Cell Signal. 2016;28(7):753–63. doi: 10.1016/j.cellsig.2016.01.007.

[45] Dong C, Virtucio C, Zemska O, Baltazar G, Zhou Y, Baia D, Jones-Iatauro S, Sexton H, Martin S, Dee J, Mak Y, Meewan M, Rock F, Akama T, Jarnagin K. Treatment of skin inflammation with benzoxaborole phosphodiesterase inhibitors: selectivity, cellular activity, and effect on cytokines associated with skin inflammation and skin architecture changes. J Pharmacol Exp Ther 2. 2016;358(3):413–22. doi: 10.1124/jpet.116.232819.

[46] Campa M, Mansouri B, Warren R, Menter A. A review of biologic therapies targeting IL-23 and IL-17 for use in moderate-to-severe plaque psoriasis. Dermatol Ther (Heidelb). 2016;6(1):1–12. DOI: 10.1007/s13555-015-0092-3.

[47] Kulig P, Musiol S, Freiberger SN, Schreiner B, Gyülveszi G, Russo G, Pantelyushin S, Kishihara K, Alessandrini F, Kündig T, Sallusto F, Hofbauer GF, Haak S, Becher B. IL-12 protects from psoriasiform skin inflammation. Nat Comm. 2016;7:13466. doi: 10. 1038/ ncomms13466.

[48] Geremia A, Biancheri P, Allan P, Corazza GR, Di Sabatino A. Innate and adaptive immunity in inflammatory bowel disease. Autoimmun Rev. 2014;13(1):3–10. doi: 10. 1016/j.autrev.2013.06.004.

[49] de Souza HS, Fiocchi C. Immunopathogenesis of IBD: current state of the art. Nat Rev Gastroenterol Hepatol. 2016;13(1):13–27. DOI: 10.1038/nrgastro.2015.186.

[50] Manrique P, Bolduc B, Walk ST, van der Oost J, de Vos WM, Young MJ. Healthy human gut phageome. Proc Natl Acad Sci U S A. 2016;113(37):10400–5. DOI: 10. 1073/pnas.160 1060113.

[51] Buela KA, Omenetti S, Pizarro TT. Cross-talk between type 3 innate lymphoid cells and the gut microbiota in inflammatory bowel disease. Curr Opin Gastroenterol. 2015;31 (6):449–55. DOI: 10.1097/MOG.0000000000000217.

[52] Goldberg R, Prescott N, Lord GM, MacDonald TT, Powell N. The unusual suspects--innate lymphoid cells as novel therapeutic targets in IBD. Nat Rev Gastroenterol Hepatol. 2016;12(5):271–83. DOI: 10.1038/nrgastro.2015.52.

[53] Reyes A, Wu M, McNulty NP, Rohwer FL, Gordon JI. Gnotobiotic mouse model of phage-bacterial host dynamics in the human gut. Proc Natl Acad Sci U S A. 2013;110 (50):20236–41. doi: 10.1073/pnas.1319470110.

[54] Khanna R, Preiss JC, MacDonald JK, Timmer A. Anti-IL-12/23p40 antibodies for induction of remission in Crohn's disease. Cochrane Database Syst Rev. 2015;5:CD007572. DOI: 10.1002/14651858.CD007572.pub2.

[55] Targan SR, Feagan B, Vermeire S, Panaccione R, Melmed GY, Landers C, Li D, Russell C, Newmark R, Zhang N, Chon Y, Hsu YH, Lin SL, Klekotka P. A randomized, double-blind, placebo-controlled phase 2 study of brodalumab in patients with moderate-to-severe Crohn's disease. Am J Gastroenterol. 2016;111(11):1599–607. doi: 10.1038/ajg.2016.298.

[56] Peckham D, Scambler T, Savic S, McDermott MF. The burgeoning field of innate immune-mediated disease and autoinflammation. J Pathol. 2017;241(2):123–39. doi: 10.1002/path.4812.

[57] McDermott MF, Aksentijevich I, Galon J, McDermott EM, Ogunkolade BW, Centola M, Mansfield E, Gadina M, Karenko L, Pettersson T, McCarthy J, Frucht DM, Aringer M, Torosyan Y, Teppo AM, Wilson M, Karaarslan HM, Wan Y, Todd I, Wood G, Schlimgen R, Kumarajeewa TR, Cooper SM, Vella JP, Kastner DL. Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. Cell. 1999;97(1):133–44.

[58] Hull KM, Shoham N, Chae JJ, Aksentijevich I, Kastner DL. The expanding spectrum of systemic autoinflammatory disorders and their rheumatic manifestations. Curr Opin Rheumatol. 2003;15(1):61–9.

[59] Hull KM, Drewe E, Aksentijevich I, Singh HK, Wong K, McDermott EM, Dean J, Powell RJ, Kastner DL. The TNF receptor-associated periodic syndrome (TRAPS): emerging concepts of an autoinflammatory disorder. Medicine (Baltimore). 2002; 81:349–68.

[60] Aksentijevich I, McDermott MF. Lessons from characterization and treatment of the autoinflammatory syndromes. Curr Opin Rheumatol. 2016. DOI:10.1097/BOR.00000000 00000362.

[61] Holzinger D, Kessel C, Omenetti A, Gattorno M. From bench to bedside and back again: translational research in autoinflammation. Nat Rev Rheumatol. 2015;11(10): 573–85. doi: 10.1038/nrrheum.2015.79.

[62] Lucherini OM, Obici L, Ferracin M, Fulci V, McDermott MF, Merlini G, Muscari I, Magnotti F, Dickie LJ, Galeazzi M, Negrini M, Baldari CT, Cimaz R, Cantarini L. First report of circulating microRNAs in tumour necrosis factor receptor-associated periodic syndrome (TRAPS). PLoS One. 2013;8(9):e73443. doi: 10.1371/journal.pone.0073443.

[63] Nedjai B, Hitman GA, Quillinan N, Coughlan RJ, Church L, McDermott MF, Turner MD. Proinflammatory action of the antiinflammatory drug infliximab in tumor necrosis factor receptor-associated periodic syndrome. Arthritis Rheum. 2009;60(2): 619–25. doi: 10.1002/art.24294.

[64] Ozen S, Bilginer Y. A clinical guide to autoinflammatory diseases: familial Mediterranean fever and next-of-kin. Nat Rev Rheumatol. 2014;10(3):135–47. doi: 10. 1038/nrrheum.2013.174.

[65] Ter Haar NM, Oswald M, Jeyaratnam J, Anton J, Barron KS, Brogan PA, Cantarini L, Galeotti C, Grateau G, Hentgen V, Hofer M, Kallinich T, Kone-Paut I, Lachmann HJ, Ozdogan H, Ozen S, Russo R, Simon A, Uziel Y, Wouters C, Feldman BM, Vastert SJ, Wulffraat NM, Benseler SM, Frenkel J, Gattorno M, Kuemmerle-Deschner JB. Recommendations for the management of autoinflammatory diseases. Ann Rheum Dis. 2015;74(9):1636–44. doi: 10.1136/annrheumdis-2015-207546.

[66] Brouckaert P, Spriggs DR, Demetri G, Kufe DW, Fiers W. Circulating interleukin-6 during a continuous infusion of tumor necrosis factor and interferon-γ. J Exp Med. 1989;169:2257–62.

[67] Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. J Exp Med. 1989(169):333–8.

[68] Sedger LM, McDermott MF. TNF and TNF-receptors: From mediators of cell death and inflammation to therapeutic giants—past, present and future. Cytokine Growth Factor Rev. 2014;25(4):453–72. doi: 10.1016/j.cytogfr.2014.07.016.

[69] Federici S, Caorsi R, Gattorno M. The autoinflammatory diseases. Swiss Med Weekly. 2012; June 19: w13602. doi: 10.4414/smw.2012.13602.

[70] Broderick L, De Nardo D, Franklin BS, Hoffman HM, Latz E. The inflammasomes and autoinflammatory syndromes. Annu Rev Pathol. 2015;10:395–424. doi: 10.1146/annurev-pathol-012414-040431.

[71] Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. Nature. 2012;481(7381):278–86. doi: 10.1038/nature10759.

[72] Canna SW, de Jesus AA, Gouni S, Brooks SR, Marrero B, Liu Y, DiMattia MA, Zaal KJ, Sanchez GA, Kim H, Chapelle D, Plass N, Huang Y, Villarino AV, Biancotto A, Fleisher

TA, Duncan JA, O'Shea JJ, Benseler S, Grom A, Deng Z, Laxer RM, Goldbach-Mansky R. An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. Nat Genet. 2014;46(10):1140–6. doi: 10.1038/ng.3089.

[73] Mortimer L, Moreau F, MacDonald JA, Chadee K. NLRP3 inflammasome inhibition is disrupted in a group of auto-inflammatory disease CAPS mutations. Nat Immunol. 2016;17(10):1176–86. doi: 10.1038/ni.3538.

[74] Rynne M, Maclean C, Bybee A, McDermott MF, Emery P. Hearing improvement in a patient with variant Muckle-Wells syndrome in response to interleukin 1 receptor antagonism. Ann Rheum Dis. 2006;65(4):553–4. doi: 10.1136/ard.2005.038091.

[75] Kingsbury SR, Conaghan PG, McDermott MF. The role of the NLRP3 inflammasome in gout. J Inflamm Res. 2011;4:39–49. doi: 10.2147/JIR.S11330.

[76] Busso N, Ea HK. The mechanisms of inflammation in gout and pseudogout (CPP-induced arthritis). Reumatismo. 2012;63 (4):230–7. doi: 10.4081/reumatismo.2011.230.

[77] Group TLMSSGaTUoBCMMA. TNF neutralization in MS. Results of a randomized, placebo-controlled multicenter study. Neurology. 1999;53(3):457–65.

[78] Abraham E, Laterre PF, Garbino J, Pingleton S, Butler T, Dugernier T, Margolis B, Kudsk K, Zimmerli W, Anderson P, Reynaert M, Lew D, Lesslauer W, Passe S, Cooper P, Burdeska A, Modi M, Leighton A, Salgo M, Van der Auwera P, Group. LS. Lenercept (p55 tumor necrosis factor receptor fusion protein) in severe sepsis and early septic shock: a randomized, double-blind, placebo-controlled, multicenter phase III trial with 1,342 patients. Crit Care Med. 2001;29(3):503–10.

[79] Papp K. Clinical development of onercept, a tumor necrosis factor binding protein, in psoriasis. Curr Med Res Opin. 2010;26(10):2287–300. doi: 10.1185/03007995.2010.507492.

[80] Kawabata H, Kadowaki N, Nishikori M, Kitawaki T, Kondo T, Ishikawa T, Yoshifuji H, Yamakawa N, Imura Y, Mimori T, Matsumura Y, Miyachi Y, Matsubara T, Yanagita M, Haga H, Takaori-Kondo A. Clinical features and treatment of multicentric Castleman's disease : a retrospective study of 21 Japanese patients at a single institute. J Clin Exp Hematop. 2013;553(1):69–77.

[81] Ringelstein M, Ayzenberg I, Harmel J, Lauenstein AS, Lensch E, Stögbauer F, Hellwig K, Ellrichmann G, Stettner M, Chan A, Hartung HP, Kieseier B, Gold R, Aktas O, Kleiter I. Long-term therapy with interleukin 6 receptor blockade in highly active neuromyelitis optica spectrum disorder. JAMA Neurol. 2015;72(7):756–63. doi: 10.1001/jamaneurol.2015.0533.

[82] Lebwohl M, Strober B, Menter A, Gordon K, Weglowska J, Puig L, Papp K, Spelman L, Toth D, Kerdel F, Armstrong AW, Stingl G, Kimball AB, Bachelez H, Wu JJ, Crowley J, Langley RG, Blicharski T, Paul C, Lacour JP, Tyring S, Kircik L, Chimenti S, Callis Duffin K, Bagel J, Koo J, Aras G, Li J, Song W, Milmont CE, Shi Y, Erondu N, Klekotka P, Kotzin B, Nirula A. Phase 3 studies comparing brodalumab with ustekinumab in psoriasis. N Engl J Med. 2015;373(14):1318–28. doi: 10.1056/NEJMoa1503824.

[83] Schmidt C. Suicidal thoughts end Amgen's blockbuster aspirations for psoriasis drug. Nat Biotechnol. 2015;33(9):894–5. doi: 10.1038/nbt0915-894b.

[84] Papp KA, Sundaram M, Bao Y, Williams DA, Gu Y, Signorovitch JE, Wang Y, Valdes JM, Mulani PM. Effects of briakinumab treatment for moderate to severe psoriasis on health-related quality of life and work productivity and activity impairment: results from a randomized phase III study. J Eur Acad Dermatol Venereol. 2014;28(6):790–8. doi: 10.1111/jdv.12177.

[85] Zhuang Y, Calderon C, Marciniak SJJ, Bouman-Thio E, Szapary P, Yang TY, Schantz A, Davis HM, Zhou H, Xu Z. First-in-human study to assess guselkumab (anti-IL-23 mAb) pharmacokinetics/safety in healthy subjects and patients with moderate-to-severe psoriasis. Eur J Clin Pharmacol. 2016;72(11):1303–10. DOI: 10.1007/s00228-016-2110-5.

[86] Gordon KB, Duffin KC, Bissonnette R, Prinz JC, Wasfi Y, Li S, Shen YK, Szapary P, Randazzo B, Reich K. A Phase 2 Trial of Guselkumab versus Adalimumab for Plaque Psoriasis. N Engl J Med. 2015;373(2):136–44. doi: 10.1056/NEJMoa1501646.

[87] Yiu ZZ, Exton LS, Jabbar-Lopez Z, Mohd Mustapa MF, Samarasekera EJ, Burden AD, Murphy R, Owen CM, Parslew R, Venning V, Ashcroft DM, Griffiths CE, Smith CH, Warren RB. Risk of serious infections in patients with psoriasis on biologic therapies: a systematic review and meta-analysis. J Invest Dermatol. 2016;136(81):1584–91. doi: 10.1016/j.jid.2016.03.035.

[88] Müller-Ladner U, Hong S, Oh C, Taylor P. Scientific rationale behind the development and approval of biosimilar infliximab (CT-P13) in Europe. Expert Rev Clin Immunol. 2015;11(Supp.1):S5–14. doi: 10.1586/1744666X.2015.1090310.

[89] Putrik P, Ramiro S, Kvien TK, Sokka T, Pavlova M, Uhlig T, Boonen A, Europe'. WGEiattorai. Inequities in access to biologic and synthetic DMARDs across 46 European countries. Ann Rheum Dis. 2014;73(2):198–206. doi: 10.1136/annrheumdis-2012-202603.

[90] Isaacs JD, Cutolo M, Keystone EC, Park W, Braun J. Biosimilars in immune-mediated inflammatory diseases: initial lessons from the first approved biosimilar anti-tumour necrosis factor monoclonal antibody. J Intern Med. 2015;279(41–59). doi: 10.1111/joim. 12432.

[91] Christl LA, Woodcock J, Kozlowski S. Biosimilars: The US Regulatory Framework. Ann Rev Med. 2. 2016; ePub. DOI: 10.1146/annurev-med-051215-031022.

[92] Park W, Hrycaj P, Jeka S, Kovalenko V, Lysenko G, Miranda P, Mikazane H, Gutierrez-Ureña S, Lim M, Lee YA, Lee SJ, Kim H, Yoo DH, Braun J. A randomised, double-blind, multicentre, parallel-group, prospective study comparing the pharmacokinetics, safety, and efficacy of CT-P13 and innovator infliximab in patients with ankylosing spondylitis: the PLANETAS study. Ann Rheum Dis. 2013;72(10):1605–12. doi: 10.1136/annrheumdis-2012-203091.

[93] Yoo DH, Hrycaj P, Miranda P, Ramiterre E, Piotrowski M, Shevchuk S, Kovalenko V, Prodanovic N, Abello-Banfi M, Gutierrez-Ureña S, Morales-Olazabal L, Tee M, Jimenez R,

Zamani O, Lee SJ, Kim H, Park W, Müller-Ladner U. A randomised, double-blind, parallel-group study to demonstrate equivalence in efficacy and safety of CT-P13 compared with innovator infliximab when coadministered with methotrexate in patients with active rheumatoid arthritis: the PLANETRA study. Ann Rheum Dis. 2013;72(10):1613–20. doi: 10.1136/annrheumdis-2012-203090.

[94] Fang J, Doneanu C, Alley WRJ, Yu YQ, Beck A, Chen W. Advanced assessment of the physicochemical characteristics of Remicade® and Inflectra® by sensitive LC/MS techniques. MAbs. 2016;8(6):1021–34. doi: 10.1080/19420862.2016.1193661.

[95] Magnenat L, Palmese A, Fremaux C, D'Amici F, Terlizzese M, Rossi M, Chevalet L. Demonstration of physicochemical and functional similarity between the proposed biosimilar adalimumab MSB11022 and Humira®. MAbs. 2016. doi: 10.1080/19420862.2016.1259046.

[96] Cho IH, Lee N, Song D, Jung SY, Bou-Assaf G, Sosic Z, Zhang W, Lyubarskaya Y. Evaluation of the structural, physicochemical, and biological characteristics of SB4, a biosimilar of etanercept. MAbs. 2015;8(6):1136–55. doi: 10.1080/19420862.2016.1193659.

[97] Velayudhan J, Chen YF, Rohrbach A, Pastula C, Maher G, Thomas H, Brown R, Born TL. Demonstration of functional similarity of proposed biosimilar ABP 501 to adalimumab. BioDrugs. 2016;30(4):339–51. doi: 10.1007/s40259-016-0185-2.

[98] Raju TS. Terminal sugars of Fc glycans influence antibody effector functions of IgGs. Curr Opin Immunol. 2008;20(4):471–8. doi: 10.1016/j.coi.2008.06.007.

[99] US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), (CVM) CfVM. Guidance for Industry, Bioanalytical Method Validation. 2013. Available from: http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

[100] Olech E. Biosimilars: Rationale and current regulatory landscape. Semin Arthritis Rheum. 2016;45(5 Suppl.):S1–10. doi: 10.1016/j.semarthrit.2016.01.001.

[101] Chapman K, Adjei A, Baldrick P, da Silva A, De Smet K, DiCicco R, Hong SS, Jones D, Leach MW, McBlane J, Ragan I, Reddy P, Stewart DI, Suitters A, Sims J. Waiving in vivo studies for monoclonal antibody biosimilar development: National and global challenges. MAbs. 2016;8(3):427–35. doi: 10.1080/19420862.2016.1145331.

[102] Hogwood CE, Bracewell DG., Smales CM. Measurement and control of host cell proteins (HCPs) in CHO cell bioprocesses. Curr Opin Biotechnol. 2014;30:153–60. doi: 10.1016/j.copbio.2014.06.017.

[103] Bracewell DG, Francis R, Smales CM. The future of host cell protein (HCP) identification during process development and manufacturing linked to a risk-based management for their control. Biotechnol Bioeng. 2015;112(9):1727–37. DOI: 10.1002/bit.25628.

[104] Feldman SR. Inflammatory diseases: Integrating biosimilars into clinical practice. Semin Arthritis Rheum. 2015;44(6 Suppl.):S16–21. DOI:10.1016/j.semarthrit.2015.04.003.

[105] Jung SK, Lee KH, Jeon JW, Lee JW, Kwon BO, Kim YJ, Bae JS, Kim DI, Lee SY, Chang SJ. Physicochemical characterization of Remsima. MAbs. 2014;6(5):1163–77. doi: 10. 4161/mabs.32221.

[106] Azevedo VF, Galli N, Kleinfelder A, D'Ippolito J, Urbano PCM. Etanercept biosimilars. Rheumatol Int. 2015;35:197–209. doi: 10.1007/s00296-014-3080-5.

[107] Buer LC, Moum BA, Cvancarova M, Warren DJ, Medhus AW, Høivik ML. Switching from Remicade® to Remsima® is safe and feasible: a prospective, open-label study. J Crohns Colitis 2016;Sept 22(pii: jjw166.). doi: 10.1093/ecco-jcc/jjw166.

[108] Ruiz-Argüello MB, Maguregui A, Ruiz Del Agua A, Pascual-Salcedo D, Martínez-Feito A, Jurado T, Plasencia C, Balsa A, Llinares-Tello F, Rosas J, Torres N, Martínez A, Nagore D. Antibodies to infliximab in Remicade-treated rheumatic patients show identical reactivity towards biosimilars. Ann Rheum Dis. 2016;75(9):1693–6. DOI: 10.1136/annrheumdis-2015-208684.

[109] Ben-Horin S, Yavzori M, Benhar I, Fudim E, Picard O, Ungar B, Lee S, Kim S, Eliakim R, Chowers Y. Cross-immunogenicity: antibodies to infliximab in Remicade-treated patients with IBD similarly recognise the biosimilar Remsima. Gut. 2016;65(7):1132–8. DOI: 10.1136/gutjnl-2015-309290.

[110] Daëron M. Fc receptor biology. Annu Rev Immunol. 1997;15:203–34. DOI: 10.1146/annurev.immunol.15.1.203.

[111] Kubagawa H, Oka S, Kubagawa Y, Torii I, Takayama E, Kang DW, Gartland GL, Bertoli LF, Mori H, Takatsu H, Kitamura T, Ohno H, Wang JY. Identity of the elusive IgM Fc receptor (FcmuR) in humans. J Exp Med. 2009;206(12):2779–93. DOI: 10.1084/jem.20091107.

[112] Cosman D, Fanger N, Borges L, Kubin M, Chin W, Peterson L, Hsu ML. A novel immunoglobulin superfamily receptor for cellular and viral MHC class I molecules. Immunity. 1997;7:273–82.

[113] Borges L, Hsu ML, Fanger N, Kubin M, Cosman D. A family of human lymphoid and myeloid Ig-like receptors, some of which bind to MHC class I molecules. J Immunol. 1997;159:5192–6.

[114] Fanger NA, Borges L, Cosman D. The leukocyte immunoglobulin-like receptors (LIRs): a new family of immune regulators. J Leukoc Biol. 1999;66:231–6.

[115] Nakajima H, Samaridis J, Angman L, Colonna M. Human myeloid cells express an activating ILT receptor (ILT1) that associates with Fc receptor gamma-chain. J Immunol. 1999;162:5–8.

[116] Woof JM, Burton DR. Human antibody-Fc receptor interactions illuminated by crystal structures. Nat Rev Immunol. 2004;4:89–99. doi:10.1038/nri1266.

[117] Tedla N, Gibson K, McNeil H.P., Cosman D, Borges L, Arm JP. The co-expression of activating and inhibitory leukocyte immunoglobulin-like receptors in rheumatoid synovium. Am J Path. 2002;160(2):425–31. DOI: 10.1016/S0002-9440(10)64861-4.

[118] Honda S, Kurita N, Miyamoto A, Cho Y, Usui K, Takeshita K, Takahashi S, Yasui T, Kikutani H, Kinoshita T, Fujita T, Tahara-Hanaoka S, Shibuya K, Shibuya A. Enhanced humoral immune responses against T-independent antigens in Fc alpha/muR-deficient mice. Proc Natl Acad Sci U S A. 2009;106(27):11230–5. doi: 10.1073/pnas.0809917106.

[119] Shima H, Takatsu H, Fukuda S, Ohmae M, Hase K, Kubagawa H, Wang JY, Ohno H. Identification of TOSO/FAIM3 as an Fc receptor for IgM. Int Immunol. 2010;22(3):149–56. DOI: 10.1093/intimm/dxp121.

[120] Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieyus S, Daëron M. Specificity and affinity of human Fc-γ receptors and their polymorphic variants for human IgG subclasses. Blood. 2009;113:3716–25. DOI: 10.1182/blood-2008-09-179754.

[121] Monteiro RC, Van De Winkel JG. IgA Fc receptors. Annu Rev Immunol. 2001;21:177–204. DOI: 10.1146/annurev.immunol.21.120601.141011.

[122] Aleyd E, Heineke MH, van Egmond M. The era of the immunoglobulin A Fc receptor FcαRI; its function and potential as target in disease. Immunol Rev. 2015;268(1):268. doi: 10.1111/imr.12337.

[123] Ben Mkaddem S, Rossato E, Heming N, Monteiro RC. Anti-inflammatory role of the IgA Fc receptor (CD89): from autoimmunity to therapeutic perspectives. Autoimmun Rev. 2013;12(6):666–9. doi: 10.1016/j.autrev.2012.10.011.

[124] Pasquier B, Launay P, Kanamaru Y, Moura IC, Pfirsch S, Ruffié C, Hénin D, Benhamou M, Pretolani M, Blank U, Monteiro RC. Identification of FcalphaRI as an inhibitory receptor that controls inflammation: dual role of FcRgamma ITAM. Immunity. 2005; 22 (1):31–42. doi: 10.1016/j.immuni.2004.11.017.

[125] Thomas SS, Borazan N, Barroso N, Duan L, Taroumian S, Kretzmann B, Bardales R, Elashoff D, Vangala S, Furst DE. Comparative immunogenicity of TNF inhibitors: impact on clinical efficacy and tolerability in the management of autoimmune diseases. A systematic review and meta-analysis. BioDrugs. 2015;29(4):241–58. doi: 10.1007/s40259-015-0134-5.

[126] Suhrbier A, Mahalingam S. The immunobiology of viral arthritides. Pharmacol Ther. 2009;124(3):301–8. doi: 10.1016/j.pharmthera.2009.09.005.

[127] Rathinam VA, Fitzgerald KA. Inflammasomes and anti-viral immunity. J Clin Immunol. 2010;30(5):632–7. doi: 10.1007/s10875-010-9431-4.

[128] Shalaby MR, Waage A, Aarden L, Espevik T. Endotoxin, tumor necrosis factor and interleukin 1 induce interleukin 6 production in vivo. Clin Immunol Immunopathol. 1989;53:488–98.

[129] Sedger LM, Shows DM, Blanton RA, Peschon JJ, Goodwin RG, Cosman D, Wiley SR. IFN-γ mediates a novel antiviral activity through dynamic modulation of TRAIL and TRAIL receptor expression. J Immunol. 1999;163(2):920–6.

[130] Ramsay AJ, Husband AJ, Ramshaw IA, Bao S, Matthaei KI, Koehler G, Kopf M. The role of interleukin-6 in mucosal IgA antibody responses in vivo. Science. 1994;264 (5158):561–3.

[131] Kim SY, Solomon DH. Tumor necrosis factor blockade and the risk of viral infection. Nat Rev Rheumatol. 2010;6:165–74. doi: 10.1038/nrrheum.2009.279.

[132] Cantini F, Niccoli L, Goletti D. Adalimumab, etanercept, infliximab, and the risk of tuberculosis: data from clinical trials, national registries, and postmarketing surveillance. J Rheumatol Suppl. 2014;91:47–55. doi: 10.3899/jrheum.140102.

[133] Tresch S, Trueb RM, Kamarachev J, French LE, Hofbauer GF. Disseminated herpes zoster mimicking rheumatoid vasculitis in a rheumatoid arthritis patient on etanercept. Dermatology. 2009;219:347–9. doi: 10.1159/000232389.

[134] Wong AK, Kerkoutian S, Said J, Rashidi H, Pullarkat ST. Risk of lymphoma in patients receiving antitumor necrosis factor therapy: a meta-analysis of published randomized controlled studies. Clin Rheumatol. 2012;31:631–6. doi: 10.1007/s10067-011-1895-y.

[135] Dinarello CA, Cannon JG, Wolff SM, Bernheim HA, Beutler B, Cerami A, Figari IS, Palladino MAJ, O'Connor JV. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. J Exp Med. 1986;164:1443–50.

[136] Smyth MJ, Kelly JM, Baxter AG, Korner H, Sedgwick JD. An essential role for tumor necrosis factor in natural killer cell-mediated tumor rejection in the peritoneum. J Exp Med. 1998;188:1611–9.

[137] Kaltsonoudis E, Voulgari PV, Konitsiotis S, Drosos AA. Demyelination and other neurological adverse events after anti-TNF therapy. Autoimmun Rev. 2014;13(1):54–8. doi: 10.1016/j.autrev.2013.09.002.

[138] Adreadou E, Kemanetzoglou E, Brokalaki C, Evangelopoulos M, Kilidireas C, Rambos A, Stamboulis E. Demyelinating disease following anti-TNFa treatment: A causal or coincidental association? Report of four cases and review of the literature. Case Reports Neurol Med. 2013; ID67935. doi: 10.1155/2013/671935.

[139] Kaltsonoudis E, Zikou AK, Voulgari PV, Konitsiotis S, Argyropoulou MI, Drosos AA. Neurological adverse events in patients receiving anti-TNF therapy: a prospective imaging and electrophysiological study. Arthritis Res Ther. 2014;16(3):R125. doi: 10.1186/ar4582.

[140] Mohan N, Edwards ET, Cupps TR, Oliverio PJ, Sandberg G, Crayton H, Richert JR, Siegel JN. Demyelination occurring during anti-tumor necrosis factor alpha therapy for inflammatory arthritides. Arthritis Rheum. 2001;44(12):2862–9.

[141] Black C, Miller BJ. Meta-analysis of cytokines and chemokines in suicidality: distinguishing suicidal versus nonsuicidal patients. Biol Psychiatry. 2015;78(1):28–37. doi: 10.1016/j.biopsych.2014.10.014.

[142] Dieperink E, Ho SB, Thuras P, Willenbring ML. A prospective study of neuropsychiatric symptoms associated with interferon-alpha-2b and ribavirin therapy for patients with chronic hepatitis C. Psychosomatics. 2003;44(2):104–12.

[143] Schaefer M, Schmidt F, Horn M, Schmid-Wendtner MH, Volkenandt M. Depression during treatment with interferon alpha. Psychosomatics. 2004;45(2):176. DOI: 10.1176/appi.psy.45.2.176.

[144] Murakami Y, Ishibashi T, Tomita E, Imamura Y, Tashiro T, Watcharanurak K, Nishikawa M, Takahashi Y, Takakura Y, Mitani S, Fujigaki H, Ohta Y, Kubo H, Mamiya T, Nabeshima T, Kim HC, Yamamoto Y, Saito K. Depressive symptoms as a side effect of Interferon-α therapy induced by induction of indoleamine 2,3-dioxygenase 1. Sci Rep. 2016;6:29920. doi: 10.1038/srep29920.

[145] Bay-Richter C, Linderholm KR, Lim CK, Samuelsson M, Träskman-Bendz L, Guillemin GJ, Erhardt S, Brundin L. A role for inflammatory metabolites as modulators of the glutamate N-methyl-D-aspartate receptor in depression and suicidality. Brain Behav Immun. 2015;43:110–7. doi: 10.1016/j.bbi.2014.07.012.

[146] Erhardt S, Lim CK, Linderholm KR, Janelidze S, Lindqvist D, Samuelsson M, Lundberg K, Postolache TT, Träskman-Bendz L, Guillemin GJ, Brundin L. Connecting inflammation with glutamate agonism in suicidality. Neuropsychopharmacology. 2013;38(5):743–52. doi: 10.1038/npp.2012.248.

[147] Clark IA, Vissel B. A neurologist's guide to TNF biology and to the principles behind the therapeutic removal of excess TNF in disease. Neural Plast. 2015;2015:358263. DOI: 10.1155/2015/358263.

[148] Clark IA, Vissel B. Excess cerebral TNF causing glutamate excitotoxicity rationalizes treatment of neurodegenerative diseases and neurogenic pain by anti-TNF agents. J Neuroinflammation. 2016;13(1):236. doi: 10.1186/s12974-016-0708-2.

[149] Ye L, Huang Y, Zhao L, Li Y, Sun L, Zhou Y, Qian G, Zheng JC. IL-1β and TNF-α induce neurotoxicity through glutamate production: a potential role for neuronal glutaminase. J Neurochem. 2013;125(6):897–908. DOI: 10.1111/jnc.12263.

[150] Tobinick EL. Perispinal delivery of CNS drugs. CNS Drugs. 2016;30(6):469–80. doi: 10.1007/s40263-016-0339-2.

[151] Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J. Structural and functional features of central nervous system lymphatic vessels. Nature. 2015;523(7560):337–41. doi: 10.1038/nature14432.

[152] Tobinick E. Rapid improvement of chronic stroke deficits after perispinal etanercept: three consecutive cases. CNS Drugs 2011 Feb;25(2):145–55 2011;25(5):145-55. doi: 10.2165/11588400-000000000-00000.

[153] Tobinick E, Kim NM, Reyzin G, Rodriguez-Romanacce H, DePuy V. Selective TNF inhibition for chronic stroke and traumatic brain injury: an observational study involving 629 consecutive patients treated with perispinal etanercept. CNS Drugs 2012;26(12):1051–70. doi: 10.1007/s40263-012-0013-2.

[154] Tobinick E, Rodriguez-Romanacce H, Levine A, Ignatowski TA, Spengler RN. Immediate neurological recovery following perispinal etanercept years after brain injury. Clin Drug Investig. 2014;34(5):361–6. doi: 10.1007/s40261-014-0186-1.

[155] Chio CC, Chang CH, Wang CC, Cheong CU, Chao CM, Cheng BC, Yang CZ, Chang CP. Etanercept attenuates traumatic brain injury in rats by reducing early microglial expression of tumor necrosis factor-α. BMC Neurosci. 2013;14:33. doi: 10.1186/1471-2202-14-33.

[156] Licastro F, Hrelia S, Porcellini E, Malaguti M, Di Stefano C, Angeloni C, Carbone I, Simoncini L, Piperno R. Peripheral inflammatory markers and antioxidant response during the post-acute and chronic phase after severe traumatic brain injury. Front Neurol. 2016;7:189. DOI: 10.3389/fneur.2016.00189.

[157] Tuttolomondo A, Pecoraro R, Pinto A. Studies of selective TNF inhibitors in the treatment of brain injury from stroke and trauma: a review of the evidence to date. Drug Des Devel Ther. 2014;8:2221–38. doi: 10.2147/DDDT.S67655.

[158] Loane DJ, Kumar A. Microglia in the TBI brain: The good, the bad, and the dysregulated. Exp Neurol. 2016 Jan;275 Pt 3:316-27. 2016;275:316–27. doi: 10.1016/j.expneurol.2015.08.018.

[159] Sairanen T, Carpén O, Karjalainen-Lindsberg ML, Paetau A, Turpeinen U, Kaste M, Lindsberg PJ. Evolution of cerebral tumor necrosis factor-alpha production during human ischemic stroke. Stroke. 2001;32(8):1750–8.

[160] Rappaport J, Volsky DJ. Role of the macrophage in HIV-associated neurocognitive disorders and other comorbidities in patients on effective antiretroviral treatment. J Neurovirol. 2015;21(3):235–41. doi: 10.1007/s13365-015-0346-y.

[161] Seilhean D, Kobayashi K, He Y, Uchihara T, Rosenblum O, Katlama C, Bricaire F, Duyckaerts C, Hauw JJ. Tumor necrosis factor-alpha, microglia and astrocytes in AIDS dementia complex. Acta Neuropathol. 1997;93(5):508–17.

[162] Han C, Lu Y, Wei Y, Wu B, Liu Y, He R. D-ribosylation induces cognitive impairment through RAGE-dependent astrocytic inflammation. Cell Death Dis. 2014;5:e1117. doi: 10.1038/cddis.2014.89.

[163] Mandal P, Schifilliti D, Mafrica F, Fodale V. Inhaled anesthesia and cognitive performance. Drugs Today (Barc). 2009;45(1):47–54. doi: 10.1358/dot.2009.45.1.1315075.

[164] Fong TG, Davis D, Growdon ME, Albuquerque A, Inouye SK. The interface between delirium and dementia in elderly adults. Lancet Neurol. 2015;14(8):823–32. DOI: 10.1016/S1474-4422(15)00101-5.

[165] Simone MJ, Tan ZS. The role of inflammation in the pathogenesis of delirium and dementia in older adults: a review. CNS Neurosci Ther. 2011;17(5):506–13. doi:10.1111/j.1755-5949.2010.00173.x.

[166] Xie Z, Dong Y, Maeda U, Moir RD, Xia W, Culley DJ, Crosby G, Tanzi RE. The inhalation anesthetic isoflurane induces a vicious cycle of apoptosis and amyloid beta-

protein accumulation. J Neurosci. 2007;27(6):1247–54. doi: 10.1523/JNEUROSCI.5320-06.2007.

[167] Zhang Y, Zhen Y, Dong Y, Xu Z, Yue Y, Golde TE, Tanzi RE, Moir RD, Xie Z. Anesthetic propofol attenuates the isoflurane-induced caspase-3 activation and Aβ oligomerization. PLoS One. 2011;6(11):e27019. doi: 10.1371/journal.pone.0027019.

[168] Jiang J, Jiang H. Effect of the inhaled anesthetics isoflurane, sevoflurane and desflurane on the neuropathogenesis of Alzheimer's disease (review). Mol Med Rep. 2015;12(1):3–12. doi: 10.3892/mmr.2015.3424.

[169] Qiao Y, Feng H, Zhao T, Yan H, Zhang H, Zhao X. Postoperative cognitive dysfunction after inhalational anesthesia in elderly patients undergoing major surgery: the influence of anesthetic technique, cerebral injury and systemic inflammation. BMC Anesthesiol. 2015;15:154. doi: 10.1186/s12871-015-0130-9.

[170] Capri M, Yani SL, Chattat R, Fortuna D, Bucci L, Lanzarini C, Morsiani C, Catena F, Ansaloni L, Adversi M, Melotti MR, Di Nino G, Franceschi C. Pre-operative, high-IL-6 blood level is a risk factor of post-operative delirium onset in old patients. Front Endocrinol (Lausanne). 2014;5:173. doi: 10.3389/fendo.2014.00173.

[171] Androsova G, Krause R, Winterer G, Schneider R. Biomarkers of postoperative delirium and cognitive dysfunction. Front Aging Neurosci 2015;7:112. doi: 10.3389/fnagi.2015.00112.

[172] Chiricozzi A, Romanelli M, Saraceno R, Torres T. No meaningful association between suicidal behavior and the use of IL-17A-neutralizing or IL-17RA-blocking agents. Expert Opin Drug Saf. 2016;5(12):1653–9. doi: 10.1080/14740338.2016.1228872.

[173] Galluzzo M, D'adamio S, Bianchi L, Talamonti M. Brodalumab for the treatment of psoriasis. Expert Rev Clin Immunol. 2016;12(12):1255–71. doi: 10.1080/1744666X.2016.1246957.

[174] Pallav P, Sagar R, Mehta M, Sharma S, Subramanium A, Shamshi F, Sengupta U, Pandey RM, Mukhopadhyay AK. Serum cytokines and anxiety in adolescent depression patients: Gender effect. Psychiatry Res. 2015;229(1–2):374–80. doi: 10.1016/j.psychres.2015.06.036.

[175] Vieira MM, Ferreira TB, Pacheco PA, Barros PO, Almeida CR, Araújo-Lima CF, Silva-Filho RG, Hygino J, Andrade RM, Linhares UC, Andrade AF, Bento CA. Enhanced Th17 phenotype in individuals with generalized anxiety disorder. J Neuroimmunol. 2010;229(1-2):212–8. doi: 10.1016/j.jneuroim.2010.07.018.

[176] Ferreira TB, Barros PO, Teixeira B, Cassano T, Centurião N, Kasahara TM, Hygino J, Vasconcelos CC, Filho HA, Alvarenga R, Wing AC, Andrade RM, Andrade AF, Bento CA. Dopamine favors expansion of glucocorticoid-resistant IL-17-producing T cells in multiple sclerosis. Brain Behav Immun. 2014;41:182–90. doi: 10.1016/j.bbi.2014.05.013.

[177] Menzella F, Lusuardi M, Galeone C, Taddei S, Zucchi L. Profile of anti-IL-5 mAb mepolizumab in the treatment of severe refractory asthma and hypereosinophilic diseases. J Asthma Allergy. 2015;8:105–14. doi: 10.2147/JAA.S40244.

[178] Nixon J, Newbold P, Mustelin T, Anderson GP, Kolbeck R. Monoclonal antibody therapy for the treatment of asthma and chronic obstructive pulmonary disease with eosinophilic inflammation. Pharmacol Ther. 2016; pii: S0163–7258(16):30195–4. doi: 10. 1016/j.pharmthera.2016.10.016.

[179] Corren J. Inhibition of interleukin-5 for the treatment of eosinophilic diseases. Discov Med. 2012;13(71):305–12.

[180] Khorasanizadeh MH, Eskian M, Assa'as AH, Camargo CAJ, Rezaei N. Efficacy and safety of benralizumab, a monoclonal antibody against IL-5Rα, in uncontrolled eosinophilic asthma. Intl Rev Immunology. 2016;35:294–311. doi: 10.3109/08830185.2015.1128901.

[181] Walsh GM. Anti-IL-4/13 based therapy in asthma. Expert Opin Emerg Drugs. 2015;20 (3):349–52. doi: 10.1517/14728214.2015.1050377.

[182] Walsh GM. Biologics targeting IL-5, IL-4 or IL-13 for the treatment of asthma – an update. Expert Rev Clin Immunol. 2016;Aug 2:1–7. doi: 10.1080/1744666X. 2016.1216316.

[183] Matsumoto H. Serum periostin: a novel biomarker for asthma management. Allergol Int. 2014;63(2):153–60. doi: 10.2332/allergolint.13-RAI-0678.

[184] Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, Shikotra A, Carter R, Audusseau S, Hamid Q, Bradding P, Fahy JV, Woodruff PG, Harris JM, Arron JR, Group. BERSoBiC-rABS. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. J Allergy Clin Immunol. 2012;130(3):647–54. doi: 10.1016/j.jaci.2012.06.025.

[185] Bujarski S, Parulekar AD, Hanania NA. Lebrikizumab in the treatment of asthma. Expert Opin Biol Ther. 2016:847–52. doi: 10.1080/14712598.2016.1182152.

[186] Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, Bucks C, Wu X, Kane CM, Neill DR, Flynn RJ, Sayers I, Hall IP, McKenzie AN. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. J Allergy Clin Immunol. 2013;132(4):933–41. doi: 10.1016/j.jaci.2013.05.012.

[187] Halim TY, Steer CA, Mathä L, Gold MJ, Martinez-Gonzalez I, McNagny KM, McKenzie AN, Takei F. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. Immunity. 2014;40(3): 425–35. doi: 10.1016/j.immuni.2014.01.011.

[188] Kim BS, Artis D. Group 2 innate lymphoid cells in health and disease. Cold Spring Harb Perspect Biol. 2015;7(5): pii: a016337. doi: 10.1101/cshperspect.a016337.

[189] Jackson RJ, Worley M, Trivedi S, Ranasinghe C. Novel HIV IL-4R antagonist vaccine strategy can induce both high avidity CD8 T and B cell immunity with greater protective efficacy. Vaccine. 2014;32(43):5703–14. doi: 10.1016/j.vaccine.2014.08.023.

[190] Ranasinghe C, Trivedi S, Stambas J, Jackson RJ. Unique IL-13Rα2-based HIV-1 vaccine strategy to enhance mucosal immunity, CD8+ T-cell avidity and protective immunity. Mucosal Immunol. 2013;6(6):1068–80. doi: 10.1038/mi.2013.1.

Chapter 12

Immunotherapeutic Biologic Agents to Treat Autoinflammatory Diseases

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Additional information is available at the end of the chapter

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Abstract

In recent years, innovative treatment for patients with autoimmune and autoinflammatory diseases has advanced in concert with our increased understanding of molecular and clinical immunology. Deeper understanding of autoimmunity has allowed for the development of cutting-edge biologic drugs for patients with relatively common autoimmune diseases. During this same period, knowledge regarding the molecular bases of autoinflammatory genetic diseases has also greatly expanded. Biologic immunotherapeutic agents developed for autoimmune diseases that primarily target cytokines that are also dysregulated in the uncommon autoinflammatory diseases are the focus of this article. In the following pages, selected genetic autoinflammatory diseases and key immunotherapeutic treatment approaches are addressed. The current understanding of these diseases and mechanisms by which therapeutic agents may benefit patients are reviewed. Indications, risks, and additional considerations for the use of these agents in treatment of autoinflammatory disorders are addressed as well.

Keywords: biologic agents, autoinflammatory diseases, cytokines, inflammasome, IL-1, TNF, IL-6, interferon

1. Introduction

Over the past 20 years, scientific work that uncovered the genetic basis of autoinflammatory diseases (AutoIDx) has expanded knowledge about pathways of the innate system. Important discoveries have linked autoinflammation to defects in the innate immune system and autoimmunity largely to changes in adaptive immune function. Immunotherapeutic agents which target cells, cytokines, and mediators of immunologic responses are part of our current "toolbox" to treat autoimmune diseases. These pharmacological agents also target and treat the excess downstream inflammatory mediators produced by genetic mutations in the innate immune

system that cause the syndromes identified as AutoIDx. This review addresses key immunotherapeutic biologic approaches for treating selected AutoIDx. Current therapeutic approaches, as well as risks and additional considerations in the use of these agents, are addressed.

2. Innate and adaptive immunity

The innate and adaptive immune systems normally work together in integrated fashion utilizing antigen-specific and antigen-independent mechanisms. The host's first line of defense is the innate system which recognizes nonspecific immunologic signals and then directs further innate system activities in concert with the adaptive immune system. In this way, the extent and complexity of the overall immunologic response is enhanced. Genetic defects in the function and control of the innate immune system cause the AutoIDx. These disorders produce unprovoked, often self-limited episodic inflammation that is not associated with antigen-specific T cell reactions or with autoantibody production. AutoIDx have no association with specific Major histocompatibility complex (MHC) alleles, unlike autoimmune diseases. Research and newer genetic techniques as exemplified by next-generation sequencing have uncovered the etiologies of multiple AutoIDx, although mutations underlying some AutoIDx remain unknown. Each discovery has furthered our understanding of pathways and therapeutic targets in the innate immune system [1].

The innate immune system is composed of effector cells, such as activated macrophages, as well as receptors, cytokines, and downstream response proteins. Surface pattern recognition receptors including Toll-like receptors (TLRs) and pathogen-associated molecular patterns (PAMPS) can activate inflammasome assembly through effects on NF-κB production and subsequent immunological signaling (Signal 1 activation). Molecules such as crystals in gout, heat-shock proteins, damaged tissue (such as with burns), as well as other PAMPS and damage-associated molecular patterns (DAMPS) (Signal 2 activation) can also provoke this response. Following Signal 1 and 2 triggers, intracellular pattern recognition proteins, including nucleotide-binding oligomerization domain-like receptors (NOD-like receptors, NLRs such as NOD-like receptor P3 (NLRP3), and NOD-like receptor C4 (NLRC4)) and cytoplasmic DNA receptors [2], are then activated. Cytoplasmic NLRs oligomerize in response to these initial signals, forming inflammasomes that are multimeric scaffolded structures that further activate cytokines [3, 4]. Inflammasomes specific to different NLR structures perpetuate cascading downstream signals. NLRP3 specifically is associated with apoptosis-associated speck-like protein containing (ASC) a caspase recruitment domain (CARD) and procaspase-1. Inflammasome NLRP3 is key as it leads to production of the central cytokine, interleukin (IL)-1, via the caspase-1 activation cascade. This is followed by conversion of IL-1β and IL-18 from inactive to enzymatically active proteins (see **Figure 1**) [5, 6]. Loss-of-function or gain-of-function gene mutations that code regulatory proteins which control inflammasome scaffold formation and subsequent cytokine activity are among the causes of AutoIDx.

Unlike AutoIDx, relatively more common autoimmune disorders, including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), are largely caused by defective tolerance,

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but the innate immune system plays a key role as well. Some autoimmune diseases, especially early-onset Crohn's disease (CrD) and sarcoidosis, are caused by innate system dysregulation occurring in concert with adaptive immune dysfunction [7]. Autoimmune diseases today are regularly treated with pharmaceutical biologic agents that target relevant inflammatory pathways. These therapies have changed the lives of the many people affected by such diseases. AutoIDx affect fewer individuals, but these populations nonetheless have benefitted from current-day immunologic therapeutic discoveries which are the focus of this review (for detailed reviews of the adaptive and innate immune systems and autoimmunity, see Refs. [8–12]).

3. Autoinflammatory diseases

The AutoIDx were identified through translational research efforts starting with uncovering the genetic cause of familial Mediterranean fever (FMF) [13] followed by identification of genes for cryopyrin-associated periodic syndromes (CAPS). AutoIDx may present with typical monthly episodes, or may be more unpredictable, with exacerbations no more than 2–3 times per year. Typical spells can be set off by seemingly innocuous triggers such as vaccination or cool environmental temperature [14]. Each disease has well-characterized features, usually including fevers, hence the former term "periodic fever syndromes" (see **Tables 1** and **2**). Below, representative conditions for which biologic therapies have been used are addressed in some detail.

3.1. Cryopyrin-associated periodic syndromes

CAPS are monogenetic dominant disorders due to the defective cold-induced autoinflammatory syndrome 1 (CIAS1) or NLRP3 gene which alters the protein cryopyrin. CAPS exhibit a range of severity based on variable penetrance of the mutations: familial cold autoinflammatory syndrome (FCAS) is the mildest, Muckle-Wells syndrome (MWS) has moderate features, and neonatal-onset multisystem inflammatory disease (NOMID, also termed chronic infantile neurological, cutaneous, articular syndrome CINCA) is the most severe and potentially life-threatening disease. Features are usually present in newborns with rash and fever; additionally, in MWS and NOMID, arthritis develops. In FCAS a cold environment precipitates exacerbations of symptoms. NOMID may cause severe arthritis with destructive bony overgrowth, as well as chronic meningitis and developmental delays. NOMID and MWS may lead to hearing loss; amyloidosis and renal failure develop in 25% of untreated individuals [1, 14].

In CAPS, gain-of-function dominant mutations occur in NLRP3, a member of the NLR protein family. Somatic mosaicism may also be associated with typical symptoms. In NOMID sporadic mutations are frequent, with up to 40% having no identifiable mutation [1]. In CAPS, spontaneous activation of cell surface TLRs and cytoplasmic sensors occurs followed by antigen unprovoked assembly of the inflammasome complex. Caspase-1 is then activated and converts both pro-IL-1ß and pro-IL-18 to operational cytokines [3, 5]. Excess activity of the assembled multimolecular inflammasome results in unregulated production of IL-1ß, causing CAPS. Overproduction of IL-1β causes further downstream excess inflammatory responses. If left untreated CAPS can lead to increased serum amyloid A (SAA) protein accumulation, amyloidosis, and renal failure [1, 14].

Disease (acronym)	Gene; heritance	Affected protein Functional changes
IL-1β activation disorders of the inflammasome		
Cryopyrin-associated periodic syndrome • Familial cold autoinflammatory syndrome (FCAS) • Muckle-Wells syndrome (MWS) • Neonatal-onset multisystem inflammatory disease (NOMID)	NLRP3, CIAS1 (1q44); AD	Cryopyrin NALP3/PYPAF1 Inflammasome activation Excess IL-1β production
Deficiency of the interleukin-1 receptor antagonist (DIRA)	IL-1RN (2q14.2); AR	Lack of IL-1Ra Unopposed IL-1 signaling
NOD-like receptor C4-MAS	NLRC4; AD	IL-1β and IL-18 produced Macrophage activation
Familial Mediterranean fever (FMF)	MEFV (16p13.3); AR (AD)	Defective pyrin (marenostrin) Increased IL-1 activation
Hypergammaglobulinemia D syndrome (HIDS)	MVK (12p24); AR	Defective mevalonate kinase IL-1β dysregulation
Pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA)	PSTPIP1 (15q24-25.1); AD	PSTPIP1 dysfunction Cytoskeletal changes stimulates inflammasome
TNF disorders of the innate immune system		
TNF receptor-associated periodic syndrome (TRAPS)	TNFRSF1A (12p13); AD	Mutant TNF receptor activates Inflammation via Il-1
Deficiency of adenosine deaminase (DADA)	CERC1; AR	Lack of activity of ADA2 Stimulate TNF dysregulation
Interferon activation disorders		
STING-associated vasculopathy of infancy (SAVI)	TMEM173; AD	STING activation Increased IFN-β transcription
Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE)	PSMB8; AR	Proteasome dysfunction Induced IFN response genes

AD, autosomal dominant; ADA, adenosine deaminase; AR, autosomal recessive; CANDLE, chronic atypical neutrophilic dermatosis with lipodystrophy/elevated temperature; CAPS, cryopyrin-associated periodic syndromes; CNS, central nervous system; DADA2, deficiency of adenosine deaminase 2; DIRA, deficiency of the IL-1 receptor antagonist; FCAS, familial cold autoinflammatory syndrome; FMF, familial Mediterranean fever; HIDS, hyper-IgD syndrome; IFN, interferon; IL-1, interleukin-1; ILD, interstitial lung disease; JAK, Janus kinase; MAB, monoclonal antibody; MAS, macrophage activation syndrome; MKD, mevalonate kinase deficiency; MWS, Muckle-Wells syndrome; NLRC4, NOD-like receptor C4; NOMID, neonatal-onset multisystem inflammatory disease; NR, not reported; PAPA, pyogenic arthritis, pyoderma gangrenosum, and acne; SAVI, STING-associated vasculopathy with onset in infancy; TNF, tumor necrosis factor; TRAPS, TNF receptor-associated periodic syndrome

Table 1. Classification of selected autoinflammatory disease (adapted from Refs. [15–17]).

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	Ethnicity	Duration of episodes	Clinical features	Amyloid
FMF	Arab, Turkish, Jewish, Armenian	1–3 days	Erysipeloid rash, serositis, peritonitis, episodic inflammatory arthritis	Variable; significant risk
TRAPS	No specific group	>7–10 days	Rash, myalgia, serositis, arthritis, conjunctivitis, periorbital edema	10%
HIDS	Dutch, French, other Europeans	3–7 days	Maculopapular rash, abdominal pain, diarrhea, polyarthritis, ulcers, adenopathy	Rare
CAPS: FCAS	Mainly European	Often <24 h	Cold triggered urticarial-like rash; nausea; arthralgia	Uncommon
CAPS: MWS	Northern European	2–3 days	Urticarial-like rash, arthritis, conjunctivitis, hearing loss	Up to 25%
CAPS: NOMID	No specific group	Continuous with flares	Urticarial-like rash, chronic arthritis and overgrowth, uveitis, meningitis, developmental delay	Up to 25%
PAPA	NR	Early joints; later skin lesions	Fever, sterile arthritis, skin ulcerations, pyoderma gangrenosum, severe cystic acne	NR
DIRA	Lebanon, Brazil, Turkey, and others	Continuous from onset	Fever, pustular neutrophilic dermatitis, aseptic osteitis	NR
SAVI	NR	Continuous from onset	Fever, ischemic skin, digital necrosis, arthritis, myositis, ILD	NR

AD, autosomal dominant; ADA, adenosine deaminase; AR, autosomal recessive; CANDLE, chronic atypical neutrophilic dermatosis with lipodystrophy/elevated temperature; CAPS, cryopyrin-associated periodic syndromes; CNS, central nervous system; DADA2, deficiency of adenosine deaminase 2; DIRA, deficiency of the IL-1 receptor antagonist; FCAS, familial cold autoinflammatory syndrome; FMF, familial Mediterranean fever; HIDS, hyper-IgD syndrome; IFN, interferon; IL-1, interleukin-1; ILD, interstitial lung disease; JAK, Janus kinase; MAB, monoclonal antibody; MAS, macrophage activation syndrome; MKD, mevalonate kinase deficiency; MWS, Muckle-Wells syndrome; NLRC4, NOD-like receptor C4; NOMID, neonatal-onset multisystem inflammatory disease; NR, not reported; PAPA, pyogenic arthritis, pyoderma gangrenosum, and acne; SAVI, STING-associated vasculopathy with onset in infancy; TNF, tumor necrosis factor; TRAPS, TNF receptor-associated periodic syndrome

Table 2. Features of selected autoinflammatory diseases (adapted from Refs. [15, 17]).

3.2. Deficiency of the IL-1 receptor antagonist (DIRA)

The naturally occurring protein, "IL-1 receptor antagonist," attenuates downstream activation of IL-1 produced by normal function of the inflammasome. Loss-of-function gene mutations cause DIRA due to dysfunctional IL-1 receptor binding to this defective protein which normally would prevent dampening of IL-1 activity [3]. DIRA differs from CAPS possibly due to uninterrupted

overactivity of both IL-1β and IL-1α in DIRA. Presentation during the newborn period is typical with fever, skin pustulosis, with neutrophilic infiltrates, and a characteristic pattern of osteitis of ribs, clavicles, vertebrae, and hips. If the excess cytokine levels are untreated in DIRA patients, there is significant morbidity and mortality due to uncontrolled IL-1 effects [16].

3.3. NLRC4-related macrophage activation syndrome (MAS)

Inflammasome dysregulation from a mutation in NOD-like receptor C4 (NLRC4) causes NLRC4-MAS. Analysis shows spontaneous inflammasome operability and activation of capsace-1 causing deregulated release of IL-1β as well as production of extremely high levels of IL-18. Fever, colitis, arthralgias, and life-threatening MAS, similar to systemic juvenile idiopathic arthritis (JIA), develop in these children [4, 18]. In MAS, presentation includes coagulopathy, pancytopenia, and hyperferritinemia, with significant morbidity and mortality. Extremely high levels of IL-18 are characteristic of this disorder but not CAPS. Anti-IL-1 therapies are currently on the market, but there are no available anti-IL-18 blockers, a limitation in optimally treating this disorder.

3.4. TNF receptor-associated periodic syndrome

TNF receptor-associated periodic syndrome (TRAPS) is due to dominant mutations in the TNFRSF1A gene [14, 19]. Irregular 2–4 week cycles occur 2–6 times a year [20, 21]. Symptoms include fever, rash, periorbital swelling, arthritis, and conjunctivitis. Serositis, similar to FMF, is reported, but in contrast, renal failure due to amyloidosis is uncommon.

Pathogenesis appears to be due to varying mutational effects on the activation of NF-κB (Signal 1) and caspace which each cause increased cytokine release. NF-κB dysregulation promotes inflammation by inducing cytokine production and also by leading to inflammatory cell apoptosis [16]. Unchecked IL-1β release in TRAPS patients is due in part to effects of exaggerated production of mitochondrial reactive oxygen species (Signal 2) as well as increased caspace-1 activity. In addition, impaired mutant TNF receptor shedding occurs. Mutant 55 kDa TNF receptor 1a surface-based receptors appear to have several defects: abnormal protein-folding responses, binding TNF less effectively causing defective TNF-associated apoptosis, prolongation of immune responses to non-mutated receptor-bound TNF, and uncontrolled downstream signaling [22]. Abnormal p55 receptors shed in TRAPS are unable to serve as naturally occurring decoys for circulating TNF [20]. These mechanisms suggest benefit of anti-TNF as well as anti-IL-1 approaches [3, 20–25].

3.5. Familial Mediterranean fever

FMF is due to recessive Mediterranean fever gene (MEFV) mutations and is the most common AutoIDx. It is characterized by self-limited episodes of fever, serositis, arthritis, and renal failure due to amyloidosis. It is theorized that pyrin has a role in IL-1 activation by suppressing pro-caspase-1 activation possibly through competition for ASC. Defective pyrin does not bind normally to ASC, weakening its negative regulator function in NLRP3 inflammasome activation [16]. Mutations hence cause uncontrolled activation of capsace-1 via the IL-1 inflammasome [23, 26]. A unique pyrin-related inflammasome also leads to activation of IL-1 and seems to be important in FMF as well [1].

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Colchicine has been a standard FMF treatment since the 1970s. Its mechanism is due to inhibition of both cytoplasmic microtubules and inflammasome activity [27]. Pyrin also binds microtubules; this pyrin-like action of colchicine in FMF may explain its efficacy in part [16]. About 10% of FMF patients fail colchicine therapy. Given the high morbidity in FMF, alternative biologic therapies addressing cytokine dysregulation are used in such cases.

3.6. Interferonopathies

A group of AutoIDx related to uncontrolled type I interferon (IFN) activity has been recently described including stimulator of IFN genes (STING) associated with vasculopathy of infancy" (SAVI)" [1, 2, 28]. Early-onset livedo reticularis, ulcerative skin lesions, pulmonary symptoms, and Raynaud's disease with vasculopathy are described. SAVI is due to mutations in STING transmembrane proteins increasing IFN levels [28]. The abnormal STING induces IFN regulatory factor that translocates to nuclei and promotes transcription of IFN [2]. The overproduction of type I IFN binds to receptors [IFN associated receptor IFNAR-1 or IFNAR-2], leading to unchecked protein kinase signaling and increased IFN-induced cytokine release. Targeting this pathway may be effective for SAVI and other interferon-driven inflammatory diseases, such as SLE, a more common primarily autoimmune disorder [1].

4. Immunotherapeutic agents

Early immunologic intervention has evolved from the use of vaccines in the late 1800s to administration of intravenous immunoglobulin in the 1980s to current-day immunotherapeutics. Advances such as production of monoclonal antibodies (MABs) by creation of hybridomas and molecular cloning have paralleled our growing understanding of immunology. Innovative work identifying receptors and signaling pathways to identify new therapeutic targets for autoimmunity paralleled the discovery of the genetic AutoIDx. Widespread production and utilization of immunotherapeutics are directly attributable to these efforts [11, 29–31].

Food and Drug Administration (FDA)-approved medications are regularly tested for applicability for additional disease processes. Therapies marketed for relatively common autoimmune diseases, such as RA, are studied as potential treatment of AutoIDx "orphan diseases." The following is a review of selected immunotherapeutic medications used to treat AutoIDx based on scientific evidence and immunologic pathways (see **Table 3**; see **Figure 1**). Side effects, toxicities, and additional considerations with the use of these therapeutic agents are also addressed.

4.1. Anti-IL-1 therapy

The central mediator for multiple AutoIDx, IL-1, was one of the first identified cytokines. It was termed the "endogenous pyrogen" since fever is one of its main consequences. Inactive IL-1β is cleaved to its active form by the IL-1 inflammasome complex. Its naturally occurring receptor antagonist, IL-1Ra, was engineered into an immunotherapeutic, anakinra, and

Generic name	Brand name	Approved indications	Type	Mechanism of action
Adalimumab	Humira	RA, Ps, AS, PsA, uveitis, CrD, JIA	Human MAB	Inhibits TNF-α
Anakinra	Kineret	RA, CAPS	Recombinant protein	IL-1 receptor antagonist
Canakinumab	Ilaris	CAPS, JIA	Human MAB	Inhibits IL-1β
Certolizumab	Cimzia	RA, AS, PsA, CrD	Humanized FAB	Inhibits TNF-α
Etanercept	Enbrel	RA, JIA, Ps, PsA, AS	Fusion receptor	Soluble TNF-α receptor antagonist
Golimumab	Simponi	RA, Ps, AS, PsA, CrD, UC	Human MAB	Inhibits TNF-α
Infliximab	Remicade	RA, AS, PsA, UC, CrD	Chimeric MAB	Inhibits TNF-α
IFN-β 1a	Rebif	MS	Cytokine inhibitor	Targets type 1 IFN
IFN-β 1b	Betaseron	MS	Cytokine inhibitor	Targets type 1 IFN
Rilonacept	Arcalyst	CAPS	Fusion receptor	Targets IL-1R1/IL-R accessory receptor
Tocilizumab	Actemra	RA, JIA, sJIA	Humanized MAB	Inhibits IL-6 receptor
Tofacitinib	Xeljanz	RA	Small molecule; JAK inhibitor	Specifically blocks JAK-STAT pathway

AS, ankylosing spondylitis; CAPS, cryopyrin associated periodic syndromes; CrD, Crohn's disease; FAB, antibody fragment; IFN, interferon; JAK, Janus kinase; MAB, monoclonal antibody; MS, multiple sclerosis; JIA, juvenile idiopathic arthritis; Ps, psoriasis; PsA, psoriatic arthritis; RA, rheumatoid arthritis; sJIA, systemic JIA; SLE, systemic lupus erythematosus; TNF, tumor necrosis factor; UC, ulcerative colitis

Table 3. Selected immunotherapeutic agents for autoinflammatory diseases (adapted from Refs. [11, 17]).

approved by the FDA in 2001 for treatment of RA. This agent is a competitive inhibitor of IL-1α and IL-1β receptor binding because the drug itself adheres to the receptor but does not produce downstream signaling. While its benefit in RA has never been dramatic, anti-IL-1 therapy is key in management of CAPS as well as other AutoIDx. Three anti-IL-1 agents are FDA approved and used in CAPS. Approved in 2009, canakinumab is a MAB to IL-1β with a long half-life that enables dosing every 1–2 months. Rilonacept is a fusion protein made from IL-1R accessory protein linked to the Fc portion of an IgG1 molecule which inhibits IL-1β and downstream signaling. It also acts as a soluble decoy for IL-1α, also preventing this cytokine from binding with the receptor. These agents control FCAS and MWS and are partly beneficial in NOMID [32–37].

Dysregulation of IL-1 as a key mediator is important in other disorders including systemic JIA and adult-onset Still's disease (AOSD) [38–40]. These diseases are also thought to be part of the spectrum of AutoIDx and respond well, sometimes dramatically, to anti-IL-1 agents. Given the presence of MAS and similarity to systemic JIA, NLRC4-MAS has been treated and responds, at least in part, to anti-IL-1 therapy [1, 3, 41]. The predominance of high IL-18 levels, which are not diminished with this approach, may explain the partial response to anti-IL-1 treatment.

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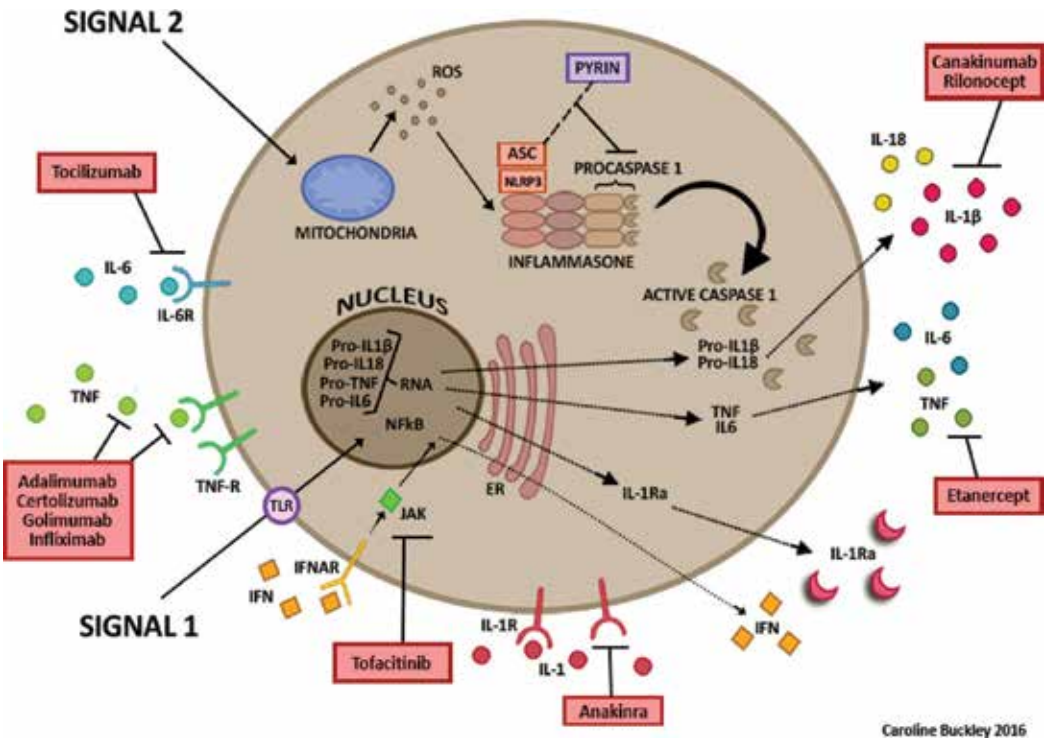

Figure 1. Targeted sites for therapeutic agents used in autoinflammatory diseases. Signal 1 inflammasome activation: surface pattern recognition receptors such as Toll-like receptors (TLR) and pathogen-associated molecular patterns stimulate production of molecules such as NF-κB and activate inflammasome assembly through downstream immunologic processes. Signal 2 inflammasome activation: molecules such as crystals in gout, heat-shock proteins, or damaged tissue (such as burns) as well as other pathogen-associated molecular patterns and damage-associated molecular patterns activate inflammasome assembly through production of reactive oxygen species (ROS) as well as downstream immunologic processes. ASC, apoptosis-associated speck protein; ER, endoplasmic reticulum; IFNAR, interferon-associated receptor; IFN, interferon; IL-1, interleukin-1; IL-1 R, IL-1 receptor; IL-1Ra, IL-1 receptor antagonist; IL-6, interleukin 6; IL-6R, IL-6 receptor; IL-18, interleukin-18; JAK, Janus kinase; NLRP3, NOD-like receptor P3; ROS, reactive oxygen species; TLR, Toll-like receptor; TNF, tumor necrosis factor; TNF-R, TNF receptor.

Unfortunately, no anti-IL-18 agents are currently marketed although efforts to develop such agents are underway. In DIRA, excessive IL-1β and IL-1α are both released. Based on pathogenesis of this disorder, and the understanding that some available agents inhibit both IL-1β and IL-1α actions, anti-IL-1 agents have been successfully used in DIRA [16].

In several other AutoIDx, pathogenesis involves activation of the IL-1 inflammasome by Signal 2 mechanisms including elevated reactive oxygen species, PAMPS and DAMPs. In TRAPS, caspace-1 is activated in vitro even while treated with anti-TNF therapy [23]. Hence, anti-IL-1 treatment may be ideal for treating these patients [42]. In FMF, defective pyrin fails to suppress inflammasome behavior, and pro-IL-1 is increasingly activated. Reports detail response to anti-IL-1 therapy in FMF [6, 14, 43]. All these observations confirm response to anti-IL-1 therapy by a spectrum of AutoIDx [3, 21, 25, 43, 44].

4.2. Anti-TNF therapy

TNF is part of a superfamily of cytokines that induce necrosis of cancer cells, leading to the term "tumor necrosis factor." TNF-α is present on the surface of cells as a transmembrane protein, and its cleavage leads to release of soluble TNF-α. Two receptors regulate this cytokine's function – TNF receptor TNFR1 55 kd and TNFR2 75 kd. The 55 kd receptor is membrane bound, and upon stimulation by TNF-α, cells release other cytokines such as IL-2 and IFN. Extracellular TNFR2 75 kd receptors deactivate soluble TNF, blunting its action. The anti-TNF agent, etanercept, an injectable biologic approved for the treatment of RA in 1998 and JIA in 1999, is an engineered fusion protein comprised of two naturally occurring soluble human 75-kd TNF receptors linked to an Fc portion of an IgG1. Etanercept mimics the natural receptor by binding extracellular TNF, limiting activation of the inflammatory response. In CrD, pathogenesis involves innate immune system activation through membrane-bound TNF. Especially in early-onset disease, NOD gene mutations and dysfunctional NF-κB activation are also pathogenic [7]. In CrD, etanercept has been found to be less effective likely due to the predominance of membrane-bound TNF in this disorder rather than soluble TNF that is inhibited by this agent [45]. Differences in receptor localization, binding, and downstream signaling explain therapeutic differences between anti-TNF agents. Infliximab is a chimeric MAB, whereas golimumab and adalimumab are humanized MABs; all three have inhibitory effects at both TNF locations. Certolizumab is an antibody fragment (FAB) that attaches to membrane-bound as well as membrane-soluble TNF. All four are more effective in CrD than is etanercept.

In TRAPS, as etanercept is a soluble p75 kd receptor, it binds extracellular, soluble TNF unbound by dysfunctional p55 mutant receptors. Some TNFR1 mutations effect cysteine residues increasing risk of amyloidosis. Successful use of etanercept minimizes this complication [20, 46]. Etanercept may be beneficial in TRAPS patients but has a variable effect; other biologic agents, especially IL-1 blockers, may be more beneficial [47]. Anti-TNF MABs such as infliximab may cause paradoxical increased inflammation due to varying effects on both TNF receptors. Infliximab is therefore not used in etanercept failures [23, 48, 49].

Anti-TNF therapy has a role in FMF in colchicine failures [27, 50]. Etanercept and MAB anti-TNF therapies have been reported to be of some benefit in these patients. Good clinical and biological responses suggest these agents are options for more resistant FMF cases to prevent amyloidosis.

4.3. Anti-IL-6 therapy

By binding to its cell surface receptor and subsequent activation of DAMPS, TLRs, and intracellular protein kinase signaling, IL-6 has been shown to be an important cytokine in the inflammatory cascade. Complex interactions exist between IL-1 and Il-6 regulatory pathways. IL-6 also stimulates the adaptive immune system through B cell immunoglobulin production, elevated inflammatory markers (C-reactive protein), and promotion of Th17 cell maturation. Tocilizumab is a humanized MAB that blocks IL-6 signaling by adhering to membrane-bound IL-6 and its soluble receptors. This agent blocks downstream activation of adhesion molecules, osteoclasts, and maturation of both T and B cells [51]. As IL-6 is a key cytokine in RA, AOSD and systemic JIA (now thought to be part of the AutoIDx family), and inhibition of its function by tocilizumab has clinical benefit, this cytokine antagonist was approved by the FDA [52].

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Tocilizumab is not as effective in CAPS as anti-IL-1 inhibitors, with lack of response in NOMID possibly due to the extremely high IL-1 levels in this disease that requires more direct blockade of IL-1 that cannot be achieved through anti-IL-6 approaches. In TRAPS and FMF, several case reports detail clinical response to tocilizumab following inadequate response to etanercept; however, cytokine levels did not change appreciably [27, 49, 53, 54]. The benefit of anti-IL-6 therapy in other AutoIDx remains to be determined.

4.4. Interferons

Induced IFNs bind to IFN-α receptors on plasmadendritic cells. These cells upregulate many IFN-induced genes and are termed the "IFN signature" [55]. These gene products have inhibiting and/or activating effects on downstream immunologic activity. As a therapeutic agent, IFN-α has been used in hepatitis C and relapsing and remitting multiple sclerosis, an autoimmune neurologic disease. IFN-α therapy in colchicine-resistant FMF was reportedly beneficial but not confirmed in subsequent studies [27]. The IFN signature correlates with disease activity in autoimmune diseases and identifies response subsets. The IFN signature may also be important in the pathogenesis of subsets of AutoIDx, as well [56]. Treatments that inhibit IFN signaling pathways continue to be sought for the more common autoimmune diseases [11, 57, 58] which should then result in additional agents for study in AutoIDx.

4.5. Cytokine signaling

Strategies that employ cytokine receptor blockers or that provide decoys for soluble cytokines have been reviewed above. Alternatively, reducing effects of cytokines can be achieved through interference with post-receptor intracellular downstream signaling pathways. As suggested above, targeting IFN pathways may be a successful approach for some AutoIDx. Recently, AutoIDx related to IFN dysregulation, the interferonopathies, have been identified. Normally, IFN binding to receptors exerts downstream effects through intracellular pathways via protein kinases. JAK-STAT signaling transmits extracellular information to nuclei influencing DNA transcription and increasing IFN and cytokine production. Tofacitinib is the first JAK inhibitor recently approved for treatment of RA. The AutoIDx SAVI is due to a mutation affecting STING membrane receptors leading to excessive IFN production. Overstimulation of downstream immunologic activity due to abnormally high levels of IFN-IFNAR binding can lead to increased signaling that can be blocked by tofacitinib JAK-inhibition. In this way, overabundant downstream IFN effects are limited. Studies show 60% improvement in inflammation in vitro. Clinical trials in patients with this rare disorder are underway [2, 28].

5. Considerations in the use of immunotherapeutics

Some patients with AutoIDx have severe illnesses, and treatment is necessary to alleviate morbidity and prevent mortality. However, concerns regarding potential toxicity of immunotherapeutics must be addressed. Risks are present with all agents: infection, induction of carcinogenesis, autoantibody formation, and development of demyelination. Additionally, infusion or injection site reactions, as well as generally transient side effects, such as increased

liver function tests, decreased blood cell counts, and abnormalities in lipid profiles, have been attributed to these agents. Dose adjustments, premedication administration, and addressing concomitant risk factors often minimize side effects. Depending on concerns due to the patient's underlying disease, toxicity fears may or may not take precedence in medication selection.

5.1. Infections

Immunotherapeutic agents all produce some degree of immunosuppression in addition to their disease controlling effects. Development of opportunistic infections with viruses, fungi, atypical bacteria, and prion associated complications (such as progressive multifocal leukoencephalopathy) are potential concerns. FDA warnings advise against prescribing these drugs for patients with active infections including hepatitis C or B [51, 59]. Continued vigilance by patients and assessment by health care providers for any possible infectious complication is necessary when using immunotherapeutic agents.

Given concerns about risk of reactivation of tuberculosis (MTB), all patients are screened for MTB prior to starting biologic therapies [60, 61]. MTB confinement in granulomas requires normal CD8+ T cells and TNF activity; infliximab and biologics that effect receptor-bound TNF inhibit this function [62]. Little data exists regarding MTB risk for agents other than anti-TNF drugs [61]; however, screening for MTB is requested for most patients prior to starting biologic therapy.

5.2. Neoplastic disease

Patients with RA, psoriatic arthritis, and CrD are at risk of developing disease-related malignancies over their lives. The concern has been raised that biologic agents increase this risk. Large epidemiologic studies have not substantiated this concern for the most part. The rate of lymphoma in RA patients treated with biologic agents is similar to those who have never been treated. Those with prior cancer diagnoses are not at increased risk of recurrence [63, 64]. In 2008, a report from the FDA raised concerns about biologic agents and neoplastic disease in pediatric CrD and JIA patients. The pediatric rheumatology community worldwide has questioned this interpretation of JIA data [65]. Continued surveillance and monitoring of malignancies is crucial post-marketing for all biologic agents as the medical community, and patients remain vigilant regarding this important issue.

5.3. Demyelination

Studies using early anti-TNF-α agents in multiple sclerosis patients detected worsening disease, and trials were halted. The development of demyelinating toxicity in RA patients also has been reported. The mechanism of this side effect may be related to blocking effects on TNF receptor2 75 kd which is required for oligodendrocyte growth. Current anti-TNF therapies block TNFR2 75 kd as well as TNFR1 55 kd; the latter of which is linked more tightly to other autoimmune diseases as well as to TRAPS. Given that some anti-TNF blockers inhibit both receptors, improved control of inflammation may be met with worsening demyelination – an unacceptable trade-off. Future agents that selectively block TNFR1 55 kd might alleviate this

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concern [11]. Current approaches include close monitoring all patients treated with anti-TNF agents for any concerning neurologic signs.

5.4. Human anti-chimeric antibody and autoantibody formation

In theory, more human-like MABs might be less immunogenic and less susceptible to human anti-chimeric antibody (HACA) formation [51, 66]. HACAs are thought to be responsible for side effects, interference with laboratory testing, as well as potential decrease in efficacy of these therapies. Decreased response to treatment due to HACAs has been of specific concern following the use of certain agents, such as infliximab in CrD and RA [67, 68]. Patients who developed anti-infliximab HACAs are also more likely to have infusion reactions and possibly reduced therapeutic benefit. Awareness of the risk of HACA formation and its consequences is crucial information for prescribing clinicians [69, 70].

Autoantibody formation in patients receiving immunotherapeutic agents is well described [70]. Development of antinuclear antibodies (ANAs) is reported in patients in biologic trials. HACAs may produce false-positive autoantibody results due to interference in laboratory tests [70]. Autoimmune syndromes have been triggered by the use of immunotherapeutic in patients with RA and, in theory, also may develop in similarly treated patients with AutoIDx [51, 71]. One must be aware of the potential for these agents to cause false-positive autoantibodies or to trigger onset of autoimmunity. These concerns must be recognized by patients and be part of surveillance by practitioners caring for individuals on biologics.

5.5. Selection of immunotherapeutics

Immunotherapeutic agents currently available are often administered parenterally. Intravenous and self-injected formulations of these agents are typically prescribed for patients. Infusion medications tend to be even more expensive due to nursing and infusion center costs in addition to the price of the drug itself, making treatment cost prohibitive for some patients. Studies also show that patient preferences regarding medication choices (oral versus injection versus infusion therapy) often do not coincide with providers' intuition about patient choices. Access to medical care, attentive nursing, insurance coverage and cost are key factors for patients' choice of therapy [72]. "Biosimilar" generic-type biologic agents are soon coming to the US market with estimated savings of 20–35% off name-brand charges. These agents will be economically advantageous to insurers and patients, assuming these "similar" medications are truly as effective as name-brand biologic agents [73]. These less expensive immunotherapeutics could potentially be accessed by more patients, improving disease control for a greater number of afflicted individuals.

6. Future directions/outlook

Safe and effective treatment options are the goals of biologic drug development and utilization. In the future, scientific advances in precision medicine and next-generation sequencing will enable precise identification of disease phenotypes/genotypes to predict response to and

toxicity from biologic agents for autoimmune diseases [74, 75]. This advance will increase our ability to tailor immunotherapeutic selection for those with AutoIDx to target recognized consequences of genetic mutations. As knowledge about autoinflammation continues to expand, new and more directed therapies will be trialed and utilized to treat these rare disorders. In this era of the "triple aim" in patient care, attention to patient experience, improving population health, and minimizing complications of disease and treatment, including cost, one can predict that the outlook for this population with rare genetic inflammatory diseases will be much brighter with greater opportunities for them to be treated with disease-specific and targeted therapies. Increasingly, these therapies have changed the lives of patients with AutoIDx in recent years and will be able to make even more of a difference in the future.

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References

[1] Stoffels M, Kastner DL: Old dogs, new tricks: monogenic autoinflammatory disease unleashed. Annu Rev Genom Hum Genet. 2016; 17:18.1–28

[2] Volpi S, Picco P, Caorsi R, et al: Type 1 interferonopathies in pediatric rheumatology. Pediatric Rheumatol. 2016; 14:35–47

[3] Hoffman HM, Broderick L: The role of the inflammasome in patients with autoinflammatory diseases. J Allergy Clin Immunol. 2016; 138:3–14

[4] Canna SW, de Jesus AA, Gouni S, et al: An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent MAS. Nat Genet. 2014; 46:1140–6

[5] Martinon F, Burns K, Tschopp J: The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of pro-IL-beta. Mol Cell. 2002; 10:417–26

[6] Moll M, Kuemmerle-Deschner JB: Inflammasome and cytokine blocking strategies in AutoIDx. Clin Immunol. 2013; 147:242–75

[7] Caso F, Galozzi P, Costa L, et al: Autoinflammatory granulomatous diseases: from Blau syndrome and early-onset sarcoidosis to NOD2-mediated disease and Crohn's disease. RMD Open. 2015; 1:e000097

[8] Mayer G: Immunology – Chapter One. Innate (Non-Specific) Immunity. www.microbiologybook.org/ghaffar/innate.htm Accessed July 19, 2015

[9] Albani S, Wedderburg LR, Prakken B: Adaptive immunity and autoimmunity: translation from bench to bedside. In: Petty R et al. Textbook of pediatric rheumatology. 7th Ed. Philadelphia: Elsevier; 2016. 33–42

[10] Gregersen PK, Behrens TW: Genetics of autoimmune diseases — disorders of immune homeostasis. Nat Rev Genet. 2006; 7:917–28

[11] O'Shea JJ, Kanno Y, Chan AC: In search of magic bullets: the golden age of immunotherapeutics. Cell. 2014; 157:227–40

[12] Marks DJ, Segal AW: Innate immunity in IBD. J Pathol. 2008; 214:260–6

[13] French FMFC: A candidate gene for FMF. Nat Genet. 1997; 17:25–31

[14] Ozen S, Bilginer Y: A clinical guide to autoinflammatory diseases: familial Mediterranean fever and next-of-kin. Nat Rev Rheumatol. 2014; 10:135–47

[15] Petty RE, Laxer RM, Lindsley CB, Wedderburn LR: Textbook of Pediatric Rheumatology 7th Ed. Periodic Fever Syndromes and other inherited AutoIDx. 2016. Chapter 47: 609–26

[16] De Jesus AA, Canna SW, Liu Y, Goldbach-Mansky R: Molecular mechanisms in genetically defined AutoIDx: disorders of amplified danger signaling. Annu Rev Immunol. 2015; 33:823–74

[17] Ostrov BE: Immunotherapeutic biologic agents in the treatment of autoimmune and autoinflammatory diseases. Immunol Invest. 2015; 44:777–802

[18] Cavalli G, Dinarello CA: Treating rheumatological diseases and co-morbidities with IL-1 blocking therapies. Rheumatology. 2015; 54:2134–44

[19] Hull KM, Drewe E, Aksentijevich I, et al: The TNF receptor-associated periodic syndrome (TRAPS): emerging concepts of an autoinflammatory disorder. Medicine (Baltimore). 2002; 81:349–68

[20] Lachmann HJ, Papa R, Gerhold K, et al: The phenotype of TNF receptor-associated autoinflammatory syndrome (TRAPS) at presentation: a series of 158 cases from the Eurofever/EUROTRAPS international registry. Ann Rheum Dis. 2014; 73:2160–7

[21] Williamson LM, Hull D, Mehta R, et al: Familial Hibernian fever. Q J Med. 1982; 51:469–80

[22] Simon A, Park H, Maddipati R, et al: Concerted action of wild-type and mutant TNF receptors enhances inflammation in TRAPS. Proc Natl Acad Sci. 2010; 107:9801–6

[23] Campbell L, Raheem I, Malemud CJ, Askar AD: The relationship between NALP3 and autoinflammatory syndromes. Int J Mol Sci. 2016; 17: 725–44

[24] Aksentijevich I, Galon J, Soares M, et al: The TRAPS: new mutations in TNFRSF1A, ancestral origins, genotype-phenotype studies and evidence for further genetic heterogeneity of periodic fevers. Am J Hum Genet. 2001; 69:301–14

[25] Mulley J, Saar K, Hewitt G, et al: Gene localization for an autosomal dominant familial periodic fever to 12p13. Am J Hum Genet. 1998; 62:884–9

[26] Chae JJ, Cho YH, Lee GS, et al: Gain of function mutations in pyrin mutations induce NLRP3 protein-independent IL-1β activation and severe autoinflammation in mice. Immunity. 2011; 34:755–68

[27] Portincasa P: Colchicine, biologic agents and more for the treatment of Familial Mediterranean Fever. The old, the new and the rare. Curr Med Chem. 2016; 23:60–86

[28] Liu Y, Jesus AA, Marrero B, et al: Activated STING in a vascular and pulmonary syndrome. N Engl J Med. 2014; 371:507–18

[29] Beck A, Wurch T, Bailly C, Corvaia N: Strategies and challenges for the next generation of therapeutic antibodies. Nat Rev Immunol. 2010; 10:345–52

[30] Cambrosio A, Keating P: Between fact and technique: the beginnings of hybridoma technology. J Hist Biol. 1992; 25: 175–230

[31] Chapman K, Pullen N, Coney L, et al: Preclinical development of monoclonal antibodies. MAbs J. 2009; 1:505–16

[32] Kuemmerle-Deschner JB, Haug I: Canakinumab in patients with cryopyrin-associated periodic syndrome: an update for clinicians. Ther Adv Musculoskelet Dis. 2013; 5:315–29

[33] Goldbach-Mansky R, Dailey NJ, Canna SW, et al: Neonatal-onset multisystem inflammatory disease responsive to interleukin-1beta inhibition. N Engl J Med. 2006; 355:581–92

[34] Goldbach-Mansky R, Shroff SD, Wilson M, et al: A pilot study to evaluate the safety and efficacy of the long-acting interleukin-1 inhibitor rilonacept (interleukin-1 Trap) in patients with familial cold autoinflammatory syndrome. Arthritis Rheum. 2008; 58:2432–42

[35] Gattorno M, Martini A: Inflammation and its mediators. In: Petty R et al. Textbook of pediatric rheumatology. 7th Ed. Philadelphia: Elsevier; 2016. 14–32

[36] Sibley CH, Chioato A, Felix S, et al: A 24-month open-label study of canakinumab in neonatal-onset multisystem inflammatory disease. Ann Rheum Dis. 2015; 74:1714–9

[37] Broderick L, Tourangeau LM, Kavanaugh A, et al: Biologic modulators in allergic and AutoIDx. Curr Opin Allergy Clin Immunol. 2011; 11:355–60

[38] Dewitt EM, Kimura Y, Beukelman T: Consensus treatment plans for new-onset systemic juvenile idiopathic arthritis. Arthritis Care Res. 2013; 64:1001–10

[39] Pouchot J, Arlet JB: Biological treatment in adult-onset Still's disease. Best Pract Res Clin Rheumatol. 2012; 26:477–87

[40] Vastert SJ, de Jager W, Noordman BJ, et al: Effectiveness of first line therapy with recombinant IL-RA in steroid-naïve patients with new onset systemic JIA: results of a prospective cohort study. Arthritis Rheum. 2014; 66:1034–43

[41] Shimizu M, Yokoyama T, Yamada K, et al: Distinct cytokine profiles of systemic JIA-associated MAS with particular emphasis on the role of IL-18 in its pathogenesis. Rheumatology. 2010; 49:1645–53

[42] Gattorno M, Martini A: Treatment of autoinflammatory syndromes. Curr Opin Pediatr. 2010; 22:771–8

[43] Eroglu FK, Besbas N, Topaloglu R, Ozen S: Treatment of colchicine-resistant Familial Mediterranean fever in children and adolescents. Rheumatol Int. 2015; 35:1733–7

[44] Ter Haar NM, Jeyaratnam J, Lachmann HL, et al: The phenotype and genotype of mevalonate kinase deficiency: series of 114 cases from the Eurofever Registry. Arthritis Rheum. 2016. doi:10.1002/art.39763

[45] Derer S, Till A, Haesler R, et al: mTNF reverse signalling induced by TNF α antagonists involves a GDF-1 dependent pathway: implications for Crohn's disease. Gut. 2013; 62:376–86

[46] Bulua AC, Mogul DB, Aksentijevich I, et al: Efficacy of etanercept in the tumor necrosis factor receptorassociated periodic syndrome: a prospective, open-label, dose-escalation study. Arthritis Rheum. 2012; 64:908–13

[47] Magnotti F, Vitale E, Rigante D, et al: The most recent advances in pathophysiology and management of TRAPS: personal experience and literature review. Clin Exp Rheumatol. 2013; 31(Suppl. 77):141–9

[48] Nedjai B, Hitman GA, Quillinan N, et al: Proinflammatory action of the anti-inflammatory drug infliximab in tumor necrosis factor receptor-associated periodic syndrome. Arthritis Rheum. 2009; 60:619–25

[49] Hosoya T, Mizoguchi F, Hasegawa H, et al: A case presenting with the clinical characteristics of tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS) without TNFRSF1A mutations successfully treated with tocilizumab. Intern Med. 2015; 54:2069–72

[50] Akgul O, Kilic E, Kilic G, Ozgocmen S: Efficacy and safety of biologic treatments in Familial Mediterranean Fever. Am J Med Sci. 2013; 346:137–41

[51] Rosman Z, Shoenfeld Y, Zandman-Goddard G: Biologic therapy for autoimmune diseases: an update. BMC Med. 2013; 11:88–100

[52] Tanaka T, Kishimoto T: Immunotherapeutic implication of IL-6 blockade. Immunotherapy. 2012; 4:87–105

[53] Koga T, Migita K, Kawakami A: Biologic therapy in FMF. Mod Rheumatol. 2016; 21: 1–5

[54] Vaitla PM, Radford PM, Tighe PJ, et al: Role of IL-6 in patients with TRAPS: assessment of outcomes following treatment with the anti-IL-6 receptor MAB tocilizumab. Arthritis Rheum. 2011; 63:1151–5

[55] Ronnblom L, Eloranta ML: The interferon signature in autoimmune diseases. Curr Opin Rheumatol. 2013; 25:248–53

[56] De Jesus AA, Deng Z, Brooks S, Kim H: Stratification of patients with autoinflammatory phenotypes by interferon (IFN) score suggests a new group of IFN mediated AutoIDx with overlapping clinical phenotypes. Pediatr Rheumatol Online J. 2015;13(Suppl. 1):O35

[57] Boyman O, Sprent J: The role of IL-2 during homeostasis and activation of the immune system. Nat Rev Immunol. 2012; 12:180–90

[58] Klatzmann D, Abbas AK: The promise of low dose IL-2 therapy for autoimmune and inflammatory diseases. Nat Rev Immunol. 2015; 15:283–94

[59] Singh JA, Cameron C, Noorbaloochi S, et al: Risk of serious infection in biological treatment of patients with rheumatoid arthritis: a systematic review and meta-analysis. Lancet. 2015; 386:258–65

[60] Rubbert-Roth A: Assessing the safety of biologic agents in patients with rheumatoid arthritis. Rheumatology. 2012; 51:v38–47

[61] Lopalco G, Rigante D, Gianni M, et al: Safety profile of anakinra in the management of rheumatologic, metabolic and autoinflammatory disorders. Clin Exp Rheumatol. 2016; 34:531–8

[62] Miller EA, Ernst JD: Anti-TNF immunotherapy and tuberculosis reactivation: another mechanism revealed. J Clin Invest. 2009; 119:1079–82

[63] De la Forest DM, Gottenberg JE, Salliot C: Safety of biologic DMARDs in RA patients in real life: a systematic literature review and meta-analyses of biologic registers. Joint Bone Spine. 2016; S1297-319X(16)30050-1

[64] Cush JJ, Kay J, Dao KH: Does rheumatoid arthritis or biologic therapy increase cancer risk?. ACR Drug Watch Q. 2012; 4:1–2

[65] Ruperto N, Martini A: JIA and malignancy. Rheumatology (Oxford). 2014; 53:968–74.

[66] Kricka LI: HACA interferences in immunological assays. Clin Chem. 1999; 45:942–56

[67] Afif W, Loftus EV, Faubion WA: Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with IBD. Am J Gastroenterol. 2010; 105:1133–9

[68] Rozin AP: Matters arising: infliximab efficiency and failure. Ann Rheum Dis. 2004; 63:751–2

[69] Alshekaili J, Li C, Cook MC: Heterophile interference accounts for method-specific dsDNA antibodies in patients receiving anti-TNF treatment. Rheumatology. 2010; 49:891–7

[70] Ostrov BE, Amsterdam D: The interference of monoclonal antibodies with laboratory diagnosis: clinical and diagnostic implications. Immunol Invest. 2013; 42:673–90

[71] Basaran O, Aydin F, Celikel BA, Uncu N, Cakar N: Coexistence of SLE and FMF in a pediatric patient. Lupus. 2016; 8:1–2

[72] Ostrov BE, Reynolds K, Scalzi LV: Patient preferences and satisfaction in a multispecialty infusion center. Patient Prefer Adherence. 2014; 8:755–61

http://dx.doi.org/10.5772/66547

[73] Mulcahy AW, Predmore Z, Mattk S: The Cost Savings Potential of Biosimilar Drugs in the US. Available at: www.rand.org/content/dam/…/RAND_PE127.pdf Accessed July 25, 2015

[74] Dennis G, Holweg CTJ, Kummerfeld SK, et al: Synovial phenotypes in RA correlate with response to biologic therapeutics. Arthritis Res Ther. 2014; 16:R90

[75] Chan AC, Behrens TW: Personalizing medicine for autoimmune and inflammatory diseases. Nat Immunol· 2013; 14:106–9

Chapter 13

Immunotherapy for Fungal Infections

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Abstract

Opportunistic fungal infections are a major health problem being appointed by some studies as the fourth main cause of hospital-acquired infection in susceptible populations. The constantly growing incidences of these diseases are associated with the growing number of susceptible individuals, such as immunocompromised individuals (leukemia, AIDS, etc) and treatment-induced immunodeficiency (hematopoietic stem cell, solid organ transplant, anticancer therapy). Furthermore, other advances in medical care, patient's long-term hospitalization and antimicrobial therapies have created several vulnerable populations to fungal infections. Currently, antifungal drug therapies are several times inefficient, and the poor outcomes are linked to difficulties in the early diagnosis of fungal infections and drug resistance among fungal pathogens. In this context, novel therapeutic approaches are welcome to stimulate efficiently the host immune response to eliminate the fungal pathogen. This chapter is intended to review advances in immunotherapy strategies for fungal infections.

Keywords: immunotherapy, vaccination, immune enhancement, fungal infections, antifungal

1. Introduction

Fungi are eukaryotic organisms ubiquitous in the environment, are considered the major decomposers in certain ecosystems, are essential to the survival of many organisms which they can be associated and are sources of food, several enzymes and drugs. There is an estimate of the existence of more than 5 million of fungal species, of that around 70,000 were described [1]. However, only a few hundred of them can also cause disease in healthy and immunocompromised humans [2, 3]. It was accepted that fungi and other pathogenic microorganisms can cause disease all by themselves through four basic conditions, also known as

"virulence factors": (i) tolerance to the host body temperature; (ii) ability to colonize and/or invade the host; (iii) production of secreted components such as toxin and proteolytic enzymes associated with the processes of lysis and absorption of host tissue and (iv) evasion and/or resistance to the host immune system [2, 4].

Based on this germ theory, antimicrobial therapies options targeted these virulence factors and have focused on the development of pharmacological products designed to kill the pathogenic microorganisms and immunological interventions that overcame the deleterious effects of their virulence factors [5]. Interestingly, the Damage-Response Framework theory, which was first proposed in 1999, emphasizes that disease state is not unidirectional and that both the pathogenic microorganism and the host contribute to pathogenicity and virulence [6]. Additionally, recent studies on the human mycobiome, that is, the collection of fungi distributed across and within the body, show that despite being as low as ≤0.1% of the total microbiota, these microorganisms can participate and modify several physiological functions of the host, including the maintenance of microbiota community structure, metabolic functions and in the development and function of the immune system [7].

Fungal infections contribute substantially to human morbidity and mortality, and despite the availability of several antifungal drugs, high rates of mortality associated with invasive fungal infections often exceed 50% [8]. In this context, in order to achieve a reduction in the global burden of fungal infections, is urgent the development of new safer and more effective antifungal drugs [9], as well as novel immunotherapeutic strategies that allow the restoration of the host immune system [10] and maintain or improve the favorable interactions between microbiota and host [11]. In this context, immunotherapy represents a therapeutic modality that attempts to augment host immune response and to control the established infection. In this chapter, we review the principles of antifungal immunotherapy.

2. Immunological aspects of fungal infections

The host defense mechanisms against fungi range from the protective mechanisms provided by skin, mucosa and innate immunity to sophisticated adaptive mechanisms (adaptive immunity), which are specifically induced during the fungal infection/disease. The activation of the innate immunity is the first line of host antifungal defenses and is mediated by phagocytic cells (polymorphonuclear and mononuclear leukocytes and dendritic cells (DCs)), cellular receptors and several humoral factors that act by (i) direct destruction of the fungi through phagocytic process or secretion of microbicide compounds and/or (ii) initiation and subsequent direction of adaptive immune responses through the production of pro-inflammatory mediators (chemokines and cytokines), induction of co-stimulatory activity by phagocytic cells and uptake, processing and presentation of antigens [12, 13].

The components of the fungal cell wall are the first structures that interact with the host innate immune system, and the response to a fungal invasion is initiated through recognition of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) present during infection by pattern recognition receptors (PRRs). The PRRs are

present in the immune cells of the myeloid lineage (dendritic cells, macrophages, monocytes and neutrophils) and nonmyeloid (epithelial and endothelial cells) [12]. The binding of PRR with fungal PAMPs, such as polysaccharides (chitin, α and β-glucans, mannans) and fungal DNA, results in the activation of intracellular signaling pathways promoting phagocytosis, cytokine production, respiratory burst and cell maturation [14].

Several families of PRRs are related to the recognition of cell wall components of fungi through the use of distinct ligand recognition domains, including Toll-like receptors (TLR), C-type lectin receptors (CLR) and proteins of galectins family [12, 15]. The PRRs present in different phagocytic cells initiate intracellular events that promote the activation of the immune system and clearance of fungi, with the specific immune response generated depending on the cell type involved (monocytes, macrophages and neutrophils) [16]. Induction of innate immunity by means of PRR activation provides the basis for developing a subsequent adaptive immune response, and dendritic cells form the interface between the innate and adaptive immune system, since these cell lineages are able to acquire antigens in peripheral tissues, mature and migrate to lymphoid organs, where they provide appropriate signals to T lymphocytes [17].

The adaptive immunity is generated by clonal selection of lymphocytes in response to specific microbial antigens, which can result in the development of immunological memory. The production of cytokines that occurs after the interaction of PRRs with fungal PAMPs drives in the differentiation of naïve T lymphocyte in different subtypes, such as T helper (Th) 1, Th2, Th17 and T regulatory (Treg). During the response process and after the immune activation, T cell subtypes express different pro- and anti-inflammatory chemokines and cytokines, which mediate different effector functions [18].

It is important to emphasize that a fine balance between pro- and anti-inflammatory signals is a prerequisite for successful control of infection. If on one hand, the early inflammation prevents or limits the fungal infection, on the other hand, the uncontrolled inflammatory response may eventually act in opposition to eradicate the disease [19]. Thus, a successful immune response against a fungal infection requires (i) resistance mechanisms, that is, ability to reduce pathogen burden through innate (e.g., dendritic cells) and adaptive (e.g., Th1, Th2, Th9, Th17 and Th22 cells) immunity and (ii) tolerance mechanisms, that is, ability to protect and/or limit the host from immune- or pathogen-induced damage (e.g., Treg cells and enzymes involved in tryptophan metabolism) [18, 20].

The consensus is that Th1 cellular responses are the main defense mechanism of the host against pathogenic fungi, whereas Th2 responses are associated with susceptibility to infections or allergic responses [21]. Th1 cells are predominantly related to protective immunity against fungi and effective antifungal vaccines [12]. Interferon-γ (IFN-γ) is the main cytokine produced by Th1 cells and is important for the stimulation of the antifungal activity of neutrophils [21]. Interleukin (IL)-4, IL-5 and IL-13 are cytokines produced during Th2 immune responses, promote an alternative route of macrophage activation, favor fungal infections and allergic responses associated with the fungus and may be related to disease recurrence [12].

Th17 responses are characterized by the production of IL-17, working in the host defense against certain extracellular bacteria and fungi [22], and are related to protective vaccine responses against experimental fungal infections [23]. However, Zelante et al. [24] described

that IL-23 and the Th17 pathway can also promote inflammation and impair antifungal resistance. Treg cells by IL-10 production have the function of moderating the inflammatory response during infection to limit damage to the host cells and restore homeostasis [25]. However, this response may limit the effectiveness of the protective immune response as a result of the reduction of pro-inflammatory activity generating persistence of the fungus in the tissue and can lead to immunosuppression [21].

3. Cell-based therapies

3.1. Granulocyte transfusion

Polymorphonuclear leukocytes are specialized cells found in the bloodstream that can directly attack microorganisms through phagocytosis, release of soluble antimicrobials and generation of neutrophil extracellular traps, playing an essential role in host defense against pathogenic bacteria and opportunistic fungal pathogens [26, 27]. Granulocyte transfusion is reserved for patients with prolonged neutropenia and life-threatening infections that are resistant to conventional treatment and is intended to improve the side effects of neutropenia and enhance repopulation of granulocytes [28–31].

Early studies of Strumia [32], Brecher et al. [33] and Freireich et al. [34] are among the pioneering research work to propose the infusion of neutrophilic granulocytes from donors as an option to enhance host defenses in patients with neutropenia. Pedersen et al. [35] reported that the combination of granulocyte transfusion with trimethoprim-sulfamethoxazole resulted in the successful treatment of a refractory *Pneumocystis carinii* pneumonia in an 11-year-old girl with chronic granulomatous disease. In a retrospective study, Bhatia et al. [36] evaluated the efficacy of granulocyte transfusion in 87 bone marrow transplant recipients during the first 100 days following the transplantation. No clinical benefit of granulocyte transfusion among 50 of these patients could be shown in the resolution of candidiasis or noncandidal infections. In a meta-analysis of 32 studies, Strauss [37] described that from 63 patients with invasive fungal infections and receiving granulocyte transfusion, only 18 (29%) had successful outcomes.

Based on these and other contradictory results, for a period of time, it has been suggested that there is no strong evidence that granulocyte transfusion consistently brings benefits for the treatment of invasive fungal infections. However, the advances in the cytapheresis technology in the last decades optimize the allogeneic or autologous collection of several types of blood leucocytes to be used in transfusion therapies, which renewed the interest in granulocyte infusions [38–40].

Price et al. [41], in a phase I/II trial of neutrophil transfusions from donors stimulated with granulocyte colony-stimulating factor (G-CSF) and dexamethasone, demonstrated that four of the seven patients with candidemia cleared the infection and, in contrast, none of the patients with aspergillosis (n = 5) or fusariosis (n = 3) were able to clear the infection. Mousset et al. [42] in a prospective, nonrandomized study demonstrated a good clinical efficacy of granulocyte transfusions to prevent recurrence of severe fungal infections during hematopoietic stem cell

transplantation or intensive chemotherapy (23 episodes). However, in a randomized phase III study with 74 neutropenic patients, Seidel et al. [29] concluded that there was no effect on survival of 55 patients with invasive fungal infection up to day 100.

Several case series and case reports [31, 43–46] provide evidence for the safety and feasibility of granulocyte transfusions in patients with severe neutropenia and uncontrolled fungal infections. Thus, although the role of therapeutic granulocyte transfusions remains controversial, future randomized controlled studies will clarify the use of the granulocyte transfusion as a life-saving treatment option [47].

3.2. Dendritic cell therapy

Dendritic cells (DCs) are the bridge between the innate and adaptive immune system by sensing the fungal pathogen via their PRRs, phagocytizing fungal particles, processing and secreting cytokines and chemokines into the environment and presenting antigens to Th cells to induce an adaptive immune response [48]. This remarkable functional plasticity of DCs has been explored for the development of fungal vaccines [49], whereas DCs transfected with yeast cells, yeast RNA or conidia (but not hyphae or hyphal RNA) induce a protective Th1 response [50].

Induction of an adoptive immunity to *Aspergillus* using DCs pulsed with live conidia or transfected with conidial RNA [51] or primed with CpG oligodeoxynucleotides and pulsed with Aspf16 antigens [52] triggers specific and protective Th1 response in murine models of hematopoietic stem cell transplantation (HSCT). DCs transduced with an adenovirus vector encoding the cDNA of IL-12 and pulsed with heat-inactivated *Aspergillus fumigatus* induce a protective response (lower fungal burdens and higher survival rate) against a model of invasive pulmonary aspergillosis [53]. Stimulation of Asp f16-specific T cell responses are more effective by using a protocol of antigen presented on DC followed by Epstein-Barr virus (EBV)-transformed B lymphoblastoid cell lines (BLCL) as antigen-presenting cells [54].

Protection against disseminated candidiasis in mice was observed with DCs pulsed with cell wall proteins expressed during infection, particularly those derived from fructose-bisphosphate aldolase, which induced a robust antibody-dependent protective responses against *Candida albicans* [55]. Bone marrow-derived dendritic cells pulsed with an acapsular *Cryptococcus gattii* strongly induced cytokine-producing CD4 T cells and multinucleated giant cells, and these were associated with a protection against a *Cryptococcus gattii* model of pulmonary cryptococcosis [56].

3.3. Adoptive T-cell transfer

Adoptive T cell transfer is an immunotherapy strategy used to treat cancer and chronic infections by intravenous injection of autologous T cells, which was, after isolated from the donor, stimulated *in vitro* with antigens or modified with a gene encoding a specific antigen receptor and expanded to a large quantity before infusion back into the patient [57]. Th1 lymphocyte responses confer significant protection against fungal infections [18], and the pivotal role of

$CD4^+$ T cell has led to increasing interest and investigation of the use of adoptive transfer of $CD4^+$ cells in the prophylaxis and treatment of invasive fungal infections [58].

Perruccio et al. [59] used heat-inactivated conidia of *A. fumigatus* to generate specific T-cell clones *in vitro*, which was infused soon after hematopoietic transplantation in 35 patients. High-frequency T-cell responses to pathogen within three weeks infusion were associated with control of *Aspergillus* antigenemia and infectious mortality. In contrast, spontaneous pathogen-specific T cells in 46 transplant recipient patients who did not receive adoptive therapy occurred in low frequency as late as 9–12 months after transplantation and displayed a nonprotective, type-2 cytokine profile. However, commonly used immunosuppressants such as cyclosporine A, mycophenolic acid and methylprednisolone decreased the number of anti-*Aspergillus* Th1 cells and the expression of CD154 by anti-*Aspergillus* Th1 cells, which may limit using this type of immunotherapy for organ transplant recipients [60].

Prolonged survival rates were observed in a model of mice invasive pulmonary aspergillosis after the adoptive transfer of splenic $CD4^+$ T cells from mice previously sensitized with a crude culture filtrate antigens of *A. fumigatus* [61]. In BALB/c mice, the cell glucanase Crf1 from *A. fumigatus* induces memory $CD4^+$ Th1 and cross-protection against lethal infection with *C. albicans* [62]. In addition to Crf1, *A. fumigatus* proteins Gel1 and Pmp20 are described as strong inducers of Th1 responses in healthy individuals [63]. Tramsen et al. [64] reported a clinical scale generation of multi-specific antifungal T cells protocol based on the use of cellular fungal extracts of *A. fumigatus, C. albicans* and *Rhizopus oryzae* that allow the generation of numerous activated memory Th1 cells that respond to a broad spectrum of fungal pathogens. The data from these and similar studies [65, 66] support the development of adoptive T-cell transfer protocols for the therapy of multiple microbial pathogens as well as in prophylaxis/vaccination protocols [67].

Despite the main role of $CD4^+$ Th1 cells for host defense against pathogenic fungi, current findings highlight the effector functions of $CD8^+$ T cells against these pathogens [10, 68]. Adoptive transfer of *Aspergillus* f16 peptide-specific $CD8^+$ T cells extended the overall survival time of *A. fumigatus*-infected immunocompromised mice [69], supporting alternative adoptive T-cell treatments for hosts with progressive depletion of $CD4^+$ T lymphocytes and at high risk of invasive fungal infections.

3.4. B cell and natural killer cell treatment

B cells and natural killer (NK) cells are other cell lineages that have been evaluated for its antimicrobial activity through adoptive transfer procedures. Hoyt et al. [70] demonstrated that adoptive transfer of B cells into mice lacking both lymphocytes and type I IFN receptor and with *Pneumocystis murina* lung infection maintained early hematopoietic progenitor activity during immune responses against the infection, thus promoting replenishment of depleted bone marrow cells in an IL-10- and IL-27-dependent manner mechanisms possibly by stimulation of dendritic cells/macrophages.

NK cells are innate lymphocytes that exhibit both adaptive and innate features and that can be activated in the presence of infected cells, allogeneic cells or transformed cells, for acting

on antigen-specific recognition and mounting rapid effector responses such as rapid cytolytic and cytokine activity and antibody secretion [71] as well as in the immunological memory process [72]. Park et al. [73] described that the NK cells mediate their protective effect in the lungs of neutropenic mice with invasive aspergillosis by acting as the most important source of IFN-γ during the early stages of infection. Additionally, the transfer of activated NK cells from a wild-type host to both IFN-γ-deficient and wild-type recipients resulted in a more rapid clearance of *A. fumigatus* from the lungs. Bouzani et al. [74], similarly, concluded that NK cells mediate anti-*Aspergillus* activity through an alternative mechanism involving IFN-γ and tumor necrosis factor (TNF)-α secretion and not through degranulation of their cytotoxic proteins.

NK cells, directly and indirectly (through IFN-γ), showed killing activity against *A. fumigatus* hyphae, but lack activity against infecting conidia [75]. Based on these observations, adoptive immunotherapy with NK cells represents a potential alternative to be used alone or in combination with other antifungal therapies, but should have limited role in prophylactic strategies against aspergillosis.

4. Cytokine therapy

Cytokines are intercellular regulatory polypeptides or glycoproteins that promote growth, differentiation and activation of normal cells and play an essential role on immunomodulation and inflammatory processes. If on one hand, the determination of a patient cytokine profile may indicate the status of the disease, on the other hand, the therapeutic administration of cytokines can result in a favorable immunomodulation for the treatment of autoimmune, neoplastic and infectious diseases [76].

4.1. Colony-stimulating factors (CSFs)

Granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF) and GM-CSF are used to accelerate myelopoiesis in neutropenic patients and as immune enhancing agents. G-CSF is widely used during chemotherapy neutropenia in clinical practice to prevent immune dysregulation and accelerated functional immune recovery [77]. G-CSF not only increases neutrophil production but also significantly enhanced polymorphonuclear-mediated killing of *A. fumigatus* and *Rhizopus arrhizus* but not against *C. albicans* [78]. In a mice model of experimental disseminated candidiasis, the treatment with recombinant G-CSF (rG-CSF) leads to significantly reduced mortality. However, it is less effective in subacute or chronic disseminated candidiasis. Additionally, combination of rG-CSF and fluconazole results in an additive effect on the reduction of fungal load in the organs [79]. Combination of G-CSF and caspofungin or caspofungin plus amphotericin B-intralipid reduced the fungal burden in organs, decreased the detection of serum galactomannan and increased survival rate up to 78.9% of infected mice with *A. fumigatus* [80]. Favorable clinical response was also observed in 15 of 18 patients with mucormycosis [81]. While G-CSF clearly reduces the neutropenic period of patients, more data about clinical outcomes in fungal infections are needed [82, 83].

Human macrophage colony-stimulating factor (hM-CSF) slightly prolonged survival of lethal *C. albicans* infection in mice and enhanced the efficacy of amphotericin B by enhancing the growth-inhibitory activities of both macrophages and neutrophils against *Candida* [84]. In a neutropenic rabbit model of pulmonary aspergillosis, M-CSF administered prophylactically significantly increased survival and decreased pulmonary injury, probably through increased phagocytosis of *A. fumigatus* conidia by alveolar macrophages [85]. Recombinant human macrophage colony-stimulating factor (rhM-CSF) associated with standard antifungal therapy, into a phase I/II trial, was administrated to 46 bone marrow transplant patients from day 0 to 28 after determination of progressive fungal disease. Survival of these patients was higher (27% *v* 5%) when compared with 58 similar historical controls [86].

GM-CSF significantly enhanced both the killing by neutrophils and monocytes and the collaboration of these cells with voriconazole for killing *C. albicans* [87]. GM-CSF blocks the *in vivo* immunosuppressive effects of dexamethasone on bronchoalveolar macrophages, killing of *A. fumigatus* conidia and suggests its use in patients at risk of pulmonary aspergillosis in the course of dexamethasone treatment [88]. Similarly, Quezada et al. [89] demonstrated that GM-CSF administered intranasally to immunosuppressed mice infected with pulmonary aspergillosis reduced the lung fungal burden compared to the control.

Wildbaum et al. [90] related a patient with chronic mucocutaneous candidiasis that quickly went to a complete clinical remission with improvement in his/her monocyte and neutrophil functions after intravenous GM-CSF treatment (leucomax, 800 mg twice a week). Safdar et al. [91] retrospectively assessed 66 patients in whom GM-CSF was given during antifungal therapy, for which more than half of partial or complete response was observed. Wan et al. [92], in a prospective multicenter randomized phase IV trial with 206 patients, showed that GM-CSG for prophylaxis of infection after allogeneic transplantation was more effective than G-CSF alone in decreasing 100-day cumulative-, transplantation- and infection-related mortalities. Further studies to assess the use and efficacy of CSFs in the treatment of invasive fungal infections are needed.

4.2. Pro-inflammatory cytokines

Several studies demonstrate that immunomodulation with a variety of cytokines can enhance the antifungal activity of neutrophils and monocytes/macrophages as well as upregulation of protective Th1 immune response [93, 94]. Of these, interferon-γ (IFN-γ) produced by T and NK cells is a key cytokine in both the innate and adaptive immune response against invasive fungal infections, for which there are randomized controlled clinical trials [95, 96].

IFN-γ, *in vitro*, effectively primed neutrophils and mononuclear cells for enhanced fungal damage against *A. fumigatus*, *Fusarium solani* and *C. albicans*, as well as the stimulation of other cytokines [97]. In experimental animal models of fungal infections, IFN-γ had efficacy in a systemic cryptococcosis infection in mice, especially in combination with amphotericin B [98], and enhances host resistance against acute disseminated *C. albicans* in mice [99] and in murine invasive models of aspergillosis [100, 101]. However, inconsistent results were observed in a murine model of candidiasis when different strains of mice were used [102].

In a phase II, double-blind, placebo-controlled study, Pappas et al. [96] evaluated the safety and antifungal activity of adjuvant therapy with recombinant IFN-γ in patients with AIDS and acute cryptococcal meningitis. The results suggested that recombinant IFN-γ induces a more rapid sterilization of the cerebrospinal fluid and better clinical outcome. Similar results were observed in a randomized controlled trial conducted by Jarvis et al. [95]. The addition of short course IFN-γ (100 or 200 μg) to conventional antifungal therapy significantly increased the rate of negative cerebrospinal fluid culture. Guidelines for management of cryptococcal disease suggest that the adjunctive immunological therapy with recombinant IFN-γ can be considered for refractory cases [103].

The adjunctive immunotherapy with recombinant IFN-γ partially restored cell-mediated immunity and enhanced antifungal immunity in a prospective case series describing eight patients with invasive *Candida* or *Aspergillus* infections [104]. Other case reports have described the successful treatment of invasive aspergillosis with the combination of IFN-γ and antifungal therapy [105–108]. Adjunctive therapy with interferon-γ, GM-CSF or G-CSF showed good functional outcome in a case of *Scedosporium apiospermum* otomastoiditis and in a case of *Mucor* sinusitis and orbital cellulitis refractories to treatment with conventional antifungals [109]. However, for invasive mold infections, more data and randomized controlled clinical trials are needed.

IFN-γ, in combination with antimicrobials drugs, is currently recommended in prophylaxis of patients with chronic granulomatous disease (CGD), a population for which invasive filamentous fungal infections are a persistent problem [108, 110].

IL-12, IL-15 and TNF-α are other pro-inflammatory cytokines that have been assessed in preclinical trials as candidate adjuvant since they also upregulate the antifungal Th1 response [94]. IL-12 plays an obligatory role for the development of a Th1 response to *Candida* [111] and *Cryptococcus neoformans* [112], enhances the antifungal capacity of monocytes against *A. fumigatus in vitro* and has also been explored in immunotherapeutic proposals for various animal models of cryptococcal infections [113]. IL-12 gene therapy enhanced the host response against experimental coccidioidomycosis [114] and accelerated the clearance of infection in a murine *Pneumocystis* pneumonia model [115]. However, the use of IL-12 as immune enhancer remains controversial because this cytokine may paradoxically increase the susceptibility of the host to fungal pathogens [116, 117].

IL-15 is involved in the innate immunity against fungal infections and, similarly like IL-12, enhances the antifungal activity of granulocyte or monocyte cells against *C. albicans, A. fumigatus, Fusarium* spp. and *Scedosporium* spp. [118–121]. Although IL-15 has been potential as a new therapeutic option against invasive fungal infections, more information from future preclinical and clinical trials is needed.

TNF-α is necessary for the development of effective immunity to fungal infections [94], enhancing the activity of granulocyte cells against *A. fumigatus, C. albicans* and *Cryptococcus neoformans* [93, 122–124]. In a murine invasive pulmonary aspergillosis model, antibody-mediated neutralization of TNF-α increases mortality, whereas the intratracheal administration of a TNF-α agonist peptide improved survival [100]. Additionally, stimulatory/protective effect of TNF-α is also described against cryptococcal infections [113].

4.3. Other cytokines

Several other cytokines have been shown to be involved in the immune process during fungal infections and may be targets for future immunotherapy proposals. Gresnigt et al. [125] described the biological relevance of IL-36 in a pathway involved in the induction of Th responses by *A. fumigatus*. Nlrp3, Asc and caspase-1 mediated *Paracoccidioides brasiliensis*-induced IL-18, and the activation of the inflammasome is associated with a strong Th1-mediated immune response and, consequently, host antifungal defense against *Paracoccidioides brasiliensis* [126]. IL-7 is potent immunotherapeutic that acts at multiple levels to improve host immunity. In mice infected intravenously with *C. albicans*, the treatment with IL-7 weakened the infection and improved the host survival rates [127]. IL-18 contributes to host defense against *Cryptococcus neoformans* and *Paracoccidioides brasiliensis* [126, 128].

5. Antibody-based therapy

Antibodies or immunoglobulins are heterodimeric proteins composed of two heavy and two light chains, which are associated with the specific humoral immunity and primary defense against several infectious diseases [129–132]. The protective potential of antibodies produced during fungal infections can be accomplished by indirect and direct mechanisms and can vary depending on certain factors, such as the isotype, subisotype and title of antibodies and major histocompatibility complex background of the host [132, 133].

The interest in the potential benefit of antibody-based therapy for invasive fungal infections starts with Dromer et al. [134] describing that the intraperitoneal administration of a monoclonal IgG1 anti-*Cryptococcus neoformans* antibody could be used as a passive serotherapy, participating in the prevention or treatment of experimental cryptococcosis and with Gigliotti and Hughes [135] describing that the use of the monoclonal antibody (mAb) M5E312 was capable of hindering the development of an experimental murine *Pneumocystis carinii* infection.

Efungumab (Mycograb®) and the 18B7 (mAb) are two examples of antifungal mAbs evaluated in clinical trials. 18B7 mAb is a murine IgG1 that demonstrated acceptable safety in a phase I dose-escalation study in subjects with treated cryptococcal meningitis [136], but efficacy data for this therapy have not been generated. However, as reviewed by Larsen et al. [136], the administration of mAb against *C. neoformans* capsular polysaccharide to infected mice prolonged survival, reduced tissue burden, enhanced granuloma formation and enhanced antifungal activity of amphotericin B, fluconazole and flucytosine. These data support the continued investigation of this mAb.

Efungumab (Mycograb®) is a human genetically recombinant antibody that binds to the *Candida* heat-shock protein 90 (HSP90), preventing a conformational change needed for fungal viability [137]. Mycograb® showed protective activity against several *Candida* species and synergized with antifungal drugs when evaluated *in vitro* and in preclinical studies [138–140]. In a clinical trial, the treatment with lipid-associated amphotericin B in combination with Mycograb® produced significant clinical improvement in outcome for patients with invasive

candidiasis [141] when compared with the lipid-associated amphotericin B monotherapy. However, due to production difficulties, safety and quality issues, the drug was refused to grant marketing authorization.

Another immunoglobulin-based strategy was the production of anti-idiotypic monoclonal antibodies [142]. These mAbs that specifically reacting with killer toxins (KT) from *Pichia anomala* and *Williopsis mrakii* have broad antimicrobial spectrum and demonstrated *in vitro* similar activity to polymorphonuclear neutrophils against the hyphae and germinated conidia of *A. fumigatus* and *in vivo* protected immunocompromised mice with invasive aspergillosis from infection [143]. Similar results were observed against *C. albicans in vitro* and in vaginal and systemic murine models of candidiasis [144, 145].

Radioimmunotherapy uses the interactions between a fungal antigen and antibodies labeled with radionuclides to deliver cytocidal amounts of ionizing radiation to the specific target [146]. The advantages attributed to this immunotherapeutic method over standard antifungal therapy include: (a) it completely destroys the target cell by lethal radiation, delivers without the need of interaction with specific metabolism of the pathogen; (b) it is less subject to drug resistance mechanisms and does not suffer the drug-drug interactions that can be observed with some antifungals and others drugs; (c) it may permit single or a limited number of doses for the treatment of fungal diseases in contrast to weeks, months or years required to combat certain mycoses with antifungal drugs; (d) mAbs can be radiolabeled to bind antigens shared by many pathogenic fungi, such as heat-shock protein 60, β-(1,3)-glucan, ceramide and melanin [146–148].

Protection against experimental *Cryptococcus neoformans* and *Histoplasma capsulatum* infections was described using the radioimmunotherapy, whose mechanisms include killing of microorganism cells by "direct hit" and "cross-fire" effects, promotion of apoptosis-like death, cooperation with macrophages and modulation of the inflammatory response [149, 150]. The effects of this therapy on bystander mammalian cells demonstrated minimal effects on host cells [151]. Reviews conducted to discuss the efficacy, toxicity, radiation resistance, radiobiological mechanisms and comparison with standard antifungal treatments [152] and the possibility of developing "panantibodies" for a universal treatment of the fungal diseases [148] suggest that this immunotherapeutic modality is a promising alternative for the treatment of invasive fungal infections.

Antibodies like anti-β-glucans demonstrated protection against *A. fumigatus*, *C. albicans* and *Cryptococcus neoformans* [153–155]. Similarly, anti-melanin antibodies inhibit the growth and protection against *C. neoformans* and *Fonsecaea pedrosoi* and potential cross-resistance against various other fungi [156, 157].

Although most studies evaluate the antibodies that neutralize or kill the fungal pathogens [132], is growing the interest in the development of antibodies that can modulate the immune system and bring benefits to the host. For instance, through different immunomodulatory processes, 3B4 antibody protected mice from *C. albicans*-induced death in passive immunization [158], an agonist antibody CD40 prolonged the survival time of mice infected with *Cryptococcus neoformans* [159], anti-CD25 treatment decreased disease severity in the progressive and regressive forms of paracoccidioidomycosis [160], anti-PD-1 and anti-PD-L1

mAbs improved survival in fungal sepsis by *C. albicans* [161] and anti-CD3 antibody rapidly reversed the pathologic immune response caused by *Pneumocystis carinii* in a murine model of pneumonia [162].

6. Antifungal vaccines

The evaluation of antifungal vaccines has a great interest, but their development is challenging [10]. Antifungal vaccines are becoming a need in clinical settings, principally of immunocompromised and debilitated patients who are more prone to develop aggressive fungal infections. However, its use is limited by the weak immune response of these patients to respond vigorously to vaccination [163]. In this way, researchers made considerable progress in the last years, leading to more specific and well-characterized vaccines. Indeed, many antifungal vaccine candidates had been reported, and some have undergone preclinical evaluation and at least two are on the phase I clinical trials [164].

Studies on vaccination against fungal infections are conducted with whole-cell inactivated, live or attenuated fungi, cell wall subunits and the transfer of passive or adoptive immunity [165]. Usually, whole-cell inactivated vaccines are poorly immunogenic and have the disadvantage of having a complex chemical composition making it difficult to standardize [163]. In contrast, live virulence-attenuated vaccines are the best immunogens to achieve protection, but are unsafe in immunocompromised or otherwise debilitated patients, and even attenuated microorganisms can sometime cause disease in this subset [165]. In this way, subunit vaccines could be the best choice with regard to standardization and safety, but it lacks the natural adjuvant properties of whole-cell or live vaccines and usually needed an adjuvant to increase immunogenicity [10]. In animal models, the use of adjuvants is not a problem, since various adjuvants, such as Freund and aluminum hydroxide among others, have been very useful, but in humans, there is a scarcity of good adjuvants suitable for use in clinical practice [165]. Several adjuvants have been tested in recent years like the use of liposomes, virosomes, fungal immunogenic moieties and other bioengineered preparations and have proven useful [165, 166].

Protective immunity triggered by vaccination depends on both T-cell responses, particularly Th1 and/or Th17, and antibody responses which in turn are dependent on the kind of immunogens delivered to immune system [165]. A study conducted with a hyphal sonicate of *A. fumigatus* administered subcutaneously in corticosteroid immunosuppressed mice demonstrated that it was capable of conferring protection against invasive pulmonary aspergillosis [167]. Several studies have been conducted using the culture hypha filtrate, sonicated or vortexed hypha and demonstrated some degree of protection [168–171]. PitiumVac®, a licensed immunotherapy to treat equine pythiosis in Brazil, is an example of the use of disrupted hyphae, and its use reaches a cure rate of 70–80% [172, 173]. Despite the good curative properties of PitiumVac®, it does not present protective activity [174].

Various investigations have reported that heat-killed yeasts (HKY) of *Saccharomyces cerevisiae* given subcutaneously in mice are protective against fungi from five genera: *Aspergillus,*

Coccidioides, Cryptococcus, Candida and *Rhizopus* [175–179]. Analysis of the underlying immune responses associated with HKY-induced protection of the host suggested that HKY vaccination induces significant and specific Th1 response and antibodies to glucan and mannan [180]. The components of HKY responsible for the cross-protective response against these infections are not known, but several homologous proteins and key cell wall glycan components shared among fungi have been described [177, 181, 182]. These results in combination with studies that demonstrated the safety of yeast-based vaccines in humans are suggestive that a pan-fungal yeast-based vaccine is possible [178, 183].

A recombinant live attenuated strain of *Blastomyces dermatitidis* null for the adhesion BAD1 (identified as a virulence factor) given subcutaneously as a vaccine protects mice from a lethal blastomycosis infection, and a Th1 response was linked with vaccine-induced resistance [184]. A study about safety, toxicity and immunogenicity of this vaccine in dogs proved to be save, well tolerated and the cytokine profile observed belonging to Th1 response (INF-γ, TNF-α and GM-CSF) [185]. Subsequent study, with this same vaccine, demonstrated that a Th1 response is dispensable and that Th17 cells are sufficient for vaccine-induced protection against a lethal pulmonary blastomycosis in mice [23]. Indeed, other two different live attenuated vaccines prepared with strains of *Histoplasma capsulatum* and *Coccidiodes posodassi* were tested and were found to protect mice from these endemic mycoses by a mechanism dependent upon Th17 cells [23, 186]. Another experimental live attenuated vaccine has been tested against hematogenously disseminated candidiasis, and it is based on a genetically engineered *C. albicans* tet-NRG1. Under certain conditions, this strain remains in the yeast phase and is nonpathogenic when administered to mice as a vaccine and resulted in substantial protection from virulent strain [187].

7. Fungal antigens

Components from fungal cell are able to modulate the Th response, particularly the molecules from their cell wall components, notably carbohydrates and glycoproteins, which are related to the induction of a Th1 and/or Th17 responses [12, 188]. These carbohydrates include β-glucans (β-1,3-linked polymers of glucose with β-1,6 branches), chitin (homopolymer of N-acetylglucosamine) and mannans (mannose chains of varying lengths and configurations added to fungal proteins through N- or O-linkages) [16]. In addition, some fungal proteins appear to activate monocytes and can be used as adjuvants in specific immunotherapy.

Fungal β-glucans activity has been researched for over 50 years with the focus principally on the glucans from yeasts (*S. cerevisiae*) and mushrooms (*Lentinan edodes, Schizophyllun commune* and *Ganoderma lucidum*) [189, 190]. Most of the fungal β-glucans markedly stimulate the immune system, and they are considered as biological response modifiers with pronounced immunomodulating activity against infectious disease and cancer [191]. Different immunological pathways have been related to the biological activities of β-glucans, as well as improvement in phagocytosis and proliferative activities of professional phagocytes (i.e., granulocytes, monocytes, macrophages and dendritic cells), T and NK cells stimulation, and

activation of the alternative pathway of complement [192]. In mammals, β-glucans are recognized by dectin-1 and complement receptor 3 (CR3), and its preparations derived from fungi have a record of safety in both preclinical and human trials [193, 194].

Torosantucci et al. [154] were the first to link the use of a β-glucan and the induction of protective antibodies against different fungal infections. In their study was used the algal glucan laminarin conjugated with the diphtheria toxoid CRM197 for mice immunization that showed the ability of this glycoconjugate vaccine to confer protection against both lethal infections of *C. albicans* and *A. fumigatus*. This ability of a nonfungal β-glucan to induce specific anti β-glucan antibodies against two different pathogenic fungi highlights the possibility of the development of a single vaccine protecting against different fungal infections [195, 196]. This β-glucan CRM197 conjugated vaccine is in preclinical phase of development for aspergillosis, candidiasis and cryptococcosis [197]. Recent works using highly purified particulate β-glucan from *S. cerevisiae* alone or conjugated with bovine serum albumin (BSA) demonstrated the pluripotent activity of this vaccine in protection against experimental aspergillosis and coccidioidomycosis [198, 199]. Once β-glucans are highly conserved molecules, common to many fungi and that are able to induce protective antibodies, this implies that β-glucan would be an important vaccine component. Therefore, all these findings provide the basis for the future development of a pan-fungal conjugate vaccine [197].

Mannans, another carbohydrate from the fungal cell wall, have been implicated a long time ago with the host protective response against candidiasis [200]. Pioneering studies demonstrated that a vaccine containing a liposome-encapsulated mannan from *C. albicans* was protective in disseminated candidiasis in mice and that the specific antibodies produced were at least partly responsible for the protection with a β-1,2-linked mannotriose being the active epitope [201, 202]. Liposomal encapsulation was required because the extract alone was poorly immunogenic, and in an attempt to improve this formulation, new studies were made by conjugating mannan extract to protein (BSA) [203, 204]. This same *C. albicans* mannan conjugated to BSA presented cross-protective activity against systemic murine aspergillosis [205]. A study with *S. cerevisiae* mannan demonstrated the change of a T-cell-independent response when the polysaccharide was administered alone for a T-cell-dependent response when it was conjugated to human serum albumin (HSA). This study was conducted in mice and showed the increase in specific IgG isotype antibodies, mainly Th1 associated (IgG_{2a} and IgG_{2b}), making it a vaccine candidate for preventive immunomodulation treatment [206]. A synthetic β-mannan trisaccharide conjugated to different peptides found in *C. albicans* cell wall proteins was demonstrated to induce protective immunity in mice against candidiasis [55]. A tricomponent conjugate vaccine that associated the β-mannan trisaccharide, tetanus toxoid and laminarin was capable to promote multiple immune pathways leading to DC activation, induction of Th17 response and a potent immunization [207].

Glucuronoxylomannan (GXM), the major capsular polysaccharide of *Cryptococcus neoformans*, exerts many immunoregulatory effects, and in humans, the development of anticapsular antibodies is correlated with improved prognosis [208]. Conjugated vaccines of GXM-tetanus toxoid elicit protective antibodies in mice [209, 210], although deleterious antibodies can also be induced [211, 212]. This contradictory effect (protective *v* deleterious antibodies) can be

avoided using GXM epitopes that elicit only protective antibodies, and it was observed coupling a derived GXM heptasaccharide to a protein carrier [212] and this vaccine is in phase I clinical trial [197]. The mimotope-based immunization can elicit an antibody response to a protective epitope on the native antigen [213]. The GXM peptide mimotope P13 conjugated to a protein carrier has demonstrated its effectiveness in prolonging survival of mice after a lethal challenge with *C. neoformans*, and the protection was associated with a reduction of serum levels of GXM and the production of antibodies to GXM [214–216].

Once carbohydrates are known to produce T-cell-independent immune responses with a poor-quality antibody response, the conjugation with immunogenic protein carriers is a strategy to overcome this poor immunogenicity [217, 218]. Immunization with glycoconjugated vaccines elicit T cell help for B cells that produce IgG antibodies and can induce memory B-cell development and T-cell memory [219]. The main carrier proteins used in licensed conjugate vaccines are the enzymatically inactive and nontoxic variant of diphtheria toxin (CRM197), diphtheria toxoid (DT) and tetanus toxoid (TT) [220, 221].

On the other hand, protein antigens are highly immunogenic, elicit a strong T-cell response and are biochemically defined, which facilitate the large-scale production of recombinant proteins [222]. Indeed, two vaccines based on recombinant proteins of *C. albicans* are under phase I clinical trials [165]. The first vaccine is based on a recombinant N-terminus of the candida adhesion, Als3p (rAls3p-N) with alum as adjuvant, and elicits Th1 and Th17 cells [223]. The target of this vaccine included systemic and mucosal candidiasis, and the study in human subjects has been performed by NovaDigm Therapeutics [224]. Data from safety and immunogenicity have been posted on the web (www.novadigm.net). The second vaccine is based on the recombinant secretory aspartyl proteinase2, Sap2 [225] and appears to confer protection by Sap-neutralizing antibodies. This active vaccine, named PEV7, is targeted to prevent recurrent vulvovaginitis and is being developed by Pevion Biotech. Initial report about safety and immunogenicity profile of PEV7 in women is strongly encouraging (www.pevion.com).

Protein and glycoprotein from different pathogenic fungi have been studied, and particularly, heat-shock proteins (HSPs) represent an attractive candidate due to their association with both innate and adaptive immunity [226, 227]. Immunization with HSPs from *Paracoccidioides brasiliensis* has been shown to provide some degree of protection against experimental disease [228, 229]. Protective immune response has been also observed with native or recombinant (r) HSP60 from *Histoplasma capsulatum*, and rHSP60 reduced fungal burden and improved survival in experimental pulmonary histoplasmosis [230, 231]. Immunization with whole glycoprotein gp43 from *P. brasiliensis* demonstrated a dual response, inducing Th1 and Th2 cells, whereas the P10 (15-mer peptide derived from gp43) elicited Th1 response that protected mice from experimental paracoccidioidomycosis [232].

Summarizing all these studies with several pathogenic fungi has shown that antibodies against cell surface molecules could be implicated in the protective response to infection. The goal is to identify which molecules or epitopes are able to produce only protective antibodies and not those isotypes that inhibit host defenses or exert a negative effect on immunity. Despite a growing medical need and all efforts made by researchers, there is still no approved vaccine against any fungal disease.

8. Conclusion

Breakthroughs in our understanding of how homeostasis is established, maintained or disrupted during fungal exposure and/or colonization should help to guide the development of new therapeutics that target specific inflammatory or metabolic end points. For example, limiting inflammation—through PRR agonism or antagonism—to stimulate a protective immune response to fungi should pave the way for the rational design of novel immunomodulatory therapies.

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References

[1] Blackwell M. The fungi: 1, 2, 3 … 5.1 million species? Am J Bot. 2011;**98**:426–438.

[2] Kohler JR, Casadevall A, and Perfect J. The spectrum of fungi that infects humans. Cold Spring Harb Perspect Med. 2015;**5**:a019273.

[3] Cui L, Morris A, and Ghedin E. The human mycobiome in health and disease. Genome Med. 2013;**5**:63.

[4] Casadevall A and Pirofski LA. Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. Infect Immun. 1999;**67**:3703–3713.

[5] Pirofski LA and Casadevall A. What is infectiveness and how is it involved in infection and immunity? BMC Immunol. 2015;**16**:13.

[6] Pirofski LA and Casadevall A. The damage-response framework of microbial pathogenesis and infectious diseases. Adv Exp Med Biol. 2008;**635**:135–146.

[7] Seed PC. The human mycobiome. Cold Spring Harb Perspect Med. 2015;**5**:a019810.

[8] Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, and White TC. Hidden killers: human fungal infections. Sci Transl Med. 2012;**4**:165rv113.

[9] Perfect JR. "Is there an emerging need for new antifungals?". Expert Opin Emerg Drugs. 2016;**21**:129–131.

[10] Iannitti RG, Carvalho A, and Romani L. From memory to antifungal vaccine design. Trends Immunol. 2012;**33**:467–474.

[11] Pflughoeft KJ and Versalovic J. Human microbiome in health and disease. Annu Rev Pathol. 2012;**7**:99–122.

[12] Romani L. Immunity to fungal infections. Nat Rev Immunol. 2011;**11**:275–288.

[13] Steele C and Wormley FL, Jr. Immunology of fungal infections: lessons learned from animal models. Curr Opin Microbiol. 2012;**15**:413–419.

[14] Netea MG, Brown GD, Kullberg BJ, and Gow NA. An integrated model of the recognition of *Candida albicans* by the innate immune system. Nat Rev Microbiol. 2008;**6**:67–78.

[15] Thiemann S and Baum LG. Galectins and immune responses-just how do they do those things they do? Annu Rev Immunol. 2016;**34**:243–264.

[16] Levitz SM. Innate recognition of fungal cell walls. PLoS Pathog. 2010;**6**:e1000758.

[17] Wuthrich M, Deepe GS, Jr., and Klein B. Adaptive immunity to fungi. Annu Rev Immunol. 2012;**30**:115–148.

[18] Borghi M, Renga G, Puccetti M, Oikonomou V, Palmieri M, Galosi C, Bartoli A, and Romani L. Antifungal Th Immunity: Growing up in Family. Front Immunol. 2014;**5**:506.

[19] Perfect JR. The impact of the host on fungal infections. Am J Med. 2012;**125**:S39–51.

[20] Romani L. Immune resistance and tolerance to fungi. G Ital Dermatol Venereol. 2013;**148**:551–561.

[21] van de Veerdonk FL and Netea MG. T-cell subsets and antifungal host defenses. Curr Fungal Infect Rep. 2010;**4**:238–243.

[22] Bar E, Whitney PG, Moor K, Reis e Sousa C, and LeibundGut-Landmann S. IL-17 regulates systemic fungal immunity by controlling the functional competence of NK cells. Immunity. 2014;**40**:117–127.

[23] Wuthrich M, et al. Vaccine-induced protection against 3 systemic mycoses endemic to North America requires Th17 cells in mice. J Clin Invest. 2011;**121**:554–568.

[24] Zelante T, et al. IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. Eur J Immunol. 2007;**37**:2695–2706.

[25] Belkaid Y and Tarbell K. Regulatory T cells in the control of host-microorganism interactions. Annu Rev Immunol. 2009;**27**:551–589.

[26] Hickey MJ and Kubes P. Intravascular immunity: the host-pathogen encounter in blood vessels. Nat Rev Immunol. 2009;**9**:364–375.

[27] Shoham S and Levitz SM. The immune response to fungal infections. Br J Haematol. 2005;**129**:569–582.

[28] Grigull L, et al. Secondary prophylaxis of invasive fungal infections with combination antifungal therapy and G-CSF-mobilized granulocyte transfusions in three children with hematological malignancies. Support Care Cancer. 2006;**14**:783–786.

[29] Seidel MG, Peters C, Wacker A, Northoff H, Moog R, Boehme A, Silling G, Grimminger W, and Einsele H. Randomized phase III study of granulocyte transfusions in neutropenic patients. Bone Marrow Transplant. 2008;**42**:679–684.

[30] Price TH. Granulocyte transfusion: current status. Semin Hematol. 2007;**44**:15–23.

[31] Ang AL and Linn YC. Treatment of severe neutropenic sepsis with granulocyte transfusion in the current era--experience from an adult haematology unit in Singapore. Transfus Med. 2011;**21**:13–24.

[32] Strumia MM. The effect of leukocytic cream injections in the treatment of the neutropenias. Am J Med Sci. 1934;**187**:527–544.

[33] Brecher G, Wilbur K, and Cronkite E. Transfusion of separated leukocytes into irradiated dogs with aplastic marrows. Exp Biol Med. 1953;**84**:54–56.

[34] Freireich EJ, Levin RH, Whang J, Carbone PP, Bronson W, and Morse EE. The function and fate of transfused leukocytes from donors with chronic myelocytic leukemia in leukopenic recipients. Ann N Y Acad Sci. 1964;**113**:1081–1089.

[35] Pedersen FK, Johansen KS, Rosenkvist J, Tygstrup I, and Valerius NH. Refractory *Pneumocystis carinii* infection in chronic granulomatous disease: successful treatment with granulocytes. Pediatrics. 1979;**64**:935–938.

[36] Bhatia S, McCullough J, Perry EH, Clay M, Ramsay NK, and Neglia JP. Granulocyte transfusions: efficacy in treating fungal infections in neutropenic patients following bone marrow transplantation. Transfusion (Paris). 1994;**34**:226–232.

[37] Strauss RG. Granulocyte transfusion therapy. Hematol Oncol Clin North Am. 1994;**8**:1159–1166.

[38] Strauss RG. Role of granulocyte/neutrophil transfusions for haematology/oncology patients in the modern era. Br J Haematol. 2012;**158**:299–306.

[39] Chanock SJ and Gorlin JB. Granulocyte transfusions. Time for a second look. Infect Dis Clin North Am. 1996;**10**:327–343.

[40] Bensinger WI, et al. The effects of daily recombinant human granulocyte colony-stimulating factor administration on normal granulocyte donors undergoing leukapheresis. Blood. 1993;**81**:1883–1888.

[41] Price TH, Bowden RA, Boeckh M, Bux J, Nelson K, Liles WC, and Dale DC. Phase I/II trial of neutrophil transfusions from donors stimulated with G-CSF and dexamethasone for treatment of patients with infections in hematopoietic stem cell transplantation. Blood. 2000;**95**:3302–3309.

[42] Mousset S, et al. Prophylactic and interventional granulocyte transfusions in patients with haematological malignancies and life-threatening infections during neutropenia. Ann Hematol. 2005;**84**:734–741.

[43] Seidel MG, et al. Granulocyte transfusions in children and young adults: does the dose matter? J Pediatr Hematol Oncol. 2009;**31**:166–172.

[44] Carter KB, Jr., Loehrl TA, and Poetker DM. Granulocyte transfusions in fulminant invasive fungal sinusitis. Am J Otolaryngol. 2012;**33**:663–666.

[45] Kadri SS, Remy KE, Strich JR, Gea-Banacloche J, and Leitman SF. Role of granulocyte transfusions in invasive fusariosis: systematic review and single-center experience. Transfusion (Paris). 2015;**55**:2076–2085.

[46] Safdar A, et al. Recombinant interferon gamma1b immune enhancement in 20 patients with hematologic malignancies and systemic opportunistic infections treated with donor granulocyte transfusions. Cancer. 2006;**106**:2664–2671.

[47] Yoshihara S, Ikemoto J, and Fujimori Y. Update on granulocyte transfusions: accumulation of promising data, but still lack of decisive evidence. Curr Opin Hematol. 2016;**23**:55–60.

[48] Ramirez-Ortiz ZG and Means TK. The role of dendritic cells in the innate recognition of pathogenic fungi (*A. fumigatus, C. neoformans* and *C. albicans*). Virulence. 2012;**3**:635–646.

[49] Roy RM and Klein BS. Dendritic cells in antifungal immunity and vaccine design. Cell Host Microbe. 2012;**11**:436–446.

[50] Perruccio K, Bozza S, Montagnoli C, Bellocchio S, Aversa F, Martelli M, Bistoni F, Velardi A, and Romani L. Prospects for dendritic cell vaccination against fungal infections in hematopoietic transplantation. Blood Cells Mol Dis. 2004;**33**:248–255.

[51] Bozza S, et al. A dendritic cell vaccine against invasive aspergillosis in allogeneic hematopoietic transplantation. Blood. 2003;**102**:3807–3814.

[52] Bozza S, Gaziano R, Lipford GB, Montagnoli C, Bacci A, Di Francesco P, Kurup VP, Wagner H, and Romani L. Vaccination of mice against invasive aspergillosis with recombinant *Aspergillus* proteins and CpG oligodeoxynucleotides as adjuvants. Microbes Infect. 2002;**4**:1281–1290.

[53] Shao C, Qu J, He L, Zhang Y, Wang J, Zhou H, Wang Y, and Liu X. Dendritic cells transduced with an adenovirus vector encoding interleukin-12 are a potent vaccine for invasive pulmonary aspergillosis. Genes Immun. 2005;**6**:103–114.

[54] Zhu F, Ramadan G, Davies B, Margolis DA, and Keever-Taylor CA. Stimulation by means of dendritic cells followed by Epstein-Barr virus-transformed B cells as antigen-presenting cells is more efficient than dendritic cells alone in inducing *Aspergillus* f16-specific cytotoxic T cell responses. Clin Exp Immunol. 2008;**151**:284–296.

[55] Xin H, Dziadek S, Bundle DR, and Cutler JE. Synthetic glycopeptide vaccines combining beta-mannan and peptide epitopes induce protection against candidiasis. Proc Natl Acad Sci U S A. 2008;**105**:13526–13531.

[56] Ueno K, et al. Dendritic cell-based immunization ameliorates pulmonary infection with highly virulent *Cryptococcus gattii*. Infect Immun. 2015;**83**:1577–1586.

[57] Kalos M and June CH. Adoptive T cell transfer for cancer immunotherapy in the era of synthetic biology. Immunity. 2013;**39**:49–60.

[58] Papadopoulou A, Kaloyannidis P, Yannaki E, and Cruz CR. Adoptive transfer of *Aspergillus*-specific T cells as a novel anti-fungal therapy for hematopoietic stem cell transplant recipients: Progress and challenges. Crit Rev Oncol Hematol. 2016;**98**:62–72.

[59] Perruccio K, et al. Transferring functional immune responses to pathogens after haploidentical hematopoietic transplantation. Blood. 2005;**106**:4397–4406.

[60] Tramsen L, Schmidt S, Roeger F, Schubert R, Salzmann-Manrique E, Latge JP, Klingebiel T, and Lehrnbecher T. Immunosuppressive compounds exhibit particular effects on functional properties of human anti-*Aspergillus* Th1 cells. Infect Immun. 2014;**82**:2649–2656.

[61] Cenci E, Mencacci A, Bacci A, Bistoni F, Kurup VP, and Romani L. T cell vaccination in mice with invasive pulmonary aspergillosis. J Immunol. 2000;**165**:381–388.

[62] Stuehler C, et al. Cross-protective TH1 immunity against *Aspergillus fumigatus* and *Candida albicans*. Blood. 2011;**117**:5881–5891.

[63] Stuehler C, Nowakowska J, Bernardini C, Topp MS, Battegay M, Passweg J, and Khanna N. Multispecific *Aspergillus* T cells selected by CD137 or CD154 induce protective immune responses against the most relevant mold infections. J Infect Dis. 2015;**211**:1251–1261.

[64] Tramsen L, Schmidt S, Boenig H, Latge JP, Lass-Florl C, Roeger F, Seifried E, Klingebiel T, and Lehrnbecher T. Clinical-scale generation of multi-specific anti-fungal T cells targeting *Candida, Aspergillus* and mucormycetes. Cytotherapy. 2013;**15**:344–351.

[65] Khanna N, Stuehler C, Conrad B, Lurati S, Krappmann S, Einsele H, Berges C, and Topp MS. Generation of a multipathogen-specific T-cell product for adoptive immunotherapy based on activation-dependent expression of CD154. Blood. 2011;**118**:1121–1131.

[66] Kumaresan PR, et al. Bioengineering T cells to target carbohydrate to treat opportunistic fungal infection. Proc Natl Acad Sci U S A. 2014;**111**:10660–10665.

[67] Zheng M, et al. Corrigendum. $CD4^+$ T cell-independent DNA vaccination against opportunistic infections. J Clin Invest. 2015;**125**:1364.

[68] Cutler JE, Deepe GS, Jr., and Klein BS. Advances in combating fungal diseases: vaccines on the threshold. Nat Rev Microbiol. 2007;**5**:13–28.

[69] Sun Z, Zhu P, Li L, Wan Z, Zhao Z, and Li R. Adoptive immunity mediated by HLA-A*0201 restricted Asp f16 peptides-specific $CD8^+$ T cells against *Aspergillus fumigatus* infection. Eur J Clin Microbiol Infect Dis. 2012;**31**:3089–3096.

[70] Hoyt TR, Dobrinen E, Kochetkova I, and Meissner N. B cells modulate systemic responses to *Pneumocystis murina* lung infection and protect on-demand hematopoiesis via T cell-independent innate mechanisms when type I interferon signaling is absent. Infect Immun. 2015;**83**:743–758.

[71] Bezman NA, et al. Molecular definition of the identity and activation of natural killer cells. Nat Immunol. 2012;**13**:1000–1009.

[72] Cerwenka A and Lanier LL. Natural killer cell memory in infection, inflammation and cancer. Nat Rev Immunol. 2016;**16**:112–123.

[73] Park SJ, Hughes MA, Burdick M, Strieter RM, and Mehrad B. Early NK cell-derived IFN-{gamma} is essential to host defense in neutropenic invasive aspergillosis. J Immunol. 2009;**182**:4306–4312.

[74] Bouzani M, Ok M, McCormick A, Ebel F, Kurzai O, Morton CO, Einsele H, and Loeffler J. Human NK cells display important antifungal activity against *Aspergillus fumigatus*, which is directly mediated by IFN-gamma release. J Immunol. 2011;**187**:1369–1376.

[75] Schmidt S, Tramsen L, Hanisch M, Latge JP, Huenecke S, Koehl U, and Lehrnbecher T. Human natural killer cells exhibit direct activity against *Aspergillus fumigatus* hyphae, but not against resting conidia. J Infect Dis. 2011;**203**:430–435.

[76] Decker WK and Safdar A. Cytokine adjuvants for vaccine therapy of neoplastic and infectious disease. Cytokine Growth Factor Rev. 2011;**22**:177–187.

[77] Volpi I, et al. Postgrafting administration of granulocyte colony-stimulating factor impairs functional immune recovery in recipients of human leukocyte antigen haplotype-mismatched hematopoietic transplants. Blood. 2001;**97**:2514–2521.

[78] Liles WC, Huang JE, van Burik JA, Bowden RA, and Dale DC. Granulocyte colony-stimulating factor administered *in vivo* augments neutrophil-mediated activity against opportunistic fungal pathogens. J Infect Dis. 1997;**175**:1012–1015.

[79] Kullberg BJ, Netea MG, Vonk AG, and van der Meer JW. Modulation of neutrophil function in host defense against disseminated *Candida albicans* infection in mice. FEMS Immunol Med Microbiol. 1999;**26**:299–307.

[80] Sionov E, Mendlovic S, and Segal E. Experimental systemic murine aspergillosis: treatment with polyene and caspofungin combination and G-CSF. J Antimicrob Chemother. 2005;**56**:594–597.

[81] Roden MM, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin Infect Dis. 2005;**41**:634–653.

[82] Smith TJ, et al. 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. J Clin Oncol. 2006;**24**:3187–3205.

[83] Ringden O, et al. Treatment with granulocyte colony-stimulating factor after allogeneic bone marrow transplantation for acute leukemia increases the risk of graft-versus-host disease and death: a study from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol. 2004;**22**:416–423.

[84] Kuhara T, Uchida K, and Yamaguchi H. Therapeutic efficacy of human macrophage colony-stimulating factor, used alone and in combination with antifungal agents, in mice with systemic *Candida albicans* infection. Antimicrob Agents Chemother. 2000;**44**:19–23.

[85] Gonzalez CE, et al. Recombinant human macrophage colony-stimulating factor augments pulmonary host defences against *Aspergillus fumigatus*. Cytokine. 2001;**15**:87–95.

[86] Nemunaitis J, et al. Long-term follow-up of patients with invasive fungal disease who received adjunctive therapy with recombinant human macrophage colony-stimulating factor. Blood. 1993;**82**:1422–1427.

[87] Vora S, Purimetla N, Brummer E, and Stevens DA. Activity of voriconazole, a new triazole, combined with neutrophils or monocytes against *Candida albicans*: effect of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. Antimicrob Agents Chemother. 1998;**42**:907–910.

[88] Brummer E, Maqbool A, and Stevens DA. *In vivo* GM-CSF prevents dexamethasone suppression of killing of *Aspergillus fumigatus* conidia by bronchoalveolar macrophages. J Leukoc Biol. 2001;**70**:868–872.

[89] Quezada G, Koshkina NV, Zweidler-McKay P, Zhou Z, Kontoyiannis DP, and Kleinerman ES. Intranasal granulocyte-macrophage colony-stimulating factor reduces the *Aspergillus* burden in an immunosuppressed murine model of pulmonary aspergillosis. Antimicrob Agents Chemother. 2008;**52**:716–718.

[90] Wildbaum G, Shahar E, Katz R, Karin N, Etzioni A, and Pollack S. Continuous G-CSF therapy for isolated chronic mucocutaneous candidiasis: complete clinical remission with restoration of IL-17 secretion. J Allergy Clin Immunol. 2013;**132**:761–764.

[91] Safdar A, Rodriguez G, Zuniga J, Al Akhrass F, Georgescu G, and Pande A. Granulocyte macrophage colony-stimulating factor in 66 patients with myeloid or lymphoid neoplasms and recipients of hematopoietic stem cell transplantation with invasive fungal disease. Acta Haematol. 2013;**129**:26–34.

[92] Wan L, et al. Effect of granulocyte-macrophage colony-stimulating factor on prevention and treatment of invasive fungal disease in recipients of allogeneic stem-cell transplantation: a prospective multicenter randomized phase IV trial. J Clin Oncol. 2015;**33**:3999–4006.

[93] Netea MG, Kullberg BJ, and Van der Meer JW. Proinflammatory cytokines in the treatment of bacterial and fungal infections. Biodrugs. 2004;**18**:9–22.

[94] Antachopoulos C and Roilides E. Cytokines and fungal infections. Br J Haematol. 2005;**129**:583–596.

[95] Jarvis JN, et al. Adjunctive interferon-gamma immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. AIDS. 2012;**26**:1105–1113.

[96] Pappas PG, Bustamante B, Ticona E, Hamill RJ, Johnson PC, Reboli A, Aberg J, Hasbun R, and Hsu HH. Recombinant interferon- gamma 1b as adjunctive therapy for AIDS-related acute cryptococcal meningitis. J Infect Dis. 2004;**189**:2185–2191.

[97] Gaviria JM, van Burik JA, Dale DC, Root RK, and Liles WC. Comparison of interferon-gamma, granulocyte colony-stimulating factor, and granulocyte-macrophage colony-stimulating factor for priming leukocyte-mediated hyphal damage of opportunistic fungal pathogens. J Infect Dis. 1999;**179**:1038–1041.

[98] Clemons KV, Lutz JE, and Stevens DA. Efficacy of recombinant gamma interferon for treatment of systemic cryptococcosis in SCID mice. Antimicrob Agents Chemother. 2001;**45**:686–689.

[99] Kullberg BJ, van't Wout JW, Hoogstraten C, and van Furth R. Recombinant interferon-gamma enhances resistance to acute disseminated *Candida albicans* infection in mice. J Infect Dis. 1993;**168**:436–443.

[100] Mehrad B, Strieter RM, and Standiford TJ. Role of TNF-alpha in pulmonary host defense in murine invasive aspergillosis. J Immunol. 1999;**162**:1633–1640.

[101] Shao C, Qu J, He L, Zhang Y, Wang J, Wang Y, Zhou H, and Liu X. Transient overexpression of gamma interferon promotes *Aspergillus* clearance in invasive pulmonary aspergillosis. Clin Exp Immunol. 2005;**142**:233–241.

[102] Garner RE, Kuruganti U, Czarniecki CW, Chiu HH, and Domer JE. *In vivo* immune responses to *Candida albicans* modified by treatment with recombinant murine gamma interferon. Infect Immun. 1989;**57**:1800–1808.

[103] Perfect JR, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of America. Clin Infect Dis. 2010;**50**:291–322.

[104] Delsing CE, et al. Interferon-gamma as adjunctive immunotherapy for invasive fungal infections: a case series. BMC Infect Dis. 2014;**14**:166.

[105] Al-Tawfiq JA and Al-Abdely HM. Vertebral osteomyelitis due to *Aspergillus fumigatus* in a patient with chronic granulomatous disease successfully treated with antifungal agents and interferon-gamma. Med Mycol. 2010;**48**:537–541.

[106] Kelleher P, Goodsall A, Mulgirigama A, Kunst H, Henderson DC, Wilson R, Newman-Taylor A, and Levin M. Interferon-gamma therapy in two patients with progressive chronic pulmonary aspergillosis. Eur Respir J. 2006;**27**:1307–1310.

[107] Saulsbury FT. Successful treatment of *Aspergillus* brain abscess with itraconazole and interferon-gamma in a patient with chronic granulomatous disease. Clin Infect Dis. 2001;**32**:E137–139.

[108] Estrada C, Desai AG, Chirch LM, Suh H, Seidman R, Darras F, and Nord EP. Invasive aspergillosis in a renal transplant recipient successfully treated with interferon-gamma. Case Rep Transplant. 2012;**2012**:493758.

[109] Abzug MJ and Walsh TJ. Interferon-gamma and colony-stimulating factors as adjuvant therapy for refractory fungal infections in children. Pediatr Infect Dis J. 2004;**23**:769–773.

[110] Segal BH and Romani LR. Invasive aspergillosis in chronic granulomatous disease. Med Mycol. 2009;**47:**S282–290.

[111] Romani L, Puccetti P, and Bistoni F. Interleukin-12 in infectious diseases. Clin Microbiol Rev. 1997;**10**:611–636.

[112] Decken K, Kohler G, Palmer-Lehmann K, Wunderlin A, Mattner F, Magram J, Gately MK, and Alber G. Interleukin-12 is essential for a protective Th1 response in mice infected with *Cryptococcus neoformans*. Infect Immun. 1998;**66**:4994–5000.

[113] Antachopoulos C and Walsh TJ. Immunotherapy of *Cryptococcus* infections. Clin Microbiol Infect. 2012;**18**:126–133.

[114] Jiang C, Magee DM, and Cox RA. Construction of a single-chain interleukin-12-expressing retroviral vector and its application in cytokine gene therapy against experimental coccidioidomycosis. Infect Immun. 1999;**67**:2996–3001.

[115] Ruan S, McKinley L, Zheng M, Rudner X, D'Souza A, Kolls JK, and Shellito JE. Interleukin-12 and host defense against murine *Pneumocystis* pneumonia. Infect Immun. 2008;**76**:2130–2137.

[116] Romani L, Bistoni F, Mencacci A, Cenci E, Spaccapelo R, and Puccetti P. IL12 in *Candida albicans* infections. Res Immunol. 1995;**146**:532–538.

[117] Toren A, Or R, Ackerstein A, and Nagler A. Invasive fungal infections in lymphoma patients receiving immunotherapy following autologous bone marrow transplantation (ABMT). Bone Marrow Transplant. 1997;**20**:67–69.

[118] Vazquez N, Walsh TJ, Friedman D, Chanock SJ, and Lyman CA. Interleukin-15 augments superoxide production and microbicidal activity of human monocytes against *Candida albicans*. Infect Immun. 1998;**66**:145–150.

[119] Winn RM, Gil-Lamaignere C, Roilides E, Simitsopoulou M, Lyman CA, Maloukou A, and Walsh TJ. Selective effects of interleukin (IL)-15 on antifungal activity and IL-8 release by polymorphonuclear leukocytes in response to hyphae of *Aspergillus* species. J Infect Dis. 2003;**188**:585–590.

[120] Winn RM, Gil-Lamaignere C, Roilides E, Simitsopoulou M, Lyman CA, Maloukou A, and Walsh TJ. Effects of interleukin-15 on antifungal responses of human polymorphonuclear leukocytes against *Fusarium* spp. and *Scedosporium* spp. Cytokine. 2005;**31**:1–8.

[121] Musso T, et al. Interleukin-15 activates proinflammatory and antimicrobial functions in polymorphonuclear cells. Infect Immun. 1998;**66**:2640–2647.

[122] Roilides E, Dimitriadou-Georgiadou A, Sein T, Kadiltsoglou I, and Walsh TJ. Tumor necrosis factor alpha enhances antifungal activities of polymorphonuclear and mononuclear phagocytes against *Aspergillus fumigatus*. Infect Immun. 1998;**66**:5999–6003.

[123] Kawakami K, Qureshi MH, Koguchi Y, Zhang T, Okamura H, Kurimoto M, and Saito A. Role of TNF-alpha in the induction of fungicidal activity of mouse peritoneal exudate cells against *Cryptococcus neoformans* by IL-12 and IL-18. Cell Immunol. 1999;**193**:9–16.

[124] Mencacci A, Cenci E, Bacci A, Montagnoli C, Bistoni F, and Romani L. Cytokines in candidiasis and aspergillosis. Curr Pharm Biotechnol. 2000;**1**:235–251.

[125] Gresnigt MS, Rosler B, Jacobs CW, Becker KL, Joosten LA, van der Meer JW, Netea MG, Dinarello CA, and van de Veerdonk FL. The IL-36 receptor pathway regulates *Aspergillus fumigatus*-induced Th1 and Th17 responses. Eur J Immunol. 2013;**43**:416–426.

[126] Ketelut-Carneiro N, Silva GK, Rocha FA, Milanezi CM, Cavalcanti-Neto FF, Zamboni DS, and Silva JS. IL-18 triggered by the Nlrp3 inflammasome induces host innate resistance in a pulmonary model of fungal infection. J Immunol. 2015;**194**:4507–4517.

[127] Unsinger J, Burnham CA, McDonough J, Morre M, Prakash PS, Caldwell CC, Dunne WM, Jr., and Hotchkiss RS. Interleukin-7 ameliorates immune dysfunction and improves survival in a 2-hit model of fungal sepsis. J Infect Dis. 2012;**206**:606–616.

[128] Kawakami K, Qureshi MH, Zhang T, Okamura H, Kurimoto M, and Saito A. IL-18 protects mice against pulmonary and disseminated infection with *Cryptococcus neoformans* by inducing IFN-gamma production. J Immunol. 1997;**159**:5528–5534.

[129] Schroeder HW, Jr. and Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol. 2010;**125**:S41–52.

[130] Strugnell RA and Wijburg OL. The role of secretory antibodies in infection immunity. Nat Rev Microbiol. 2010;**8**:656–667.

[131] Parren PW, Poignard P, Ditzel HJ, Williamson RA, and Burton DR. Antibodies in human infectious disease. Immunol Res. 2000;**21**:265–278.

[132] Casadevall A and Pirofski LA. Immunoglobulins in defense, pathogenesis, and therapy of fungal diseases. Cell Host Microbe. 2012;**11**:447–456.

[133] Rivera J and Casadevall A. Mouse genetic background is a major determinant of isotype-related differences for antibody-mediated protective efficacy against *Cryptococcus neoformans*. J Immunol. 2005;**174**:8017–8026.

[134] Dromer F, Charreire J, Contrepois A, Carbon C, and Yeni P. Protection of mice against experimental cryptococcosis by anti-*Cryptococcus neoformans* monoclonal antibody. Infect Immun. 1987;**55**:749–752.

[135] Gigliotti F and Hughes WT. Passive immunoprophylaxis with specific monoclonal antibody confers partial protection against *Pneumocystis carinii* pneumonitis in animal models. J Clin Invest. 1988;**81**:1666–1668.

[136] Larsen RA, Pappas PG, Perfect J, Aberg JA, Casadevall A, Cloud GA, James R, Filler S, and Dismukes WE. Phase I evaluation of the safety and pharmacokinetics of murine-derived anticryptococcal antibody 18B7 in subjects with treated cryptococcal meningitis. Antimicrob Agents Chemother. 2005;**49**:952–958.

[137] Karwa R and Wargo KA. Efungumab: a novel agent in the treatment of invasive candidiasis. Ann Pharmacother. 2009;**43**:1818–1823.

[138] Matthews RC, Rigg G, Hodgetts S, Carter T, Chapman C, Gregory C, Illidge C, and Burnie J. Preclinical assessment of the efficacy of mycograb, a human recombinant antibody against fungal HSP90. Antimicrob Agents Chemother. 2003;**47**:2208–2216.

[139] Hodgetts S, Nooney L, Al-Akeel R, Curry A, Awad S, Matthews R, and Burnie J. Efungumab and caspofungin: pre-clinical data supporting synergy. J Antimicrob Chemother. 2008;**61**:1132–1139.

[140] Nooney L, Matthews RC, and Burnie JP. Evaluation of Mycograb, amphotericin B, caspofungin, and fluconazole in combination against *Cryptococcus neoformans* by checkerboard and time-kill methodologies. Diagn Microbiol Infect Dis. 2005;**51**:19–29.

[141] Pachl J, et al. A randomized, blinded, multicenter trial of lipid-associated amphotericin B alone versus in combination with an antibody-based inhibitor of heat shock protein 90 in patients with invasive candidiasis. Clin Infect Dis. 2006;**42**:1404–1413.

[142] Magliani W, Conti S, Gerloni M, Bertolotti D, and Polonelli L. Yeast killer systems. Clin Microbiol Rev. 1997;**10**:369–400.

[143] Cenci E, et al. Protection of killer antiidiotypic antibodies against early invasive aspergillosis in a murine model of allogeneic T-cell-depleted bone marrow transplantation. Infect Immun. 2002;**70**:2375–2382.

[144] Polonelli L, Magliani W, Conti S, Bracci L, Lozzi L, Neri P, Adriani D, De Bernardis F, and Cassone A. Therapeutic activity of an engineered synthetic killer antiidiotypic antibody fragment against experimental mucosal and systemic candidiasis. Infect Immun. 2003;**71**:6205–6212.

[145] Polonelli L, et al. Yeast killer toxin-like candidacidal Ab6 antibodies elicited through the manipulation of the idiotypic cascade. PLoS One. 2014;**9**:e105727.

[146] Nosanchuk JD and Dadachova E. Radioimmunotherapy of fungal diseases: the therapeutic potential of cytocidal radiation delivered by antibody targeting fungal cell surface antigens. Front Microbiol. 2011;**2**:283.

[147] Bryan RA, Jiang Z, Howell RC, Morgenstern A, Bruchertseifer F, Casadevall A, and Dadachova E. Radioimmunotherapy is more effective than antifungal treatment in experimental cryptococcal infection. J Infect Dis. 2010;**202**:633–637.

[148] Bryan RA, et al. Toward developing a universal treatment for fungal disease using radioimmunotherapy targeting common fungal antigens. Mycopathologia. 2012;**173**:463–471.

[149] Dadachova E and Casadevall A. Treatment of infection with radiolabeled antibodies. Q J Nucl Med Mol Imaging. 2006;**50**:193–204.

[150] Dadachova E and Casadevall A. Radioimmunotherapy of infectious diseases. Semin Nucl Med. 2009;**39**:146–153.

[151] Bryan RA, Jiang Z, Morgenstern A, Bruchertseifer F, Casadevall A, and Dadachova E. Radioimmunotherapy of *Cryptococcus neoformans* spares bystander mammalian cells. Future Microbiol. 2013;**8**:1081–1089.

[152] Dadachova E and Casadevall A. *Cryptococcus neoformans* as a model for radioimmunotherapy of Infections. Interdiscip Perspect Infect Dis. 2011;**2011**:830286.

[153] Torosantucci A, et al. Protection by anti-beta-glucan antibodies is associated with restricted beta-1,3 glucan binding specificity and inhibition of fungal growth and adherence. PLoS One. 2009;**4**:e5392.

[154] Torosantucci A, et al. A novel glyco-conjugate vaccine against fungal pathogens. J Exp Med. 2005;**202**:597–606.

[155] Rachini A, et al. An anti-beta-glucan monoclonal antibody inhibits growth and capsule formation of *Cryptococcus neoformans in vitro* and exerts therapeutic, anticryptococcal activity *in vivo*. Infect Immun. 2007;**75**:5085–5094.

[156] Rosas AL, Nosanchuk JD, and Casadevall A. Passive immunization with melanin-binding monoclonal antibodies prolongs survival of mice with lethal *Cryptococcus neoformans* infection. Infect Immun. 2001;**69**:3410–3412.

[157] Alviano DS, Franzen AJ, Travassos LR, Holandino C, Rozental S, Ejzemberg R, Alviano CS, and Rodrigues ML. Melanin from *Fonsecaea pedrosoi* induces production of human antifungal antibodies and enhances the antimicrobial efficacy of phagocytes. Infect Immun. 2004;**72**:229–237.

[158] Li W, et al. Host defence against *C. albicans* infections in IgH transgenic mice with V(H) derived from a natural anti-keratin antibody. Cell Microbiol. 2007;**9**:306–315.

[159] Zhou Q, Gault RA, Kozel TR, and Murphy WJ. Immunomodulation with CD40 stimulation and interleukin-2 protects mice from disseminated cryptococcosis. Infect Immun. 2006;**74**:2161–2168.

[160] Felonato M, Pina A, de Araujo EF, Loures FV, Bazan SB, Feriotti C, and Calich VL. Anti-CD25 treatment depletes Treg cells and decreases disease severity in susceptible and resistant mice infected with *Paracoccidioides brasiliensis*. PLoS One. 2012;**7**:e51071.

[161] Chang KC, et al. Blockade of the negative co-stimulatory molecules PD-1 and CTLA-4 improves survival in primary and secondary fungal sepsis. Crit Care. 2013;**17**:R85.

[162] Bhagwat SP, Wright TW, and Gigliotti F. Anti-CD3 antibody decreases inflammation and improves outcome in a murine model of *Pneumocystis* pneumonia. J Immunol. 2010;**184**:497–502.

[163] Pikman R and Ben-Ami R. Immune modulators as adjuncts for the prevention and treatment of invasive fungal infections. Immunotherapy. 2012;**4**:1869–1882.

[164] Lin L, et al. Th1-Th17 cells mediate protective adaptive immunity against *Staphylococcus aureus* and *Candida albicans* infection in mice. PLoS Pathog. 2009;**5**:e1000703.

[165] Cassone A and Casadevall A. Recent progress in vaccines against fungal diseases. Curr Opin Microbiol. 2012;**15**:427–433.

[166] Hamad M. Antifungal immunotherapy and immunomodulation: a double-hitter approach to deal with invasive fungal infections. Scand J Immunol. 2008;**67**:533–543.

[167] Ito JI and Lyons JM. Vaccination of corticosteroid immunosuppressed mice against invasive pulmonary aspergillosis. J Infect Dis. 2002;**186**:869–871.

[168] Santurio JM, Leal AT, Leal AB, Festugatto R, Lubeck I, Sallis ES, Copetti MV, Alves SH, and Ferreiro L. Three types of immunotherapics against pythiosis insidiosi developed and evaluated. Vaccine. 2003;**21**:2535–2540.

[169] Reichard U, Herrmann S, and Asif AR. Vaccination approaches against opportunistic fungal infections caused by *Aspergillus fumigatus*. Curr Protein Pept Sci. 2014;**15**:424–429.

[170] Wormley FL, Jr., Cutright J, and Fidel PL, Jr. Multiple experimental designs to evaluate the role of T-cell-mediated immunity against experimental vaginal *Candida albicans* infection. Med Mycol. 2003;**41**:401–409.

[171] Wanachiwanawin W, et al. Efficacy of immunotherapy using antigens of *Pythium insidiosum* in the treatment of vascular pythiosis in humans. Vaccine. 2004;**22**:3613–3621.

[172] Watanabe MJ, de Moura Alonso J, Alves ALG, Yamada ALM, Bosco SdMG, Rodrigues CA, and Hussni CA. Equine pythiosis: report of 28 cases from São Paulo State, Brazil. Semina: Ciências Agrárias. 2015;**36**:909–916.

[173] Santos C, Santurio J, Colodel E, Juliano R, Silva J, and MARQUES LC. Contribution to the study of cutaneous pythiosis in equidae. Ars Veterinaria. 2011;**27**:134–140.

[174] Santos CE, Marques LC, Zanette RA, Jesus FP, and Santurio JM. Does immunotherapy protect equines from reinfection by the oomycete *Pythium insidiosum*? Clin Vaccine Immunol. 2011;**18**:1397–1399.

[175] Liu M, Capilla J, Johansen ME, Alvarado D, Martinez M, Chen V, Clemons KV, and Stevens DA. *Saccharomyces* as a vaccine against systemic aspergillosis: 'the friend of man' a friend again? J Med Microbiol. 2011;**60**:1423–1432.

[176] Capilla J, Clemons KV, Liu M, Levine HB, and Stevens DA. *Saccharomyces cerevisiae* as a vaccine against coccidioidomycosis. Vaccine. 2009;**27**:3662–3668.

[177] Liu M, Clemons KV, Johansen ME, Martinez M, Chen V, and Stevens DA. *Saccharomyces* as a vaccine against systemic candidiasis. Immunol Invest. 2012;**41**:847–855.

[178] Luo G, Gebremariam T, Clemons KV, Stevens DA, and Ibrahim AS. Heat-killed yeast protects diabetic ketoacidotic-steroid treated mice from pulmonary mucormycosis. Vaccine. 2014;**32**:3573–3576.

[179] Majumder T, Liu M, Chen V, Martinez M, Alvarado D, Clemons KV, and Stevens DA. Killed Saccharomyces cerevisiae protects against lethal challenge of *Cryptococcus grubii*. Mycopathologia. 2014;**178**:189–195.

[180] Liu M, Clemons KV, Bigos M, Medovarska I, Brummer E, and Stevens DA. Immune responses induced by heat killed *Saccharomyces cerevisiae*: a vaccine against fungal infection. Vaccine. 2011;**29**:1745–1753.

[181] Champer J, et al. Protein targets for broad-spectrum mycosis vaccines: quantitative proteomic analysis of Aspergillus and *Coccidioides* and comparisons with other fungal pathogens. Ann N Y Acad Sci. 2012;**1273**:44–51.

[182] Stevens DA, Clemons KV, and Liu M. Developing a vaccine against aspergillosis. Med Mycol. 2011;**49**(Suppl 1):S170–176.

[183] DiMiceli L, Pool V, Kelso JM, Shadomy SV, Iskander J, and Team VAERS. Vaccination of yeast sensitive individuals: review of safety data in the US vaccine adverse event reporting system (VAERS). Vaccine. 2006;**24**:703–707.

[184] Wüthrich M, Filutowicz HI, and Klein BS. Mutation of the WI-1 gene yields an attenuated Blastomyces dermatitidis strain that induces host resistance. J Clin Invest. 2000;**106**:1381–1389.

[185] Wuthrich M, Krajaejun T, Shearn-Bochsler V, Bass C, Filutowicz HI, Legendre AM, and Klein BS. Safety, tolerability, and immunogenicity of a recombinant, genetically engineered, live-attenuated vaccine against canine blastomycosis. Clin Vaccine Immunol. 2011;**18**:783–789.

[186] Wang H, LeBert V, Hung CY, Galles K, Saijo S, Lin X, Cole GT, Klein BS, and Wuthrich M. C-type lectin receptors differentially induce th17 cells and vaccine immunity to the endemic mycosis of North America. J Immunol. 2014;**192**:1107–1119.

[187] Saville SP, Lazzell AL, Chaturvedi AK, Monteagudo C, and Lopez-Ribot JL. Use of a genetically engineered strain to evaluate the pathogenic potential of yeast cell and filamentous forms during *Candida albicans* systemic infection in immunodeficient mice. Infect Immun. 2008;**76**:97–102.

[188] Goodridge HS, Wolf AJ, and Underhill DM. Beta-glucan recognition by the innate immune system. Immunol Rev. 2009;**230**:38–50.

[189] Vetvicka V and Vetvickova J. Beta 1,3-glucan silver bullet or hot air? Open Glycoscience. 2010;**3**:1–6.

[190] Giavasis I. Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. Curr Opin Biotechnol. 2014;**26**:162–173.

[191] Novak M and Vetvicka V. Glucans as biological response modifiers. Endocr Metab Immune Disord Drug Targets. 2009;**9**:67–75.

[192] Novak M and Vetvicka V. Beta-glucans, history, and the present: immunomodulatory aspects and mechanisms of action. J Immunotoxicol. 2008;**5**:47–57.

[193] Weitberg AB. A phase I/II trial of beta-(1,3)/(1,6) D-glucan in the treatment of patients with advanced malignancies receiving chemotherapy. J Exp Clin Cancer Res. 2008;**27**:40.

[194] Williams DL, Sherwood ER, Browder IW, McNamee RB, Jones EL, and Di Luzio NR. Pre-clinical safety evaluation of soluble glucan. Int J Immunopharmacol. 1988;**10**:405–414.

[195] Casadevall A and Pirofski L-a. Polysaccharide-containing conjugate vaccines for fungal disease. Trends Mol Med. 2006;**12**:6–9.

[196] Bromuro C, et al. Beta-glucan-CRM197 conjugates as candidates antifungal vaccines. Vaccine. 2010;**28**:2615–2623.

[197] Astronomo RD and Burton DR. Carbohydrate vaccines: developing sweet solutions to sticky situations? Nat Rev Drug Discov. 2010;**9**:308–324.

[198] Clemons KV, et al. Whole glucan particles as a vaccine against murine aspergillosis. J Med Microbiol. 2014;**63**:1750–1759.

[199] Clemons KV, Antonysamy MA, Danielson ME, Michel KS, Martinez M, Chen V, and Stevens DA. Whole glucan particles as a vaccine against systemic coccidioidomycosis. J Med Microbiol. 2015;**64**:1237–1243.

[200] Garner RE and Domer JE. Lack of effect of *Candida albicans* mannan on development of protective immune responses in experimental murine candidiasis. Infect Immun. 1994;**62**:738–741.

[201] Han Y and Cutler JE. Antibody response that protects against disseminated candidiasis. Infect Immun. 1995;**63**:2714–2719.

[202] Han Y, Kanbe T, Cherniak R, and Cutler JE. Biochemical characterization of *Candida albicans* epitopes that can elicit protective and nonprotective antibodies. Infect Immun. 1997;**65**:4100–4107.

[203] Han Y, Ulrich MA, and Cutler JE. *Candida albicans* mannan extract-protein conjugates induce a protective immune response against experimental candidiasis. J Infect Dis. 1999;**179**:1477–1484.

[204] Paulovičová E and Machová E. *Candida albicans* mannan–protein conjugate as vaccine candidate. Immunol Lett. 2003;**85**:251–255.

[205] Liu M, Machova E, Nescakova Z, Medovarska I, Clemons KV, Martinez M, Chen V, Bystricky S, and Stevens DA. Vaccination with mannan protects mice against systemic aspergillosis. Med Mycol. 2012;**50**:818–828.

[206] Paulovičová E, Bystrický S, Masárová J, Machová E, and Mislovičová D. Immune response to *Saccharomyces cerevisiae* mannan conjugate in mice. Int Immunopharmacol. 2005;**5**:1693–1698.

[207] Lipinski T, Fitieh A, St Pierre J, Ostergaard HL, Bundle DR, and Touret N. Enhanced immunogenicity of a tricomponent mannan tetanus toxoid conjugate vaccine targeted to dendritic cells via Dectin-1 by incorporating beta-glucan. J Immunol. 2013;**190**:4116–4128.

[208] Vecchiarelli A. Immunoregulation by capsular components of *Cryptococcus neoformans*. Med Mycol. 2000;**38**:407–417.

[209] Casadevall A, Mukherjee J, Devi SJ, Schneerson R, Robbins JB, and Scharff MD. Antibodies elicited by a Cryptococcus neoformans-tetanus toxoid conjugate vaccine have the same specificity as those elicited in infection. J Infect Dis. 1992;**165**:1086–1093.

[210] Devi SJ. Preclinical efficacy of a glucuronoxylomannan-tetanus toxoid conjugate vaccine of *Cryptococcus neoformans* in a murine model. Vaccine. 1996;**14**:841–844.

[211] Mukherjee J, Nussbaum G, Scharff MD, and Casadevall A. Protective and nonprotective monoclonal antibodies to *Cryptococcus neoformans* originating from one B cell. J Exp Med. 1995;**181**:405–409.

[212] Oscarson S, Alpe M, Svahnberg P, Nakouzi A, and Casadevall A. Synthesis and immunological studies of glycoconjugates of *Cryptococcus neoformans* capsular glucuronoxylomannan oligosaccharide structures. Vaccine. 2005;**23**:3961–3972.

[213] Datta K and Pirofski LA. Towards a vaccine for *Cryptococcus neoformans*: principles and caveats. FEMS Yeast Res. 2006;**6**:525–536.

[214] Maitta RW, Datta K, Lees A, Belouski SS, and Pirofski LA. Immunogenicity and efficacy of *Cryptococcus neoformans* capsular polysaccharide glucuronoxylomannan peptide mimotope-protein conjugates in human immunoglobulin transgenic mice. Infect Immun. 2004;**72**:196–208.

[215] Fleuridor R, Lees A, and Pirofski L. A cryptococcal capsular polysaccharide mimotope prolongs the survival of mice with *Cryptococcus neoformans* infection. J Immunol. 2001;**166**:1087–1096.

[216] Datta K, Lees A, and Pirofski LA. Therapeutic efficacy of a conjugate vaccine containing a peptide mimotope of cryptococcal capsular polysaccharide glucuronoxylomannan. Clin Vaccine Immunol. 2008;**15**:1176–1187.

[217] Ada G and Isaacs D. Carbohydrate-protein conjugate vaccines. Clin Microbiol Infect. 2003;**9**:79–85.

[218] Avci FY, Li X, Tsuji M, and Kasper DL. Carbohydrates and T cells: a sweet twosome. Semin Immunol. 2013;**25**:146–151.

[219] Anish C, Schumann B, Pereira CL, and Seeberger PH. Chemical biology approaches to designing defined carbohydrate vaccines. Chem Biol. 2014;**21**:38–50.

[220] Shinefield HR. Overview of the development and current use of CRM 197 conjugate vaccines for pediatric use. Vaccine. 2010;**28**:4335–4339.

[221] Tontini M, et al. Comparison of CRM 197, diphtheria toxoid and tetanus toxoid as protein carriers for meningococcal glycoconjugate vaccines. Vaccine. 2013;**31**:4827–4833.

[222] Hole CR and Wormley FL, Jr. Vaccine and immunotherapeutic approaches for the prevention of cryptococcosis: lessons learned from animal models. Front Microbiol. 2012;**3**:291.

[223] Spellberg B, Ibrahim AS, Lin L, Avanesian V, Fu Y, Lipke P, Otoo H, Ho T, and Edwards Jr JE. Antibody titer threshold predicts anti-candidal vaccine efficacy even though the mechanism of protection is induction of cell-mediated immunity. J Infect Dis. 2008;**197**:967–971.

[224] Hennessey Jr J, Schmidt C, Ibrahim A, Filler S, White C, Yeaman M, Fu Y, and Edwards J. A Phase 1 clinical evaluation of NDV3, a vaccine to prevent disease caused by *Candida*

spp. and *Staphylococcus aureus*. In 51st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago. Washington, DC: American Society for Microbiology. 2011; of Conference; 2011 p.

[225] Sandini S, La Valle R, Deaglio S, Malavasi F, Cassone A, and De Bernardis F. A highly immunogenic recombinant and truncated protein of the secreted aspartic proteases family (rSap2t) of *Candida albicans* as a mucosal anticandidal vaccine. FEMS Immunol Med Microbiol. 2011;**62**:215–224.

[226] Segal BH, Wang XY, Dennis CG, Youn R, Repasky EA, Manjili MH, and Subjeck JR. Heat shock proteins as vaccine adjuvants in infections and cancer. Drug Discov Today. 2006;**11**:534–540.

[227] Assis-Marques MA, Oliveira AF, Ruas LP, dos Reis TF, Roque-Barreira MC, and Coelho PS. *Saccharomyces cerevisiae* expressing Gp43 protects mice against *Paracoccidioides brasiliensis* infection. PLoS One. 2015;**10**:e0120201.

[228] Ribeiro AM, Bocca AL, Amaral AC, Souza ACC, Faccioli LH, Coelho-Castelo AA, Figueiredo F, Silva CL, and Felipe MSS. HSP65 DNA as therapeutic strategy to treat experimental paracoccidioidomycosis. Vaccine. 2010;**28**:1528–1534.

[229] Ribeiro AM, Bocca AL, Amaral AC, Faccioli LH, Galetti FC, Zarate-Blades CR, Figueiredo F, Silva CL, and Felipe MS. DNAhsp65 vaccination induces protection in mice against *Paracoccidioides brasiliensis* infection. Vaccine. 2009;**27**:606–613.

[230] Gomez FJ, Allendoerfer R, and Deepe GS, Jr. Vaccination with recombinant heat shock protein 60 from *Histoplasma capsulatum* protects mice against pulmonary histoplasmosis. Infect Immun. 1995;**63**:2587–2595.

[231] Deepe Jr GS and Gibbons RS. Cellular and molecular regulation of vaccination with heat shock protein 60 from *Histoplasma capsulatum*. Infect Immun. 2002;**70**:3759–3767.

[232] Taborda CP, Juliano MA, Puccia R, Franco M, and Travassos LR. Mapping of the T-cell epitope in the major 43-kilodalton glycoprotein of *Paracoccidioides brasiliensis* which induces a Th-1 response protective against fungal infection in BALB/c mice. Infect Immun. 1998;**66**:786–793.

Section 4

Experimental Immunotherapy

Chapter 14

Immunotherapy with Dialyzable Leukocyte Extracts Containing Transfer Factor

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Additional information is available at the end of the chapter

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Abstract

Dialyzable leukocyte extracts (DLE) are complexes consisting of a large number of low molecular weight substances. These extracts possess immunomodulatory properties, which are mainly attributed to small peptides with molecular weight of 3.5–6.0 kDa called "transfer factor." This chapter reviews the nature and immunological characteristics of DLE containing transfer factor (TF), their mechanism of action and the possible uses as immunomodulators in human and veterinary medicine. A main advantage of TF-preparations as immunotherapeutic agents is that they induce a rapid immune response against the pathogen (within 24 h) and thereby reduce the time for the patient immune response by 9–13 days. The low level of difficulty of the process of obtaining protocols determines their relatively low cost and the possibility to combine them with other therapeutic agents during treatment makes them subject to medical applications in the future, including against some new diseases.

Keywords: animal models, cytokines, chromatography, diseases, dialyzable leukocyte extract, lymphocytes, transfer factor, ultrafiltration

1. Introduction

The immune system is extremely effective when it protects the organism from foreign (or own, but modified) genetic substances. However, some antigens that trigger the immune system—microbial or cancer cells, have devised a number of techniques to avoid the immune reaction. This means that the immune system constantly adapts to new attacks and eliminates the antigens by identifying each cell.

Autoimmune diseases occur when the recognition process of cells breaks down, resulting in the destruction of healthy cells and tissues. That is, when the immune system is essentially "popular up," which can be difficult to treat.

This requires the search for new approaches to immunotherapy of infectious, oncological and autoimmune diseases. In the recent years, preparations, called "dialyzable leukocyte extracts" (DLE), were introduced to immunotherapy.

The dialyzable leukocyte extracts are complexes that are built up by approximately 200 substances that have low molecular weight [1–3]. DLE have immunomodulatory properties, which are due to small peptides—the "transfer factor" (TF). The rest of the components in the extracts have different biochemical and immunological properties and contribute to the immunomodulatory effect carried by TF.

In 1955, Dr. H. Sherwood Lawrence defined the transfer factor stating that cell-mediated immune response to antigens (allergens) can be passively transferred by DLE of viable human leucocytes from a donor of immunity to a naïve recipient [4]. He prepared an intracellular extract from circulating leucocytes of patients who had been exposed to tuberculosis (TB) and then injected the leukocyte extract into non-exposed to TB volunteer patients. Using a delayed type hypersensitivity test, Dr. Lawrence demonstrated that the immune system of non-exposed to TB patients treated with leukocyte extract can recognize TB and response to it, as if has already fought it. Therefore, the immune response to a certain antigen can be "transferred" from one person to another using a leukocyte extract. The transfer factor can instruct the immune system to do several different things, thereby influencing immunity via different paths and can come in three different sizes. Thus, the induction of a number of different effects on the immune system released by leukocyte extracts implies the presence of more than one active factor in it, and there seem to be multiple factors involved in transferring immunity [5]. One activity is the presence of the inducer and helper functions (the so-called inducer factor and antigen-specific factor). An additional activity is the presence of the suppressory (regulatory) function (the so-called suppressor factor) [6].

Later on, it was found that the transfer of immunity by leukocyte derivatives is also possible from one species to another. This universal ability makes it possible to get the TF-preparations from a species which is different to the recipient species.

The discovery of this phenomenon gave new opportunities in the field of medicine. Since cell-mediated immunity (CMI) is crucial for controlling infections, as well as cancer, autoimmune diseases, immunodeficiencies and allergies, the transfer factor can be used in the treatment of these cases [1, 3, 7–10].

The author will review the nature and immunological characteristics of DLE-containing TF, their mechanism of action and the possible uses as immunomodulators in human and veterinary medicine.

2. Composition, physicochemical and biochemical properties of DLE

Transfer factor, the main component of the DLE, has a molecular weight of 3.5–6.0 kDa. It consists of small peptides and oligoribonucleotides [3]. The ribonucleotide is attached to the amino terminus of the peptide. The specific activity of TF is due to the peptides weighing 5.0 kDa [11].

Transfer factor peptides have the same amino acid sequence, regardless of their origin and species belonging. In 2000, while analyzing the peptide partial sequences of transfer factors, Kirkpatrick [12] found a novel amino acid consensus sequence LLYAQDL/VEDN, which is found in each of the analyzed TF-preparations. This novel sequence binds with high affinity to specific receptors of target cells (the so-called TF receptors). However, tyrosine and glycine are always more concentrated in TF [9]. The N-terminal region of these peptides is very similar to some neuropeptides, such as the enkephalins [13].

Apart from transfer factor, the extracts also contain cyclic nucleotides, ascorbate, prostaglandins, histamine, serotonin, nicotinamide and some amino acids and purine bases [14]. DLE preparations are transparent, pyrogen-free, light yellow fluids, with pH 5.5–7.0 or lower (pH 5.6–6.8) depending on the method of obtaining [2, 10, 15]. The ratio OD_{260}/OD_{280} (absorbance index, which represents the ratio of nucleotides to peptides) of the extracts ranges from 1.8 to 3.0 depending on the method of preparation [15–18]. The index values show that nucleotides are relatively predominant over peptides. The osmolarity of DLE preparation was measured by Grob et al. [15] and has a value of 520 ± 90 mOsm/L.

TF is resistant to treatment with DNase, pancreatic RNase and trypsin, but cannot withstand snake venom phosphodiesterase. It is stable in conditions of deep freezing, but it is not over certain temperatures. In particular, DLE preparations are biologically active for several years when kept at temperatures ranging between −20°C and −70°C. The fact that the double-stranded nucleic acid can melt determines TF's heat sensitivity [10]. As it is a low molecular weight mixture, the dialyzable leukocyte extracts are not immunogenic and contain no histocompatibility antigens [3, 10].

3. Mechanism of action and immunological properties of the transfer factor

The transfer factor is produced by CD4+Th1 cells during the immune response to an antigen. Its biological activity includes different, sometimes opposite effects. Transfer factor contains the following constituents: inducer, antigen-specific and suppressor factors [6, 10].

The inducer factor sets the immune system in a state of readiness by sending a specific signal to the cells. When nonimmune leukocyte populations are cultured with inducer factor, they acquire the capacity to respond to a specific antigen [6]. It enhances the antigenic stimulus, which causes the production of interferon gamma (IFN-γ), interleukin (IL)-2 and tumor necrosis factor alpha (TNF-α) by CD4+Th1 cells. As a consequence, cell-mediated immune response develops against the target antigen [10] and it includes interleukins (Il-6 and Il-8) formed by activated monocytes [19, 20].

The regulation of the production of TNF-α, Il-6 and Il-8 is associated with effects on toll-like receptors (TLR2 and TLR4) expression and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and cyclic adenosine monophosphate activities [19]. Subsequently, however, the role of the ligands for TLR4 as regulators of the production of these cytokines was excluded [21, 22].

The antigen-specific components help the immune system to identify the antigens by using a variety of tags that identify them. They are informational molecules that are involved in the immune recognition of antigens that have penetrated the organisms and are also included in the formation of the immune memory.

When immune leukocyte populations are cultured with suppressor factor, their response to a specific antigen is blocked [6]. The suppressor factor maintains the balance in the immune system, preventing its overactivity in the absence of any new threats to the body. This helps to control the autoimmune diseases and to improve the adaptability of the immune system. In general, the mechanism of action of the suppressor factor can be defined as a catalytic immune response. Suppressor fractions are involved in the regulation and weakening of the immune response to the antigen by stimulating the formation of IL-10 and inhibiting cytokines by Th2 cells [6, 10, 23]. According to Burger et al. [23], a component of TF (fraction IV from exclusion chromatography on Sephadex G-25) possess immunosuppressive activity. This component was identified as nicotinamide. In our study on the immunological activity of rabbit DLE, we found that suppressor ingredients of the extract have different molecular masses (in peaks I, II, III, V and VI)—**Figure 1**.

In the dialyzable leukocyte extracts, molecules with adjuvant-like activity can also be identified. This component of DLE possesses a nonspecific activity expressed by enhancing the immune response to other antigens or allergens [24]. These fractions are with molecular weight <3.5 kDa and cause two types of immune response: (1) amplification of the response to antigens to which the donor has preexisting immunity and (2) the induction of inflammatory response histologically resembling delayed hypersensitivity in the absence of an added antigen. The substances mediating these responses could be divided into unique components by the use of a long (1 × 150 cm) G-10 column or by hydroxylapatite chromatography. We found fractions with adjuvant-like activity in rabbit DLE in peak IV in gel filtration Sephadex G-25 (**Figure 1**) [25].

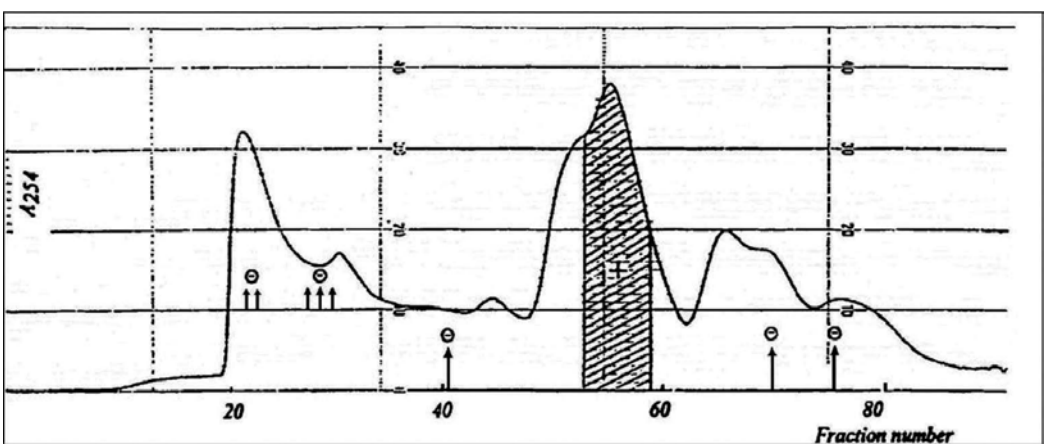

Figure 1. Elution profile of DLE, obtained from rabbit lymph nodes and spleen after gel-filtration through Sephadex G-25 (column 2.6/70). Liquid chromatography system Pharmacia. The fraction with adjuvant-like activity is marked with ⊕ and suppressor fractions are marked with ⊖ [25].

Fractions with hematopoietic activity can also be detected in the extracts [26] as well as with in vitro antibacterial activity [27].

In vivo, DLE enhanced recovery of the pool of granulocyte-macrophage hemopoietic progenitor cells (GM-CFC) in the bone marrow of normal or sublethally irradiated mice and increased survival of mice exposed to a lethal radiation dose. In vitro, sera of mice treated with DLE-induced GM-CFC colony formation in cultures of normal mouse bone marrow cells, i.e., produced colony-stimulating activity (CSA) [26].

Low molecular weight fraction of bovine DLE (below 3.5 kDa, the so-called fraction S) has bactericidal and bacteriostatic activity in vitro against pathogenic bacterial strains *Staphylococcus aureus, Streptococcus pyogenes, Lysteria monocytogenes, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*. These results showed a remarkable in vitro antibacterial property of bovine DLE against several pathogenic bacteria [27].

DLE regulate the expression of the hBD-2 and LL-37 genes [28]. Since the two peptides (hBD-2 and LL-37) have antibacterial action, DLE play a major role in the innate immune defense against invasive bacterial infections and inflammations. The probability that a microorganism shows resistance to these peptides is much lower than presented by antibiotics. However, these peptides have the ability to immunoregulate the acquired immune response, thus promotes inflammation without an eye injury.

Furthermore, the lymphocytes of a naïve recipient can serve as a replicator of transfer factor. This is achieved by integrating the specifics of the injected TF in the recipient lymphocytes, which effectively means that the recipient can be seen as a TF donor as well. This phenomenon is known as "the black box effect" and allows us to obtain TF-preparations from donors which have been infected with an unknown pathogen [29].

Therefore, TF develops CMI in patients who are suffering from immunodeficient infectious diseases, as well as in disorder with certain anergies. It is agreed that transfer factor is more efficient in educating naïve cells about the approaching danger [10]. It takes its part in the whole process of activation of the immune response by controlling and preventing immune overreaction and mistargeted reaction in the development of autoimmune diseases.

4. Obtaining transfer factor preparations

TF-preparations may be obtained from animal and human sources by injecting them with certain pathogen to produce specific transfer factor [10]. The first TF-preparations were obtained in the laboratory of Dr. Lawrence [4] though dialysis of human leukocyte cryolysates. Furthermore, dialysis as a separation method of low-molecular-weight components was replaced by ultrafiltration [8, 26, 30]. Membrane filters with a cutoff of <12 kDa are used in the procedure. Apart from human leucocytes, TF-preparations are obtained from ultrafiltrated animal cryolysed leukocytes or lymphoid organs (lymph nodes and spleen) [17, 18, 26, 31, 32].

Transfer factor can also be obtained from bird eggs and the method is patented [33]. TF with activity against HBV was extracted from egg yolk [34].

Another source of TF is bovine colostrum. A method for extracting TF from colostrum was prepared, which currently has very wide application [35]—the reason is that by using a relatively simple procedure, large amounts of TF can be obtained and the donor cows can be immunized with different antigens depending on the purpose. TF which has been obtained from bovine colostrum is patented as a commercial product (4life TF) [36].

DLE may be further subjected to purification using column chromatography, high-performance liquid chromatography [10, 37] and molecular exclusion liquid chromatography [38]. Through these procedures not only a better purification of the preparations can be achieved, but also different fractions can be isolated. The figure below presents the elution profile of DLE after gel-filtration on Sephadex G-25 (**Figure 1**).

Six peaks were obtained as the active fractions were eluted in peak IV. Some fractions in peaks I, II, III, V and VI possess suppressive activity.

5. Application of DLE preparations in human and veterinary medicine

The application of DLE or TF preparations in medicine is based on the influence of transfer factor and other components of dialyzable leukocyte extracts on the function of a number of components of the immune system and on regulating the synthesis of cytokine. When the immune system of an organism interacts with a pathogen, at least one TF is created in every instant and this reaction applies to all pathogens [39].

The rapidity of the reaction against pathogens is a strong advantage of TF-preparations as therapeutic agents (the response is within 24 h). This is a fraction of the time for a complete cell-mediated response of the immune system to a pathogen, which is 10–14 days.

Cancer, heart diseases, Alzheimer's, rheumatoid arthritis, hepatitis and other major diseases are caused by abnormalities in the formation of the transfer factor in CD4+Th1 cells. This determines the wide range of possibilities to use TF preparations in the field of medicine.

Another possibility of immunotherapy with DLE and TF is the use of the so-called the black box effect. There are two ways to use this effect: (a) initially receiving TF from patients recovering from infection with an unknown pathogen that replicate to naïve experimental animals or in tissue culture of lymphoblastoid cell line. The immune system of the experimental animals and lymphoblastoid cells from the tissue culture function as an effective copier, which can produce specific transfer factor activity to the unknown pathogen; (b) unidentified pathogen isolated from the tissues of the patient is injected into a naïve experimental animals that produce antigen-specific TF. This method has been used for the preparation of the first human immunodeficiency virus (HIV)-specific preparation TF in1983 before it is established viral etiology of AIDS [40].

In our studies in the 1990s, we have received and tested dialyzable leukocyte extracts of blood and lymphoid organs of pigs from farms with considerable respiratory and gastrointestinal diseases in newborn and young animals. Given the polyetiological character of these diseases, the approach of obtaining DLE through the "black box effect" proved successful—mortality and morbidity of these diseases on the farm that was treated with DLE were significantly reduced.

5.1. Cancer

There are about 100 reports on the effect of TF on cancer, either in patients suffering from cancer, or on animal and in vitro models.

Pineda et al. [41] studied the efficacy of TF as immunotherapy to treat experimental glioblastoma (brain cancer involving glial cells) in rats. TF was obtained from immunized swine and administered at a dose of 4×10^6, 8×10^5 and 1.6×10^5 cells, respectively. The best dose was 4×10^6 cells. TF was also combined with carmustine for experimental therapy in rats with C6 malignant glioma. The authors observed that treating rats reduces the size of the tumor and increases the CD2+, CD4+, CD8+ and natural killer cell counts. The percentage of apoptotic tumor cells and the percentage of tumor tissue expressing Th1 cytokines were also increased. The study demonstrated the beneficial effects of using both TF and chemotherapy, this application having a synergic effect. Therefore, it is possible to decrease the doses of chemotherapy while preserving the same effect of the treatment.

In vitro research has revealed that the ability of lymphocytes to kill cancer cells is aided by transfer factor. DNA fragmentation in MCF-7 breast cancer cells can be caused by DLE obtained from cattle. These DLE can also induce the cytotoxic effect and suppression of some proteins that are associated with apoptosis (TP53, Bag-1, c-Myc, Bax, Bcl-2 and Bad) at the level of mRNA expression in MCF-7 breast cancer cells [42]. Bim mRNA expression was not detected. The extract did not affect the viability of normal mononuclear cells. The extract had dose-dependent cytotoxic effects and demonstrated an IC50 at a dosage of 0.06 U/mL. It induced DNA fragmentation in cancer cells at doses of 0.06 and 0.13 U/mL.

Treatments against cancer with TF were performed about 40 years ago. Fudenberg [43] found that treatment of patients with osteosarcoma with transfer factor derived from selected donors increased cell-mediated cytotoxicity. It appears that treatment with TF can provide prophylaxis against metastases when administered to patients without clinically apparent metastases at the time of surgical removal of the primary tumor. However, initial results from the viewpoint of whether immunotherapy with TF is more effective than chemotherapy were unproven and controversial.

When testing a TF preparation (Transferón®) as an adjuvant to chemotherapy in patients with osteosarcoma in stages III and IV, Juarez [44] recorded an increase in the number of CD3+ CD8+, CD16+ and CD56+ in the blood of the patients. The author also observed that the patients that were treated with Transferón® remained at the same stage without any new metastatic lesions.

Pizza et al. [45] conducted a follow-up investigation, ranging from 1 to 9 years. They were treated with TF 50 patients with prostate cancer unresponsive to conventional therapy. In 44% of them, a beneficial effect was observed (higher survival rates). The investigation showed that complete remission was achieved in 2 patients, partial remission in 5.1, and no progression of metastatic disease in 14. The median survival was 126 weeks, higher than the survival rates reported in the literature for patients of the same stage.

Immunotherapy with TF has been tested in lung cancer patients also. The rationale for using TF in this type of cancer is that the possibility of improving their cell-mediated immunity to tumor associated antigens may improve their survival. Pilotti et al. [46] obtained beneficial results regarding the treatment of lung cancer with TF used as an adjuvant. TF was extracted from the lymphocytes of blood bank donors. During 11 years, 99 non–small cell lung cancer (NSCLC) resected patients were monthly treated with TF. In the same period, 257 NSCLC-resected patients were considered as non-treated controls. The survival rates of the treated patients were significantly improved compared to untreated both for patients in stages 3a and 3b and patients with histological subtype large cell carcinoma. Survival of treated patients is also significantly higher for patients with lymph node involvement (N2 disease). These results suggest that the administration of TF to NSCLC resected patients may improve their survival.

Continuing their previous research, Franco-Molina et al. [47] showed that adjuvant immunotherapy with bovine dialyzable leukocyte extract (in the form of the preparation IMMUNEPOTENT CRP) against lung cancer can cause an immunomodulatory effect. Twenty-four NSCLC patients were included in the study and divided into two groups. Group 1 received a conventional treatment of 5400 cGy external radiotherapy in 28 fractions and chemotherapy consisting of intravenous cisplatin 40 mg/m^2 delivered weekly for 6 weeks. Group 2 received the conventional treatment plus IMMUNEPOTENT CRP (5U) administered daily. The administration of IMMUNEPOTENT CRP induced immunomodulatory activity—increasing the total leukocytes and T-lymphocyte subpopulations CD4+, CD8+, CD16+ and CD56+ and maintaining DHT) and increased the quality of the patients' lives, suggesting immunologic protection against chemotherapeutic side effects in NSCLC patients. These results suggest the possibility of using IMMUNEPOTENT CRP alongside radiation and chemotherapy for maintaining the immune system and increasing the quality of life of the patients.

Therefore, it can be concluded that the TF-preparations, alone or in combination with conventional anticancer treatments can be applied successfully against some types of cancer. However, the scope of research on immunotherapeutic effect of the TF should expand to include other cancers.

5.2. Human infectious, parasitic and allergic diseases

5.2.1. Viral infections

5.2.1.1. Retroviral infections (AIDS and simian AIDS)

Since CMI plays a major role in the control of AIDS, it is considered that TF preparations can be favorable for patients with this disease. DLE can reduce the transcription of HIV-1 and inactivate the NF-κB signaling pathway [32].

The first clinical trial on a TF preparation for AIDS treatment was conducted by Viza et al. [40]. Transfer factor was prepared by immunization of mice with leukocytes from an AIDS patient and replicated in the LDV/7 cell line. In the study, three patients took TF orally for 3–5 months. The results of the treatment were overall clinical improvement and restoration of the skin test reactivity of the patients and a slight increase in their CD4+ cell counts.

Similar results were obtained in the treatment of AIDS patients with non-HIV-specific TF preparations where DTH was restored [48–50] (as mentioned in Ref. [29]). This is associated with the expression of IL-2 receptors of T-lymphocytes [49].

In later research, Pizza et al. treated 25 seropositive patients with mouse-derived HIV-specific transfer factor, which was taken orally by the patients for different time periods ranging from 60 to 1870 days. The results of the treatment were generally favorable; DTH was restored to recall antigen and CD4+ cell counts in 11 of the patients and CD8+ cell counts in 15 of the patients was increased [51]. Such an increase in CD8+ (as well as an increase in the total leukocyte number and the Il-2 level) in AIDS patients after treatment with HIV-specific TF has also been reported by other authors [52].

The cited data indicate that further studies on immunotherapy of HIV infection should be focused on stimulating of cellular, rather than the humoral immune response. This is because despite of the higher expenses for research, the initial hopes and expectations of obtaining an effective anti-HIV vaccine have been dashed for more than 30 years [29].

Both HIV-specific and non-HIV-specific TF preparations can be used as an adjunctive treatment for AIDS. Since the resistance to HIV infection depends on the functional condition of the T cytotoxic lymphocytes, transfer factor can make them "instructed" to resist more effectively. Recent data support this thesis. Resistance to HIV depends on genes of the HLA complex that play a role in the immune recognition of the virus by the T lymphocytes, and the presentation of the viral capsid to the CD8+ T cells is crucial in this process [29].

The simian immunodeficiency virus (SIV) infection, producing simian AIDS (SAIDS) in macaques, is the most accessible model, and presents similarities to that of HIV.

Thus, SIV-specific TF was used to investigate its effect on experimental infections with SIV on macaques model. SIV-specific TF was obtained by Viza et al. [53] from the helper and/or the cytotoxic lymphocyte subpopulations, as well as the total lymphocyte population of mice immunized with SIV and reproduced in cell culture by the LDV/7 cells. During a 108-day observation period, the authors found that several hematological and immunological parameters of treated macaques were significantly different from those of the nontreated with TF. The CD4/CD8 ratio, as well as the CD4 cells and platelet counts, showed significant variations between the treated and the non-treated macaques. The animals treated with TF derived from cells enriched with extracts of CD8+ T showed best results.

5.2.1.2. Herpesvirus infections

TF-preparations have been tested on infections with the *Herpes simplex virus* (HSV), Cytomeg*alovir*us (CMV), *Varicella zoster*, Hodgkin's lymphoma and Epstein-Barr virus (EBV) in order to prove their efficiency against them.

Khan et al. [54] were the first to treat HSV with specific TF. In 1981, 16 patients suffering from recurrent herpes—HSV-1 (cold sores) and HSV-2 (genital) were injected with TF on a weekly or monthly basis. The results of this treatment were encouraging—eight patients stopped having outbreaks altogether whereas the remaining eight exhibited a significant reduction in the frequency of outbreaks.

It was also found that the treatment with TF has led to an increase in the number of T lymphocytes; while in approximately half of the patients, it had been reduced prior to the treatment.

The effects of TF against *Varicella zoster* infections have been clinically studied in many works [55–57]. In summary, it has been established that TF serves both as protection and therapy against this type of infections. Compared to untreated patients, patients treated with TF show an increase of CD4+ cells, the γ-interferon level and the CD4/CD8 ratio. The main advantage of TF compared to other antiviral agents is that it induces the production of γ-interferon, which is crucial in the treatment of this infection.

In other herpes infections, transfer factor preparations have shown the best effect against the CMV infection. The treatment with TF develops a cell mediated immune response against CME and disappearance of viremia which leads to dramatic clinical improvements in the patients [29].

The treatment with a specific TF preparation may prevent the re-induction of EVB-induced diseases, but it has no clinical effect against Hodgkin's lymphoma [29].

5.2.1.3. Other viral infections

The effect of specific TF preparations has been tested in cases of infections with other viral diseases, such as viral hepatitis and the human papilloma virus. After treatment of patients with hepatitis B, the biopsy results were encouraging and at the same time, the data were supported by a number of biochemical and immunological indices [58, 59].

Another application of specific TF preparations is their use as an alternative to vaccines against newly occurred deadly influenza viruses. This is due to the fact that they can be produced considerably quickly and because they do not carry the risk of accidents during the production of recombinant vaccine strains of influenza viruses in laboratories [29].

5.2.1.4. Mycobacterial infection

These diseases result from the defect or absence of a cell-mediated immune response to mycobacteria. Thus, it can be presumed that TF could be beneficial in cases of these infections, enhancing the CMI.

Patients who did not respond to conventional therapy were used to trial the effects of TF against *Mycobacterium tuberculosis*. The test was conducted over 40 years ago and the results of the treatment included enhanced CMI reactivity and overall improvement of the participants' clinical condition [60]. Later on, Viza et al. [29] proved that the therapeutic effect of TF against *M. tuberculosis* is dose-dependent. Moreover, TF can be used as adjuvant in cases of ganglionic and cutaneous tuberculosis resistant to conventional treatments [61].

The mechanisms of action of TF against *M. tuberculosis* were studied using the mouse model. The specific TF was produced from tuberculous BALB/c mice following intra-tracheal infection. The treatment with TF leads to restoration of the expression of the Th1 cytokine pattern, whereas the increase of delayed type hypersensitivity leads to inhibition of bacterial proliferation and animal survival [62].

TF preparations against *Mycobacterium leprae*, *Mycobacterium fortuitum pneumonia* and *Mycobacterium xenori* were also tested (quote by Viza et al.) [29]. Treatments with TF preparations to patients suffering from leprosy were performed for a fairly long time (over 40 years). Improvement in the condition of patients was achieved, but the results were not convincing.

5.2.1.5. Fungal infection

TF preparations were obtained and tested against chronic mucocutaneous candidiasis, coccidiodomycosis and fungal keratitis.

The treatment showed a positive effect on *Candida albicans*, increasing the immunological reactivity of the treated patients [63].

Graybill et al. [64] treated three patients with progressive coccidioidomycosis. Prolonged clinical remission was found in two of them. Comparable results have been obtained by Catanzaro et al. [65].

Thirty-three-year-old man with fungal keratitis was treated with dialyzable leukocyte extract as adjuvant. After TF therapy, it was observed diminished infiltration of the corneal stroma and epithelial healing; interestingly, the systemic Immunological changes characterized by increased frequency of IFN-g+ cells were coincident with the clinical progress observed in the patient [66].

5.2.1.6. Parasitoses and allergies

TF have been used in cutaneous leishmaniosis, cryptosporidiosis (in AIDS patients) and echinococcosis [29, 67–70].

Several months of therapy against leishmaniosis with TF has led to a considerable healing of the lesions in patients whose disease had persisted for 8–30 years [67].

When treated with TF against cryptosporidiosis, a decrease in bowel movement frequency was found, as well as a significant weight gain, with eradication of oocytes from the stool in two of them [70].

Influencing these diseases is associated with increasing the CMI response [68], inducing the formation of IFN-γ and inhibiting of IL-5 synthesis [69].

Transfer factor may be a beneficial adjuvant in the treatment of allergic rhinitis [71] and atopic dermatitis [72].

TF-preparation (Transferon) induced some low-frequency non-serious adverse events during adjuvant treatment of patients with immune-mediated diseases [73].

5.3. Animal infectious and parasitic diseases

The large economic losses caused by outbreaks in domestic animals necessitated the search for new approaches to reduce and control them. That is why almost immediately after the first attempts to use transfer factor in human medicine, experiments with TF were also initiated in veterinary medicine [2, 7]. The first experiments carried out in this area were with TF against parasites and intracellular microorganisms.

The first publications on testing TF preparations in the field of animal health are for treating coccidiosis. Liburg et al. [74] have shown that treatment with specific DLE from rats, which have been experimentally infected with *Eimeria nieschulzi*, causes a reduction in the number of oocysts in their feces. Similar results were received for other types of coccidia in domestic animals by Klesius and Kristensen [75] and Klesius and Giamborne [76]—the oocysts in the feces were reduced. Specific TF preparations were used to treat *Eimeria bovis* in calves and *Eimeria tenella* in chickens.

The specific DLE preparations can be successfully used in ruminants to prevent nematodoses. Furthermore, *Trichostrongylus axei, Trichostrongylus colubriformis, Ostertagia circumcinta, Ostertagia ostertagi* and *Haemonchus controtus* were the focus of research on the action of both nondialyzed leucocytes lysate and dialyzable TF preparations. The worm burden was significantly reduced as a result of the treatment with DLE. Furthermore, nondialyzed leucocytes lysates proved to be more effective compared to dialyzable transfer factor preparations [7].

Dialyzable leukocyte extracts were also tested for their ability to prevent salmonella infections in domestic animals. The first successful studies on the protective effect of TF against *Salmonella typhimurium* were performed on mouse model [17, 77]. Later, Mikula et al. [78] and Mikula et al. [2] reported bacteriological, immunological and clinical trials of specific and non-specific DLE on calves which have been experimentally infected with a virulent strain *Salmonella typhimurium*. Injecting the calves intravenously with specific DLE protected them against the experimental infection with a pathogenic strain *S. typhimurium* 4/5. The number of salmonellas in the feces of the calves was reduced, the phagocytic activity of the leukocytes was activated, the number of lymphocytes in the peripheral blood was increased and specific CMI was developed—which proves the protective action of the specific DLE.

The activity of specific preparations against experimental infection with *Salmonella choleraesuis* was tested using the mouse model [25]. The treating of the mice with DLE induced a high specific protective effect (70%). Diffuse proliferation of activated macrophages in the lamina propria of the small intestine at the place of penetration of Salmonellas has been observed. The proliferation of activated macrophages is a manifestation of cell-mediated immune response to Salmonellas in the penetrated tissues [79].

DLE-preparation has also been tested against natural Salmonella infection in pigs by us. Treatment of animals has led to limiting morbidity and coping with the infection at the farm. The effectiveness of specific DLE against infections with *Salmonella enterica* subsp. *enteritidis*

in chickens has also been examined [80]. It has been found that the treatment of chickens with DLE reduces the presence of *S. enterica* in the caecum.

The protective effect of the transfer factor preparation against some viral and bacterial infections in piglets was examined [81]. The preparation was obtained from the lymph nodes of pigs immunized against the causative agents of respiratory and gastrointestinal diseases as piglets. Treatment with the preparation displayed a very good protective effect, triggering a significant reduction in morbidity and mortality in the treated piglets.

Similar results were obtained recently in Mexico, where a research group has received highly purified swine dialyzable spleen extract with antiviral activity tested on mouse model [82].

When applied to pigs, DLE considerably increased the IFN-γ concentration in their serum as measured 30 days after the treatment. This can be used to limit the cases of diarrhea and respiratory diseases among the animals—which would lead to definite improvements since these are both problematic diseases in weaned pigs. The application of porcine DLE has a good effect on weaned pigs [31].

It was received and tested TF preparation against avian influenza [83]. It has been proven that the treatment of chickens with specific TF, alone or combined with a vaccine against avian influenza, induces the expression of IFN-γ and IL-2.

6. Dosage of TF preparations

One unit of TF preparation is equal to the product obtained from 5×10^8 leukocytes. The dosage of TF in immunotherapy depends on the patient's characteristics, the disease and the therapeutic response.

Berrón-Pérez et al. [9] offer a few basic schemes for immunotherapy with TF—for initial treatment for acute and chronic diseases and for short- and long-term maintenance treatment. The authors offer various schemes in infectious, non-infectious diseases (allergic and autoimmune) and special diseases (sepsis, major surgery and cancer).

7. Conclusion

It can be summarized that dialyzable leukocyte extracts containing transfer factor are promising immunotherapeutic agents. In a large number of experiments, their protective and therapeutic effect has been proven against a number of diseases in humans and domestic animals. Understanding the mechanism of action of DLE and determining appropriate dosages determine the positive effects of their application against a very wide range of diseases—neoplasm, immune deficiencies, infectious, parasitic and autoimmune diseases.

The low level of difficulty of the process of obtaining protocols determines their relatively low cost and the possibility to combine them with other therapeutic agents during treatment makes them subject to medical applications in the future, including against some new diseases.

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References

[1] Fudenberg HH, Fudenberg HH. Transfer factor: past, present and future. Ann Rev Pharmacol Toxicol. 1989;**29**:475-516.

[2] Mikula I, Pistl J, Snirc J. Leukocyte dialysate in veterinary practice [in Slovak]. Slov Vet Cas. 1994;**19(4)**:194–199.

[3] Sanchez-Gonzalez DJ, Sosa-Luna CA, Vasquez-Moctezuma I. Transfer factors in medical therapy [in Spanish]. Med Clin-Barcelona. 2011;**137(6)**:273–277.

[4] Lawrence HS. The transfer in human of delayed skin sensitivity to Streptococcal M substance and tuberculin with disrupts leucocytes. J Clin Investig. 1955;**34**:219–230.

[5] White A. A guide to transfer factors and immune system health, 2nd ed. North Charleston, SC: BookSurge Publishing; 2009. 294 p.

[6] Lawrence HS, Borkowsky W. Transfer factor—current status and future properties. Biotherapy. 1996;**9**:1–5.

[7] Kirkpatrick CH. Transfer factor: perspectives in human and veterinary medicine. J Exp Pathol. 1987;**3(4)**:383–398.

[8] Fudenberg HH, Pizza G. Transfer factor: new frontiers. Prog Drug Res. 1994;**42**:309–400.

[9] Berrón-Pérez R, Chávez-Sánchez R, Estrada-García I, Espinosa-Padilla S, Cortez-Gómez R, Serrano-Miranda E, Ondarza-Aguilera R, Pérez-Tapia M, Pineda BO,

Jiménez-Martínez MC, Portugués A, Rodríguez A, Cano L, Pacheco PU, Barrientos J, Chacón R, Serafín J, Mendez A P, Monges NA, Cervantes E, Estrada-Parra S. Indications, usage, and dosage of the transfer factor. Rev Alerg Mex. 2007;**54(4)**:134–139.

[10] Krishnaveni M A review on transfer factor an immune modulator. Drug Invent Today. 2013;**5(2)**:153–156.

[11] Kirkpatrick CH. Structural nature and function of transfer factor. Ann NY Acad Sci. 1993;**685(1)**:362–368.

[12] Kirkpatrick CH. Transfer factors: identification on conserved sequences in transfer factor molecules. Mol Med. 2000;**6(4)**:332–341.

[13] Sudhir KS, Sizemore RS, Gottlieb AA. Immunomodulatory components present in IMREG-1, an experimental immunosupportive biologic. Nat Biotechnol. 1988;**6**:810–815.

[14] Klesius PH, Fudenberg HH, Smith CL. Comparative studies on dialyzable leukocyte extracts containing transfer factor—a review. Comp Immunol Microbiol Infect Dis. 1980;**3(3)**:247–260.

[15] Grob PJ, Reymond JF, Hacki MA and Frcy-Wettstein M. Some physicochemical and biological properties of a transfer factor preparation and its clinical application. In: Proceedings of the Second International Workshop on Basic Properties and Clinical Applications of Transfer Factor; 5–8 October 1975; Frederick (MD). New York: Academic Press; 1976. p. 247–262.

[16] Ashorn R, Uotila A, Kuokkanen K, Räsänen L, Karhumäki E, Krohn K. Cellular immunity in acne vulgaris during transfer factor treatment. Ann Clin Res. 1984;**17(4)**:152–155.

[17] Mikula I, Pistl J. The use of mouse model for the determination of protective activity in Salmonella-specific leucocyte dialyzate. Acta Vet Brno. 1989;**58(2)**:281–296.

[18] Arnaudov A, Daskalova S. Physicochemical and immunological analysis of antisalmonella dialyzable leucocyte extracts [in Bulgarian]. Vet Med. 1997;**3(1–2)**:46–49.

[19] Ojeda MO, Van't Veer C, Fern-andez-Ortega CB, Rosainz MDJA & Buurman W. Dialyzable leukocyte extract differentially regulates the production of TNFα, IL-6, and IL-8 in bacterial component-activated leukocytes and endothelial cells. Inflamm Res. 2005;**54(2)**:74–81.

[20] Robles-Contreras A, Vizuet L, Rivera E, Serafin-López J, Estrada-Garcia I, Estrada-Parra S, Chávez R, Garfias Y, Perez-Tapia M, Jiménez-MartínezMC. Down regulation of IL-8 and IL-6 in human limbal epithelial cells cultured with human dialyzable leukocyte extracts. Rev Alerg Mex. 2011;**58**:147–154.

[21] Roberto-Avila F, Wong-Baeza I, Serafin-Lopez J, Isibasi-Araujo A, Lopez-Macias C, Perez-Tapia S, Estrada-Garcia IC, Estrada-Parra SA. Human dialyzable leukocyte extracts (DLE) have ligands for TLR-2 but not for TLR-4 [Meeting Abstracts]. J Immunol. 2007;**178**:S89.

[22] García-Hernández U, Robledo-Avila FH, Alvarez-Jiménez VD, Rodríguez-Cortés O, Wong-Baeza I, Serafín-López J, Aguilar-Anguiano LM, Estrada-Parra S, Estrada-García I, Pérez-Tapia SM, Chacón-Salinas R. Dialyzable leukocyte extracts activate TLR-2 on monocytes. Nat Prod Commun. 2014;**9(6)**:853–856.

[23] Burger DR, Vandenbark AA., Daves D, Anderson WA, Vetto, RM, Finke P. Nicotinamide: suppression of lymphocyte transformation with a component identified in human transfer factor. J Immunol. 1976;**117(3)**:797–801.

[24] Gottlieb AA, Maziarz GA, Tamaki N, Sutcliffe SB. The effects of dialyzable products from human leukocyte extracts on cutaneous delayed-hypersensitivity response. J Immunol. 1980;**124(2)**:885–892.

[25] Arnaudov A, Tziporkov N. Some properties and protective activity of specific DLE against *Salmonella cholerae* suis infection. Biotherapy. 1996;**9**:105–108.

[26] Hromas J, Vacek A, Hofer M, Luksíková E, Svoboda J, Schneiderová H. Hemopoiesis-stimulating effects and enhanced survival of irradiated mice after peroral or intraperitoneal administration of ultrafiltered pig leukocyte extract (UPLE, IMUNOR). Immunopharmacol Immunotoxicol. 2002;**24(4)**:651–664.

[27] Franco-Molina MA, Mendoza-Gamboa E, Castillo-Tello P, Tamez-Guerra RS, Villarreal-Treviño L, Tijerina-Menchaca R, Castillo-León L, Zapata-Benavides P, Rodríguez-Padilla C. In vitro antibacterial activity of bovine dialyzable leukocyte extract. Immunopharmacol Immunotoxicol. 2006;**28(3):**471–483.

[28] Robles-Contreras A, Rivera-Mendez E, Vizuet-Garcia L, Perez-Tapia SM, Bautista-de Lucio VM, Estrada-Parra SA, Serafin-Lopez J, Jimenez-Martinez MC. Dyalizable leukocyte extracts regulate the expression of HBD-2 and LL37 in human limbal epithelial cells [abstract]. Invest Ophthalmol Vis Sci. 2011;**52(6)**:1121.

[29] Viza D, Fudenberg HH, Palareti A, Ablashi D, De Vinci C, Pizza G. Transfer factor: an overlooked potential for the prevention and treatment of infectious diseases. Folia Biol-Praha. 2013;**59**:53–67.

[30] Perepechkina NP, Perepechkin LP. Efficient molecular mass fractionation of leukocyte extract by membrane separation. J Membr Sci. 1999;**160(1)**:1–6.

[31] Hernandez-Peralta P, Perez-Tapia SM, Limon-Flores AY, Vazquez-Leyva S, Estrada-Parra S, Sanchez-Betancourt I, Navarro-Hernandez JA, Cobos-Marin L. Dialyzable leukocyte extract as inductor of interferon gamma concentration in serum of weaned pigs [in Spanish]. Archivos de Medicina Veterinaria. 2014;**46(3)**:425–430.

[32] Lara HH, Ixtepan-Turrent L, Garza-Trevino EN, Badillo-Almaraz JI, Rodriguez-Padilla C. Antiviral mode of action of bovine dialyzable leukocyte extract against human immunodeficiency virus type 1 infection. BMC Res Notes. 2011;**4(1)**:474–482.

[33] Hennen WJ, Lisonbee DT, inventors. US Patent and Trademark Office, assignee. Methods for obtaining transfer factor from avian sources, compositions including avian generated transfer factor, and methods of use. United States patent US. 2002;6, 468, 534.

[34] Xu YP, Zou WM, Zhan XJ, Yang SH, Xie, DZ, Peng SL. Preparation and determination of immunological activities of anti-HBV egg yolk extraction. Cell Mol Immunol. 2006;**3(1)**:67–71.

[35] Wilson GB, Poindexter C, Fort JD & Ludden KD. De Novo initiation of specific cell-mediated immune responsiveness in chickens by transfer factor (specific immunity inducers) obtained from bovine colostrum and milk. Acta Virol. 1988;**32**:6–18.

[36] Wilson GB, Paddock GV, inventors. Amtron, Inc., assignee. Process for obtaining transfer factor from colostrum, transfer factor so obtained and use thereof. United States patent US. 1988;4, 816, 563.

[37] Kirkpatrick CH, Greenberg LE, Petersen EA. Transfer factor. Lymphokines. 1983;**8**:1–39.

[38] Estrada-Parra S, Estrada-Garcia ICE, Perez-Tapia SM, inventors. US Patent and Trademark Office, assignee. Method for obtaining a dialyzable leukocyte extract. United States patent US. 2014; 20, 140, 357, 840.

[39] Kirkpatrick Ch. Activities and characteristics of transfer factors. Biotherapy. 1996;**9**:13–16.

[40] Viza D, Lefesvre A, Patrasco M, Phillips J, Hebbrecht N, Laumond G, Vich JM. A preliminary report on three AIDS patients treated with anti-HIV specific transfer factor. J Exp Pathol. 1987;**3**:653–659.

[41] Pineda B, Estrada-Parra S, Pedraza-Medina B, Rodriguez-Ropon A, Pérez R, & Arrieta O. Interstitial transfer factor as adjuvant immunotherapy for experimental glioma. J Exp Clin Cancer Res. 2005;**24(4)**:575–583.

[42] Franco-Molina MA, Mendoza-Gamboa E, Miranda-Hernández D, Zapata-Benavides P, Castillo-León L, Isaza-Brando C, Tamez-Guerra RS, Rodríguez-Padilla C. In vitro effects of bovine dialyzable leukocyte extract (bDLE) in cancer cells. Cytotherapy. 2006;**8(4)**:408–414.

[43] Fudenberg HH. Dialyzable transfer factor in the treatment of human osteosarcoma: an analytic review. Ann NY Acad Sci. 1976;**277(1)**:545–557.

[44] Juarez PC. Effect of Transferon as an adjuvant in the treatment of osteosarcoma [in Spanish] [thesis]. Mexico City: National Polytechnic Institute; 2011.

[45] Pizza G, De Vinci C, Cuzzocrea D, Menniti D, Aiello E, Maver P, Corrado G, Romagnoli P, Dragoni E, LoConte G, Riolo U, Palareti A, Zucchelli P, Fornarola V, Viza D. A preliminary report on the use of transfer factor for treating stage D3 hormone-unresponsive metastatic prostate cancer. Biotherapy. 1996;**9**:123–132.

[46] Pilotti V, Mastrorilli M, Pizza G, De Vinci C, Busutti L, Palareti A, Gozzetti G, Cavallari A. Transfer factor as an adjuvant to non-small cell lung cancer (NSCLC) therapy. Biotherapy. 1996;**9**:117–121.

[47] Franco-Molina MA, Mendoza-Gamboa E, Zapata-Benavides P, Vera-García ME, Castillo-Tello P, García de la Fuente A, Mendoza RD, Garza RG, Támez-Guerra RS, Rodríguez-Padilla C IMMUNEPOTENT CRP (bovine dialyzable leukocyte extract) adjuvant immunotherapy: a phase I study in non-small cell lung cancer patients. Cytotherapy. 2008;**10**:490–496.

[48] Carey JT, Lederman MM, Toossi Z, Edmonds K, Hodder S, Calabrese LH, Proffitt MR, Johnson CE, Ellner JJ. Augmentation of skin test reactivity and lymphocyte blastogenesis in patients with AIDS treated with transfer factor. JAMA. 1987;**257(5)**:651–655.

[49] Gottlieb MS, Zackin RA, Fiala M, Henry DH, Marcel AJ, Combs KL, Vieira J, Liebman HA, Cone LA, Hillman KS, Gottlieb AA. Response to treatment with the leukocyte-derived immunomodulator IMREG-1 in immunocompromised patients with AIDS-related complex: a multicenter, double-blind, placebo-controlled trial. Ann Intern Med. 1991;**115(2)**: 84–91.

[50] Gottlieb AA Clinical and immunologic observations in patients with AIDS-related complex treated with IMREG-1. Int J Immun. 1991;**13**:29–32.

[51] Pizza G, Chiodo F, Colangeli, Pizza G, Gritti F, Raise E, Fudenberg HH, De Vinci C, Viza D. Preliminary observations using HIV-specific transfer factor in AIDS. Biotherapy. 1996;**9**:41–47.

[52] Raise E, Guerra L, Viza D, Pizza G, De Vinci C, Schiattone ML, Rocaccio L, Cicognani M, Gritti F. Preliminary results in HIV-1-infected patients treated with transfer factor (TF) and zidovudine (ZDV). Biotherapy. 1996;**9**:49–54.

[53] Viza D, Vich JM, Minarro A, Ablashi DV, Salahuddin SZ. Soluble extracts from a lymphoblastoid cell line modulate SAIDS evolution. J Virol Met. 1988;**21**:241–253.

[54] Khan A, Hansen B, Hill NO, Loeb E, Pardue AS & Hill JM Transfer factor in the treatment of herpes simplex types 1 and 2. Dermatology. 1981;**163(2)**:177–185.

[55] Steele RW, Myers MG, Vincent MM. Transfer factor for the prevention of varicella-zoster infection in childhood leukemia. N Engl J Med. 1980;**303(7)**:355–359.

[56] Bowden RA, Siegel MS, Steele RW, Day LM & Meyers JD. Immunologic and clinical responses to Varicella-Zoster virus-specific transfer factor following marrow transplantation. J Infect Dis. 1985;**152**:1324–1327.

[57] Estrada-Parra S, Nagaya A, Serrano E, Rodriguez O, Santamaria V, Ondarza R, Chavez R, Correa B, Monges A, Cabezas R, Calva C, Estrada-Garcia I. Comparative study of transfer factor and acyclovir in the treatment of herpes zoster. Int J Immunopharmacol. 1998;**20(10)**:521–535.

[58] Pizza G, Viza D, Roda A, Aldini R, Roda E & Barbara L. Transfer factor for the treatment of chronic acute hepatitis. N Engl J Med. 1979;**300 (23)**:1332.

[59] Roda E, Viza D, Pizza G, Mastroroberto L, Phillips J, De Vinci C & Barbara L. Transfer factor for the treatment of HbsAg-positive chronic active hepatitis. Exp Biol Med. 1985;**178(3)**:468–475.

[60] Whitcomb ME, Rocklin RE. Transfer factor therapy in a patient with progressive primary tuberculosis. Ann Intern Med. 1973;**79(2)**:161–166.

[61] Vidal L, Palafox D. Transfer factor may provide immunomodulation in cutaneous tuberculosis. In: Poster session of WAO Symposium on Immunotherapy and Biologics; 13–14 December 2013; Chicago. Poster 2002.

[62] Fabre RA, Pérez TM, Aguilar LD, Rangel MJ, Estrada-Garcia I, Hernandez-PandoR, Estrada Parra S. Transfer factors as immunotherapy and supplement of chemotherapy in experimental pulmonary tuberculosis. Clin Exp Immunol. 2004;**136(2)**:215–223.

[63] Masi M, De Vinci C, Baricordi OR. Transfer factor in chronic mucocutaneous candidiasis. Biotherapy. 1996;**9**: 97–103.

[64] Graybill JR, Silva J, Alford RH, Thor DE. Immunologic and clinical improvement of progressive coccidioidomycosis following administration of transfer factor. Cell Immunol. 1973;**8(1)**:120–135.

[65] Catanzaro A, Spitler L, Moser KM. Immunotherapy of coccidioidomycosis. J Clin Invest. 1974;**54(3)**:690–701.

[66] Santacruz-Valdes C, Aguilar G, Perez-Tapia M, Estrada-Parra S, Jimenez-Martinez MC. Dialyzable leukocyte extracts (Transfer factor) as adjuvant therapy for fungal keratitis. Am J Case Rep. 2010;**11**:97–101.

[67] Sharma M K, Anaraki F, Ala F. Preliminary results of transfer factor therapy of persistent cutaneous leishmania infection. Clin Immunol Immunopathol. 1979;**12**:183–190.

[68] Delgado O, Romano EL, Belfort E, Pifano F, Scorza JV, Rojas Z. Dialyzable leukocyte extract therapy in immunodepressed patients with cutaneous Leishmaniosis. Clin Immunol Immunop. 1981;**19(3)**:351–359.

[69] Dvoroznakova, Porubcova J, Sevcıkova Z. Immune response of mice with alveolar echinococcosis to therapy with transfer factor, alone and in combination with albendazole. Parasitol Res. 2009;**105(4)**:1067–1076.

[70] McMeeking A, Borkowsky W, Klesius PH, Bonk S, Holzman RS, Lawrence HS. A controlled trial of bovine dialyzable leukocyte extract for cryptosporidiosis in patients with AIDS. J Infect Dis. 1990;**161(1)**:108–112.

[71] Homberg TA, Lara RI, Perez-Tapia SM, Martínez MDCJ. Dialyzable leukocyte extracts as adjuvant treatment for allergic rhinitis. In: Poster session of WAO Symposium on Immunotherapy and Biologics; 13–14 December 2013; Chicago. Poster 1008.

[72] García AE, Carreón JG, Ramírez E, Toledo M, Parra SE, Tapia MP, Martínez, MDCJ. Randomized clinical trial to evaluate oral dializable leukocyte extracts as immunomodulator treatment in atopic dermatitis. In: Poster session of WAO Symposium on Immunotherapy and Biologics; 13–14 December 2013; Chicago. Poster 1026.

[73] Homberg T, Sáenz V, Galicia-Carreón J, Lara I, Cervantes-Trujano E, Andaluz MC, Vera E, Pineda O, Ayala-Balboa J, Estrada-García A, Estrada-Parra S, Pérez-Tapia M, Jiménez-Martínez MC. The adverse event profile in patients treated with Transferon TM (Dialyzable leukocyte extracts): a preliminary report. Pharmacol Pharm. 2015;**6(2)**:65–74.

[74] Liburd EM, Pabst HF, Armstrong WD. Transfer factor in rat coccidiosis. Cell Immunol. 1972;**5(3)**:487–489.

[75] Klesius PH, Kristensen F. Bovine transfer factor: effect on bovine and rabbit coccidiosis. Clin Immunol Immunopathol. 1977;**7(2)**:240–252.

[76] Klesius PH, Giambrone JJ. Adoptive transfer of delayed hypersensitivity and protective immunity to *Eimeria tenella* with chicken-derived transfer factor. Poult Sci. 1984;**63(7)**:1333–1337.

[77] Smith RA, Esa A, Stiff M. Transfer of Salmonella resistance and delayed hypersensitivity with murine-derived transfer factor. Infect Immun. 1982;**36(1)**: 271–276.

[78] Mikula I, Pistl J, Rosocha J. Dialyzable leukocyte extract used in the prevention of Salmonella infection in calves. Vet Immunol Immunop. 1992;**32(1)**:113–124.

[79] Arnaudov A. A study on the protective action of Salmonella-specific dialyzable leukocyte extract. Folia Vet. 2000;**44(2)**:76–79.

[80] Kokincakova T, Herich R, Levkutova M. Effect of application of dialyzable leukocyte extract on experimental salmonellosis in chickens. Folia Veterinaria. 2008;**1**:36–37.

[81] Ignatov G, Bojadjiev S, Arnaudov A. Specific polyvalent immune stimulant for pigs, containing transfer factor. Efficiency against some bacterial and viral infections. In: Proceedings of Eighth Congress of the Microbiologist in Bulgaria; 21–23 October 1993; Varna. Sofia: USB Publishing Group; 1994. p. 61–65.

[82] Merchand-Reyes G, Pavón L, Pérez-Sánchez G, Vázquez-Leyva S, Salinas-Jazmín N, Velasco-Velázquez M, Medina-Rivero E & Pérez-Tapia SM. Swine dialyzable spleen extract as antiviral prophylaxis. J Med Food. 2015;**18(11)**:1239–1246.

[83] Bravo-Blas A, Tellez R, Uribe S, Salmerón F, Valdes L, Estrada-Parra S. & Cobos-Marín L. Transfer factor acting as IFN-gamma and IL-2 mRNA expression inductor in chicken vaccinated against avian influenza. Archivos de Medicina Veterinaria. 2010; **42(1)**:67–71

Chapter 15

Exopolysaccharides from Bacteria with Novel Application

Tsveteslava Ignatova-Ivanova

Additional information is available at the end of the chapter

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Abstract

The physiological role of EPS depends on the ecological niches and the natural environment in which microorganisms have been isolated. In this chapter, data on EPS production and the effect of EPS on corrosion of steel produced by *Lactobacillus* sp. are presented and discussed. *Lactobacillus plantarum* Ts was obtained from the Collection of Department of Biology, Shumen University. It was tested for its ability to produce exopolysaccharides when cultivated in a medium containing 10% sucrose. It could be underlined that 10% sucrose in the medium stimulated the process of protection of corrosion. Also, the biofilm in vitro in the combined cultivation of *Staphylococcus aureus* and the *Lactobacillus plantarum* Ts probiotic bacterium on the surface of different metal materials for fixed dental prostheses was investigated [unpublished results]. The structure of layer over steel plates was analyzed by scanning electron microscopy (SEM) JSM 5510. In our opinion, more detailed research is needed to be done in the future, and the possibilities should be analyzed for the creation of a thin biofilm from a probiotic bacterium or an exopolysaccharide this bacterium has produced, which would protect the implants against the growth of a pathogenic biofilm.

Keywords: exopolysaccharides, corrosion, microbial biofilms

1. Introduction

According to Reyes et al. reported one of the most complex topics within bacterial anatomy and physiology is that of exopolysaccharides (EPSs). These molecules have various structures and functions and also provide different types of advantages to their producing microorgan-

isms, including surface variability, resistance to innate and acquired immunity mechanisms, the ability to adhere to different surface and cell types, and resistance to antibiotic activity [1].

Exopolysaccharides (EPSs) are a term first used by Sutherland [2] "to describe high-molecular-weight carbohydrate polymers produced by marine bacteria." EPSs can be found as in capsular material or as dispersed slime in the surrounding environment with no obvious association to any one particular cell [3].

Many microorganisms produce exopolysaccharides as a strategy for growing, adhering to solid surfaces, and surviving adverse conditions.

Considerable progress has been made in discovering and developing new microbial EPSs that possess novel industrial significance [4].

Bacterial EPSs by Reyes [1] are "believed to play an important role in the environment by promoting survival strategies such as bacterial attachment to surfaces and nutrient trapping, which facilitate processes of biofilm formation and development. These microbial biofilms have been implicated in corrosion of metals, bacterial attachment to prosthetic devices, fouling of heat exchange surfaces, toxicant immobilization, and fouling of ship hulls." Corrosion of metals is one of the most serious and challenging problems faced by industries worldwide. Biofilms composed of a secreted polymeric substance containing microbial population have shown to inhibit corrosion in metals [5, 6]. Fang et al. and Chongdar et al. reported that "kinetics of corrosion processes of metals, mineral, and polymeric materials can be influenced by biofilms. Products of their metabolic activities including enzymes, exopolymers, organic and inorganic acids, as well as volatile compounds such as ammonia or hydrogen sulfide can affect cathodic and/or anodic reactions, thus altering electrochemistry at the biofilm/metal interface. This phenomenon is often referred to as 'biocorrosion' or 'microbially influenced corrosion'. Microbiologically, influenced corrosion has been documented for metals exposed to sea water, fresh water, demineralized water, process chemicals, food stuffs, soils, aircraft fuels, human plasma, and sewage" [7, 8].

In this paper, data on EPS production and the effect of EPS on corrosion of steel produced by *Lactobacillus* sp. are presented and discussed. The adhesion in the combined cultivation of *Staphylococcus aureus* and the *Lactobacillus plantarum* Ts probiotic bacterium on the surface of different metals is presented and discussed. Also, the biofilm in vitro in the combined cultivation of *Staphylococcus aureus* and the *Lactobacillus plantarum* Ts probiotic bacterium on the surface of different metal materials for fixed dental prostheses (Magnum Splendidum; Magnum Ni-Cr-Fe, Ruby Alloy–P, Ruby Alloy–C, and Ruby Alloy) is investigated [unpublished results].

2. Materials and methods

Strains: *Staphylococcus aureus* 745 were obtained from the Collection of the Department of General and Applied Microbiology, Sofia University. The isolate was checked for purity and

maintained in slant of Nutrient agar. Nutrient agar (Biolife 272-20128, Milano, Italia) was the medium used as the growth medium for the microbe.

Lactobacillus plantarum Ts was obtained from Collection of Department of Biology, Shumen University. The strain cultivated in medium of MRS (de Mann Rogosa Sharpe, Biolife 272-20128, Milano, Italia). The pH of medium was adjusted to 6.5 with 1M NaOH. The basic medium was sterilized by autoclaving at 121°C for 20 min.

Media for study of corrosion protection: The strain cultivated in media of MRS (de Mann Rogosa Sharpe, Biolife 272-20128, Milano, Italia) in composition, g/l: Tween 80–1; peptone from casein–10.0; meat extract–8.0; yeast extract–4.0; K_2HPO_4–2.0; sodium acetate–5.0; ammonium citrate–2.0; $MgSO_47H_2O$–0.2; and $MnSO_4$–0.05. The pH of the medium was adjusted to 6.5 with 1 M NaOH. The basic medium was sterilized by autoclaving at 121°C for 20 min, and carbohydrates supplemented were sterilized using 0.22-μM filters (Manisart®). The basic MRS broth was supplemented with 10% sucrose to be tested [9–11].

Media for study of microbial biofilm: The strain cultivated in media of MRS (de Mann Rogosa Sharpe, Biolife 272-20128, Milano, Italia) with 5% sucrose and congo red. Positive results are indicated by black colonies with a dry crystalline consistency.

Study of the corrosive stability: The study of the corrosive stability of steel samples was conducted with the gravimetric method. Before use, steel panels (10 × 4 × 0.2 mm) were treated with 70% C_2H_5OH, washed with water and dried in an oven, cooled in a desiccator, weighed on a balance, and kept in a desiccator unit used. The weight of the samples was measured using analytical balances. The dimensions of the samples were measured with micrometer. Initially, the steel samples were added in two variants: deproteinized supernatant and free cell supernatant. Then, the steel samples were added in HCl as control probe, and a dilution (3:100) of the cultural media of the studied strain was added as inhibitor of the corrosion. The duration of the procedure was 120 h at 18°C. After the treatment, the steel samples were washed with water and dried to constant weight. The structure of layer over steel plates was analyzed by scanning electron microscopy (SEM) JSM 5510 [9–11].

Study of bacterial adhesion: Before the assays, the strains *L. plantarum* Ts and *S. aureus* 745 were twice pre-cultured in MRS broth and Nutrient broth, respectively, for 24 h at 37°C. Exponential cultures in broths were used as inoculum for the adhesion analysis.

Preparation of the metal samples: The steel plates made of low carbon steel are weighed with an allowance of 0.0001 g with an assay balance. The precise weighing (with an allowance of 0.0001 g) of the metal plates before and after the treatment found a minimum negative change in their weight, which may be caused by reduction resulting from corrosion processes, on one hand, or growth because of the forming of a biofilm, on the other. They are put sterilely in a liquid ambient which contains a mixture of *L. plantarum* and *S. aureus* 745 in a proportion 1:1. The samples were incubated at 37°C for 24 h. The structure of the layer over the metal plates was analyzed by scanning electron microscopy (SEM) JSM 5510. All experiments were performed in triplicate [12].

3. Results and discussion

Corrosion process causes great economic losses in various industries, shipbuilding, jewelry, archaeological monuments, railway, water channels, and all countries of the world. For handling this problem are normally applied different physical and chemical methods, but they often prove toxic. A perspective in this regard can be the application example of exopolysaccharides produced by the so-called good bacteria–probiotics. The presence of EPS associated with bacterial cells can be recognized by the formation of colonies in mucous solid medium. Therefore, the presence of a translucent or creamy material involving a mucoid colony is indicative of EPS production potential. When cultivated in a medium with high content of saccharides such as 10% sucrose solutions, strain *L. plantarum* Ts synthesized exopolysaccharides (**Figure 1A**).

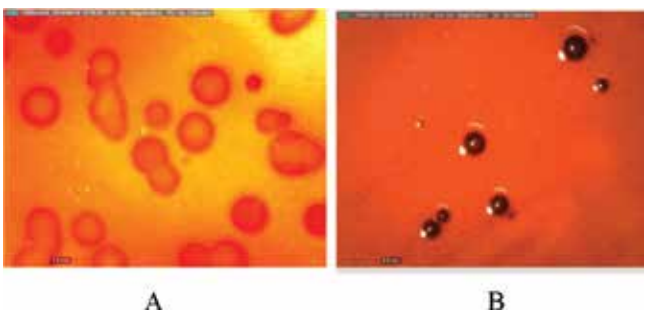

Figure 1. Congo red agar method exopolysaccharides (EPSs) produced by *L. plantarum* Ts cultivated in a medium containing 10% sucrose, which are secreted in the culture medium. (A) Red colonies show non-EPSs producers; (B) black colonies show biofilm formation of EPSs *L. plantarum* Ts. The pictures were taken using stereomicroscope OPTIKA (Italy).

When they develop microbial biofilm, the organisms are much more resistant and treating them is much more difficult. For investigation of the microbial biofilm, using different methods had been proposed, but in our work, we used the method by using the staining congo red.

"Qualitative assessment of biofilm formation is the microorganisms are grown in agar with 5% sucrose and congo red" [13]. Positive results are indicated by black colonies with a dry crystalline consistency. When cultivated in a medium with high content of saccharides such as 10% sucrose solutions, with 5% congo red, strain *L. plantarum* Ts formed biofilm (**Figure 1B**).

In the presence of high concentrations of sugars (as in our case 10% sucrose), lactic acid bacteria synthesize extracellular exopolysaccharide (**Figure 1A**), which is displayed as mucoid colonies on agar medium. By adding the staining congo red, the exopolysaccharides produced by lactic acid bacteria are displayed in black (**Figure 1B**).

Our studies also show that the use of sugar supplementation (sucrose was normally used though similar results were obtained using 5% glucose) is essential for the detection of slime production using the congo red medium. "The congo red method is rapid, sensitive, and reproducible and has the advantage that colonies remain viable on the medium" [13].

Similar experiments have also been demonstrated by other authors [14, 15]. Homopolysaccharides produced by generally recognized as safe (GRAS) lactic acid bacteria are often synthesized by a single extracellular sucrose enzyme, using only sucrose as substrate [15]. The structure of the layer over the steel plates was analyzed by scanning electron microscopy. The results from this procedure are shown in **Figure 2**.

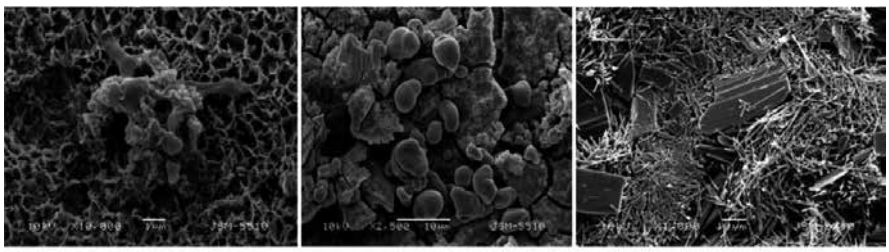

Figure 2. Biofilm formed by *L. plantarum* Ts on the surface of mild steel, visualized using SEM. (A) Biofilm formed by EPS from lactic acid bacteria; (B) biofilm formed by lactic acid bacteria; (C) control–steel plates after corrosion in HCl.

Microscope techniques provide information about the morphology of microbial cells and colonies, their distribution on the surface, and the nature of corrosion products (crystalline or amorphous). They can also reveal the type of attack (e.g., pitting or uniform corrosion) by visualizing changes in microstructure and surface features after removal of the covering and corrosion products (**Figure 2**).

The pictures in **Figure 2B** show that there is a biofilm formed on the steel surface which is an indicator of the good adhesive capacity of *L. plantarum* Ts type. The biofilm makes it not easily corrodible in 10% HCl, supplemented with cultivated ambient from the same strain grown in a composite of 10% sucrose (**Figure 2A**). **Figure 2C** shows a picture of a steel surface sample treated directly with 10% HCl. The observed lamellae are most probably $FeCl_2$ crystals, product of the corrosion.

Microorganisms can interact with the metal surfaces differently. Most often they form biofilms on contacting surfaces, but can also react with various metals to form complex compounds. For this reason, we think that different techniques have to be used to clarify the corrosion process influenced by microorganisms. When the corrosion process starts, the surface of metals is deposited large quantities of ferrousions, which are very harmful for all steel materials. If lactic acid bacteria can immobilized in the microbial biofilm, these ferrousions that could explain why exopolysaccharides produced by these bacteria protect the metal surface from corrosion. Similar to our van Geel-Schutten "biofilm of a polysaccharide-producing culture", delta marina was found to act as a strong corrosion inhibitor with almost complete passivation of mild steel, reducing the corrosion rate by 95% [16].

In our previous studies [9–11, 17–20], it was shown that the presence of high concentration of lactose (5 to 15%), high concentration of sucrose 4%, mixed sucrose 4 and 2% maltose, mixed sucrose 5 and 5% maltose, mixed 5% sucrose and 5% fructose, and mixed 5% sucrose and 5%

fructose, high concentration of lactose, sucrose and fructose (10%) the strains *Lactobacillus delbrueckii* B5, *L. delbrueckii* K27, *L. delbrueckii* B8, *L. delbrueckii* O43, *L. delbrueckii* K3, *L. delbrueckii* K17, and *L. delbrueckii* K15 and *Lactobacillus fermentum* Ts synthesized exopolysaccharides which have inhibitory properties. Moreover, we have shown that some of the end products of the fermentation process are also able to form a protective layer on the metal surfaces [20]. The observed inverse relationship between EPS and the corrosion rate of mild steel suggests that such a metal-polysaccharide complex was probably involved in developing a protective film on the metal surface in natural environment.

In recent years, the development of new technologies in medicine and dentistry leads to the production of various medical materials and prostheses.On these materials, however, when introduced in the human body, are deposited large number of microorganisms. According to van Geel-Schuten, 'biofilms are a major cause of systemic infections (e.g., nosocomial infections) in humans [16]. It is known that the human body consists about 3–4 kg microorganisms–mostly useful–but there are also the so- called 'pathogens'. In surgery, the probability of contamination with microorganisms and especially with *Staphilococcus* sp is rather big. The microorganisms, in order to survive and to form stable microbial population, create a microbial biofilm, which, however, makes them much more stable against antibiotics compared to when they are in a free state, thereby causing biomaterial-centered infections (BCI). The ability of the microorganisms to adher to different surfaces is determined on the one hand for the species and their metabolism and on the other by the type and elemental composition of the material itself. A powerful tool for the removal of BCIs is could be the use of nanocover of 'good bacteria' —probiotics.

The search for biomaterials that are able to provide for the optimal resistance to the infection can be based only on the deep understanding of the interactions between bacteria and biomaterials' [23].

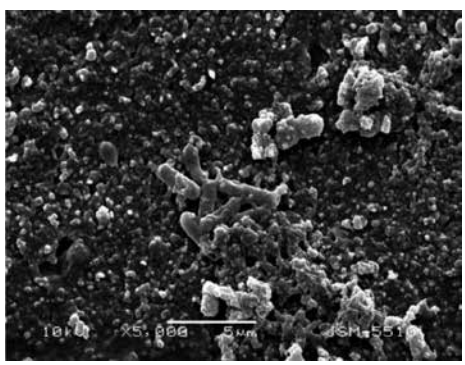

Figure 3. SEM of the tested samples of steel plates.

The adhesion in the combined cultivation of *Staphylococcus aureus* and the *Lactobacillus plantarum* Ts probiotic bacterium on the surface of different metals is investigated [12]. Also, the biofilm in vitro in the combined cultivation of *Staphylococcus aureus* and the *Lactobacillus*

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plantarum probiotic bacterium on the surface of different metal materials for fixed dental prostheses (Magnum Splendidum; Magnum Ni-Cr-Fe, Ruby Alloy–P, Ruby Alloy–C, and Ruby Alloy) is investigated [unpublished results].

The results obtained from the SEM analysis of the adhesion ability of the tested microorganisms on the different metals are shown in **Figure 3**. When a combined culture is used on the surface of the steel plates, only the probiotic bacterium adheres.

The results obtained from the SEM analysis of the adhesion ability of the tested microorganisms on the different dental prostheses are shown in **Figure 4**.

The ability of microorganisms to adhere to the surface of various surfaces is determined by various physicochemical interaction of forces, such as–Lifshitz –van der Waals forces, Brownian motion forces, and electrostatic forces. These results are discussed by other authors [21, 22]. On the other hand, the microbial adhesion may be due to the presence of specific active group sin the microbial exopolysaccharides.

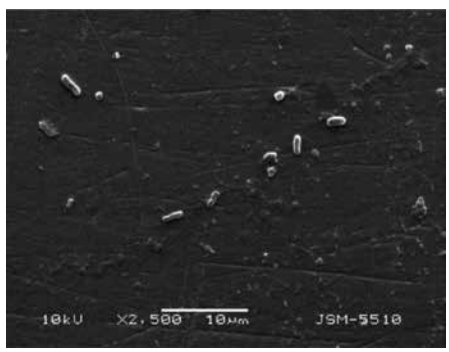

Figure 4. SEM of the tested samples of implants for tooth prosthesis.

After adhesion to biomaterials, most microorganisms start secreting slime and embed themselves in a slime layer, the glycocalix, which is an important virulence factor for BCI and which explains the extraordinary prevalence of slime producing *S. epidermidis* in BCI" [21]. According to Costerson et al., "the glycocalix provides protection against humoral and excreted cellular immune components, as these can not readily diffuse through the slime layer" [23]. Why it is only on the some plate that a biofilm of the beneficial bacteria is formed is a question difficult to answer at this stage. In our opinion, differences are also likely to appear in the adhesion process under in vitro and in vivo conditions because other processes are going to have an impact in the living organism, too. Kristopher P. et al. [24] concluded that "hydrophobic and photo-induced superhydrophilic surface coatings both have potential as a means of reducing microbial fouling of surfaces."

According to "the updated paradigm for biocompatibility", as redrawn by Williams, a biomaterial should perform its designed function eliciting the most appropriate tissue response [25].

The various metabolic ways and the various end metabolic products of the two types of bacteria: *Staphylococcus aureus* and *Lactobacillus plantarum*, could explain to a certain extent the different biofilms formed on the different metal surfaces. Different types of complex compounds are probably formed between the secreted exopolysaccharides or the end metabolic products and the metal surfaces. The mechanism of this process is still to be explained. The adhesion according to Page et al. "of microbes to surfaces can be affected by numerous physicochemical factors, and the complexity of microbial adhesion has been demonstrated. There is no one clear explanation for the behavior of all of the materials with regard to adhesion of microbes to their surface" [3]. Anti-infective biomaterials need to be tailored according to the specific clinical application. All their properties have to be tuned to achieve the best anti-infective performance together with safe biocompatibility and appropriate tissue interactions. The lack of well-structured prospective multicenter clinical trials hinders the achievement of conclusive data on the efficacy and comparative performance of anti-infective biomaterials [12].

4. Conclusion

In our opinion, more detailed research is needed to be done in the future and the possibilities should be analyzed for the creation of a thin biofilm from a probiotic bacterium or an exopolysaccharide this bacterium has produced, which would protect the implants against the growth of a pathogenic biofilm.

On the other hand, conduction of more detailed studies on the application of exopolysaccharides and the development of nanolayers as potential inhibitors of the corrosion process are needed.

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References

[1] Reyes RE, Gonzalez CR, Jimunez RC, Herrera MO, Andrade AA. Mechanisms of O-antigen structural variation of bacterial lipopolysaccharide (LPS). 2012. INTECH. http://dx.doi.org/10.5772/48147. Chapter 3.

[2] Sutherland IW. Bacterial exopolysaccharides. Advances in Microbial Physiology. 1972; 8: 143–213. doi:10.1016/S0065-2911(08)60190-3.

[3] Sutherland IW. Biosynthesis of microbial exopolysaccharides. Advances in Microbial Physiology. 1982; 23: 79–150. doi:10.1016/S0065-2911(08)60336-7.

[4] Nicolaus B, Kambourova M, Oner ET. Exopolysaccharides from extremophiles: from fundamentals to biotechnology. Environmental Technology. 2010; 31(10):1145–1158. doi:10.1080/09593330903552094

[5] Finkenstadt V, Willett CJ. Agricultural polymers for corrosion protection of metals. 236th National Meeting of the American Chemical Society. 2008.

[6] Finkenstadt V, Willett CJ. Corrosion protection of low-carbon steel using exopolysaccharides coating from Leuconostoc mesenteroides. Biotechnology Letters. 2011; 33: 1093–1100. doi:10.1007/s10529-011-0539-2

[7] Fang HHP, Xu LC, Chan KY. Effects of toxic metals and chemicals on biofilm and biocorrosion. Water Research. 2002; 36: 4709–4716. doi:10.1016/S0043-1354(02)00207-5.

[8] Chongdar S, Gunasekaran G, Kumar P. Corrosion inhibition of mild steel by aerobic biofilm. Elecrtrochimica Acta. 2005; 50: 4655–4665. doi:10.1016/j.electacta.2005.02.017.

[9] Ignatova-Ivanova Ts, Ivanov R. Exopolysaccharides from lactic acid bacteria as corrosion inhibitors. Journal of Life Sciences. 2014; 8: 940–945. doi:10.17265/1934-7391/2014.12.001

[10] Ignatova-Ivanova Ts, Ivanov R. Study of biofilm formed by lactic acid bacteria on the surface of mild steel. Journal of Life Sciences. 2014; 8: 799–804. doi:10.17265/1934-7391/2014.10.001

[11] Ignatova-Ivanova Ts, Ivanov R. Exopolysaccharides from lactic acid bacteria as corrosion inhibitors. Acta Scientifica Naturalis. 2016; 3(1): 51–59. DOI: 10.1515/asn-2016-0008

[12] Ignatova-Ivanova Ts, Ibrjam S, Ismailov I, Christov V, Ivanov R. Adhesion and surface growth of *Staphylococcus aureus* and *Lactobacillus plantarum* on various metals. Journal of IMAB–Annual Proceeding (Scientific Papers). 2015; 21(2): 793–796. doi:10.5272/jimab.2015212.793

[13] Freeman J, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. Journal of Clinical Pathology. 1989; 42:872–874. doi: 10.1136/jcp.42.8.872

[14] Jayaraman A, Earthman JC, Wood TK. Corrosion inhibition by aerobic biofilms on SAE 1018 steel. Applied Microbiology and Biotechnology. 1997; 47: 62–68. doi:10.1007/s002530050889

[15] Marshall KC. Biofilms: an overview of bacterial adhesion, activity, and control at surfaces. ASM News. 1992; 58: 202–207.

[16] Geel-Schutten GH van. Exopolysaccharide synthesis by Lactobacillus reuteri. Ph.D. thesis, University of Groningen. 2000.

[17] Ignatova-Ivanova Ts, Ivanov R, Iliev I, Ivanova I. Study anticorrosion effect of eps from now strains *Lactobacillus delbruecii*. Biotechnology and Biotechnology EQ. 2009; 23(1): 705–708. doi:10.1080/13102818.2009.10818522

[18] Ignatova-Ivanova Ts, Ivanov R, Iliev I, Ivanova I. Study of anticorrosion effect of exopolysaccharides produced *Lactobacillus delbrueckii* b5 cultivated on different carbohydrates. Biotechnology and Biotechnology EQ. 2011; 26(1): 224–227. doi: 10.5504/50YRTIMB.2011.0040

[19] Ignatova-Ivanova Ts, Ivanov R. Anticorrosion effect of biofilm forming by lactobacillus strains on metal surfaces. Bulgarian Journal of Agricultural Science. 2013; 19(2): 83–85.

[20] Ignatova-Ivanova Ts, Ibrjam S, Ivanov R. Study of the effect of lactic acid fermentation end products on the speed of the corrosion process. International Journal of Current Microbiology and Applied Sciences. 2015; 4(4): 397–401.

[21] Christensen GD, Baddour LM, Hasty DL, Lowrance JH, Simpson WA. Microbialand foreign body factors in the pathogenesis of medical device infections. In: Bisno AL, Waldvogel FA, editors. eds. Infections Associated with Indwelling Medical Devices. Washington, DC: American Society for Microbiology. 1989; 27–59.

[22] Van Oss CJ. The forces involved in bioadhesion to flat surfaces and particles–their determination and relative roles. Biofouling. 1991; 4(1–3): 25–35. doi:10.1080/08927019109378192

[23] Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999; 284(5418): 1318–1322. doi:10.1126/science.284.5418.1318

[24] Page K, Wilson M, Mordan NJ, Chrzanowski W, Knowles J, Parkin IP. Study of the adhesion of *Staphylococcus aureus* to coated glass substrates. Journal of Materials Science. 2011; 46 (19): 6355–6363. doi:10.1007/s10853-011-5582-9

[25] Arciola CR, Campoccia D, Speziale P, Montanaro L, Costerton JW. Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. Biomaterials. 2012; 33(26): 5967–5982. doi: 10.1016/j.biomaterials.2012.05.031.

Allergy

Chapter 16

Unmet Needs in Understanding Sublingual Immunotherapy to Grass Pollen

Gabriele Di Lorenzo, Maria Stefania Leto-Barone, Simona La Piana and Danilo Di Bona

Additional information is available at the end of the chapter

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Abstract

The lack of medication for allergy symptoms at the end of the last millennium has been the promoter of the idea of treating allergies as if you were treating an infectious disease, by vaccination prophylaxis. Two forms of AIT 1) subcutaneous immunotherapy (SCIT) and 2) sublingual immunotherapy (SLIT) are used in the world. Considerable interest has emerged in SLIT both scientifically and especially financially. SLIT is not a new treatment modality. First description dates back to 1900 when H. Curtis. It was relatively widely used until the late 1970's mainly in US by homeopathic therapists.

A number of case series describing experience with the oral route were published during the 1920s and 1930s, but it seems to have been perceived not as efficacious nor as well tolerated as subcutaneous immunotherapy. The companies producing allergen immunotherapy have an alliance with important opinion leaders on both shores of the Atlantic.

If SLIT did not work for 40 years, why should it work for respiratory allergic diseases today? This question is the mother of all questions in the field of respiratory allergic diseases. The purpose of this chapter is to provide past and current information about immunotherapy, and discuss controversies over efficacy and safety, and dosing considerations for SLIT to grass.

Keywords: subcutaneous immunotherapy (SCIT), Sublingual immunotherapy (SLIT), Allergic rhinitis, Grass pollen, Evidence-Based Medicine

1. Background

For everything there is a season. This sentence from the Bible indicates and explains the history of sublingual immunotherapy (SLIT) in a nutshell. We must remember that allergy

treatment is a hybrid specialty. Respiratory diseases that come to the allergist are not uniquely his, but are shared with other specialties. Of the two major manifestations of respiratory allergic diseases, asthma and hay fever, the internist and pneumologist have plenty of opportunities to observe asthma and nose-and-throat specialists have great familiarity with hay fever.

The drugs given for relief of allergic symptoms are familiar to physicians in general. After the characterization of immunoglobulin E (IgE) in 1967, a variety of *in vitro* tests to detect it and quantitative serum specific IgE have been developed. *In vitro* testing has since become more common [1].

Consequently, of two areas belong exclusively to the allergist, *in vivo* testing and allergen immunotherapy, only the latter has remained, used exclusively by allergists. Although AIT has been used to treat allergic rhinitis for over 100 years, its role remains controversial. AIT is accepted in full by most allergists, but only with many reservations by other medical specialists of diseases of the respiratory tract. Currently, two forms of AIT (1) subcutaneous immunotherapy (SCIT) and (2) sublingual immunotherapy (SLIT) are used in the world. Considerable interest has emerged in SLIT both scientifically and especially financially.

Compared with SCIT, SLIT is easy to administer, does not involve administration of injections and may be administered at home, avoiding the inconvenience of office visits and finally it can be prescribed by general practitioners, otolaryngologists and dermatologists as well as allergists [2].

In the mid-1950s, antedating by several years the more recent publications dealing with SLIT by Europeans, [3] a group of Midwestern physicians, headed by Dr. Herbert J. Rinkel, practiced a form of unconventional allergy that included a technique of administering preparations of allergenic extract beneath the tongue [4]. Rinkel changed the oral route suggested by Curtis 11 years before Noon's publication on subcutaneous immunotherapy [5].

A number of case series describing experience with the oral route were published during the 1920s and 1930s, but it seems to have been perceived not as efficacious nor as well tolerated as subcutaneous immunotherapy. Subsequently, the method fell out of favor [6, 7].

The genesis of this procedure, like all homeopathic therapies, was engendered by a strong belief, considerable imagination and although successful according to anecdotal reports from its practitioners, the technique lacked the rigor of scientific proof [8, 9].

The value of SCIT to the allergist was unquestioned until 1998. The allergist often asks skeptics of SCIT: how could SCIT has survived to this day, unless it was of genuine value? Unfortunately, the value of AIT is not to be determined by the fact that this procedure has survived a century. Bloodletting in medicine lasted for a much longer time as a therapy for pneumonia [10], but today it is not considered a rational therapy.

However, evidence-based medicine must be taken into account in the field of medicine, which is based on rigorous research and the scientific method. The companies producing allergen immunotherapy have an alliance with important opinion leaders on both shores of the Atlantic [11].

A search of published literature under the topic "sublingual immunotherapy and RCT" revealed 24 citations in English before 2000 and 102 citations after 2000 (inception to July 30, 2016), demonstrating the growing interest of companies and opinion leaders in SLIT for allergic rhinitis.

However, the medication for allergic rhinitis currently in use is highly effective and nasal steroids are of particular value [12].

In some ways it would be the same as supporting the use of cromolyn for the therapy of allergic rhinitis rather than nasal steroids. A significant obstacle in evaluating the clinical value of SLIT is choosing the best criteria to prove clinical efficacy. Our position is that the best indication of efficacy is improvement in symptoms and decrease in medication that contributes significantly to the patient's quality of life. The optimal study design for investigating clinical efficacy to evaluate SLIT includes pretreatment monitoring of symptom score for a season. This gives the advantage of elucidating the clinical relevance of allergen exposure in eliciting symptoms, representing the only possible way of ensuring an equal balance of disease severity in active and control groups. The magnitude of the clinical improvement is also important. It is of course critical to document a statistically significant difference between the active group and the control group, but *p-values* do not *per se* guarantee the effectiveness of a specific treatment. In 1991, Varney documented that immunotherapy has a clinical capacity to reduce, in actively treated patients, symptoms and drug intake by about 20% compared to the placebo-treated group [13].

If sublingual immunotherapy (SLIT) did not work for 40 years, why should it work for respiratory allergic diseases today? This question is the mother of all questions in the field of respiratory allergic diseases. The purpose of this chapter is to provide past and current information about immunotherapy and discuss controversies over efficacy and safety and dosing considerations for SLIT to grass. Allergy to grass is the most important form of seasonal pollinosis.

Several persistent misconceptions or "false beliefs" have been built up around AIT and its use in allergic rhinitis (AR), in particular regarding sublingual immunotherapy (SLIT). These misconceptions largely arose because of improper use of evidence-based medicine that was widespread in this field until the 1990s.

2. Evidence-based medicine (EBM)

Initially EBM was identified with the frequency with which you reach health interventions proven effective (more helpful than harmful) and which prevents interventions more harmful than useful. Three Doctors, an Englishman, Archie Cochrane, an American, Alan Feinstein and a Canadian David Sackett, can be considered the founders of this movement [14].

They identified the combination of carrier EBM in the interaction between research evidence with patient preferences. This paradigm was, however, revised by Sackett in 1996 in an article published in the BMJ that clarified that EBM cannot be considered without also

considering that clinical expertise. Only research or expertise alone could not be considered EBM [15].

In 2002, Haynes et al. quoted a famous phrase from Osler, "The value of experience is not much to see but see wisely" and then EBM defers to center the patient that must be studied with the findings that emerge from research and patient preferences that all should be handled with the clinical expertise of the physician [16, 17].

A few years later Shekelle et al. classified the literature data on primary sources that include expert opinion, observational studies, case studies-final control, cohort studies, clinical trials (RCT) and on secondary sources that include systematic reviews with or without meta-analysis of RCT [18].

The importance of the evidence is maximum for systematic reviews with meta-analysis and the risk is minimal for bias in systematic reviews with meta-analyses. Secondary sources are always the representation of primary studies but should be guaranteed from the critical analysis of who writes them. They use an explicit method and systematic examination of primary studies. Finally, the tertiary sources are represented by evidence-based clinical guidelines [19].

The limits of primary studies are often related to sponsorships and thus influenced by the results, as reported by Ioannidis in this article a few years ago published in PLoS Medicine [20]. EBM is in crisis for the misuse and overproduction of primary studies, often useless because they duplicate other studies and in publication of therapeutic advantages with marginal shift of attention from individual research to therapy [21].

For these reasons, the EBM approach must be reevaluated critically, trying to customize the decisions not only by referring to the available evidence or patient preferences or adherence to therapy but also considering the cultural and financial aspects of the patient who must decide, investment in training doctors both in pre and postgraduate education and indicating the use of secondary studies rather than primary ones [22–25].

3. From subcutaneous immunotherapy to sublingual immunotherapy: the return to the past

In a document published in 1993 in allergy about immunotherapy, the authors wrote in the preface: why does a diagnostic etiology in a patient with allergic respiratory disease indicate the specific therapy as the only logical solution of this specific diagnosis [26].

Immunotherapy is the scientific path that Leonard Noon published in the Lancet in 1911 [27]. The route was started by Bostock who described hay fever, i.e., allergic rhinitis and that proved to be the one that Backley demonstrated to be caused by grass season [28]. The studies of immunotherapy by Noon (who died prematurely from a form of tuberculosis), were continued by the pioneer John Freeman [29, 30]. It is necessary to remind readers that the use of antihistamines was reported in 1952 [31].

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However, the lack of medication for allergy symptoms has been the promoter of the idea of treating allergies as if you were treating an infectious disease, by vaccination prophylaxis [32].

In other words, the comparison between immunotherapy and placebo was based for many years on two symptomatic criteria: the number of days during the season in which eye symptoms were noted; the number of days in which nasal symptoms were noted [27, 29, 30].

Immunotherapy has spread rapidly around the world and the first study compared to placebo was done by Frankland and published in Lancet in 1954 [33]. Another study was performed on ragweed in the United States and published in the New England J Medicine [34].

From a purely subjective assessment of both the patient and the allergist, the outcome of the studies is beginning to consider the evaluation criteria more objectively. At the end of the 1960s, the number of antihistamine pills taken for the relief of symptoms was evaluated [35].

However, a sort of skepticism around this therapy remained until Ishizaka and Johansson discovered IgE and showed that they were the cause of allergic reactions [36, 37].

The fact that the antibodies played a role in patients treated with immunotherapy had already been suggested by Sherman et al. [38].

Finally, we must remember that chemical pharmacology was born in the nineteenth century. The first antihistamine was synthesized by Bovet and Staub in 1937, the molecule was the 2-isopropyl 5 methyl phenoxyethyldiethylamine, demonstrating mild antihistamine action and considerable toxicity. In 1949, Bovet synthesized pyrilamine maleate, a diethylamine essentially free of toxic effects. But only in 1972, did Black et al. succeed in synthesizing antihistamines selective for the different receptors [39, 40]. Later, in 1974, beclomethasone dipropionate, became, the most important goal for the treatment of allergic rhinitis after that of antihistamines [41].

Allergy has been involved in a process of globalization. Before the 1980s there was no allergen standardization; this resulted in marked variations in allergenic strength among allergen vaccine batches produced in different phases. Immunotherapy was considered "Galenic" drugs, because they were prepared upon request of the allergist for a specific patient. The article of the Committee on Safety of Medicine challenges the use of ITS in the UK antihistamines and nasal steroids are marketed because they have been validated through controlled clinical trials as effective and safe [42].

However, in a study published by Reid et al. the problem of fatal reactions as a result of ITS is now widely known [43]. Alarm about the safety of ITS, the use in clinical practice of antihistamines and nasal corticosteroids with fewer side effects, the lack of full understanding of the mechanisms of action of ITS bring specific immunotherapy into a crisis. International scientific allergy companies, such as EAACI, produce important opinion-based scientific articles that, however, enhance the use of immunotherapy [44].

European allergists request single specific allergens for immunotherapy, rather than the allergen mixtures that had previously been requested and the companies, operating in the manufacturing sector of the allergen, participate more actively in the scientific debate. The companies begin to understand that the field of respiratory allergy, particularly that of

allergic rhinitis, is very rewarding financially. They also understand, however, that it is a field of medicine that is very closed and allergists are very jealous of AIT. They do not believe that it can be shared with other doctors [45–48].

In 1998, Malling published: immunotherapy as an effective tool in allergy treatment [49] in the allergy: a symposium review. The authors of this review stated on the bases of a number of DBPC studies, they could affirm that the clinical efficacy of immunotherapy in rhinitis and asthma, when potent and standardized extracts were applied in carefully selected patients, was well documented. Immunotherapy has the potential to reduce symptoms and the need for drugs significantly and furthermore possibly prevent progression into more severe disease.

SCIT has been evaluated on an arbitrary grading of the magnitude of clinical efficacy (**Table 1**).

1	No efficacy (symptom/medication scores improved by <30%)
2	Low efficacy (improvement 30–44%)
3	Moderate efficacy (improvement 45–59%)
4	High efficacy (improvement of >60%)

Table 1. Arbitrary grading of the magnitude of clinical efficacy by Malling [49].

Malling et al. wrote that: "this grading is arbitrary and controversial, but in daily clinical practice it is more operational than statistical *P* values. Compared with the efficacy of drug treatment of allergic diseases, the grading seems sensible. A symptom/medication score amelioration of <30% does not seem to justify the immunotherapy involving a potential risk of side-effects and will probably not be considered worthwhile by patients" [49]. Fifteen RCTs investigated the efficacy of SCIT in grass-pollen allergy [13, 50–63], of which 14 proved clinical efficacy. Only the RCT of Doltz et al. demonstrated a clinical effect of 10% improvement [62].

However, we must make two important observations: (1) SCIT is clinically effective, i.e., symptom/medication scores diminished by >30% in the actively treated in 72.3% of the studies and precisely in 93.3% of RCTs that investigated the efficacy of SCIT in grass-pollen allergy, in 69.2% of SCIT in ragweed, in 66.6% of SCIT in various allergens (Mountain cedar, Parietaria, Cupressus and Cocos) and in 60% of SCIT in DHM; (2) to satisfy an accurate indication for immunotherapy, this should be done by an allergist [49].

In other words, Malling et al. reiterate that only an allergist can decide if, when and how to do AIT. Certainly, this statement has not induced large investments from companies. However, the risk that AIT would remain a niche therapy managed only by allergists, is realistic.

In the same year, Malling et al. published a position paper of the European Academy of Allergy and Clinical Immunology entitled *Local immunotherapy* in allergy [3].

In this position paper, the authors examined the noninjective administration of specific immunotherapy in allergic disorders such as rhinitis and asthma, recommending to replace the

term "alternative immunotherapy" with "local immunotherapy" since the former may generate misleading associations and confusion with other, scientifically undocumented therapies used in allergic diseases, e.g., acupuncture, hypnosis, homeopathy and other methods [64]. Local immunotherapy included local nasal (LNIT), local bronchial (LBIT), oral (OIT) and sublingual (SLIT) administration of allergen extracts.

Of these routes, both OIT and SLIT, like all homeopathic therapies, were engendered by a strong belief, considerable imagination and although successful, according to anecdotal reports from its practitioners, the technique lacked the rigor of scientific proof [8, 9].

The companies understand that SCIT would hardly be accepted by patients because the loss of time it takes is very significant—the time to go to the Allergist's office, plus the time of the turn to take the shot, then wait at least 30 minutes after the shot. Overall, the time required for a shot was about 2 hours. However, it was necessary to give credibility to alternative routes of administration of specific therapy, using the methodology of evidence-based medicine. For this reason, Malling et al. presented the inclusion criteria of the studies in the chapter, reported in **Table 2**. We will report, as for SCIT (see above), only the studies for allergy to grass pollen, performed with four choices of local immunotherapy.

1	Placebo-controlled, double-blind (PCDB) studies
2	Allergen extracts and doses defined
3	Treatment protocol and statistical analysis appropriate including an adequate sample size (over seven patients per group)
4	Studies published in peer-reviewed journals in English
5	Symptom/medication scores provided.

Table 2. Criteria used in position paper published in Allergy in 1998 [3].

3.1. Nasal immunotherapy

The authors included only five RCTs with grass allergens and all studies showing clinical efficacy [65–69]. However, Malling et al. concluded that LNIT demonstrate "The side-effects do not appear to present a significant problem" [3].

3.2. Bronchial immunotherapy

RCTs for allergy to grass pollen with local bronchial immunotherapy (LBIT) have not been published. However, the comments about LBIT were the following words: "LBIT is not sufficiently documented and there is concern about potentially serious immediate and delayed side-effects" [3].

While Crimi's RCT about LBIT concluded that: "We conclude that LIT may be an effective and safe alternative to traditional immunotherapy" [70].

3.3. Oral immunotherapy

The authors examined three RCTs with grass [71–73]. None of these RCTs demonstrates clinical efficacy. The authors concluded that: "Only two studies indicate clinically relevant efficacy with either birch pollen administered in enteric-coated capsules or after treatment with aqueous mite extract for at least 1 year" [3].

3.4. Sublingual immunotherapy

The author considered only two RCTs with grass [74, 75]. Considering these two studies, the major innovation in the field of allergy is the presence of employees of the company that manufactures and sells the SLIT in the authors of the publication. Another important consideration is that Bjorksten, coauthor of this chapter, wrote an Editorial about the RCT of Sabbah entitled: "Local immunotherapy is not documented for clinical use" [76].

The comment of the authors about sublingual immunotherapy was: "Sublingual immunotherapy has been shown to reduce rhinitis symptoms and/or medication needs in six RCTs. The documentation of efficacy is based on a limited number of studies including around 120 patients" [3].

However, Malling et al. had excluded two RCTs with grass from their review, because one did not supply data on allergen doses [77] and the other did not include a placebo group [78].

After Malling's position paper, the largest systematic reviews of sublingual immunotherapy, which reported on two primary outcomes, i.e., symptom score (SS) and medication score (MS), were performed in 2003 [79] and updated in 2010 [80] and published in the Cochrane collaboration database.

These two systematic reviews of sublingual immunotherapy suggested that the SLIT benefit in symptom improvement and drug use reduction is higher than placebo. But, the conclusions of the above mentioned meta-analyses were based on studies conducted in patients with allergies to both perennial and seasonal allergens, while the efficacy of SLIT for grass allergens was assessed by a subgroup analysis [79, 80]. However, other RCTs have been published on SLIT with grass allergens for AR [81–86]. Two RCTs presented results that remain inconsistent and the overall assessment of the treatment efficacy is still difficult to evaluate [85, 86]. Fourteen other RCTs on grass were published between 2004 and 2009 [87–100].

Our in-depth meta-analysis found that in seasonal allergic rhinoconjunctivitis, SLIT with grass allergens provided a statistically significant improvement of symptoms and a significant reduction of anti-allergic medication compared with placebo. The data from 19 RCTs representing a pooled total of 1518 patients receiving SLIT and 1453 receiving placebo, indicate the available evidence is sufficient to conclude: (1) SLIT with grass allergens improves rhinosinusitis symptoms and reduces the use of anti-allergic medications compared with placebo but the overall effect is clinically modest, (2) prolonged pre-season treatment significantly increases the response rate, and (3) a course of treatment no longer than 12 months with a monthly allergen dose of 450mg seems to be the best treatment choice. However, further studies are needed to clearly determine the role of SLIT with grass allergens in children [101].

Several reviews have reported an equivalent clinical efficacy between SCIT and SLIT for seasonal allergic rhinitis to grass pollens [102, 103]. SLIT has also been shown to be relatively safe and fairly well tolerated. These features account for the increasing use of SLIT in Europe. Improved safety and easy administration compared with SCIT are important advantages [104]. However, the relative efficacy of SCIT and SLIT has not yet been clearly shown. The only published study comparing the two treatments has been performed without placebo [85].

Therefore, to clarify this issue, we performed and published a meta-analysis to compare SCIT and SLIT with a fairly large number of double-blind, placebo-controlled trials on SCIT and SLIT (updating the previous published meta-analysis) [101] in patients with seasonal allergic rhinitis to grass pollens [105]. This meta-analysis of data from 36 RCTs, 10 with SLIT drops [75, 81, 83, 87, 88, 92, 94–96, 98], 12 with SLIT tablets [82, 84, 89, 90, 91, 93, 97, 99, 100, 106–108] and 14 with SCIT [13, 52, 54, 55, 61–63, 109–115].

These studies included a total of 3014 patients treated with immunotherapy and 2768 controls who received placebo. They provide indirect evidence that in patients with seasonal allergic rhinoconjunctivitis to grass SCIT is more effective than SLIT in the control of symptoms and in the reduction of anti-allergic medication use. There is significant heterogeneity in the results of individual RCTs, in particular for SCIT studies, which raises some concern. However, any degree of heterogeneity is acceptable if both the predefined eligibility criteria for the meta-analysis are sound and the data are correct [116].

Some of the SCIT trials included in our analysis were performed more than two decades ago on small sample sizes, but the quality of the studies has been considered sufficient to justify their inclusion not only in our meta-analysis but also in some Cochrane meta-analyses [79, 80, 117]. Our study suggests that the choice of SLIT is mainly based on safety reasons. In fact, the number of reactions requiring epinephrine was higher in SCIT RCTs (12 in 960 patients), than in SLIT RCTs (1 episode in 4046 patients). However, the number of mild to severe adverse events was higher in SLIT than in SCIT (**Table 3**).

	SCIT		**SLIT**	
	Treated	**Placebo**	**Treated**	**Placebo**
Total EAs, no.	960	456	4046	1856
Total AEs/patients	0.86	0.50	2.13	0.99
Withdrawals for AE, no.	18	5	78	25
Withdrawals for AEs/patients %	0.0019	0.005	0.04	0.0013
Anaphylactic reactions, no.	12	2	1	0

Table 3. Total adverse events (AE) related to SCIT and SLIT [105].

Moreover, our data provide indirect evidence that SCIT with grass allergens is more effective than SLIT to improve symptoms and reduce antiallergic medication for seasonal allergic rhinoconjunctivitis. However, trials directly comparing the two different routes of immunotherapy are needed to confirm these data [105]. An ideal comparative study would be a

randomized, placebo-controlled, double-blind, double-dummy study that enrolls a large number of patients from a single center or a single country or a few countries with similar pollen exposure and patients of similar ethnicity. The treatment should be started at least 16 weeks before the expected beginning of the pollen season and last 1 or 2 years. A vaccine with a dose of the main extract ≥275 mg for SLIT should be used. The ideal dose for SCIT vaccines has yet to be determined. Nonetheless, no one should be surprised by the criticism of our meta-analyses because the critics are authors who supported the use of SLIT [118, 119].

The convenience and safety of sublingual immunotherapy (SLIT) are likely factors for its widespread use in Europe, where it is now the preferred route of administration of ASI and were licensed as drugs in September 2009 (Grazax®, Alk-Abellò; Oralair®, Stallergenes) [120].

The US Food and Drug Administration (FDA) announced approval of the five-grass pollen sublingual tablet (Oralair®) in April 2014, followed by the Timothy grass pollen sublingual tablet (Grazax®/ALK-Abellò, marketed by Merck in the US under the name of Grastek®) [121–123]. SLIT with liquid allergen extracts had been used off-label in the US before FDA approval [2].

Our previous meta-analysis showed that SLIT was effective for seasonal allergic rhinoconjunctivitis to grass, but its cl vinical benefit compared to placebo was modest [101, 105]. That data also showed that SLIT tablets are more effective than drops, probably because of a higher allergen content. All of the RCTs of SLIT published at that time had been performed in Europe [82, 84, 89–92, 97, 99, 100, 106, 124]. But since then five additional RCTs have been published, all conducted in North America. [107, 108, 125–127].

In our meta-analysis on SLIT tablets [128], data on symptom score were available in 13 RCTs [82, 84, 89, 90, 91, 92, 97, 99, 107, 108, 125–127], and data on medication score in twelve studies [82, 89, 90, 91, 92, 97, 99, 107, 108, 125–127]. We excluded the Caffarelli study, because he used an allergoid, Lais [106] and Horak study because it was performed in an allergen challenge chamber [100] and the Halken study [124], which is a secondary analysis on a previously published dataset [102]. The 13 RCTs included a total of 4659 patients. Seven studies were conducted in Europe [82, 84, 89, 90, 92, 97, 99] and five in North America [107, 108, 125–127] and one in both Europe and Canada [91].

The SS and MS were assessed as outcome measures of the treatment effect. Outcome data were continuous, but different scoring systems and scales for symptoms and medication were used by the authors. To compare the results, analyses were performed by the method of standardized mean difference (SMD), expressing the differences in means between SLIT and placebo in terms of units of the pooled SD. The overall SMD among patients treated with SLIT and placebo was estimated using models based on both fixed effects and random effects assumptions [129]. The magnitude of the overall effect was classified according to Cohen's guidelines: effect size of 0.2, 0.5, and 0.8 correspond to small, medium and large effects [130]. Since 11 out of 13 RCTs used the same SS ranging from 0 to 18 points (the higher the score the worse the disease severity) as outcome measure, we compared the results of these studies using the original SS, reporting the results as mean difference (MD) of SS-points. Excluding the studies by Pradalier [82] and Smith [84], we could compare the studies using the original SS, which is easier to interpret. Using this method the mean difference between SLIT and placebo was −0.83 SS points (95%CI −1.03, −0.63, $p = 0.0001$) without significant heterogeneity ($I^2 = 16\%$). The SMD excluding these two studies did not change compared to the main

analysis performed with 13 studies (SMD −0.28, 95%CI, −0.39, −0.18; p < 0.0001), indicating that SMD of −0.28 corresponds to a MD of −0.83 SS points.

Data on medication score were obtained for 12 RCTs (4558 patients) [82, 89, 90, 91, 92, 97, 99, 107, 108, 125–127]. A statistically significant difference between SLIT and placebo was observed only in seven RCTs [89–92, 97, 125, 127]. The pooled estimate of treatment on medication score was statistically significant (SMD −0.24; 95%CI, −0.31, −0.17; $p < 0.0001$). An analysis using the original medication score was not performed due to the highly different scoring systems used. A total of 1817/2597 (70.0%) of patients receiving SLIT vs. 1137/2555 (44.5%) of subjects receiving placebo complained of adverse events. Probable treatment-related adverse events were reported in 9 out of 13 studies and there were three times as many adverse events in patients receiving SLIT (1384/2259, 61.2%) than in those receiving placebo (477/2279, 20.9%). Most AEs were moderately severe for both groups. The withdrawal rate for an AE was higher in the SLIT group (159 patients, 6.0%) than in the placebo group (56 patients, 2.2%). No episode of anaphylaxis was reported in the RCTs; but nine adverse events requiring epinephrine were reported in the SLIT group, of which seven were treatment related. Three serious adverse events requiring epinephrine were also reported in the placebo group, but none of them were treatment related (**Table 4**). The forest plot and the funnel plot of the data reported above can only be seen in the original publication due to copyright [128].

	SLIT	**Placebo**	**OR**	***P***
Total AE, # patients (%)	1817/2597 (70)	1137/2555 (45)	2.91	<0.0001
TRAE, # patients (%)[†]	1384/2259 (61)	477/2279 (21)	5.98	<0.0001
Withdrawals for AE, # patients (%)	159/2658 (6)	58/2587 (2)	2.77	<0.0001
Anaphylactic reactions	0	0	–	n.s.
AE requiring adrenaline	9	3	–	n.s.
TRAE requiring adrenaline	7	0	–	n.s.

Note: AE, adverse events; TRAE, treatment-related adverse events.

Table 4a. Adverse events during treatment [128].

TRAD	**SLIT *n* (%)**	**Placebo**	***p***	**#Studies**
Oral pruritus	689/2228 (30.9)	84/2126 (3.9)	<0.00001	11/13
Throat irritation	418/2045 (20.4)	71/2006 (3.5)	<0.00001	9/13
Mouth edema	226/2105 (10.7)	17/2033 (0.8)	<0.00001	9/13
Ear pruritus	181/1524 (11.9)	32/1444 (2.2)	<0.00001	6/13
Eye pruritus	81/852 (9.5)	20/768 (2.6)	<0.00001	4/13
Oropharyngeal pain	122/1306 (9.3)	33/1309 (2.5)	<0.00001	4/13

Note: Other side effects, such as headache, cough, tongue pruritus, sneezing, rhinorrhea, nasal discomfort and nasopharyngitis, have not been reported in the table since they were reported in less than four studies.

Table 4b. Most common treatment-related adverse events (TRAE), occurring in at least 5% of patients in the treatment group.

4. The methodological problem used to evaluate the tablets grass

In the last part of this chapter, we will focus on the most critical methodological defect of the SLIT RCTs, which is the metric that has been used in RCTs to assess the clinical benefit. This metric is mathematically incorrect because, as clearly will explained in the study, it calculates the percentage difference between SLIT and placebo, not taking into account the symptom score (SS) scale range and leading to a huge magnification of the difference between groups.

By using this metric, a 1-point difference will be the same percentage difference in an 18-point scale (the te common SS scale used), a 100-point scale, or any other scale, and this is mathematically unacceptable (a detailed explanation has been reported in **Figure 1**).

Example study	SLIT	Placebo	Difference	Percentage improvement
RTSS (baseline)	15	15		
Mean SS during treatment	3	4	-1	(3 - 4)/4=**25%** **(not including the scale)**
Difference	-12	-11	-1	
Percentage improvement	80%	74%		80%-74%=**6%** **(11 - 12)/15=6%** **(including the scale)**

Figure 1. 18-point scale.

The correct metric, which takes into account the scale range, was indicated by the World Allergy Organization (WAO) [104].

Recommendations for standardization of clinical trials with allergen and is based on the comparison between the pretreatment and post treatment SSs of the active and placebo groups. Using this metric, we showed a small difference between SLIT and placebo, which is less than the US Food and Drug Administration (FDA) (15%) and WAO (20%) thresholds of efficacy. The baseline in the case of SLIT RCTs is the retrospective (prior year) total symptom score (RTSS), which is used by the investigators of the original RCTs as inclusion criteria. In other words, the RTSS is assumed by the investigators as the SS that the patients would have in the absence of any treatment (corresponding to the inclusion criteria). We acknowledge that the RTSS might be imprecise, but it should be similar to the SS of the treatment season, especially if the pollen count of the two consecutive seasons is similar, and we have shown for the Cox study, performed with 300IR 5-grass pollen sublingual tablet (Oralair®) in only US sublingual study, that this possible imprecision does not affect the results [102].

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In our meta-analysis [128], we reported the difference between SLIT and placebo not only in terms of the standardized mean difference (SMD) but also in terms of the mean difference (MD), which is the difference in SS points between SLIT and placebo. We showed that this difference is −0.83 SS points (95% CI, −1.03 to −0.63). In a recent work, Devillier and the Stallergenes©, an industry that market the SLIT, estimated the minimally important difference, which is defined as the smallest improvement considered worthwhile by a patient, as 1.1 to 1.3 SS points in patients with grass pollen-related rhinoconjunctivitis [131].

Therefore, according to the Devillier estimation, the difference that we found (−0.83 SS points) between SLIT and placebo is not perceived by the patients as clinically important, confirming the conclusions of our previous study. In **Figure 2**, we reported the 95% CI for the mean value (−1.03 to −0.63 for the random effect and even smaller for the fixed effect). This implies that we are 95% confident that this interval contains the true value of the parameter. Therefore −1.03 could be a value for the population parameter: even if it was the true value (the most favorable extreme to SLIT), the probability of observing a value of less than −1.1 is only 0.25 (25% of patients could benefit significantly from SLIT). In contrast, if the true value was that reported in our study as a point estimate, less than 0.5% of patients can show an improvement of greater than −1.1. This is in accordance with the calculation using our metric reporting an SS reduction to less than the WAO (20%) and FDA (15%) thresholds of efficacy.

Example study	SLIT	Placebo	Difference	Percentage improvement
RTSS (baseline)	95	95		
Mean SS during treatment	3	4	-1	(3 - 4)/4=**-25%** **(not including the scale)**
Difference	-92	-91	-1	
Percentage improvement	96.85%	95.8%		95.8%-96.9%=**-1.05%** **(91 - 92)/95=-1.05%** **(including the scale)**

Figure 2. Hypothetical 100-point scale, with a hypothetical RTSS (baseline score) = 95, congruent with a 100-point scale.) RTSS, retrospective total symptom score (baseline). With the calculation shown in RCTs (horizontal arrow) only the mean SS during the treatment is considered, ignoring the scale range. In WAO indicated calculation, the same we propose (vertical arrow), the scale range is included. The inclusion of the scale in the calculation changes the percentage of the improvement, even if the difference between the two groups remains the same.

Regarding safety of the AIT, on the basis of what is reported in RCTs the majority of adverse events are mild to moderate and that "both SCIT and SLIT are very safe" [132] but as we showed in our previous meta-analysis [105] indirectly comparing SCIT and SLIT, the withdrawal rate for adverse events was higher in the SLIT group (78 patients; 0.04% vs 0.013% in the placebo group) than in the SCIT group (18 patients; 0.019% vs 0.005% in the placebo group). This evidence should also be considered.

5. Conclusions

Regarding the physician-patient dialog to respect patient preference according to evidence-based medicine principles [15, 16], we believe that in the case of SLIT, the patient has to be informed correctly about the small benefit of the treatment.

In the interest of patients, caution must be exercised when such a small treatment benefit is reported, especially if one considers that sponsored studies (as in the case of all SLIT RCTs) always show greater benefit compared with independent studies using the same drugs or devices [133, 134].

This chapter shows that there is an increasing interest in risk-sharing schemes by both payers and manufacturers, as they serve as mechanisms for reduce uncertainty in collecting evidence once a new drug is already being used in a health care system. In principle, they could provide additional options to payers and manufacturers, to boost overall efficiency [135]. The ambitious goal is to help reduce the likelihood of payers adopting technologies that turn out not to be cost effective, while at the same time helping manufacturers earn profitable prices to invest in future innovative technologies. Italy is one of the countries that started early with these agreements: AIFA, the Italian drug agency, agreed on its first contract in July 2006 [136].

The regulatory authorities such as the US Food and Drug Administration (FDA), the European Medicines Agency (EMA) and the Japanese Medical Device Agency are responsible for the approval of any drug. Academia (including universities, scientific institutions and societies) is another major stakeholder with an important role in influencing the behavior of prescribers. Patients and consumer associations have also an important role: patients are involved in RCTs, consumers associations are consulted in decision concerning research priorities.

Finally, pharmaceutical companies maintain a sort of monopoly in development of new drugs and promote the drug and the sales [137]. In the European Union (EU), as well as in US, medicines are authorized by the European Commission (EC) and Federal Trade Commission, respectively. After a positive evaluation by the European Medicine Agency (EMA), Food Drug Administration (FDA) uses the centralized procedure or the national agencies through decentralized procedures. According to the EU legislation and provisions of the FDA, the evaluation of medicines seeking marketing authorization is only based on their quality, safety and efficacy. No information is required on their comparative efficacy with respect to drugs already available. In our case, SLIT has been compared only with placebo in all RCTs, the indirect comparison between SCIT and SLIT has shown that the SCIT is superior to SLIT [105]. After our meta-analysis, other studies have been published that concluded that SLIT has at least non-inferior efficacy and comparable safety compared to SCIT, but a lower annual cost [138, 139].

Our review provides moderate-grade evidence to support that SCIT is superior to SLIT for reduction of allergic rhinoconjunctivitis symptoms. Finally, we do not discuss the considerations on the disease-modifying effects of AIT, because they have been evaluated in another study that is difficult to get published because of the obvious conflict of interest in peer review which is responsible for reviewing the manuscript [140].

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References

[1] Ryan MW, Marple BF, Leatherman B, Mims JW, Fornadley J, Veling M, Lin SY. Current practice trends in allergy: results of a United States survey of otolaryngologists, allergist-immunologists and primary care physicians. Int Forum Allergy Rhinol. 2014; 4:789–95.

[2] Saporta D. Sublingual immunotherapy: a useful tool for the allergist in private practice. Biomed Res Int. 2016; 2016:9323804.

[3] Malling HJ, Abreu-Nogueira J, Alvarez-Cuesta E, Björkstén B, Bousquet J, Caillot D, Canonica GW, Passalacqua G, Saxonis-Papageorgiou P, Valovirta E. Local immunotherapy. Allergy. 1998; 53: 933–44.

[4] Herbert J. Rinkel, 1896–1963. J Allergy Clin Immunol. 1963; 34: 556–557.

[5] Curtis HH. The immunizing cure of hayfever. Med News (NY). 1900; 77: 16–18.

[6] Zeller M. Oral ragweed pollen therapy. J Allergy. 1939; 10: 579–589.

[7] London M. Combined oral and subcutaneous treatment for ragweed pollinosis. J Allergy. 1939; 10: 453–458.

[8] Golbert TM. A review of controversial diagnostic and therapeutic techniques employed in allergy. J Allergy Clin Immunol. 1975; 56: 170–90.

[9] American College of Physicians. Clinical ecology. Ann Intern Med. 1989; 111: 168–178.

[10] Thomas DP. The demise of bloodletting. J R Coll Physicians Edinb. 2014; 44: 72–77.

[11] Bousquet J, Van Cauwenberge P. A critical appraisal of 'evidence-based medicine' in allergy and asthma. Allergy. 2004; 59 (Suppl 78): 12–20.

[12] Berger WE, Meltzer EO. Intranasal spray medications for maintenance therapy of allergic rhinitis. Am J Rhinol Allergy. 2015; 29: 273–282.

[13] Varney VA, Gaga M, Frew AJ, Aber VR, Kay AB, Durham SR. Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by antiallergic drugs. BMJ 1991; 302: 265–269.

[14] Evidence-Based Medicine Working Group. Evidence-based medicine. A new approach to teaching the practice of medicine. JAMA. 1992; 268: 2420–2425.

[15] Sackett DL, Rosenberg WM, Gray JA, Haynes RB, Richardson WS. Evidence based medicine: what it is and what it isn't. BMJ. 1996; 312: 71–72.

[16] Haynes RB, Devereaux PJ, Guyatt GH. Physicians' and patients' choices in evidence based practice. BMJ. 2002; 324: 1350 (In 1993 JAMA publishes the guide on the search of the medical literature).

[17] Guyatt GH, Rennie D. Users' guides to the medical literature. JAMA. 1993; 270: 2096–2097.

[18] Shekelle PG, Woolf SH, Eccles M, Grimshaw J. Clinical guidelines: developing guidelines. BMJ. 1999; 318: 593–6.

[19] Hirsh J, Guyatt G. Clinical experts or methodologists to write clinical guidelines? Lancet 2009; 374: 273–275.

[20] Ioannidis JP. Why most published research findings are false. PLoS Med. 2005; 2: e124.

[21] Greenhalgh T, Howick J, Maskrey N. Evidence based medicine renaissance group. Evidence based medicine: a movement in crisis? BMJ. 2014; 348: g3725.

[22] Faulkner C, Fidler F, Cumming G. The value of RCT evidence depends on the quality of statistical analysis. Behav Res Ther. 2008; 46: 270–281.

[23] Alderson P, Gliddon L, Chalmers I. Academic recognition of critical appraisal and systematic reviews in British postgraduate medical education. Med Educ. 2003; 37: 386.

[24] Doucet M, Sismondo S. Evaluating solutions to sponsorship bias. J Med Ethics. 2008; 34: 627–630.

[25] Sismondo S. Pharmaceutical company funding and its consequences: a qualitative systematic review. Contemp Clin Trials. 2008; 29:109–113.

[26] Malling HJ, Weeke B. Position paper: immunotherapy. Allergy 1993; 48 (Suppl 14): 9–35.

[27] Noon L. Prophylactic inoculation against hay fever. Lancet 1911; 1: 1572–1573.

[28] Kay A.B. Landmarks in allergy during the 19th century. Bergmann K-C, Ring J (eds) In History of Allergy. Chem Immunol Allergy. Based, Karger 2014; 100: 21–26.

[29] Freeman J. Further observation on the treatment of hay fever by hypodermic inoculations of pollen vaccine. Lancet 1911; 2: 814–817.

[30] Freeman J. Rush inoculation. Lancet 1930; 2: 744.

[31] Reports of societies. BMJ. 1952; 1: 380.

[32] Dunbar WP. The present state of our knowledge of hay-fever. J Hyg. 1913; 13: 105–136.

[33] Frankland AW, Augustin R. Prophylaxis of summer hay-fever and asthma: a controlled trial comparing crude grass-pollen extracts with the isolated main protein component. Lancet. 1954; 266:1055–1057.

[34] Lowell FC, Franklin W. A double-blind study of the effectiveness and specificity of injection therapy in ragweed hay fever. N Engl J Med. 1965; 273: 675–679.

[35] Fontana VJ, Holt LE Jr, Mainland D. Effectiveness of hyposensitization therapy in ragweed hay-fever in children. JAMA. 1966; 195: 985–992.

[36] Ishizaka K, Ishizaka T, Hornbrook MM. Physico-chemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. J Immunol. 1966; 97: 75–85.

[37] Johansson SG. Raised levels of a new immunoglobulin class (IgND) in asthma. Lancet. 1967; 2: 951–953.

[38] Sherman WB, Stuli A, Cooke RA. Serologic change in hay fever treated over a period of years. J Allergy. 1940; 11: 225–244.

[39] Ungar G, J.L. Parrot, D. Bovet, Inhibition des effets de l'histamine sur l'intestineisole′ du cobaye par quelques substances sympathomime′ tiques et sympathicolytiques. C R Soc Biol., 1937; 124: 445–446.

[40] Black JW, Duncan WA, Durant CJ, Ganellin CR, Parsons EM. Definition and antagonism of histamine H2-receptors. Nature. 1972; 236: 385–390.

[41] Mygind N. Local effect of intranasal beclomethasone dipropionate aerosol in hay fever. Br Med J. 1973; 4: 464–466.

[42] CSM Update: desensitising vaccines. BMJ 1986; 293: 948.

[43] Reid MJ, Lockey RF, Turkeltaub PC, Platts-Mills TA. Survey of fatalities from skin testing and immunotherapy 1985–1989. J Allergy Clin Immunol. 1993; 92: 6–15.

[44] Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. J Allergy Clin Immunol. 1998; 102: 558–562.

[45] Holt LE. A nonallergist looks at allergy. NEJM. 1967; 276: 1449–1454.

[46] Lowell FC, Franklin W. Injection therapy. J Allergy Clin Immunol. 1968; 41: 174–175.

[47] Eiser N. Desensitisation today. BMJ. 1990; 300: 1412–1413.

[48] Bousquet J. Desensitisation today. BMJ. 1990; 301: 293.

[49] Malling HJ. Immunotherapy as an effective tool in allergy treatment. Allergy. 1998; 53: 461–472.

[50] Weyer A, Donat N, L'Heritier C, et al. Grass pollen hyposensitization versus placebo therapy. I. Clinical effectiveness and methodological aspects of a pre-seasonal course of desensitization with a four-grass pollen extract. Allergy. 1981; 36: 309–317.

[51] Grammer LC, Shaughnessy MA, Suszko IM, Shaughnessy JJ, Patterson R. A double-blind histamine placebocontrolled trial of polymerized whole grass for immunotherapy of grass allergy. J Allergy Clin Immunol. 1983; 72: 448–453.

[52] Ortolani C, Pastorello E, Moss RB, et al. Grass pollen immunotherapy: a single year double-blind, placebo controlled study in patients with grass pollen-induced asthma and rhinitis. J Allergy Clin Immunol. 1984; 73: 283–290.

[53] Grammer LC, Shaughnessy MA, Fmkle SM, Shaughnessy JJ, Patterson R. A double-blind placebo-controlled trial of polymerized whole grass administered in an accelerated dosage schedule for immunotherapy of grass pollinosis. J Allergy Clin Immunol. 1986; 78: 1180–1184.

[54] Bousquet J, Frank E, Soussana M, Hejjaoui A, Maasch HJ, Michel FB. Double-blind, placebo-controlled immunotherapy with a high-molecular-weight, formalinized allergoid in grass pollen allergy. Int Arch Allergy Appl Immunol. 1987; 82: 550–552.

[55] Bousquet J, Hejjaoui A, Skassa Brociek W, et al. Double blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. I. Rush immunotherapy with allergoids and standardized orchard grass-pollen extract. J Allergy Clin Immunol. 1987; 80: 591–598.

[56] Bousquet J, Maarch H, Martinot B, Hejjaoui A, Wahl R, Michel FB. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. II. Comparison between parameters assessing efficacy of immunotherapy. J Allergy Clin Immunol. 1988; 82: 439–446.

[57] Bousquet J, Maasch HJ, Hejjaoui A, et al. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. III. Efficacy and safety of unfractionated and high-molecular-weight preparations in rhinoconjunctivitis and asthma. J Allergy Clin Immunol. 1989; 84: 546–556.

[58] Bousquet J, Hejjaoui A, Soussana M, Michel F-B. Doubleblind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. IV. Comparison of the safety and efficacy of two dosages of a high-molecular-weight allergoid. J Allergy Clin Immunol. 1990; 85: 490–497.

[59] Bousquet J, Becker WM, Hejjaoui A, et al. Differences in clinical and immunologic reactivity of patients allergic to grass pollens and to multiple-pollen species. II. Efficacy of a double-blind, placebo-controlled, specific immunotherapy with standardized extracts. J Allergy Clin Immunol. 1991; 88: 43–53.

[60] Machiels JJ, Somville MA, Jacquemin MG, Saint-Remy JMR. Allergen-antibody complexes can efficiently prevent seasonal rhinitis and asthma in grass pollen hypersensitive patients. Allergy. 1991; 46: 335–348.

[61] Pastorello EA, Pravettoni V, Incorvaia C, et al. Clinical and immunological effects of immunotherapy with alum-absorbed grass allergoid in grass-pollen-induced hay fever. Allergy. 1992; 47: 281–290.

[62] Dolz 1, Martinez-C6cera C, Bartolome JM, Cimarra M. A double-blind, placebo-controlled study of immunotherapy with grass-pollen extract Alutard SO during a 3-year period with initial rush immunotherapy. Allergy. 1996; 51: 489–500.

[63] Zenner HP, Baumgarten C, Rasp G, et al. Short-term immunotherapy: a prospective, randomized, double-blind, placebo-controlled multicenter study of molecular standardized grass and rye allergens in patients with grass pollen-induced allergic rhinitis. J Allergy Clin Immunol. 1997; 100: 23–29.

[64] Watkins AD. The role of alternative therapies in the treatment of allergic disease. Clin Exp Allergy. 1994; 24: 813–825.

[65] Johansson SG, Deuschl H, Zetterström O. Use of glutaraldehyde-modified timothy grass pollen extract in nasal hyposensitisation treatment of hay fever. Int Arch Allergy Appl Immunol. 1979; 60: 447–460.

[66] Georgitis JW, Reisman RE, Clayton WF, Mueller UR, Wypych JI, Arbesman CE. Local intranasal immunotherapy for grass-allergic rhinitis. J Allergy Clin Immunol. 1983; 71: 71–76.

[67] Georgitis JW, Clayton WF, Wypych JI, Barde SH, Reisman RE. Further evaluation of local intranasal immunotherapy with aqueous and allergoid grass extracts. J Allergy Clin Immunol. 1984; 74: 694–700.

[68] Andri L, Senna G, Betteli C, et al. Local nasal immunotherapy with extract in powder form is effective and safe in grass-pollen rhinitis. A double blind study. J Allergy Clin Immunol. 1996; 97: 34–41.

[69] Bardare M, Zani G, Novembre E, Vierucci A, Local nasal immunotherapy with a powder extract for grass pollen induced rhinitis in pediatric ages: a controlled study, J Investig Allergol Immunol. 1996; 6: 359–363.

[70] Crimi E, Voltolini S. Troise C, et al. Local immunotherapy with *Dermatophagoides* extract in asthma. J Allergy Clin Immunol. 1991; 87: 721–728.

[71] Cooper PJ, Darbyshire J, Nunn AJ, Warner JO. A controlled trial of oral hyposensitization in pollen asthma and rhinitis in children. Clin Allergy. 1984; 14: 541–550.

[72] Taudorf E, Laursen LC, Djurup R, et al. Oral administration of grass pollen to hay fever patients. An efficacy study in oral hyposensitization. Allergy. 1985; 40: 321–335.

[73] Mosbech H, Dreborg S, Madsen F, et al. High dose grass pollen tablets used for hyposensitization in hay fever patients. A one-year double blind placebo controlled study. Allergy. 1987; 42: 451–455.

[74] Sabbah A, Hassoun S, Le Sellin J, Andrd C, Sicard H. A double blind placebo controlled trial by the sublingual route of immunotherapy with a standardized grass pollen extract. Allergy. 1994; 49: 309–313.

[75] Feliziani V, Lattuada G, Parmiani S, Dall'Aglio PP. Safety and efficacy of sublingual rush immunotherapy with grass allergen extracts. A double blind study. Allergol Immunopathol (Madr). 1995; 23: 224–230.

[76] Björkstén B. Local immunotherapy is not documented for clinical use. Allergy. 1994; 49: 299–301.

[77] van Niekerk CH, De Wet Jl. Efficacy of grass-maize pollen oral immunotherapy in patients with seasonal hay fever: a double blind study. Clin Allergy 1987; 17: 507–513.

[78] Feliziani V, Marfisi RM. Parmiani S. Rush immunotherapy with sublingual administration of grass allergen extract. Allergol Immunopathol (Madr). 1993; 21: 173–178.

[79] Wilson DR, Torres LI, Durham SR. Sublingual immunotherapy for allergic rhinitis. Cochrane Database Syst Rev. 2003; 2: CD002893.

[80] Radulovic S, Calderon MA, Wilson D, Durham S. Sublingual immunotherapy for allergic rhinitis. Cochrane Database Syst Rev. 2010; 12: CD002893.

[81] Hordijk GJ, Antvelink JB, Luwema RA. Sublingual immunotherapy with a standardized grass pollen extract; a double-blind placebo-controlled study. Allergol Immunopathol (Madr). 1998; 26: 234–240.

[82] Pradalier A, Basset D, Claudel A, Couturier P, Wessel F, Galvain S, et al. Sublingual-swallow immunotherapy (SLIT) with a standardized five-grass-pollen extract (drops and sublingual tablets) versus placebo in seasonal rhinitis. Allergy. 1999; 54: 819–828.

[83] Torres-Lima M, Wilson D, Pitkin L, Roberts A, Nouri-Aria K, Jacobson M, et al. Grass pollen sublingual immunotherapy for seasonal rhinoconjunctivitis: a randomized controlled trial. Clin Exp Allergy. 2002; 32: 507–14.

[84] Smith H, White P, Annila I, Poole J, Andre C, Frew A. Randomized controlled trial of high-dose sublingual immunotherapy to treat seasonal allergic rhinitis. J Allergy Clin Immunol. 2004; 114: 831–837.

[85] Quirino T, Iemoli E, Siciliani E, Parmiani S, Milazzo F. Sublingual versus injective immunotherapy in grass pollen allergic patients: a double blind (double dummy) study. Clin Exp Allergy. 1996; 26:1253–1261.

[86] Clavel R, Bousquet J, André C. Clinical efficacy of sublingual-swallow immunotherapy: a double-blind, placebo-controlled trial of a standardized five-grass-pollen extract in rhinitis. Allergy. 1998; 53: 493–498.

[87] Rolinck-Werninghaus C, Wolf H, Liebke C, Baars JC, Lange J, Kopp MV, et al. A prospective, randomized, double-blind, placebo-controlled multi-centre study on the efficacy and safety of sublingual immunotherapy (SLIT) in children with seasonal allergic rhinoconjunctivitis to grass pollen. Allergy. 2004; 59: 1285–1293.

[88] Bufe A, Ziegler-Kirbach E, Stoeckmann E, Heidemann P, Gehlhar K, Holland-Letz T, et al. Efficacy of sublingual swallow immunotherapy in children with severe grass pollen allergic symptoms: a double-blind placebo-controlled study. Allergy. 2004; 59: 498–504.

[89] Dahl R, Kapp A, Colombo G, de Monchy JG, Rak S, Emminger W, Rivas MF, Ribel M, Durham SR. Efficacy and safety of sublingual immunotherapy with grass allergen tablets for seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol. 2006; 118: 434–440.

[90] Dahl R, Stender A, Rak S. Specific immunotherapy with SQ standardized grass allergen tablets in asthmatics with rhinoconjunctivitis. Allergy. 2006; 61: 185–190.

[91] Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once-daily grass allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol. 2006; 117: 802–809.

[92] de Blay F, Barnig C, Kanny G, Purohit A, Leynadier F, Tunon de Lara JM, et al. Sublingual-swallow immunotherapy with standardized 3-grass pollen extract: a double-blind, placebo-controlled study. Ann Allergy Asthma Immunol. 2007; 99: 453–461.

[93] Didier A, Malling HJ, Worm M, Horak F, Jager S, Montagut A, et al. Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. J Allergy Clin Immunol. 2007; 120: 1338–1345.

[94] Mosges R, Bruning H, Hessler HJ, Gotz G, Knaussmann HG. Sublingual immunotherapy in pollen-induced seasonal rhinitis and conjunctivitis: a randomized controlled trial. Acta Dermatovenerol Alp Panonica Adriat. 2007; 16: 143–148.

[95] Roder E, Berger MY, Hop WC, Bernsen RM, de Groot H, Gerth van Wijk R. Sublingual immunotherapy with grass pollen is not effective in symptomatic youngsters in primary care. J Allergy ClinImmunol. 2007; 119: 892–898.

[96] Pfaar O, Klimek L. Efficacy and safety of specific immunotherapy with a high-dose sublingual grass pollen preparation: a double-blind, placebo-controlled trial. Ann Allergy Asthma Immunol. 2008; 100: 256–263.

[97] Wahn U, Tabar A, Kuna P, Halken S, Montagut A, de Beaumont O, et al. Efficacy and safety of 5-grass-pollen sublingual immunotherapy tablets in pediatric allergic rhinoconjunctivitis. J Allergy ClinImmunol. 2009; 123: 160–166.

[98] Ott H, Sieber J, Brehler R, Fölster-Holst R, Kapp A, Klimek L, et al. Efficacy of grass pollen sublingual immunotherapy for three consecutive seasons and after cessation of treatment: the ECRIT study. Allergy. 2009; 64: 1394–1401.

[99] Bufe A, Eberle P, Franke-Beckmann E, Funck J, Kimmig M, Klimek L, et al. Safety and efficacy in children of an SQ-standardized grass allergen tablet for sublingual immunotherapy. J Allergy ClinImmunol. 2009; 123: 167–173.

[100] Horak F, Zieglmayer P, Zieglmayer R, Lemell P, Devillier P, Montagut A, et al. Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. J Allergy ClinImmunol. 2009; 124: 471–477.

[101] Di Bona D, Plaia A, Scafidi V, Leto-Barone MS, Di Lorenzo G. Efficacy of sublingual immunotherapy with grass allergens for seasonal allergic rhinitis: a systematic review and meta-analysis. J Allergy Clin Immunol. 2010; 126: 558–66.

[102] Cox L, Wallace D. Specific allergy immunotherapy for allergic rhinitis: subcutaneous and sublingual. Immunol Allergy Clin North Am. 2011; 31: 561–599.

[103] Calderon MA, Casale TB, Togias A, Bousquet J, Durham SR, Demoly P. Allergen specific immunotherapy for respiratory allergies: from meta-analysis to registration and beyond. J Allergy ClinImmunol. 2011; 127: 30–38.

[104] Canonica GW, Baena-Cagnani CE, Bousquet J, Bousquet PJ, Lockey RF, Malling HJ, et al. Recommendations for standardization of clinical trials with Allergen Specific Immunotherapy for respiratory allergy. A statement of a World Allergy Organization (WAO) taskforce. Allergy. 2007; 62: 317–324.

[105] Di Bona D, Plaia A, Leto-Barone MS, La Piana S, Di Lorenzo G. Efficacy of subcutaneous and sublingual immunotherapy with grass allergens for seasonal allergic rhinitis: a meta-analysis-based comparison. J Allergy Clin Immunol. 2012; 130: 1097–1107.e2.

[106] Caffarelli C, Sensi LG, Marcucci F, Cavagni G. Preseasonal local allergoid immunotherapy to grass pollen in children: a double-blind, placebo-controlled, randomized trial. Allergy. 2000; 55: 1142–1147.

[107] Blaiss M, Maloney J, Nolte H, Gawchik S, Yao R, Skoner DP. Efficacy and safety of timothy grass allergy immunotherapy tablets in North American children and adolescents. J Allergy ClinImmunol. 2011; 127: 64–71.

[108] Nelson HS, Nolte H, Creticos P, Maloney J,Wu J, Bernstein DI. Efficacy and safety of timothy grass allergy immunotherapy tablet treatment in North American adults. J Allergy Clin Immunol. 2011; 127: 72–80.

[109] Brewczynski PZ, Kroon AM. Efficacy and safety of immunotherapy with modified grass pollen allergens. Results of a placebo controlled study [Wirksamkeit und vertraglichkeit einer immuntherapie mit modifizierten graserpollenallergenen]. Allergologie. 1999; 22: 411–420.

[110] Drachenberg KJ, Wheeler AW, Stuebner P, Horak F. A well-tolerated grass pollen specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. Allergy. 2001; 56: 498–505.

[111] Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol. 2005; 116: 608–613.

[112] Corrigan CJ, Kettner J, Doemer C, Cromwell O, Narkus A. Study Group. Efficacy and safety of pre seasonal-specific immunotherapy with an aluminium-adsorbed six-grass pollen allergoid. Allergy. 2005; 60: 801–807.

[113] Frew AJ, Powell RJ, Corrigan CJ, Durham SR. UK Immunotherapy Study Group. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol. 2006; 117: 319–325.

[114] DuBuske LM, Frew AJ, Horak F, Keith PK, Corrigan CJ, Aberer W, et al. Ultrashort-specific immunotherapy successfully treats seasonal allergic rhinoconjunctivitis to grass pollen. Allergy Asthma Proc. 2011; 32: 239–247.

[115] Pfaar O, Urry Z, Robinson DS, Sager A, Richards D, Hawrylowicz CM, et al. A randomized placebo-controlled trial of rush preseasonal depigmented polymerized grass pollen immunotherapy. Allergy. 2012; 67: 272–279.

[116] Higgins JP. Commentary: heterogeneity in meta-analysis should be expected and appropriately quantified. Int J Epidemiol. 2008; 37: 1158–1160.

[117] Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for seasonal allergic rhinitis. Cochrane Database Syst Rev. 2007; 1: CD001936.

[118] Calderon MA, Andersen JS, Nelson HS. Are meta-analysis-based comparisons solid evidence? J Allergy ClinImmunol. 2013; 132: 506–508.

[119] Di Bona D, Plaia A, Di Lorenzo G. Reply: To PMID 23021885 (Are meta-analysis-based comparisons solid evidence?). J Allergy Clin Immunol. 2013; 132: 508–510.

[120] Canonica GW, Cox L, Pawankar R, et al. Sublingual immunotherapy: World Allergy Organization position paper 2013 update. World Allergy Organ J. 2014; 7: 6.

[121] US Food and Drug Administration approval notification.

[122] [http://www.fda.gov/downloads/BiologicsBloodVaccines/Allergenics/UCM391580.pdf (Accessed on April 04, 2014).]

[123] [US FDA approval letter. http://www.fda.gov/biologicsbloodvaccines/allergenics/ucm 393185.htm (Accessed on April 15, 2014).]

[124] Halken S, Agertoft L, Seidenberg J, et al. Five-grass pollen 300IR SLIT tablets: efficacy and safety in children and adolescents. Pediatr Allergy Immunol. 2010; 21: 970–976.

[125] Cox LS, Casale TB, Nayak AS, et al. Clinical efficacy of 300IR 5-grass pollen sublingual tablet in a US study: the importance of allergen-specific serum IgE. J Allergy Clin Immunol. 2012; 130: 1327–1334.e1.

[126] Murphy K, Gawchik S, Bernstein D, et al. A phase 3 trial assessing the efficacy and safety of grass allergy immunotherapy tablet in subjects with grass pollen-induced allergic rhinitis with or without conjunctivitis, with or without asthma. J Negat Results Biomed. 2013; 12: 10–19.

[127] Maloney J, Bernstein DI, Nelson H, et al. Efficacy and safety of grass sublingual immunotherapy tablet, MK-7243: a large randomized controlled trial. Ann Allergy Asthma Immunol. 2014; 112:146–153.

[128] Di Bona D, Plaia A, Leto-Barone MS, La Piana S, Di Lorenzo G. Efficacy of Grass Pollen Allergen Sublingual Immunotherapy Tablets for Seasonal Allergic Rhinoconjunctivitis: A Systematic Review and Meta-analysis. JAMA Intern Med. 2015; 175: 1301–1309.

[129] DerSimonian R, Laird N. Meta-analysis in clinical trials. Controlled Clin Trials. 1986; 7: 177–188.

[130] Cohen J. Statistical power analysis for the behavioral sciences. 2nd edition. Hillsdale (NJ): Lawrence Erlbaum Associates; 1988.

[131] Devillier P, Chassany O, Vicaut E, de Beaumont O, Robin B, Dreyfus JF, et al. The minimally important difference in the Rhinoconjunctivitis Total Symptom Score in grass-pollen-induced allergic rhinoconjunctivitis. Allergy. 2014; 69: 1689–1695.

[132] Aasbjerg K, Dalhoff KP, Backer V. Adverse events during immunotherapy against grass pollen-induced allergic rhinitis—differences between subcutaneous and sublingual treatment. Basic Clin Pharmacol Toxicol. 2015; 117: 73–84.

[133] Lundh A, Sismondo S, Lexchin J, Busuioc OA, Bero L. Industry sponsorship and research outcome. Cochrane Database Syst Rev 2012; 12:MR000033.

[134] Drugs and devices look more effective in studies sponsored by industry. BMJ 2012; 345: e8386.

[135] Towse, A., Garrison, L.P.: Can't get no satisfaction? Will pay for performance help? Pharmacoeconomics. 2010; 28: 93–102.

[136] Agenzia Italiana del Farmaco. L'uso dei farmaci in Italia. Rapporto nazionale anno 2012. Osservatorio Nazionale sull'impiego dei Medicinali, OsMed. Roma, (2013). http://www.agenziafarmaco.gov.it/it/content/rapporti-osmed-luso-dei-farmaci-italia. Accessed 19 Mar 2014

[137] Goldacre, B. Bad Pharma: How Drug Companies Mislead Doctors and Harm Patients (Fourth Estate, 2012. 364)

[138] Chelladurai Y, Suarez-Cuervo C, Erekosima N, Kim JM, Ramanathan M, Segal JB, Lin SY. Effectiveness of subcutaneous versus sublingual immunotherapy for the treatment of allergic rhinoconjunctivitis and asthma: a systematic review. J Allergy Clin Immunol. Pract 2013; 1: 361–369.

[139] Dranitsaris G, Ellis AK. Sublingual or subcutaneous immunotherapy for seasonal allergic rhinitis: an indirect analysis of efficacy, safety and cost. J Eval Clin Pract. 2014; 20: 225–238.

[140] Manchikanti L, Kaye AD, Boswell MV, Hirsch JA. Medical journal peer review:process and bias. Pain Physician. 2015; 18:E1–E14.

Chapter 17

Allergen-Specific Immunotherapy Follow-Up by Measuring Allergen-Specific IgG as an Objective Parameter

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Additional information is available at the end of the chapter

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Abstract

The clinical efficacy of the allergen-specific immunotherapy (AIT) has been well-documented using inhalant or hymenoptera-derived allergens in atopic patients with corresponding specific IgE antibodies. AIT is considered as the unique treatment that is capable of modifying the natural course of the allergic disease because it induces a variety of immunological mechanisms, with emphasis in the production of blocking IgG antibodies by IL-10-stimulated B cells due to the generation of Treg, Breg, or even Th2 cells. Thus, the measurement of specific IgG subclasses, particularly IgG4, to the crude extract or more importantly to allergen components, might be a useful and potential tool to follow-up objectively the patients undergoing AIT in addition to clinical parameters. In this chapter, the authors have emphasized a very sensitive and highly specific reverse ELISA, developed by them, to measure IgG subclasses directed to clinically relevant natural allergens that are undoubtedly better when compared to those obtained with recombinant counterparts. Such a technique may produce more authentic results taking into account the IgG subclass binding capacity to a particular allergen and might be a valuable and alternative method for monitoring activation of tolerance-inducing mechanisms in patients under AIT.

Keywords: allergen-specific immunotherapy, immunotherapy follow-up, blocking antibody, IgE, IgG4

1. Introduction

Allergen-specific immunotherapy (AIT) is indicated for atopic patients with IgE-mediated allergic diseases, particularly in allergic rhinitis, mild or moderate asthma and hymenoptera sting allergy. AIT is an effective treatment that aims to induce changes in immune response against specific allergen components derived from causal agents instead of the exteriorized symptoms, helping for modifying the natural course of the allergic disease and improving the patients' quality of life by the reduction of symptoms and medication use when naturally exposed to sensitized allergens. It involves a build-up phase that consists of the administration of gradually increasing levels of specific allergens until an effective dose that enables the reduction of the severity of the disease is reached, even in the presence of the natural allergen exposure [1].

On the one hand, the classical respiratory allergic disease is mediated by IgE antibodies to indoor or outdoor inhalant allergens through the development of Th2 cells that produce a well-known cytokine profile, including IL-4 and IL-13 [2]. These cytokines are crucial to cause antibody class switch on B cells to induce the synthesis of IgE antibodies, which in turn bind to mast cells and basophils that possess Fc epsilon receptor (FcεR types I or II) on their membranes, inducing the sensitization phase. In subsequent contacts, allergens containing genuine- or cross-reactive epitopes capable to cross-link to IgE bound to target cells can activate these cells, with consequent release of preformed and newly formed vasoactive mediators. The preformed mediators (histamine) are responsible for early phase symptoms and newly formed those (leukotrienes and cytokines) for inducing a late-phase response, characterizing the type I hypersensitivity reaction [3].

On the other hand, the administration of allergens by AIT has been proved to cause early allergen-specific mast cell desensitization, likely as a consequence of the development of regulatory T cells (Tr1 cells) that particularly produce IL-10, which induces antibody class switch on B cells to produce IgG4 antibody subclass. An alternative way to produce other subclasses of IgG can be achieved due to the fact that AIT can provoke immune deviation from Th2 in favor of Th1 responses that culminate in the production of IFN-γ, which induces B cells to produce IgG1 subclass [4]. In the initial phase of AIT the immunological response involves the production of IgG1 antibodies whereas IgG4 is the dominant subclass in prolonged AIT. Therefore, IgG antibodies induced by AIT may act as blocking antibodies, reflecting in the reduction of mast cell activation and degranulation as well as competing with IgE antibodies for allergen binding, blocking IgE-dependent mast cell activation and inhibiting IgE-facilitated allergen presentation [1].

Currently, there is no routine laboratorial test for the detection of allergen-specific IgG antibodies, particularly IgG1 and IgG4 subclasses. Physicians, who assist patients with respiratory allergy that have been submitted to AIT, are following the treatment of such patients only by subjective clinical parameters. The possibility of following such patients under AIT by laboratorial evaluation of allergen-specific IgG1 and/or IgG4 levels has stimulated researchers to develop objective methods for quantifying allergen-specific IgG antibodies.

The detection of IgG antibodies, particularly IgG1 and IgG4 subclasses, against specific allergenic components, such as the major allergens of *Dermatophagoides pteronyssinus* (Der p 1 and

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Der p 2) would indicate the development of a physiological response, i.e., a defense response against dust mite allergens [5]. Production of specific IgG4 antibodies to relevant allergenic components has been associated with the protective activity due to its function as blocking antibody through mechanisms of competition for allergen between IgG4 and cell-bound IgE antibodies [6]. Thus, the role of specific serum IgG subclasses, particularly IgG4, might be considered as a good marker of protective or blocking antibody that may be useful for monitoring activation of tolerance-inducing mechanisms in patients under AIT.

Therefore, it becomes particularly interesting the development of a method for quantifying IgG subclasses against clinically relevant allergens. These antibodies can be detected in the serum or other biological fluids, such as saliva from patients with allergic respiratory disease using an immunoenzymatic technique (reverse ELISA) and allergen component-specific monoclonal antibodies for monitoring patients under AIT. This assay represents a potential tool for monitoring patients with respiratory allergy, especially during AIT.

2. Allergic response

2.1. Sensitization phase

The balance of the different subsets of T helper cells such as Th1, Th2 and Treg with their cytokine profiles supports the maintenance of the homeostasis of the immune system. The breakdown of this balance among Th1, Th2 and Treg cells leads to excessive activation of Th1 or Th2 cells, culminating in the development of autoimmune diseases or induction of IgE-mediated allergic diseases, respectively [2]. Allergies are one of the most prevalent diseases in the world, once they are a result of a breakdown in the immune tolerance that individuals usually have to food, inhalant and insect venom allergens [7, 8]. These diseases have a mechanism of response based on an interaction of the innate and adaptive immune system, with interaction of various cell types, cytokines, chemokines and costimulatory signals responsible for different T-cell responses [9].

Th2-cell subset is induced in a classical respiratory allergic disease, triggering a pathogenesis related to several indoor or outdoor inhalant allergens as excretions of house dust mite and cockroaches, animal dander, pollens and fungal spores, among others [10]. In addition, the dose and function of the allergen are relevant for allergic sensitization [7]. This step is the first event of the classical pathogenesis, which is mediated by producing specific IgE antibodies directed to epitopes derived from inhalant allergens through the development of Th2 cells. First of all, the allergens can pass through the epithelial tissue cells of the respiratory tract or directly bind in receptors of innate immune cells. Then, allergens are uptaken and processed by professional antigen-presenting cells (APCs), as dendritic cells (DCs), that present peptides through class II major histocompatibility complexes (MHC II) to naive CD4+ T cells located in the submucosal layer, driving to effector and memory T cells of the Th2 phenotype (**Figure 1**) [11]. For that, APCs mediate the production and secretion of crucial cytokines as IL-4, characterizing the occurrence of the third signal of the immune response, which will be responsible for the STAT-6 activation and subsequently GATA-3 (GATA-binding protein 3

transcription factor) upregulation [12]. Besides the antigenic peptide presentation (first signal of the immune response), the participation of costimulatory molecules (second signal) is necessary to reach the development of Th2 cells by increasing the expression of genes encoded on 5q31-33 chromosome (**Figure 1**). These genes are associated to IL-3, IL-4, IL-5, IL-9, IL-13 cytokines and granulocyte-macrophage colony stimulating factor (GM-CSF) codification, related to Th2 pathway [13]. Some of these cytokines, such as IL-4 and IL-13, are responsible for switching the antibody class on B cell to induce the synthesis of IgE antibodies, which bind certain target cells that possess Fc epsilon receptor (FcεR) type I (high-affinity) or type II (low-affinity) on their membranes like mast cells and basophils, leading to the establishment of the sensitization phase [14].

Some allergens, as proteolytic protein or lipopolysaccharide (LPS), can stimulate other bias of Th2 response, once the linkage of proteolytic allergens to pattern recognition receptors (PRRs) like protease-activator receptors (PARs), or a linkage of LPS to toll-like receptors (TLRs), both localized in barrier epithelial cells, or even the production of reactive oxygen species (ROS) by damaged cells can promote various effects that drive to a proinflammatory response. For instance, PARs and TLRs can be a trigger to epithelial cells to produce cytokines, like thymic stromal lymphopoietin (TSLP), interleukin-25 (IL-25) and IL-33 related to allergic inflammation (**Figure 1**) [15, 16]. IL-25 and IL-33 can upregulate NFκ-B, together with TSLP that activate

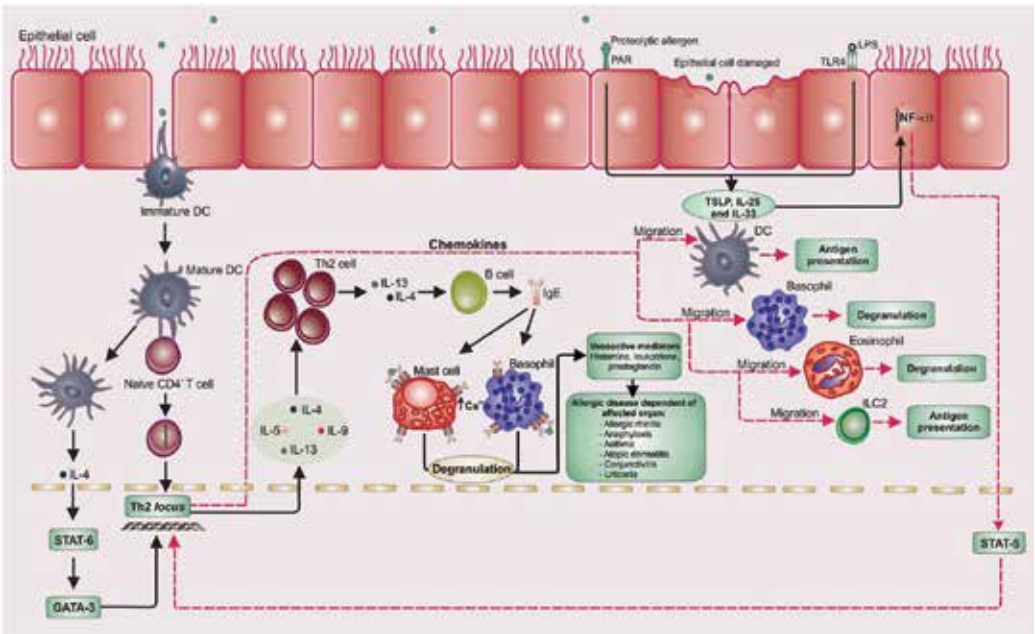

Figure 1. Innate and adaptive allergic immune response. Sequential events for allergen sensitization and triggering the immune response that generates different allergic diseases depending on the affected organs are shown. $CD4^+$: cluster of differentiation 4; DC: dendritic cell; GATA-3: GATA-binding protein 3 transcription factor; ILC2: group 2 innate lymphoid cell; LPS: lipopolysaccharide; NF-κB: nuclear factor kappa B; PAR: protease-activated receptor; STAT-5: signal transducer and activator of transcription 5; STAT-6: signal transducer and activator of transcription 6; TLR4: Toll-like receptor type 4; TSLP: thymic stromal lymphopoietin.

STAT-5 promoting an increase of Th2 genes regulation. This way stimulates the production of chemokines and cytokine release that contribute to cell migration, especially DCs, basophils and eosinophils as well as group 2 innate lymphoid cells (ILC2) involved in allergic responses (**Figure 1**) [17, 18].

Taken together, there are mechanisms that promote a Th2 pathway by GATA-3 upregulation induced especially by IL-4-activated STAT-6, or a Th2 route in which GATA-3 expression is induced in an IL-4 and STAT-6-independent manner [2]. Thus, the maintenance of Th2 responses by environmental allergens is related to the type of recognition of the allergens in the epithelial barrier, which promotes the linkage of innate and adaptive responses [19].

2.2. Effector phase

Allergic subjects besides mast cells and eosinophils with a greater number of IgE receptors, have an increase of IgE-producing B cells stimulated by IL-4 and IL-13-secreting Th2 subset [20]. In a subsequent contact with allergens that contain genuine- or cross-reactive epitopes capable to cross-link IgE bound to target cells, calcium-dependent activation of these cells can occur with release of preformed vasoactive mediators as histamine responsible for the early phase symptoms and newly formed vasoactive mediators like leukotrienes and cytokines for late phase symptoms (**Figure 1**) [21]. These mediators are characterized by the maintenance of long-lasting symptoms due to the continued tissue inflammation and injury, characterizing typically the type I hypersensitivity reaction. Therefore, maturation of eosinophils induced especially by IL-5 and basophils by IL-3 and IL-4 are the main secreting effector cells of inflammatory mediators observed in the classical allergic response [12]. Local symptoms or systemic anaphylaxis may be observed depending the affected organ and tissues in a particular individual response to sensitized allergens (**Figure 1**) [21].

The intensity of the immune response to allergens is crucial to develop an allergic condition mediated by IgE antibody, or a healthy condition depending on the individual gene susceptibility, environmental pollutants, features of allergens, among others [22–24]. Other antibody classes have been analyzed because of this variation of response between allergic and healthy subjects, such as IgA and IgG subclasses [23–25]. In healthy individuals, B cell response to house dust mite allergens ranges from no response to predominantly production of IgG antibodies specific to allergens, particularly IgG1 or IgG4 subclass, in the absence or low concentration of IgE. Differently, IgG levels, particularly IgG4 subclass, have also been detected in allergic subjects in addition to high levels of IgE, but IgG1 levels have been found at similar levels in both healthy and allergic individuals [26, 27].

3. Cellular and molecular mechanisms of AIT

Allergen-specific immunotherapy (AIT) is performed by the administration of increasing concentrations of allergens (build-up phase) up to maintenance doses, mainly given by subcutaneous, epicutaneous, oral, sublingual, or recently by intralymphatic route. AIT aims to induce

changes on the immune response of allergic individuals, drawing a state of allergen-specific tolerance, which contributes with a curative effect for a long period of time [28–32].

The cellular and molecular mechanisms of AIT are diverse, involving the very early mast cell and basophil desensitization, effect on antigen-presenting cells, modulation of T and B cell repertories as well as modification of allergen-specific antibody responses (**Figure 2**) [32].

Although AIT reduces the allergic inflammation mediated by IgE-dependent mechanism over the time, a very early effect on basophil and mast cell activation status is observed just after the initiation of the therapeutic regimen, leading to a lower risk to develop anaphylactic manifestations [33–35]. The subjacent mechanism of basophil and mast cell desensitization has not been elucidated yet; however, some clues highlight this issue. AIT leads to a controlled releasing of histamine and leukotrienes by basophil and mast cells after allergen administration, producing a gradual reduction of granule content of the inflammatory mediators in these cells in patients submitted to immunotherapy [31, 33, 36], although there is not a direct evidence of diminution of intracellular vasoactive mediator amount by histological analysis. Short-term venom immunotherapy induces desensitization of FcεRI-mediated basophil response. The levels of mRNA and FcεRI cell-surface expression decreased in basophil cells from patients submitted to venom immunotherapy, indicating that the reduction in FcεRI

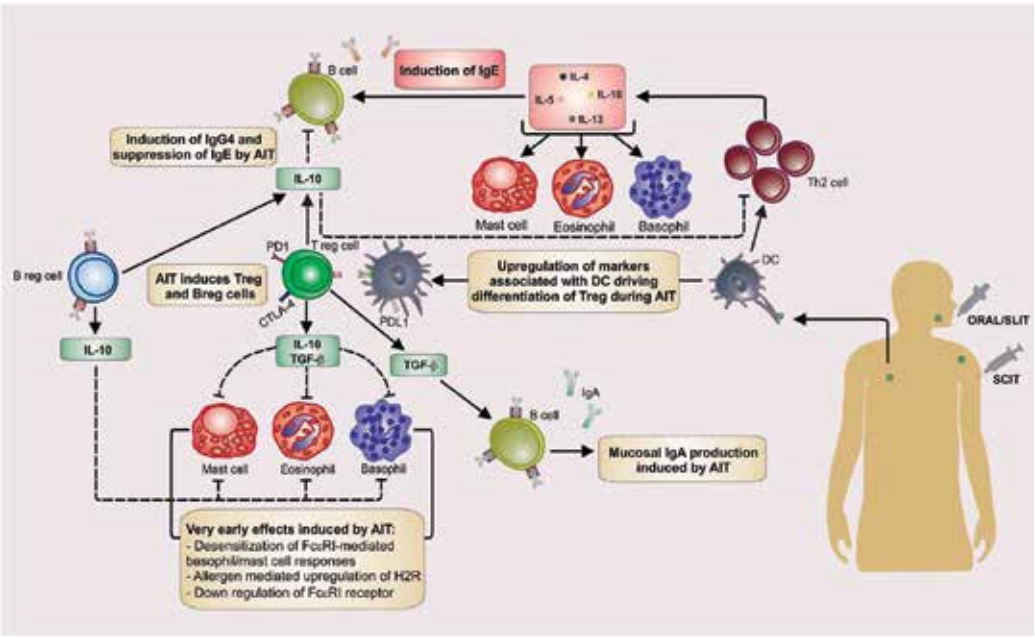

Figure 2. Immunological changes induced by allergen-specific immunotherapy (AIT). Desensitization of basophil/mast cells and upregulation of markers associated with dendritic cells (DCs) driving differentiation of IL-10-producing Treg and Breg cells and subsequent activation of B cells to synthesize allergen-blocking factors, particularly IgG4 and suppression of IgE antibodies during AIT are shown. AIT: allergen-specific immunotherapy; Breg cell: regulatory B cell; CTLA-4: cytotoxic T lymphocyte antigen-4; DC: dendritic cell; FcεRI: high-affinity receptor for the Fc region of immunoglobulin E (IgE); H2R: histamine H2 receptor; PD1: programmed death-1 receptor; PDL1: programmed death ligand-1; SCIT: subcutaneous immunotherapy; ORAL/SLIT: oral/sublingual immunotherapy; Treg cell: regulatory T cell.

expression contributes to the phenomenon of the early basophil desensitization observed after AIT [37, 38]. On the other hand, AIT also provokes an allergen-mediated upregulation of the type 2 histamine receptor (H2R) gene, which was associated with the suppression of FcεRI-mediated basophil activation, inducing a tolerogenic response (**Figure 2**) [34, 39]. The engagement of H2R with its agonists prevents further histamine and leukotriene releasing as well as IL-4 and IL-8 production by basophil cells [34]. The molecular mechanism involved in H2R-dependent basophil desensitization is supposed to be mediated by the cAMP pathway because the stimulation with H2R agonist or with a direct cAMP inducer was able to inhibit the FcεRI-mediated basophil activation [34]. In this way, the increase of concentration of cAMP activates PKA (Protein Kinase A, the principal intracellular target of cAMP), which in turn decreases the intracellular calcium influx, thus preventing FcεRI-dependent basophil and mast cell degranulation [22].

Antigen-presenting cells, particularly dendritic cells (DC), display an important role in the induction of allergic diseases driving Th2 responses and the IgE-dependent pathophysiologic mechanism. Some evidences reveal that AIT can affect directly the phenotype of the antigen-presenting cells correlating with clinical improvement in patients with allergic diseases [40–42]. A regulatory dendritic cell signature correlating with the clinical efficacy after allergen-specific sublingual immunotherapy (SLIT) has been observed in peripheral blood mononuclear cells (PBMCs) from clinical allergic responders in comparison with nonresponders or patients that received only placebo [40]. Likewise, a report using transcriptomic and proteomic approaches demonstrated that PBMCs from allergic patients downregulate the expression of markers related with DC driving the differentiation of Th2 cells, whereas upregulate markers associated with DC driving differentiation of T regulatory cells, after only 4 months of SLIT. These results indicate that AIT has an early effect on antigen-presenting cells that trigger the Th2 downregulation [42]. Therefore, the changes evoked during AIT regimen on antigen-presenting cells, with a predominance of DC tolerogenic subsets inducing the development of T regulatory (Treg) cells, may be part of the mechanism behind of the therapeutic efficacy observed in AIT (**Figure 2**).

The induction of the allergen-specific tolerance is a pivotal event required in AIT procedures by generating allergen-specific Treg cells [43], responsible for maintaining immune homeostasis. Treg cells have been characterized by stable expression of CD25, CD4 and FOXP3 (Forkhead box protein 3), expression of suppressive surface molecules, such as cytotoxic T lymphocyte antigen-4 (CTLA-4) and programed death 1 (PD1) and secretion of IL-10 and TGF-β cytokines [31]. Accordingly, increased numbers of Treg cells were also detected in nasal mucosa correlating with clinical efficacy after AIT, supporting the importance of these cells on tolerogenic phenomenon observed in patients upon AIT [44].

TGF-β produced by Treg cells is a potent inhibitor of Th2, Th1 and Th17 effector response and has been associated with the suppression of seasonal allergic inflammation [31] and production of mucosal allergen-specific IgA after AIT [30, 45, 46]. Likewise, IL-10 produced by Tr1, Treg and Breg cells were markedly increased after AIT in allergic individuals and those cells were also associated with suppressor effect observed in several immunotherapeutical protocols [47–49].

IL-10 acts as a potent suppressor cytokine, reducing the production of proinflammatory cytokines by mast cells, decreasing the eosinophil functions and also downregulating the expression of MHC class II and costimulatory molecules on surface of monocytes/macrophages and DCs, thus preventing allergen-induced Th2 activation [50–53]. Importantly, IL-10 is related with the antibody class switch on B cells, favoring the production of IgG4 subclass, a dominant antibody subclass in late phase response of AIT, which is associated with a gradual decreasing of IgE levels. Alternatively, AIT can also provoke immune deviation from Th2 in favor of Th1 responses that culminate in the production of IFN-γ, inducing preferentially B cells to produce IgG1 subclass directed to allergenic components present in the formulation of the AIT [31].

Therefore, allergen-specific IgG antibodies induced during AIT may act as blocking antibodies, reflecting in the reduction of mast cell activation and degranulation due to its competition with IgE antibodies for allergen binding and inhibiting IgE-facilitated allergen presentation [54].

4. Immunotherapy follow-up

In addition to clinical parameters like improvement in symptoms and medication scores that are subjective, it should be very helpful if the medical assistant had also objective parameters such as complementary laboratorial tests for the follow-up of allergic patients under AIT.

Considering the several cellular and molecular mechanisms involved in AIT described before, such as the determination of the type 2 histamine receptor (H2R), basophil activation test, or a procedure for measuring regulatory dendritic cell signature, all of which can be correlated with the clinical efficacy of AIT, it becomes evident that complex methods should be employed and certainly would be difficult to be applied in the routine analysis.

Therefore, we can accomplish that measurement of specific IgG, particularly of the IgG4 subclass, might be used for monitoring patients receiving AIT, since it will be more simple and feasible in any clinical analysis laboratory. In this context, a previous study has demonstrated a lack of correlation between venom-specific total IgG levels and prediction of systemic reactions, concluding that measuring specific IgG antibodies is not useful for monitoring AIT. In this study, the authors postulated that IgG subclasses could be probably involved, since the clinical improvement is not necessarily reflected in the total IgG antibody titre [55]. However, other investigators have found a correlation between low levels of venom-specific IgG and a greater risk of anaphylaxis in patients submitted to venom allergen immunotherapy during 4 years and the opposite was also true, a lower risk of systemic reaction could be observed in those patients with high levels of venom-specific IgG, concluding that the measurement of specific IgG is useful and beneficial, especially for advising greater risk of anaphylaxis in patients who present low levels of specific IgG [56]. An interpretation that we can point out is the existence of two groups of patients who are receiving AIT; one group includes the good responders and another those patients that are non- or low-responders and such fact can be associated to their intrinsic genetic features, particularly related to the specific type of HLA (human leukocyte antigen). Also, we need to consider the presence of pre-existing levels of allergen-specific IgG subclasses before AIT, since the patients themselves may present

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stimulation of their immune system for attempting to synthesize blocking antibodies as an autoregulatory mechanism.

Recent experimental study using a high-dose cutaneous exposure to *Dermatophagoides pteronyssinus* mite extract has shown to induce effective blocking IgG production, supporting that the detection of increased IgG antibody titres is a promisor marker of clinical efficacy of AIT [57].

In addition, a study employing a nonclassical allergen intralymphatic immunotherapy using a modular antigen transporter Fel d 1 (MAT-Fel d 1) has found a strong increase in allergen-specific IgG4 levels and some increase in IgG2 antibody subclasses, but this procedure was not able to stimulate the production of IgG1 and IgG3 subclasses [58].

The production of specific IgG antibodies to allergens, especially IgG4 subclass, is the most important immunological change induced by AIT [59–62]. However, in some studies there is a lack of correlation between increased IgG4 titres and clinical improvement [55], since the induction of IgG4 blocking antibodies may not be reflected in serum or other biological fluid samples, requiring bioassays as the inhibition of IgE-facilitated allergen presentation for its possible detection [63]. Accordingly, production of specific IgG4 antibodies to relevant allergenic components has been associated with the protective activity due to its function as blocking antibody through mechanisms of competition for allergen between IgG4 and cell-bound IgE antibodies [6]. In this context, several other investigators have found that clinical improvement after mite AIT was associated with increased levels of serum specific IgG4 or ratio of specific IgG4/IgG1 [64–66].

Unfortunately, there is no current routine laboratorial test for the detection of allergen-specific IgG antibodies, particularly IgG1 and IgG4 subclasses, against crude allergen extract and/or clinically relevant allergen components that could be used as a useful tool for monitoring AIT. Physicians, who assist patients with respiratory allergy that have been submitted to AIT, are following the treatment of such patients only by clinical parameters (symptoms and medication scores) that are very subjective. The possibility of following such patients under AIT using allergen-specific IgG1 and/or IgG4 antibody measurements will enable to monitor those patients by using objective parameters in association with subjective clinical parameters. This fact has stimulated researchers to develop objective methods for quantifying those allergen-specific IgG antibodies.

In 2001, our group has developed a reverse ELISA technique for quantifying Der p 2 allergen-specific IgE antibodies, using capture Der p 2-specific monoclonal antibodies. This technique was developed with the intention of helping the allergy diagnosis by means of a molecular allergen component, since the presence of Der p 2 allergen-specific IgE antibodies indicates the occurrence of an allergic response in the patient. It has also been demonstrated that this technique has a higher sensitivity related to conventional ELISA [67].

On the other hand, the detection of specific IgG antibodies or particularly IgG1 and IgG4 subclasses, against Der p 2, or against any other specific allergenic component, would indicate the development of a physiological response, i.e., a defense response against dust mite allergens.

Thus, on the basis of the information described above, it becomes particularly interesting to develop a method for quantifying IgG antibody subclasses against clinically relevant allergens. These antibodies can be detected in the serum or other biological fluids, such as saliva from patients with allergic respiratory disease using an immunoenzymatic technique (reverse ELISA) and relevant monoclonal antibodies for monitoring patients under AIT.

5. Method for measuring allergen-specific IgG subclasses

Part of our group has developed a reverse ELISA technique as described in the European patent application registered as EP 2232265, providing a method for measuring allergen-specific IgG antibody subclasses, including IgG1, IgG2, IgG3 and IgG4, for monitoring patients with allergic diseases under AIT [68]. As illustrated in **Figure 3**, allergen-specific monoclonal antibodies, for example, anti-Der p 1 or anti-Der p 2, are bound to ELISA microtitration plates in order to capture the corresponding natural allergens, Der p 1 or Der p 2, respectively, present in the crude *D. pteronyssinus* extract, which subsequently interacts with specific IgG antibodies existent in serum samples or other biological fluids from allergic patients. Those antibodies are later detected by the addition of mouse monoclonal antibodies, against human IgG subclasses, preferentially IgG4, labeled with biotin and, subsequently are incubated with the streptavidin-peroxidase enzymatic conjugate. Reaction is revealed by the addition of the enzymatic substrate (hydrogen peroxide) diluted in a chromogenic buffer [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) – ABTS] and absorbance is determined in a microtitration plate reader, at 405 nm.

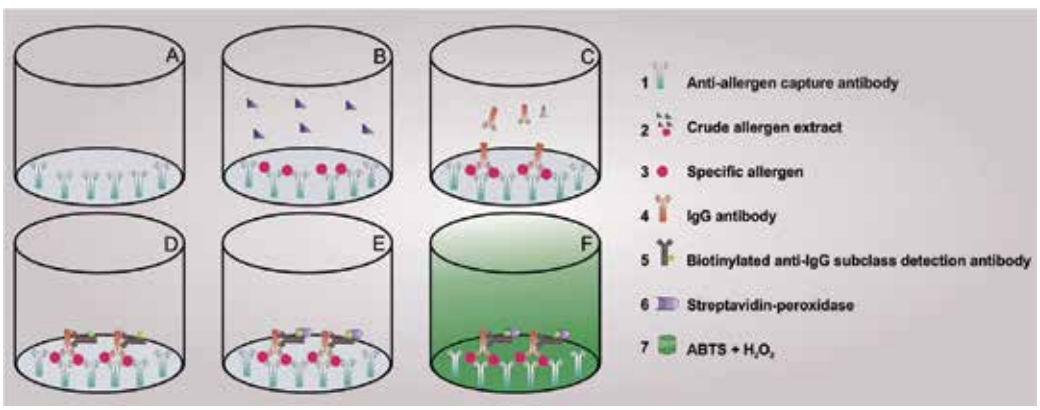

Figure 3. Representative diagram (sequential steps A to F) of the reverse enzyme-linked immunosorbent assay (ELISA). (1) Capture allergen-specific (Der p 1 or Der p 2) monoclonal antibody; (2) crude *Dermatophagoides pteronyssinus* extract; (3) allergen (Der p 1 or Der p 2) present in crude extract; (4) allergen-specific IgG antibody present in serum samples or other biological fluids from allergic patients; (5) monoclonal antibody against human IgG subclass (preferentially IgG4) labeled with biotin; (6) streptavidin-peroxidase enzymatic conjugate; (7) reaction is revealed by the addition of enzymatic substrate (hydrogen peroxide) diluted in a chromogenic buffer (ABTS) and absorbance is determined in a plate reader at 405 nm (Taketomi EA and Silva DAO, 2016, found in European Patent Office EP 2232265 [68]).

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The reverse ELISA (rELISA) technique for the detection of IgG antibody subclasses has a great advantage over others that use indirect ELISA [69, 70]. It does not require purified allergens or antigens, which are often too expensive or difficult to obtain in a purified and isolated form, since the natural allergen components present in the crude allergen extract are bound on the microtitration plate by the capture allergen-specific monoclonal antibody. Another advantage of this assay is that it does not require specific and exclusive equipment, avoiding a direct dependence between the producers of the diagnostic kits or the diagnostic equipment and the consumers.

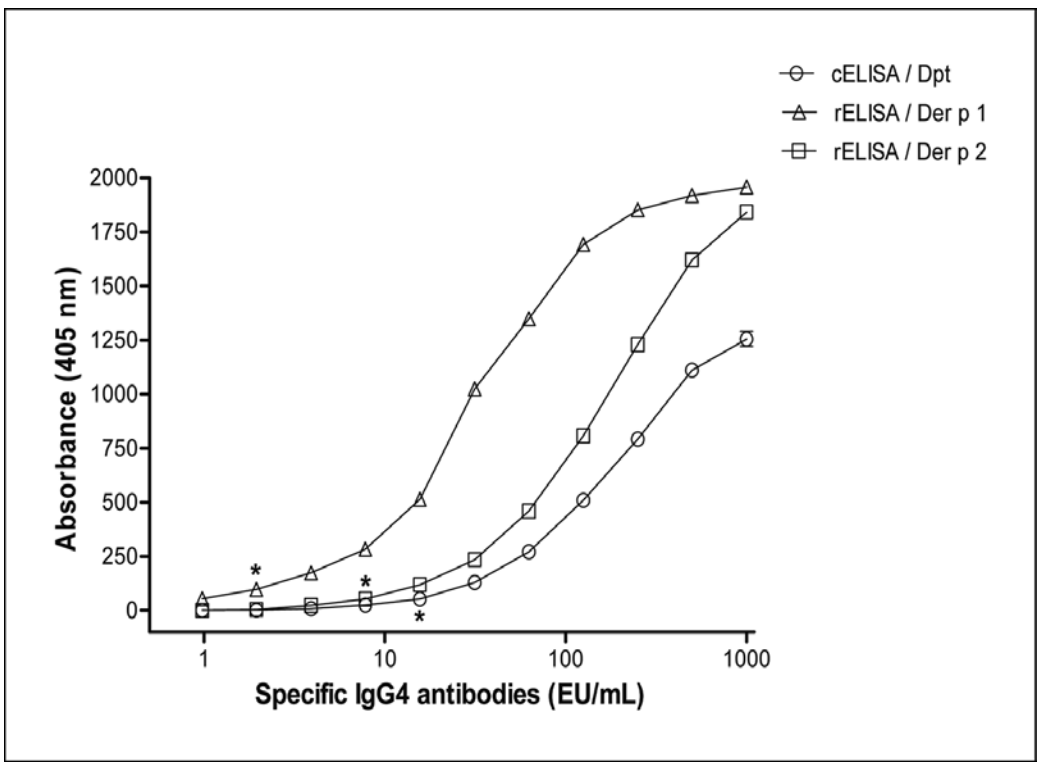

Figure 4. Sensitivity of cELISA and rELISA for the measurement of IgG4 antibodies to the crude extract of *Dermatophagoides pteronyssinus* (Dpt) and its major allergens (Der p 1 and Der p 2). The sensitivity of each assay is indicated by the asterisk (Taketomi EA and Silva DAO, 2016, found in European Patent Office EP 2232265 [68]).

The rELISA assay also demonstrated higher sensitivity than the conventional ELISA (cELISA) in the measurement of allergen-specific IgG subclasses, particularly IgG4 antibodies, to the crude *D. pteronyssinus* (Dpt) extract and its major allergens (Der p 1 and Der p 2), using a pool of reference sera obtained from mite-allergic patients (**Figure 4**). The sensitivity of each assay was 15.6 EU/mL for cELISA-Dpt, 1.9 EU/mL for rELISA-Der p 1 and 7.8 EU/mL for rELISA-Der p 2. Likewise, specificity of rELISA for the measurement of allergen-specific IgG subclasses, particularly IgG4 antibodies to the major allergens (Der p 1 and Der p 2) was shown

to be higher than cELISA for the detection of IgG4 to crude Dpt extract as determined by inhibition assays (**Figure 5**). All assays showed a dose-dependent manner inhibition when a pool of reference sera containing allergen-specific IgG4 antibodies was incubated with increasing concentrations (0.15–15,000 AU/mL) of the crude Dpt extract as inhibitor antigen. Inhibition was higher than 80% for all assays, with 88% for cELISA-Dpt, 82% for rELISA-Der p 1 and 89% for rELISA-Der p 2, when the highest concentration of Dpt allergen extract was used.

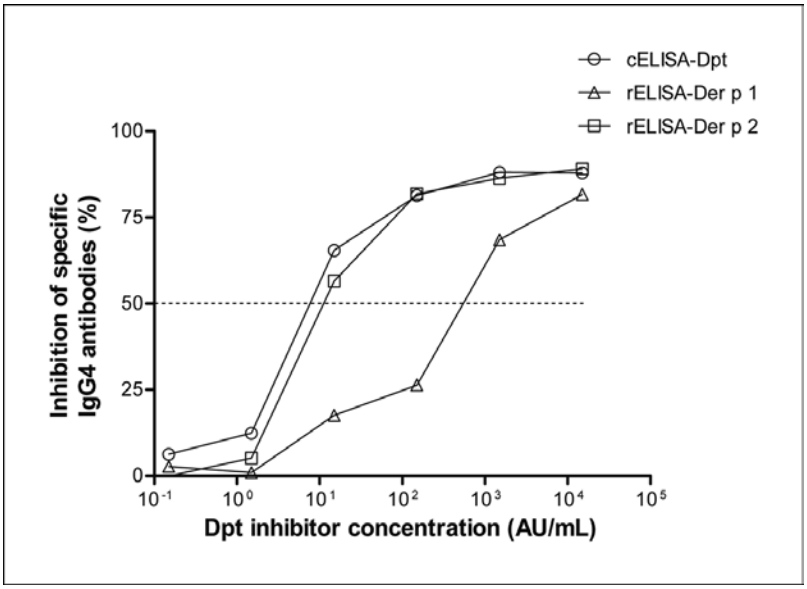

Figure 5. cELISA and rELISA specificity for IgG4 antibodies to the crude extract of *Dermatophagoides pteronyssinus* (Dpt) and its major allergens (Der p 1 and Der p 2) using competitive inhibition assays. Mite-allergic patient sera were preadsorbed with different concentrations of Dpt inhibitor antigen and then assayed in each cELISA and rELISA for measurement of specific IgG4 antibodies. Data represent the percentage of inhibition in each assay (Taketomi EA and Silva DAO, 2016, found in European Patent Office EP 2232265 [68]).

In our previous study [71], rELISA was also employed for monitoring specific IgG4 levels to *D. pteronyssinus* major allergens (Der p 1 and Der p 2) along with cELISA for measuring IgG4 levels to crude Dpt extract in serum samples of two groups of mite-allergic patients under AIT by subcutaneous route: one active DPT group, receiving the *D. pteronyssinus* extract and another placebo group. Serum samples were analyzed in two time-points, day 0 and after 1 year of treatment. As shown in **Figure 6**, patients of the active group (DPT) had increased levels of IgG4 to *D. pteronyssinus* extract and its major allergens, particularly to the Der p 1 allergen component, after 1 year of therapy as compared to patients without active immunotherapy (placebo group). Also, there was a significant increase of serum IgG1 levels to *D. pteronyssinus* extract and Der p 1 allergen component in patients that had received active immunotherapy in contrast with those patients belonging to the placebo group [71].

Furthermore, we were also able to show a significant increase in IgG1 and IgG4 levels to *D. pteronyssinus*, Der p 1 or Der p 2 allergen components after 12 and 18 months of sublingual immunotherapy using *D. pteronyssinus* extract. In contrast, patients receiving placebo did not

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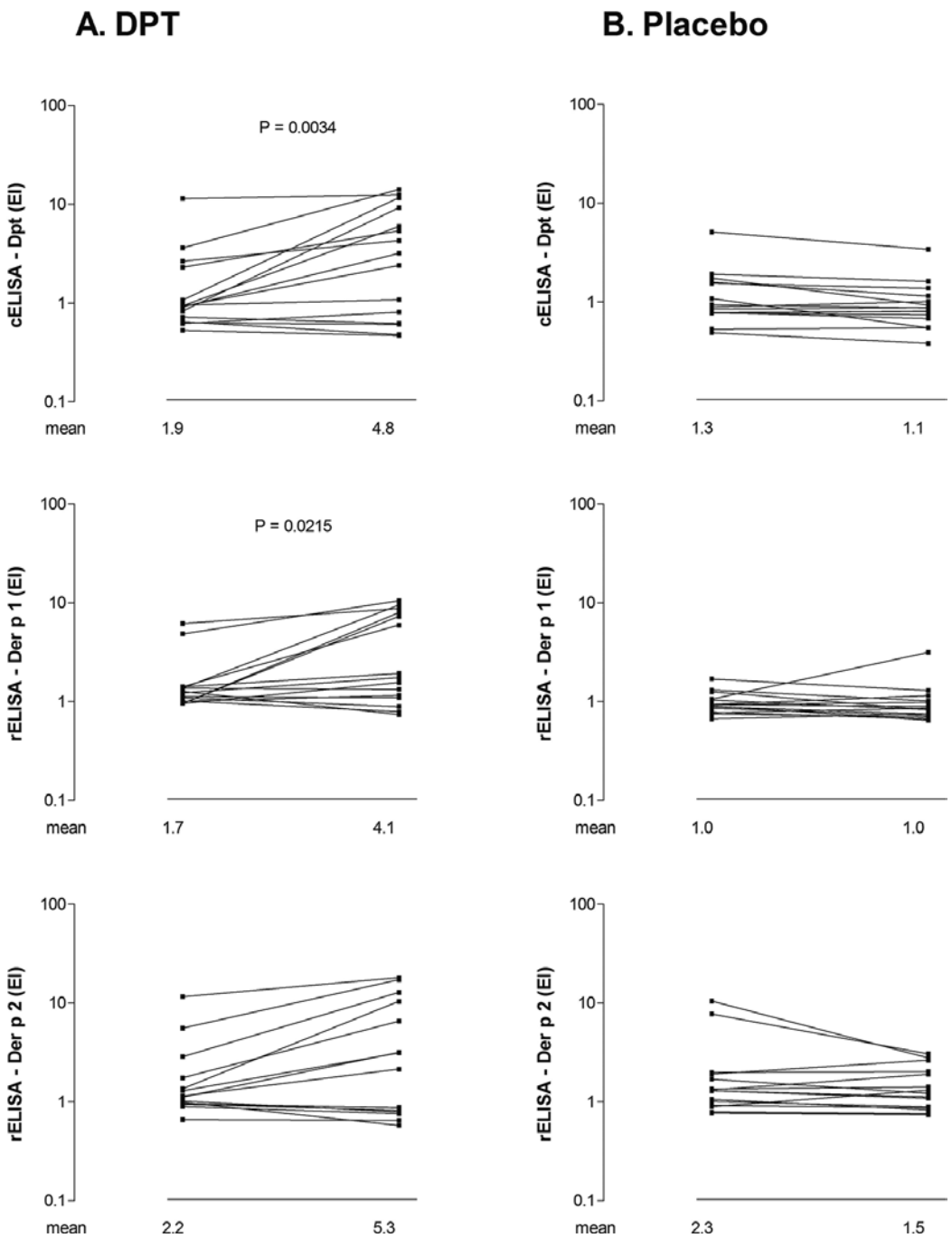

Figure 6. Levels of IgG4 antibodies to the crude extract of *Dermatophagoides pteronyssinus* (Dpt) and its major allergens (Der p 1 and Der p 2) determined by cELISA and rELISA in sera from patients randomized to two treatment groups: (A**)** active DPT (Dpt extract; n = 15) and (B**)** Placebo (n = 15). Antibody levels are expressed in ELISA indices (EI) as individual values on day 0 and after 1 year of treatment and connected with a line; the mean EI values for each of those two time-points are also indicated. Significant differences before and after treatment within the groups were determined by the Wilcoxon signed-rank test (Taketomi EA and Silva DAO, 2016, found in European Patent Office EP 2232265 [68]).

show any increases in IgG1 or IgG4 antibody levels to crude *D. pteronyssinus* extract or its major allergen components in that studied period of time [30].

Thus, our studies have shown that increased levels of allergen-specific IgG subclasses, particularly IgG4 and IgG1, can be detected after variable period of AIT in the serum of patients receiving mite AIT, using major natural components in the ELISA technique that allow better reaction than their modified or recombinant counterparts without the need of purified allergen components. For this reason, the measurement of specific serum IgG subclasses, particularly IgG4, should be considered as a good marker of protective or blocking antibody that may be useful for monitoring activation of tolerance-inducing mechanisms in patients under AIT.

Therefore, according to the results described above, reverse ELISA has shown to be a sensitive and alternative method for measuring natural allergen-specific serum IgG antibody subclasses, especially IgG4, providing valuable information for monitoring patients with allergic respiratory disease during AIT with peptides or native or recombinant allergens of clinical relevance.

6. Conclusion

We can conclude that IgE-mediated allergic patients submitted to AIT usually demonstrate immunological changes, in particular, induction of allergen-specific IgG that may act as blocking factors competing with IgE antibodies and thus contributing for ameliorating the clinical symptoms. In this context, we recommend follow these patients under AIT using clinical (symptoms and medication scores) and laboratorial (allergen-specific IgG subclass measurement) parameters since this technique has shown to be a potential tool for monitoring these patients.

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References

[1] Akdis M, Akdis CA. Therapeutic manipulation of immune tolerance in allergic disease. Nature reviews drug discovery. 2009;**8**:645–660. DOI: 10.1038/nrd2653

[2] Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? Nature Reviews Immunology. 2010;**10**:225–235. DOI: 10.1038/nri2735

[3] Wynn TA. Type 2 cytokines: mechanisms and therapeutic strategies. Nature Reviews Immunology. 2015;**15**:271–282. DOI: 10.1038/nri3831

[4] Matsuoka T, Shamji MH, Durham SR. Allergen immunotherapy and tolerance. Allergology International: Official Journal of the Japanese Society of Allergology. 2013;**62**:403–413. DOI: 10.2332/allergolint.13-RAI-0650

[5] Thomas WR. House dust mite allergens: new discoveries and relevance to the allergic patient. Current Allergy and Asthma Reports. 2016;**16**:69. DOI: 10.1007/s11882-016-0649-y

[6] Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology. 2009;**39**:469–477. DOI: 10.1111/j.1365-2222.2009.03207.x

[7] Traidl-Hoffmann C, Jakob T, Behrendt H. Determinants of allergenicity. The Journal of Allergy and Clinical Immunology. 2009;**123**:558–566. DOI: 10.1016/j.jaci.2008.12.003

[8] Calderon MA, Casale TB, Togias A, Bousquet J, Durham SR, Demoly P. Allergen-specific immunotherapy for respiratory allergies: from meta-analysis to registration and beyond. The Journal of Allergy and Clinical Immunology. 2011;**127**:30–38. DOI: 10.1016/j.jaci.2010.08.024

[9] Jutel M, Akdis CA. T-cell subset regulation in atopy. Current Allergy and Asthma Reports. 2011;**11**:139–145. DOI: 10.1007/s11882-011-0178-7

[10] Shakib F, Ghaemmaghami AM, Sewell HF. The molecular basis of allergenicity. Trends in Immunology. 2008;**29**:633–642. DOI: 10.1016/j.it.2008.08.007

[11] Holgate ST. Innate and adaptive immune responses in asthma. Nature Medicine. 2012;**18**:673–683. DOI: 10.1038/nm.2731

[12] Holgate ST, Polosa R. Treatment strategies for allergy and asthma. Nature Reviews Immunology. 2008;**8**:218–230. DOI: 10.1038/nri2262

[13] Cousins DJ, Lee TH, Staynov DZ. Cytokine coexpression during human Th1/Th2 cell differentiation: direct evidence for coordinated expression of Th2 cytokines. Journal of Immunology. 2002;**169**:2498–2506. DOI: 10.4049/jimmunol.169.5.2498

[14] Wu LC, Scheerens H. Targeting IgE production in mice and humans. Current Opinion in Immunology. 2014;**31**:8–15. DOI: 10.1016/j.coi.2014.08.001

[15] Bulek K, Swaidani S, Aronica M, Li X. Epithelium: the interplay between innate and Th2 immunity. Immunology and Cell Biology. 2010;**88**:257–268. DOI: 10.1038/icb.2009.113

[16] Georas SN, Rezaee F. Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation. The Journal of Allergy and Clinical Immunology. 2014;**134**:509–520. DOI: 10.1016/j.jaci.2014.05.049

[17] Lambrecht BN, Hammad H. Allergens and the airway epithelium response: gateway to allergic sensitization. The Journal of Allergy and Clinical Immunology. 2014;**134**:499–507. DOI: 10.1016/j.jaci.2014.06.036

[18] Divekar R, Kita H. Recent advances in epithelium-derived cytokines (IL-33, IL-25 and thymic stromal lymphopoietin) and allergic inflammation. Current Opinion in Allergy and Clinical Immunology. 2015;**15**:98–103. DOI: 10.1097/ACI.0000000000000133

[19] Romeo MJ, Agrawal R, Pomes A, Woodfolk JA. A molecular perspective on TH2-promoting cytokine receptors in patients with allergic disease. The Journal of Allergy and Clinical Immunology. 2014;**133**:952–960. DOI: 10.1016/j.jaci.2013.08.006

[20] van Ree R, Hummelshoj L, Plantinga M, Poulsen LK, Swindle E. Allergic sensitization: host-immune factors. Clinical and Translational Allergy. 2014;**4**:1–9. DOI: 10.1186/2045-7022-4-12

[21] Ravetch JV, Kinet JP. Fc receptors. Annual Review of Immunology. 1991;**9**:457–492. DOI: 10.1146/annurev.iy.09.040191.002325

[22] Serra-Pages M, Olivera A, Torres R, Picado C, Mora F, Rivera J. E-prostanoid 2 receptors dampen mast cell degranulation via cAMP/PKA-mediated suppression of IgE-dependent signaling. Journal of Leukocyte Biology. 2012;**92**:1155–1165. DOI: 10.1189/jlb.0212109

[23] Batard T, Hrabina A, Bi XZ, Chabre H, Lemoine P, Couret MN, et al. Production and proteomic characterization of pharmaceutical-grade *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* extracts for allergy vaccines. International Archives of Allergy and Immunology. 2006;**140**:295–305. DOI: 10.1159/000093707

[24] Jackola DR, Pierson-Mullany LK, Liebeler CL, Blumenthal MN, Rosenberg A. Variable binding affinities for allergen suggest a 'selective competition' among immunoglobulins in atopic and non-atopic humans. Molecular Immunology. 2002;**39**:367–377. DOI: 10.1016/S0161-5890(02)00108-6

[25] Batard T, Basuyaux B, Laroze A, Lambin P, Bremard-Oury C, Aucouturier P, et al. Isotypic analysis of grass-pollen-specific immunoglobulins in human plasma. 2. Quantification of the IgE, IgM, IgA class and the IgG subclass antibodies. International Archives of Allergy and Immunology. 1993;**102**:279–287. DOI: 10.1111/j.1365-2222.1996.tb00528.x

[26] Akdis M. Healthy immune response to allergens: T regulatory cells and more. Current Opinion in Immunology. 2006;**18**:738–744. DOI: 10.1016/j.coi.2006.06.003

[27] Kemeny DM, Urbanek R, Ewan P, McHugh S, Richards D, Patel S, et al. The subclass of IgG antibody in allergic disease: II. The IgG subclass of antibodies produced following natural exposure to dust mite and grass pollen in atopic and non-atopic individuals.

Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology. 1989;**19**:545–549. DOI: 10.1111/j.1365-2222.1989.tb02431.x

[28] Senti G, Prinz Vavricka BM, Erdmann I, Diaz MI, Markus R, McCormack SJ, et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**:17908–17912. DOI: 10.1073/pnas.0803725105

[29] Senti G, Graf N, Haug S, Ruedi N, von Moos S, Sonderegger T, et al. Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. The Journal of Allergy and Clinical Immunology. 2009;**124**:997–1002. DOI: 10.1016/j.jaci.2009.07.019

[30] Queiros MG, Silva DA, Siman IL, Ynoue LH, Araujo NS, Pereira FL, et al. Modulation of mucosal/systemic antibody response after sublingual immunotherapy in mite-allergic children. Pediatric Allergy and Immunology: Official Publication of the European Society of Pediatric Allergy and Immunology. 2013;**24**:752–761. DOI: 10.1111/pai.12163

[31] Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. The World Allergy Organization Journal. 2015;**8**:17. DOI: 10.1186/s40413-015-0063-2

[32] Schmid JM, Nezam H, Madsen HH, Schmitz A, Hoffmann HJ. Intralymphatic immunotherapy induces allergen specific plasmablasts and increases tolerance to skin prick testing in a pilot study. Clinical and Translational Allergy. 2016;**6**:19. DOI: 10.1186/s13601-016-0107-x

[33] Jutel M, Muller UR, Fricker M, Rihs S, Pichler WJ, Dahinden C. Influence of bee venom immunotherapy on degranulation and leukotriene generation in human blood basophils. Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology. 1996;**26**:1112–1118. DOI: 10.1111/j.1365-2222.1996.tb00496.x

[34] Novak N, Mete N, Bussmann C, Maintz L, Bieber T, Akdis M, et al. Early suppression of basophil activation during allergen-specific immunotherapy by histamine receptor 2. The Journal of Allergy and Clinical Immunology. 2012;**130**:1153–1158 e2. DOI: 10.1016/j.jaci.2012.04.039

[35] Erzen R, Kosnik M, Silar M, Korosec P. Basophil response and the induction of a tolerance in venom immunotherapy: a long-term sting challenge study. Allergy. 2012;**67**:822–830. DOI: 10.1111/j.1398-9995.2012.02817.x

[36] Eberlein-Konig B, Ullmann S, Thomas P, Przybilla B. Tryptase and histamine release due to a sting challenge in bee venom allergic patients treated successfully or unsuccessfully with hyposensitization. Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology. 1995;**25**:704–712. DOI: 10.1111/j.1365-2222.1995.tb00007.x

[37] Celesnik N, Vesel T, Rijavec M, Silar M, Erzen R, Kosnik M, et al. Short-term venom immunotherapy induces desensitization of FcepsilonRI-mediated basophil response. Allergy. 2012;**67**:1594–1600. DOI: 10.1111/all.12044

[38] Celesnik Smodi N, Silar M, Erzen R, Rijavec M, Kosnik M, Korosec P. Down-regulation of FcepsilonRI-mediated CD63 basophil response during short-term VIT determined venom-nonspecific desensitization. PloS One. 2014;**9**:e94762. DOI: 10.1371/journal.pone.0094762

[39] Akdis CA, Blaser K. Histamine in the immune regulation of allergic inflammation. Journal of Allergy and Clinical Immunology. 2003;**112**:15–22. DOI: 10.1067/mai.2003.1585

[40] Zimmer A, Bouley J, Le Mignon M, Pliquet E, Horiot S, Turfkruyer M, et al. A regulatory dendritic cell signature correlates with the clinical efficacy of allergen-specific sublingual immunotherapy. The Journal of Allergy and Clinical Immunology. 2012;**129**:1020–1030. DOI: 10.1016/j.jaci.2012.02.014

[41] Lundberg K, Rydnert F, Broos S, Andersson M, Greiff L, Lindstedt M. Allergen-specific immunotherapy alters the frequency, as well as the FcR and CLR expression profiles of human dendritic cell subsets. PloS One. 2016;**11**:e0148838. DOI: 10.1371/journal.pone.0148838

[42] Gueguen C, Bouley J, Moussu H, Luce S, Duchateau M, Chamot-Rooke J, et al. Changes in markers associated with dendritic cells driving the differentiation of either TH2 cells or regulatory T cells correlate with clinical benefit during allergen immunotherapy. The Journal of Allergy and Clinical Immunology. 2016;**137**:545–558. DOI: 10.1016/j.jaci.2015.09.015

[43] Jutel M, Kosowska A, Smolinska S. Allergen immunotherapy: past, present and future. Allergy, Asthma and Immunology Research. 2016;**8**:191–197. DOI: 10.4168/aair.2016.8.3.191

[44] Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa. The Journal of Allergy and Clinical Immunology. 2008;**121**:1467–1472, 1472. e1. DOI: 10.1016/j.jaci.2008.03.013

[45] Pilette C, Nouri-Aria KT, Jacobson MR, Wilcock LK, Detry B, Walker SM, et al. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-β expression. The Journal of Immunology. 2007;**178**:4658–4666. DOI: 10.4049/jimmunol.178.7.4658

[46] Gloudemans AK, Lambrecht BN, Smits HH. Potential of immunoglobulin A to prevent allergic asthma. Clinical and Developmental Immunology. 2013;**2013**:542091. DOI: 10.1155/2013/542091

[47] Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. Journal of Allergy and Clinical Immunology. 2003;**111**:1255–1261. DOI: 10.1067/mai.2003.1570

[48] Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, et al. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. The Lancet. 2004;**363**:608–615. DOI: 10.1016/s0140-6736(04)15592-x

[49] Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance and immune deviation. The Journal of Allergy and Clinical Immunology. 2007;**120**:707–713. DOI: 10.1016/j.jaci.2007.06.013

[50] O'Garra A, Vieira PL, Vieira P, Goldfeld AE. IL-10-producing and naturally occurring CD4+ Tregs: limiting collateral damage. Journal of Clinical Investigation. 2004;**114**:1372–1378. DOI: 10.1172/jci23215

[51] Wu K, Bi H, Sun K, Wang C. IL-10-producing type 1 regulatory T cells and allergy. Cellular and Molecular Immunology. 2007;**4**:269–275. DOI: 10.1002/9780471773719.ch13

[52] Taylor A, Akdis M, Joss A, Akkoc T, Wenig R, Colonna M, et al. IL-10 inhibits CD28 and ICOS costimulations of T cells via src homology 2 domain-containing protein tyrosine phosphatase 1. The Journal of Allergy and Clinical Immunology. 2007;**120**:76–83. DOI: 10.1016/j.jaci.2007.04.004

[53] Akdis CA, Akdis M. Mechanisms of immune tolerance to allergens: role of IL-10 and tregs. The Journal of Clinical Investigation. 2014;**124**:4678–4680. DOI: 10.1172/JCI78891

[54] Siman IL, de Aquino LM, Ynoue LH, Miranda JS, Pajuaba AC, Cunha-Junior JP, et al. Allergen-specific IgG antibodies purified from mite-allergic patients sera block the IgE recognition of *Dermatophagoides pteronyssinus* antigens: an *in vitro* study. Clinical and Developmental Immunology. 2013;**2013**:657424. DOI: 10.1155/2013/657424

[55] Ewan PW, Deighton J, Wilson AB, Lachmann PJ. Venom-specific IgG antibodies in bee and wasp allergy: lack of correlation with protection from stings. Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology. 1993;**23**:647–660. DOI: 10.1111/j.1365-2222.1993.tb01791.x

[56] Golden DB, Lawrence ID, Hamilton RH, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Clinical correlation of the venom-specific IgG antibody level during maintenance venom immunotherapy. The Journal of Allergy and Clinical Immunology. 1992;**90**:386–393. DOI: 10.1016/S0091-6749(05)80019-3

[57] Hirai T, Yoshioka Y, Takahashi H, Handa T, Izumi N, Mori T, et al. High-dose cutaneous exposure to mite allergen induces IgG-mediated protection against anaphylaxis. Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology. 2016;**46**:992–1003. DOI: 10.1111/cea.12722

[58] Freiberger SN, Zehnder M, Gafvelin G, Gronlund H, Kundig TM, Johansen P. IgG4 but no IgG1 antibody production after intralymphatic immunotherapy with recombinant MAT-Feld1 in human. Allergy. 2016;**71**:1366–1370.DOI: 10.1111/all.12946

[59] Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. Journal of Allergy and Clinical Immunology. 1998;**102**:157–164. DOI: 10.1016/S0091-6749(98)70079-X

[60] Ebner C. Immunological mechanisms operative in allergen-specific immunotherapy. International Archives of Allergy and Immunology. 1999;**119**:1–5. DOI: 24168

[61] Ewan PW. New insight into immunological mechanisms of venom immunotherapy. Current Opinion in Allergy and Clinical Immunology. 2001;**1**:367–374. DOI: 10.1097/00130832-200108000-00015

[62] Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. Current Opinion in Allergy and Clinical Immunology. 2004;**4**:313–318. DOI: 10.1097/01.all.0000136753.35948.c0

[63] van Neerven RJ, Arvidsson M, Ipsen H, Sparholt SH, Rak S, Wurtzen PA. A double-blind, placebo-controlled birch allergy vaccination study: inhibition of CD23-mediated serum-immunoglobulin E-facilitated allergen presentation. Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology. 2004;**34**:420–428. DOI: 10.1111/j.1365-2222.2004.01899.x

[64] Wang JY, Lei HY, Hsieh KH. The change of allergen-specific IgG subclass antibodies during immunotherapy in mite-sensitive asthmatic children. Asian Pacific Journal of Allergy and Immunology/Launched by the Allergy and Immunology Society of Thailand. 1992;**10**:11–18.

[65] Einarsson R, Dreborg S, Hammarstrom L, Lofkvist T, Smith CI, Svensson G. Monitoring of mite *Dermatophagoides farinae* allergen-specific IgG and IgG subclass distribution in patients on immunotherapy. Allergy. 1992;**47**:76–82. DOI: 10.1111/j.1398-9995.1992.tb05092.x

[66] Pastorello EA, Incorvaia C, Gerosa A, Vassellatti D, Italia M, Pravettoni V. Allergen specific IgG subclass antibody response in hyposensitization with *Dermatophagoides pteronyssinus* extract. New England and Regional Allergy Proceedings. 1987;**8**:417–421. DOI: 10.2500/108854187778999630

[67] Silva DA, Gervasio AM, Sopelete MC, Arruda-Chaves E, Arruda LK, Chapman MD, et al. A sensitive reverse ELISA for the measurement of specific IgE to Der p 2, a major *Dermatophagoides pteronyssinus* allergen. Annals of Allergy, Asthma & Immunology: Official Publication of the American College of Allergy, Asthma and Immunology. 2001;**86**:545–50. DOI: 10.1016/S1081-1206(10)62903-1

[68] Taketomi EA, Silva DAO. Method for quantifying allergen-especific human IgG subclasses for the control and attendance of specific immunotherapy. European patent office EP 2232265. 2016.

[69] Miyazawa H, Inouye S, Sakaguchi M, Koizumi K. A reverse-sandwich ELISA for IgG antibody to a pollen allergen. The Journal of Allergy and Clinical Immunology. 1988;**82**:407–413. DOI: 10.1016/0091-6749(88)900139

[70] Miyazawa H, Bannai H, Yanase T, Morita C, Satoh S, Sugiyama J, et al. A reverse-sandwich enzyme-linked immunosorbent assay for verocytotoxin 1 and 2 antibodies in human and bovine sera. Clinical and Diagnostic Laboratory Immunology. 1999;**6**:701–704.

[71] Queirós MGJ, Silva DAO, Alves R, Chiba HF, Amaral VBS, Almeida KC, et al. Mite-specific immunotherapy using allergen and/or bacterial extracts in atopic patients in Brazil. Journal of Investigational Allergology and Clinical Immunology. 2008;**18**:84–92.

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This is another attempt of InTech to continue the dissemination of international knowledge and experience in the field of immunology. The present book includes a number of modern concepts of specialists and experts in the field of immunotherapy, covering the major topics and analyzing the history, current stage, and future ideas of application of modern immunomodulation. It is always a benefit, but also a compliment, to gather a team of internationally distinguished authors and to motivate them to reveal their expertise for the benefit of medical science and health practice. On behalf of all readers, immunologists, immunogeneticists, biologists, oncologists, microbiologists, virologists, hematologists, chemotherapists, health-care experts, as well as students and medical specialists, also on my personal behalf, I would like to extend my gratitude and highest appreciation to InTech for giving me the unique chance to be the editor of this exclusive book.

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