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Biological Therapy for Inflammatory Bowel Disease

Edited by Raquel Franco Leal and Tristan Torriani

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Meet the editors

Raquel Franco Leal is Associate Professor at the Department of Surgery, Colorectal Surgery Unit, School of Medical Sciences, University of Campinas (UNICAMP). She has graduated in Medical Sciences, specialized in General Surgery and Coloproctology, and received her MSc and PhD degree at the University of Campinas. She was a Postdoctoral fellow at the University of Chicago and at the Institut D'Investigacions August Pi I Sunyer Biomèdiques, Hospital Clínic of Barcelona. She is member of the Brazilian Society of Coloproctology and the International Society of University Colon and Rectal Surgeons (ISUCRS), an honorary member of the Brazilian Association of Ulcerative Colitis and Crohn's Disease (ABCD), and a member of the Study Group of Inflammatory Bowel Disease in Brazil (GEDIIB), the European Crohn's and Colitis Organisation (ECCO), and the Pan American Crohn's Colitis Organisation (PANCCO). As a researcher she has been devoted to biomedical science and in particular to the study of inflammatory bowel diseases for over 14 years. Dr Franco Leal is the Coordinator of the Inflammatory Bowel Disease (IBD) Research Laboratory at the UNICAMP, developing basic and clinical research on IBD.

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Contents

Preface

The treatment of inflammatory bowel disease (IBD) has posed a major challenge since its appearance. Biomedical researchers, physicians, gastroenterologists, and surgeons have struggled to improve the quality of life of their patients and have sought, above all else, to keep the disease under remission for as long as possible. Blockers for tumoral necrosis factor alpha (TNF-α) were the first biological drugs to be discovered and for this reason they played a crucial role in the subsequent evolution of IBD treatment. The aim of this book is to provide an overview of such drugs and the latest developments in IBD immunopathology. Our contributors discuss the main indications, efficacy, and possible side effects of the different types of drugs available today for IBD treatment.

The book is divided into four parts. Section 1 deals with the immunopathological aspects of IBD with a special focus, in the second chapter, on the pathogenic role of TNF-α. Section 2 contains a single chapter with a critical assessment of corticoids and immunosuppressants, which preceded biological therapy. Section 3 discusses anti-TNF-α therapy in its general aspects of indication and action mechanisms. More specifically, it also deals with practical aspects concerning measures that should be taken before initiating biological therapy and indications for surgery. Lastly, Section 4 examines new types of biological therapy that have come after anti-TNF-α and that are now available in most countries around the world.

We hope that the reader will find this book to be a practical and concise resource on the clinical treatment of IBD that is also helpful in daily clinical practice. I would like to thank Prof. Dr. Tristan Torriani, co-editor of this book, for his assistance in the linguistic revision of the chapters, and also to Ms. Katarina Pausic for her attentive work throughout the planning and editing process.

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Section 1

Immunopathological Aspects

Chapter 1

Mucosal Immunology in the Inflammatory Bowel Diseases

Giovanna Rosa Degasperi

Abstract

Inflammatory bowel disease (IBD) includes two major phenotypes, Crohn's disease and ulcerative colitis, which have different clinical characteristics and immune response profiles. Dysregulation of the intestinal immune response with elevated secretion of proinflammatory cytokines is a hallmark of IBD. In this chapter, we will characterize the cells of the innate and adaptive immunity involved in the pathogenesis of IBD. Innate lymphoid cells as well as dendritic cells, neutrophils, macrophages, B cells and T cells, including Th1 and Th2, Th9 and Th17 cells will be specifically characterized in this scenario. The cross talks and cytokine-mediated regulation of these cells with emphasis on cytokines IL-17, IL-22 and IL-23 will also be emphasized.

Keywords: inflammatory bowel disease, innate immune response, adaptive immune response, T regulatory cells, toll-like receptors

1. Introduction

Inflammatory bowel disease has become a worldwide health burden with increasing incidence and prevalence, contributing to the increased risk of colorectal cancer development [1]. IBD encompasses both Crohn's disease (CD) and ulcerative colitis (UC). Its etiopathology is still unknown, although it is believed that it may be a combination of genetic and environmental factors, as well as microbiota, diet and immune response.

Evidence suggests that abnormalities in both the innate and adaptive immune responses against intestinal microbiota, harmful antigens or extrinsic pathogens which may have crossed the intestinal barrier play an important role in the inflammatory process associated with the disease in genetically susceptible individuals. Several components of the mucosal immune system are implicated in the pathogenesis of IBD, including innate lymphoid cells, innate immune response (macrophages, neutrophils, and dendritic cells), and adaptive immune response (T and B cells) cells, as well as different cytokine and chemokine types which are secreted by these cells [2].

$TCD4^+$ lymphocytes from the intestinal mucosa, through the production of pro-inflammatory cytokines, play a central role both in the induction and in the persistence of chronic inflammation which are characteristic of CD and UC. These cells are key components of the adaptive immune response able to secrete specific cytokines in response to the recognition of peptides in MHC Class II in antigen-presenting cells (ACP), several cytokines and the expression of transition factors, in a process known as differentiation of $TCD4^+$ or Th0 cells, which results in the

generation of T helper lymphocytes (Th) Th1, Th2, Th17, and Th9. These cells have the peculiarity of secreting specific cytokines. These subsets of differentiated T helper lymphocytes perform a number of functions. However, immune responses executed in a dysregulated manner by some of these subsets result in chronic inflammation and tissue damage [3].

In the intestinal mucosa, APCs such as dendritic cells can induce differentiation of *naïve* $TCD4^+$ lymphocytes in one of the specific subsets of T helper which will be responsible for altering intestinal homeostasis, contributing to the setting in of chronic inflammation in the intestine which is a hallmark of IBD. While CD is mediated by Th1 cells, UC has been identified as a disease associated to Th2 cells. Studies indicate that, in CD, the Th1-related cytokines, such as the tumoral necrosis factor α (TNF-α), interferon-γ (IFN-γ), interleukin-12 (IL-12), as well as those associated to Th17 such as IL-17A, IL-21, and IL-23, are increased in the intestinal mucosa [4]. In UC, it has been demonstrated that there is an increase in the production of IL-5 and IL-13 which are Th2 identity cytokine [5].

In addition to Th17 cells, IL-9-secreting Th9 cells can also promote exacerbate inflammatory diseases such as IBD [6, 7]. Th9 cells are also known to be involved in immunity against helminth parasites [8]. Moreover, results from colitis animal models and studies in humans indicate a role for innate lymphoid cells (ILC) in the pathogenesis of chronic intestinal inflammation in IBD. The ILC are a population of lymphocytes present in regions of the mucosa, in which they perform the function of protecting against pathogens, including extracellular bacteria, helminths, and viruses. The ILCs are cells with a high degree of plasticity depending on the exposition to cytokines from the microenvironment in which they are present.

2. General features of the colon mucosal: barriers of protection and intestinal immune system

The intestinal epithelium has important functions, such as absorption, secretion and digestion. In the epithelium, in addition to enterocytes, some other epithelial cells, such as goblet cells, perform a protective function through the secretion of mucus. This protective action may be verified in experiments with animal models which show that MUC2-null mice developed spontaneous colitis [9]. In addition to goblet cells, Paneth cells also display protective action, since they produce defensins, which are antimicrobial peptides that modulate the composition of the intestinal microbiota [10].

The epithelium forms a mucous barrier with tight junctions between the enterocytes preventing the entrance of a myriad of substances. Defects in the epithelial integrity may contribute to the development of IBD, allowing the passage of microorganisms through the epithelial layer. In chronic inflammatory disorders, such as IBD, the microbial components of the microbiota are translocated through the damaged barrier of the mucosa and, through the interaction with cells of the immune system in the lamina propria, trigger an inflammatory response [11].

The intestinal epithelium is located between the lumen and the lamina propria. In the lamina propria there are cells of the immune system, and, in the lumen, the microbiota consists of commensal microorganisms, including bacteria, viruses and fungi. The most abundant cells in the epithelial compartment are absorptive cells, which not only provide a physical barrier against luminal antigens, but also mediate the crosstalk between the intestinal microbiome and the immune system of the host, particularly through the innate immune receptors, specifically, the pattern recognition receptors (PRR), known as Toll-like receptors (TLR), which are expressed throughout the intestinal tract. The healthy human small intestine

expresses TLR-2 and TLR-4 [12]. Cells of the innate immune compartment which reside in the lamina propria are sentinels which detect invading pathogens through their TLR. These cells are part of the mononuclear phagocytic system, including macrophages and dendritic cells which encompass and process microbial antigens in the *naïve* $TCD4^+$ lymphocytes from Peyer's patches, through major histocompatibility complex type II (MHC II). The $TCD4^+$ lymphocytes produce cytokines which activate B cells into transforming into plasmocytes which selectively produce immunoglobulin A (IgA).

The IgA is an abundant isotype in blood serum, in which it is normally present in concentrations of 1 to 3 mg/ml. In circulation, IgA is generally found as a monomer IgA [13, 14]. Dimeric IgA is the predominant antibody in secretions of the gastrointestinal tract. In this format, IgA is generated by the union of two molecules of monomeric IgA. Its production is mediated by the plasmocytes located in the lamina propria of the mucosa, and, despite being a protein, IgA present in the secretions of the lumen is quite resistant to proteolysis by the gastric and intestinal enzymes [15].

The process of transport and secretion of this immunoglobulin of the plasmocytes located in the lamina propria from the mucosa to the intestinal lumen occurs through the connection to receptors for immunoglobulins which are expressed in the mucosal epithelial cells' basal layer. Once the connection is made, the complex formed is endocytosed by the epithelial cell and transported to the apical portion of the cell membrane, where it is then liberated in the lumen with the extracellular fragment of the receptor, thus forming secretory IgA (sIgA) [16].

In the lumen, these IgA have the capacity to connect to antigens from the mucosa surface, preventing their penetration and adherence to the epithelial layer of the mucosa. The formation of the antigen-sIgA complex favors the retention of pathogenic microorganisms to the mucus and stimulates its secretion, facilitating the enzymatic degradation and antigen elimination without having to activate the inflammatory response [17].

In patients with IBD, the damage of the barrier function of the intestinal epithelial layer results in an influx of IgA-opsonized bacteria. Interestingly, it has been demonstrated that the presence of these immune IgA complexes in the lamina propria contributes to inflammation induced by FcαRI [13]. Recent findings have demonstrated that co-stimulation of FcαRI strongly affects pro-inflammatory cytokine production by some immune system cells such as phagocytes. FcαRI is also expressed in immune cells such as eosinophils and dendritic cells [18].

Thus, there is ample evidence of defense against intestinal pathogens. The epithelial layer, mucus, antimicrobial peptides, immune system cells in the lamina propria, and IgA together help to establish a beneficial environment to tolerate the diverse community of bacteria of the microbiota and food antigens, as well as to elaborate a response against pathogenic microorganisms.

3. TLRS: key immune sensors of microbiota in the gut

Throughout the gastrointestinal mucosa there are receptors which specialize in identifying pathogenic microorganisms. The process of recognition of pathogens is highly specific and occurs through the connection between pathogen-associated molecular patters (PAMP) and PRR. Known PRR are classified as: TLR, NOD-like receptors (NLR), RIG-1-like receptors (RLR), of which the TLR are the most correlated to IBD.

In mammals, TLR comprise a family of 13 types of receptors, of which TLR 1–9 are more easily found in cells from the small and large intestines. In humans, only

TLR 2, 3, 4, 5, and 9 have been consistently identified, highlighting that TLR-3 and TLR-5 are present in larger numbers in the enterocytes. The TLR are found in the plasma membrane or in the endosomal intracellular compartments. The activation of these receptors is made by PAMP which have relative specificity to distinct TLR. The TLR-2, for example, identifies peptidoglycans and lipoproteins; TLR-3 identifies viral RNA; TLR-4 recognizes lipopolysaccharide (LPS); TLR-5 recognizes flagellin, and TLR-9 connects to bacterial DNA. Despite the small number of receptors, this distribution reflects the elevated capacity for identifying molecular patters in a number of pathogens [19].

Once activated, TLR become dimerized and trigger the subsequent activation of downstream signaling cascades, e.g., the activation of NF-κB which leads to the induction of a variety of inflammatory cytokines. Except for TLR3, other TLR signaling pathways depend on MyD88 to activate NF-κB and produce pro-inflammatory cytokines. The TLR signaling pathway is quite similar to the interleukin (IL)-1R family, since TLR contains the domain Toll/Interleukin-1 (TIR). The TIR domain contains the TIRAP adaptor protein. When TLR-1, 2 or 6 are activated, the domain containing TIRAP lying downstream of these TLR and recruits the adaptor protein from the primary myeloid response 88 (MyD88) which leads to the activation of the kinase associated with the IL-1 receptor (IRAK). The activation of IRAK, in turn, induces the activation of serine and threonine kinases which are responsible for the degrading of IκBα, known as an inhibitor of the nuclear transcription factor κB or NF-κB. The degrading of IκBα allows for the migration of the NF-κB from cytoplasm to the nucleus. In the nucleus, this nuclear factor induces the production of pro-inflammatory cytokines and chemokines which will trigger the innate and, subsequently, the adaptive immune responses [20].

Furthermore, there is an alternate signaling pathway to MyD88 which involves TLR-3 and TLR-4. This alternate pathway is mediated by the activation of the TIR-domain-containing adapter-inducing Interferon-β (TRIF). Thus, signaling TLR is divided in two pathways: one dependent on MyD88 and the other independent of MyD88, but dependent on TRIF. Downstream of the TLR signaling pathways, activated NF-κB and interferon regulatory factor (IRF) to the production of pro-inflammatory cytokines [20].

Additionally, TLRs provides a connection between innate and adaptive immunity. Dendritic cells that is innate immune response cell, can sense microbes by these receptors in their surface. In this way, this cell controls microbial driven T lymphocyte polarization to Th1, Th2, Th9 or Th17 in lymphoid tissues. After interaction with microbial components, immature dendritic cell migrate to the draining lymphoid tissues to present microbial antigens to T lymphocytes [21]. It was hypothesized that an abnormal pattern of bacterial recognition by these cells through TLRs alter its activation and cytokine production which may underlie chronic inflammatory processes, such as IBD [22].

A number of studies have shown a correlation between TLR and IBD, be it enabling or inhibiting the disease. Interestingly, it has been demonstrated that TLR-2 must form heterodimers with TLR1 or TLR6 in order to trigger intracellular signaling pathways. The inhibition of TLR2/6 signaling has played a beneficial role by slowing down IBD progression. It was also reported that TLR6 was overexpressed in the intestines of IBD patients and might promote experimental colitis in mice [23]. In this case, it was proved that TLR-6 was important and activated the polarization of Th1 and Th17 of $TCD4^+$ lymphocytes. Also, considering that TLR4 gene expression was upregulated in the intestinal epithelia of patients with active UC, TLR4 might be a participant in UC disease development. Moreover, it was demonstrated that TLR8 is upregulated in patients with active UC and that the expression of the genes TLR2, 4, 8 and 9 is positively regulated in these patients.

Contrary to the evidence presented above, which show TLR supporting IBD, studies show that the activation of TLR-9 prevented the development of inflammation of the mucosa, and fomented healing of wounds in models of colitis [24]. Still others presented data that TLR3, TLR7, or TLR9 agonists could induce type I IFN, which can prevent experimental colitis [25].

4. The link between innate and adaptive immune response in intestine: the role of macrophages and dendritic cells

Macrophages and intestinal dendritic cells which reside in the lamina propria are APCs that act as sentinels to the maintenance of intestinal homeostasis. They are capable of establishing an interaction between the innate and adaptive immunity by means of the presentation of antigens to the *naïve* $TCD4^+$, via MHC II [26].

The recognition of microorganisms for phagocytosis occurs by means of PRR. Macrophages also express PRR which recognize PAMP present on the surface of invading intestinal microorganisms. It is through this interaction that immune cells distinguish between commensal microorganisms and pathogens, thus designing an appropriate immune response program. Captured antigens from pathogenic microorganisms are presented to *naïve* $TCD4^+$ lymphocytes via MHC class II. In 1998, it was described that intestinal macrophages in mice carrying colitis present low levels of MHC class II expression, which hinder adaptive immune response in the inflammatory condition established by this disease [27].

With relation to dendritic cells, they also have the function of transporting antigens to mesenteric lymph nodes and Peyer's patches, and, subsequently, inducing the generation of responses by intestinal $TCD4^+$ lymphocytes specific to the antigen. They act as sentinels, acquiring antigens in peripheral tissues before migrating to secondary lymphoid organs. Dendritic cells can recognize antigens through the emission of their extensions in the luminal region. Alternatively, this recognition may occur through M cells which are also considered as presenting antigens. The M cells can recognize antigens in the intestinal lumen, internalize them and present them to the dendritic cells located in the lamina propria of the mucosa [28, 29].

Dendritic cells and macrophages are characterized according to their expression of specific membrane markers [30]. The intestinal dendritic cells may be divided in $CD103^+CD11b^+$ and $CD103^+CD11b^-$ in mice, or $CD103^+Sirp\alpha^+$ and $CD103^+Sirp\alpha^-$ in humans [31]. Dendritic cells, both in mice and humans, stimulate the differentiation of Th1 and Th17 lymphocytes subtypes [32]. Regarding intestinal macrophages, they are identified by their expression of the F4/80, CD64, CD11b and CX3CR1 markers [33, 34]. In these macrophages, despite their ample phagocytic activity, the expression of co-stimulatory molecules CD40, CD80 and CD86 are decreased, as well as innate immune response receptors such as LPS or CR3 [35, 36].

Macrophages residing in the lamina may still be differentiated in two distinct phenotypes characterized as M1 and M2. Specific combinations of cytokines induce the polarization to one of these phenotypes. IFN-γ induces the appearance of the M1 phenotype, which has as its identity the secretion of TNF-α, IL-12, IL-6 and IL-23 pro-inflammatory cytokines. These cytokines are present in the context of inflammatory intestinal diseases. The M2 macrophages arise in microenvironments rich in IL-4 and produce large quantities of IL-10 [37]. It is known that mice deficient in IL-10 develop spontaneous colitis [38]. Moreover, mutations in genes which codify proteins in the IL10R subunit have been found in patients with early-onset enterocolitis [39]. Generally, while M1 macrophages cause tissue damage and hinder cell proliferation, M2 macrophages support proliferation and tissue repair [40]. It was shown that M1 macrophages which invade intestinal tissues contribute directly

to break the epithelial barrier by means of disruption of tight junction proteins and induction of apoptosis of epithelial cells, thus supporting intestinal inflammation which is characteristic of IBD [41].

While mononuclear phagocytes perform an important role in the induction of inflammation in several tissues by means of the production of pro-inflammatory cytokines, chemokines and oxygen-free radicals, residing macrophages as well as intestinal dendritic cells exhibit a tolerogenic phenotype mediating tolerance to commensal microorganisms [42, 43].

Thus, macrophages phagocyte intestinal pathogens efficiently, although they do not cause an exacerbated inflammatory response. This is a characteristic which distinguishes intestinal macrophages from those found in other compartments. When macrophages present disorders in the recognizing microorganisms in the intestine, an inflammatory reaction may be established. This condition has been observed in IBD. In such situations, these macrophages produce high, significant quantities of IL-1β, IL-6, TNF-α and IL-23 [44]. Among them, IL-23 can stimulate the production of IL-22 under several infectious conditions [45]. IL-22 is essential for preventing the integrity of the intestinal barrier and inducing the production of antimicrobial peptides and chemokines which recruit cells such as, e. g., neutrophils [46].

5. Old and new lymphocyte players in inflammatory bowel disease

5.1 Revisiting TH1 and TH2 lymphocytes

Naïve TCD4$^+$ lymphocytes have a high degree of plasticity and the capacity for differentiating into subsets of effector or regulatory T cells during the process of activation. For approximately two decades, it was believed that these lymphocytes could only divide into the subtypes Th1 or Th2 [47].

The effector T lymphocytes subtypes Th1/Th2 were the first to be described in scholarly literature, leading to the comprehension of how TCD4$^+$ could shape the appropriate response to different pathogens. Subsequently, the identification of effector T lymphocytes Th17, T regulatory (Treg) and Th9 changed the Th1/Th2 historical paradigm.

These subtypes express distinct factors of transcription and secret different cytokines. In response to antigenic stimuli, TCD4$^+$ lymphocytes express transcription factors which determine specific signaling pathways. These are responsible for the production of cytokines to each of these T cell patterns. The differentiation to a particular type of effector T lymphocytes is intimately related to interleukins which are available in the microenvironment in which a *naïve* TCD4$^+$ lymphocytes is exposed. Th1 cells have as signature the production of IFN-γ, TNF-α and IL-12, which are responsible for cellular immunity and host defense against a number of pathogens, especially intracellular organisms. Interleukin-12 acting via the transcription factor STAT4, in concert with another transcription factor, T-bet, are critical for Th1 differentiation. On the contrary, the development of Th2 cells is initiated by the signaling of IL-4 with a participation of the STAT6 and GATA3 transcription factors. Classically, the Th2 lineage is specialized in the elimination of parasitic infections (such as helminths). IL-4, along with IL-5 and IL-13 produced by this lineage, are potent activators of B cells which, in this condition, produce IgE immunoglobulin and recruit eosinophils [48].

CD is a disease mediated by Th1, while it is believed that UC is mediated by Th2 response. A significant increase of Th1 cytokines has been demonstrated in inflamed mucosa of CD, whereas the in inflamed areas of UC as increased

cytokines were present in a Th2 profile [49]. Another study showed that the T cells in the mucosa of DC patients secret high amounts of IFN-γ and IL-2 than from T-lymphocytes from UC patients [50, 51]. Furthermore, it has been demonstrated that UC patients produce increased amounts of IL-5 [52]. Data from biopsies of both DC and UC patients showed high *ex vivo* levels of IFN-γ and lower levels of IL-13 have been found in UC as compared to DC patients [53]. In addition, it has been demonstrated that IL-5, IL-13, IL-15 and IL-33 mRNA levels in DC patients were significantly increased when compared to both DC and control [5]. Interestingly, it was shown that pediatric CD is characterized by Th1 in the terminal ileum and Th1/Th17 immune response in the colon [54]. However, currently it is considered that Th1 and Th2 immune responses do not represent the complexity of immune responses measured by intestinal T cells. In such a context, as will be discussed in the next section, more recent studies have demonstrated the involvement of the Th17 pathway in the physiopathological processes of IBD [55].

5.2 TH17: friend and foe

Studies suggest that Th17 cells perform an important role in the host's defense against extracellular pathogens which are not effectively countered by Th1 or Th2 cells. They are also known by their action in the physiopathology of autoimmune diseases and recently have been identified in the scenario of IBD.

Th17 cells require specific cytokines and transcription factors for their differentiation. They are dependent on IL-6 and TGF-β for their differentiation and are defined by expression of the transcription factor RORγt orphan nuclear receptor [56]. Interestingly, in the absence of IL-6, the cytokine TGF-β promotes the differentiation of FoxP3 innate regulatory T cells (iTreg). The expression of IL-23R is low in *naïve* T lymphocytes, although, in the presence of IL-6 and TGF-β, there is an increase in the expression of the IL-23 receptor. IL-23 is not necessary for the appearance of the Th17 phenotype, although it is important for its maintenance and expansion [57]. The signaling of TGF-β hinders IL-23R and antagonizes RORyt, contributing also to the appearance of iTreg [58].

Signal transduction downstream of IL-6 and TGF-β, including JAK/STAT3 activation, induces expression of RORγt, which is the master transcription factor defining Th17 cells as a distinct lineage and promotes transcription of IL-17. The cytokines produced belong to the IL-17 family and are known as IL-17A (commonly known as IL-17), IL-17B, IL-17C, IL-17D, IL-17E (or IL-25) and IL-17F [57]. Cytokines are characterized as pro-inflammatory if they induce the recruitment of neutrophils. However, Th17 cells are also capable of secreting IL-21 and IL-22, which perform the important role of host defense on the mucosa surface as well as acting against extracellular pathogens, such as fungi and bacteria. In addition to Th17 cells, others have been characterized as secreting IL-17 and IL-22, such as innate lymphoid cells (ILCs), natural killer cells, NKT cells, mast cells, as well as phagocytes that are recruited to the site of infection [59].

Some evidence show that interleukins IL-17 and IL-22 may perform a protective function by inducing the production of antimicrobial peptides, as well as acting in the recruitment of neutrophils to act in the defense against fungi and bacteria [60–62]. It is known that in intestinal epithelial cells IL-17 stimulates the expression of tight junction claudin proteins [63]. In an experimental animal model of dextran sulfate sodium (DSS)-induced colitis, it was demonstrated that IL-17 regulates the localization of the tight junction protein occludin and also reduces gut permeability following epithelial injury [64]. In the IBD scenario, Th17 cells appear as protagonists in the inflammatory process [65]. It was demonstrated that IL17R knockout

mice were protected against the induction of colitis by trinitrobenzenesulfonic acid (TNBS). In another study, a high expression of IL-17A was reported in blood serum and in the colon of IBD patients [66]. Other groups indicated a positive correlation between the severity of the disease and the levels of IL-17 in ulcerative colitis patients, or even that lymphocytes which produce IL-17 and IL-23 were increased in colitis and DC patients [67].

Thus, this protector role contradicts the pro-inflammatory role of Th17 cells in IBD and the distinguishing factor between beneficent and pathogenic Th17 is still unclear. Additional studies are required to clarify if Th17 lymphocytes at any moment lose this protecting role in the course of IBD or if the inflammatory role in these diseases is due to a Th17 pro-inflammatory cell response which is boosted by recently activated *naïve* $TCD4^+$ lymphocytes.

5.3 T regulatory cells in maintaining homeostasis at the intestinal lamina propria

Two types of T_{regs} cells are well characterized in the literature such as natural T_{regs} (nT_{regs}) cells which are generated in the thymus through IL-2 signaling and as induced or adaptive T_{regs} (iT_{regs}) arising in peripheral tissues [68, 69]. The key cytokine for the induction of T_{reg} cells, especially the iT_{regs}, is the TGF-β and the FOXP3 transcription factor is considered as an identity and the main regulator for the differentiation and function of these cells [69]. T_{reg} cells produce IL-10 and themselves also produce large amounts of TGF-β.

These cells play a role in maintaining peripheral tolerance to their own antigens [70]. In the intestinal lamina propria they are important for the maintenance of tissue homeostasis through the negative regulation of T effector cells (T_{eff} cells). This regulation occurs through the production of the immunosuppressive cytokine IL-10 and the expression of CTLA-4, which is able to deplete CD80/CD86 [71]. The CD80 and CD86 expressed by APCs provide essential co-stimulatory signals to T lymphocytes through ligation of CD28 in addition to T cell receptor (TCR) signaling [72]. CTLA-4 also appears to play a particularly important immunoregulatory role in the human intestine. It has been shown that treatment with anti-CTLA-4 Ipilimumab for cancer, increases the immune response against the disease by decreasing T_{reg} cell function. However, data shows that this treatment can result in potentially lethal colitis in a number of patients [73, 74].

Abnormalities in the functions as well as the presence of these cells in the intestine contribute to the establishment of IBD [75, 76]. The inhibitory molecule CTLA-4 is highly expressed on the surface of T_{reg} cells and plays a critical role in the inhibitory function both *in vitro* and *in vivo* of T_{reg} cells by limiting availability of CD80 and CD86 (Slavik et al., 1996). CD80 and CD86 expressed by APCs supply essential co-stimulatory signals to T cells via ligation of CD28 in addition to TCR signaling [77].

Inflammation in IBD may occur as a function of an imbalance between Th17 cells and Treg cells. It is known that both Th17 and iTregs are from $TCD4^+$ lymphocytes in the presence of TGF-β. However, when IL-6 cytokine levels are elevated in the gut, TGF-β and TCR signaling result in upregulation of RORγt and therefore in the appearance of Th17 cells with pro-inflammatory profile. As discussed above, the role of lymphocytes in IBD is unclear. Several studies have shown them to be either pathogenic or protective [78].

A decrease in T_{reg} and increase in Th17 cells was observed in the peripheral blood of IBD patients [79]. Additionally, the ability of T_{reg} cells to suppress autologous T-cell proliferation was reduced in IBD patients [80].

5.4 TH9: new lymphocyte players in IBD

In addition to the previously discussed T lymphocyte subtypes Th1, Th2 and Th17, studies have confirmed the existence of a new one denominated Th9, which are characterized by the expression of high amounts of IL-9. Initially, it was believed that IL-9 was produced by the Th2 subtype; however, it has been discovered that Th9 lymphocytes do not express the GATA-3 transcription factor in comparable levels to the Th2 lymphocyte, and not even other transcription factor, such as T-bet, RORγt and FOXP-3, characteristic of Th1, Th17 and Treg, respectively.

Naïve T cells differentiate into Th9 if they are exposed simultaneously to IL-4 and TGF-β. The transcription factor STAT6 protein, activated by IL-4, stimulates an increase of IL-9 in Th9 cells [81]. Interestingly, it was shown that IL-4 and STAT6 are responsible for downregulating T_{reg} cells by the inhibition of FOXP3 expression, which results IL-9 production [82].

Still, a complicated network of transcription factors, such as Interferon 4 (IRF4) regulating factor and Smads are essential to adequate induction of this phenotype. Additionally, PU.1 transcription factor is critically involved in the signaling mediated by TGF-β. TGF-β is also important to the signaling pathways which culminate in the activation of Smad2, Smad3 and Smad4 transcription factors, which are necessary to appearance of the Th9 phenotype [83].

Several experimental pieces of evidence suggest that Th9 cells are involved in the pathogenesis of IBD. It has been demonstrated that mice which received *in vitro* cultivated T cells with TGF-β and IL-4 developed severe colitis [84]. Nalleweg et al. investigated the expression of IL-9 and IL-9R in peripheral blood, biopsies and surgical samples from patients with ulcerative colitis. Among other results, they showed that mRNA expression was significantly increased in inflamed samples from these patients. Additionally, it was shown that IL-9R was overexpressed on gut epithelial cells and IL-9 induced STAT5 activation in these cells. Considering the results, it was suggested that targeting IL-9 might become a therapeutic option for patients with ulcerative colitis also suggest that Th9 cells represent a likely target for the treatment of chronic intestinal inflammation [85]. The authors found that in patients with ulcerative colitis are more T cells expressing the transcription factor PU.1 and interleukin 9 (IL-9). In this study, the mice whose T cells were deficient in PU.1 were protected from colitis, which was even suppressed when these animals were treated with antibody to IL-9.

Additionally, a study which analyzed IL-9 in venous blood samples de CD and UC patients, it became evident that there was a significant correlation between disease severity and IL-9 in the CD patients, but not in the UC [86].

Th9 cells also regulate the intestinal mucosa's barrier function. The exacerbated intestinal IL-9 production breaks the intestinal epithelial barrier and compromises tolerance to certain commensal microorganisms, which enables the occurrence of inflammation. In an animal experimental model of TNBS-induced colitis, the expression of tight junction molecules was investigated in the inflamed colon. It was observed that some of these molecules were up regulated in the colon of TNBS-treated IL-9 KO mice [87].

6. Innate lymphoid cells (ILCS): innate counterparts of T-helper lymphocytes

A decade after their discovery, ILCs are currently recognized as performing a regulator function of intestinal homeostasis, and alterations in these cells'

responses are related to IBD [88]. They represent a family of immune system cells which derive from a progenitor known as Id2 and process the morphologic characteristics of lymphocytes, although they do not have rearrangements at the antigen receptors. The cells of these groups are able to produce cytokines which correspond to the profile of those produced by the $TCD4^+$ subtypes [89]. ILC are categorized in three groups, detailed bellow.

Group 1 ILC are comprised of ILC1 and natural killer cells. The Tbet transcription factor and the IL-12, IL-15 and IL-18 cytokines are responsible for the generation of these cells which have as a characteristic the production of Th1 cytokines, particularly IFN-γ [90]. The cells in the Group 02 are characterized as ILC-2 and are dependent on GATA and RORyt transcription factor, as well as the stimulus of IL-25 and IL-33 cytokines. These cells produce Th2 cytokines, such as IL-5 and IL-13 [91]. In Group 3, ILC3 and lymphoid tissue inducer (LTi) cells are RORyt dependent, and, similarly to Th17, have the ability of secreting IL-17 and IL-22 through the same stimulus with IL-1β and IL-23 [92]. ILC3 are the most abundant in the gastrointestinal tract [93, 94].

The ILC3, in the intestine, in addition to interacting directly with the microbiota, act together with other cells to ensure and maintain local homeostasis. Studies have revealed that ILC of this group express MHC II and can process and present antigens. However, when in contact with $TCD4^+$ lymphocytes by MHC II, instead of inducing the proliferation of these cells, the ILC act by limiting the response theses lymphocytes to commensal bacteria. It has been demonstrated that, in the

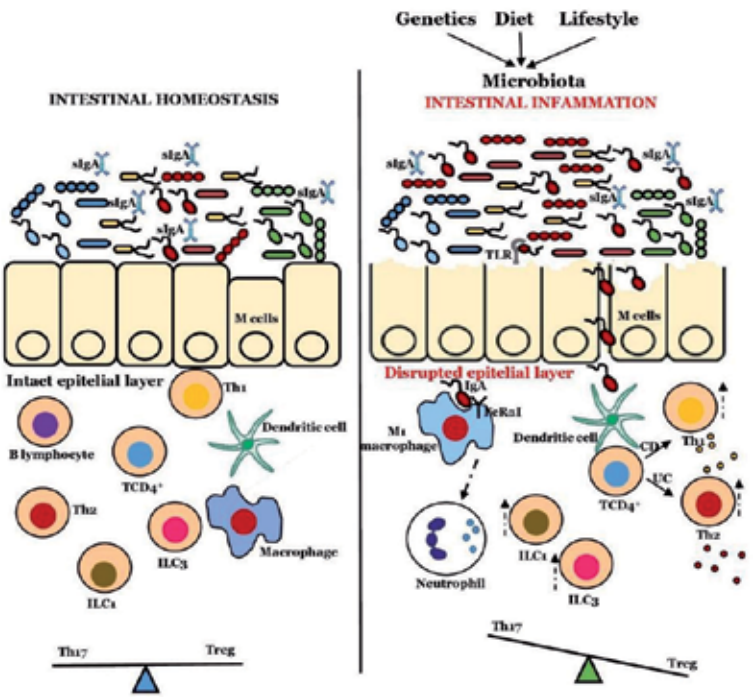

Figure 1.
During intestinal inflammation, such as IBD, barrier permeability is impaired, allowing the passage of luminal antigens into the lamina propria. These antigens can be recognize by TLR or captured by M cells. The exposure of immune cells to the luminal content induces $TCD4^+$ activation, differentiation and inflammatory cytokine release as well as neutrophil recruitment. IgA-opsonized bacteria contributes to the inflammation induced by FcαRI. Several environmental factors (diet, genetics, lifestyle) can modulate the microbiota composition and the activation of immune cells in the gut. UC, Ulcerative Colitis; DC, Crohn's Disease; ILCs, Innate Lymphoid Cells, Th, T helper cells; T_{arg}, T Regulatory Cells, IgA, Immunoglobulin A; sIgA, Secretory IgA, TLR, Toll Like Receptor; FcαRI, FcαReceptor I.

absence of MHC II, the ILC of murines induce deregulated responses in TCD4$^+$ cells for commensal bacteria, causing, thus, spontaneous intestinal inflammation [95]. In addition, it has been proved that pediatric Crohn's disease patients have reduced levels of MHC II$^+$ ILC3 [96].

The ILC3 have also been described as key effector cells in immunity against pathogens [97]. This protector effect occurs mainly through the secretion of IL-22 and IL-17, which induce epithelial cells and produce antimicrobial peptides against pathogens. The lack of ILC3 in the intestine leads to a decrease of IL-22 and hinders the production of antimicrobial peptides [88].

However, ILC3 seems to act as a double-edged sword. It was demonstrated that inappropriate activation of ILC3 causes intestinal damage through the excessive production of IL-22. This may induce epithelial cells and generate chemokines which attract neutrophils, which leads to the accumulation of these cells and to the tissue destruction [98]. Additionally, it was shown that colonic ILC3 from UC and CD patients showed higher expression of IL-22 when compared to healthy individuals [99].

Although ILC3 are smaller in number in the gastrointestinal tract, studies on ILC1 accumulate in inflamed mucosal tissues. It was shown that the frequency of the ILC1 subset was higher in inflamed intestine of CD patients, which indicates a role for these IFN-γ-producing ILC1 in the pathogenesis of gut mucosal inflammation [100, 101]. Forkel et al., also identified an increase in the ILC1 subset frequency in DC patients when diagnosed with the disease.

In conclusion, recently, a new population of ILC has been discovered and identified as ILCreg. During the intestinal inflammatory process, these cells may be induced to suppress the activation of ILC1 and ILC3, through IL-10, resulting in protection against the inflammatory process **Figure 1** [102].

Abbreviations

APC	antigen presenting cells
CTLA-4	cytotoxic T lymphocyte antigen 4
DC	Crohn's disease
TIR	toll-interleukin 1 receptor
TIRAP	toll-interleukin 1 receptor (TIR) domain-containing adapter protein
IBD	inflammatory bowel disease
UC	ulcerative colitis
DSS	dextran sulfate sodium
FcαRI	FC alpha receptor I
FOXP3	forkhead box P3
IRAK	IL-1 receptor-associated kinase
IKBα	transcription factor inhibitor κB
IFN	interferon
ILCS	innate lymphoid cells
IRF-4	interferon regulatory factor 4
LTI	lymphoid tissue inducer
IRF	interferon regulatory factor
MHC CLASS II	major histocompatibility complex type II
MYD88	myeloid differentiation protein
NOD	NOD-like receptors
NF-KB	transcription nuclear factor
NK CELL	natural killer cell

PAMP	pathogen-associated molecular pattern
PRR	pattern recognition receptors
RLR	RIG-1-like receptors
RORγT	transcription factor orphan nuclear receptor
T_{eff}	T effector cells
Th	T helper cells
TCR	T cell receptor
TLR	toll-like receptors
T_{reg}	regulatory T cells
TNB	2,4,6 trinitrobenzenesulfonic acid
TGF-β	transforming growth factor beta
TIR domain	containing adapter-inducing beta interferon

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Chapter 2

The Role of TNF in the Pathogenesis of Inflammatory Bowel Disease

Martina Perše and Ana Unkovič

Abstract

Tumor necrosis factor (TNF) is a pleotropic cytokine involved in a wide range of pathological processes, including inflammatory bowel disease (IBD). In the past, TNF was recognized as a pro-inflammatory cytokine with deleterious effects. This has led to the development of anti-TNF drugs, which revolutionized the treatment of inflammatory disorders such as Crohn's disease. However, in the past 20 years, clinical studies have shown that anti-TNF drugs are not always effective. Moreover, in some rare cases, anti-TNF drugs can even cause an aggravation of the disease. Nowadays, there is increasing evidence that TNF is not only detrimental but can also play an important role in health and the maintenance of homeostasis. The aim of this chapter is to briefly summarize the literature demonstrating the complex dichotomous role of TNF in IBD and discuss the role of anti-TNF drugs in the treatment of IBD.

Keywords: tumor necrosis factor, inflammatory bowel disease, side effects, TNF inhibitors, paradoxical side effects, homeostasis

1. Introduction

Inflammatory bowel disease (IBD) is a chronic, relapsing inflammatory condition of gastrointestinal tract with high incidence and prevalence in Western countries (North America, Europe, the highest in Scandinavia, and the United Kingdom) [1]. It is estimated that IBD affects 2.5–3 million people in Europe [2].

IBD consist primarily of Crohn's disease (CD) and ulcerative colitis (UC), which are distinguished by the location and the nature of the inflammation [3]. Patients with IBD experience many symptoms, such as abdominal pain, fever, vomiting, diarrhea, rectal bleeding, anemia, and weight loss, which have significant impact on their quality of life. Symptoms vary depending on the location and severity of inflammation and can be very painful and disruptive and in some cases even life-threatening (CD patients have 40% risk of mortality) [3].

IBD affects a young population, in the second and third decades of life or even in late adolescence [4]. The majority of patients with IBD progress to relapsing and chronic disease and need lifelong treatment and care. The health economic burden and permanent work disability in IBD are high in Europe with a total yearly direct healthcare cost of 4.6–5.6 billion Euros [2]. In recent years, the management of IBD has improved, due to the fact that the new treatments with anti-TNF drugs induce

not only clinical remission but also a significant endoscopic improvement or even disappearance of the intestinal lesions [5, 6].

However, in the past two decades, clinical studies have shown that anti-TNF drugs are not always effective. Moreover, in some rare cases, anti-TNF drugs can even cause an aggravation of the disease. Therefore, this chapter aims to briefly summarize the detrimental role of TNF in the pathogenesis of IBD and to highlight the beneficial role of TNF, which is too often overlooked in the health and the disease.

2. Dual identification of TNF (cachectin)

Tumor necrosis factor (TNF, also known as TNFa, cachectin, or cachexin) was identified/named in 1975 by Carswell et al. who demonstrated that the serum of endotoxin-treated mice, rat, and rabbits, previously infected with *Mycobacterium bovis* strain Bacillus Calmette-Guerin caused hemorrhagic necrosis of various tumors in mice. They found that hemorrhagic necrosis of tumors in vivo was caused by so-called tumor necrosis factor (TNF) released from host cells, very likely macrophages, in response to injected endotoxin. They showed that both, a TNF-positive serum and endotoxin, were effective in causing necrosis of similar spectrum of transplanted tumors and at a similar phase of their growth. Moreover, a TNF-positive serum had cytotoxic effects on mouse and human tumor cells in vitro as well [7].

In 1985, human TNF was purified, characterized, and cloned, which enabled production of large quantities of a highly purified TNF protein for extensive investigations [8, 9]. Since recombinant TNF has shown antitumor activity in both transplantable murine tumors and human tumor xenografts, TNF was quickly launched into clinical trials as a potential anticancer agent. Recombinant human TNF has been tested in several phase I and phase II clinical trials in the 1980s and 1990s. However, the initial enthusiasm for the use of TNF as a systemic treatment has waned in the face of significant toxicities and a lack of evidence for therapeutic benefit. Systemic TNF treatment was found to cause dose-dependent toxicities such as fever, hypotension, and tachycardia [10–12].

Independently, other groups of researchers investigated metabolic basis for cachexia and endotoxin-induced septicemia and septic shock syndrome. Hypertriglyceridemia in animals injected with endotoxin was found to result from defective triglyceride clearance due to systemic suppression of the enzyme lipoprotein lipase. Finally, the substance responsible for specific suppression of lipoprotein lipase activity was identified and named cachectin [13, 14]. Interestingly, soon after the characterization of human TNF in 1985, it was recognized that the TNF and cachectin are the same single protein with the complex dual role [8, 9, 15].

Nevertheless, direct evidence that cachectin is a mediator of the pathology/septicemia induced by endotoxin was demonstrated by Beutler and colleagues [16, 17]. They showed that passive immunization with rabbit antiserum or purified Ig against murine TNF protected the mice from the lethal effect of the endotoxin lipopolysaccharide [16]. The same group then showed that injection of recombinant human TNF into rats in quantities similar to those produced endogenously in response to endotoxin caused hypotension, metabolic acidosis, hemoconcentration, and death of animals within minutes to hours. Thus, effects similar to those are induced by injection of endotoxin [17]. These observations led to the speculation that neutralization of TNF may be beneficial in life-threatening septicemia. Despite increased interest in the use of anti-TNF drugs for the treatment of sepsis, numerous clinical trials have showed only a small survival benefit (3.6%) [18]. The likely

reason for the failure of anti-TNF drugs in sepsis can be found in the original animal study, where it was clearly demonstrated that neutralization of TNF was efficient in preventing death in mice only when administered before a very short time after the injection of endotoxin [16].

Nevertheless, the effort invested in the development of anti-TNF drugs, originally intended for the treatment of sepsis, enabled the use of anti-TNF therapy in the chronic inflammatory diseases, including IBD. However, the investigations and hopes regarding the use of anti-TNF drugs in sepsis and the use of TNF as an anticancer agent are still in progress [10, 19].

3. A link between TNF and IBD

The first evidence showing a link between TNF and IBD were publications reporting that patients with IBD have increased levels of TNF in serum, stool, or mucosal biopsy specimens [20–23]. However, the initial hopes for the use of TNF as a marker of IBD have waned when it was recognized that TNF can be increased also during infectious colitis [24] or TNF may even not be increased in patients with IBD [25] or TNF can be reduced in response to certain medication such as cyclosporine A [22, 26]. Nevertheless, a published reports about successful treatment of CD patients with TNF chimeric monoclonal antibodies (cA2 or infliximab) [27] established clear association of TNF involvement in the pathogenesis of IBD and caused extensive investigation of TNF role in IBD and production of various genetic models, including transgenic mice with persistent TNF overproduction in various tissues.

It was clearly demonstrated that persistent systemic overproduction of TNF ($TNF^{\Delta ARE/\Delta ARE}$ mice) can cause severe systemic health problems in mice, such as severe chronic polyarthritis, profound inflammatory changes in the terminal ileum and occasionally in the proximal colon, hypoplastic thymus with atrophied and disorganized cortical and medullary areas, and occasional mild inflammation in the liver and lung. These alterations were first detected in homozygous mice between 1 and 4 weeks of their age. Heterozygous mice developed the same health problems but later in their life inflammatory arthritis at 6–8 weeks of age and severe inflammatory bowel disease extending into muscular layers of the bowel wall at 4–7 months of their age. Homozygous mice never exceeded the body weight of 3-week-old mice and died between 5 and 12 weeks of their age [28]. It was also demonstrated that chronic intestinal inflammation can be triggered by persistent local TNF overproduction. Mice homozygous for persistent overproduction of TNF in the intestinal epithelium ($TNF^{i\Delta ARE/i\Delta ARE}$ mice) developed chronic ileitis by the age of 16–20 weeks and had increased mucosal and systemic protein levels of TNF. No inflammation in other tissues was found. No histological signs of joint injury were observed. Heterozygous mice ($TNF^{i\Delta ARE/+}$) develop only mild villous blunting with scarce inflammation (not significant) [29]. In addition, mice with persistent myeloid cell-specific TNF overproduction also developed symptoms of weight loss and ileitis by the age of 5 months (homo and heterozygous) but with more severe symptoms in the homozygous mice. Interestingly, mice with persistent T lymphocyte-specific TNF overproduction developed mild symptoms of IBD but only on homozygous background. On the other hand, mice with persistent B lymphocyte-specific TNF overproduction did not show any signs of IBD by the age of 15 months [30]. Results of numerous animal studies gave tacit confirmation that persistent systemic or local TNF overproduction is detrimental and responsible for intestinal inflammation, serious health problems, and even death [31].

The introduction of anti-TNF therapies in the 1998 affected the treatment of many chronic inflammatory disorders, including rheumatoid arthritis, ankylosing spondylitis, and IBD. Five therapeutic agents have been licensed in the USA and most other parts of the world. Randomized controlled trials demonstrated the efficacy and safety of induction and maintenance therapy for moderate-to-severe IBD. Subsequent studies have demonstrated that infliximab treatment results in a positive clinical response as well as in a significant endoscopic improvement, confirmed also by histological examination as a complete reduction in the inflammation infiltrate. The breakthrough in the treatment of patients with IBD with anti-TNF therapy has firmly established the dogma that TNF is a major cytokine in this disease [32, 33]. Anti-TNF drugs such as infliximab, adalimumab, and etanercept are nowadays commonly used in the treatment of a variety of inflammatory and autoimmune diseases (IBD, rheumatoid arthritis, psoriasis, psoriasiform arthritis, and ankylosing spondylitis). Nevertheless, with the increasing use and longer follow-up periods, more information about effectiveness and side effects of anti-TNF therapy in IBD has been published.

4. Side effects of anti-TNF drugs

First reported/known adverse events of anti-TNF drugs were mainly immunogenicity leading to acute and delayed infusion reactions and loss of response, infectious complication, and concerns about tumor induction or progression [34, 35].

Today, after two decades of clinical experience with anti-TNF drugs and 2 million treated patients, it is widely known that around 30% of patients do not respond to anti-TNF therapy (primary nonresponders) and almost half of patients with initial response develop secondary loss of response within the first year. Among nonresponders, some may have low serum drug levels which could be explained by under-dosing or high drug clearance. Development of immunogenicity against the anti-TNF drugs is also associated with loss of response. In such cases, consideration of switch in anti-TNF drugs or dose escalation following loss of response may be an effective strategy [32]. However, some patients on anti-TNF drugs experience primary or secondary nonresponse despite adequate serum drug levels and the absence of neutralizing antibodies. Recently, it was proposed that such nonresponders may have upregulated other alternative inflammatory pathways independent of TNF [36]. Nevertheless, despite all complications and high costs of anti-TNF drugs, economic evaluation studies have shown that the benefit of anti-TNF drugs is still higher than the costs [37].

4.1 Anti-TNF drugs and risk of infection and malignancy

Susceptibility to infection and risk of malignancy has been a significant concern from the beginning of anti-TNF drug use. In the past, it was widely reported that anti-TNF therapy was associated with increased susceptibility to infections, particularly tuberculosis and hepatitis B. However, when it was recognized that anti-TNF drugs trigger the reactivation of latent infections [38], screening for tuberculosis and hepatitis B in clinical settings was implemented. Soon, reports about tuberculosis or hepatitis infections associated with anti-TNF therapy diminished [34]. Interestingly, recent publications report that anti-TNF therapy alone does not increase the risk of serious infection in IBD patients [39, 40]. Moreover, a systematic review (5528 patients) reported that the rate of serious infection was significantly lower among pediatric patients with IBD treated with anti-TNF than those treated with steroids or adults with IBD who received anti-TNF therapy [39].

In contrast, increasing number of reports about other untypical opportunistic infectious diseases, such as cytomegalovirus infection, histoplasmosis, aspergillosis appeared [34, 40]. Importantly, recent population-based study (190,694 patients with IBD) found that anti-TNF monotherapy was associated with increased risk of serious infection, mycobacterial infection, and bacterial infection but with decreased risk of opportunistic viral infection when compared with thiopurine monotherapy. However, when anti-TNF drugs are part of combination therapy with other immunosuppressive drugs, particularly thiopurines, the risk of serious infection and opportunistic infection increases [34, 41].

Anti-TNF drugs have been associated with the increased risk for malignancy [34]. In the past, few studies reported T-cell non-Hodgkin's lymphoma or hepatosplenic T-cell lymphoma in IBD patients using anti-TNF drugs [42], while more recent studies found no association between anti-TNF drugs and hematologic malignancies. It was reported that the risk of lymphoma was no greater among children with IBD who received anti-TNF drugs than those treated with other IBD therapies or adults treated with anti-TNF drugs [39]. REFURBISH study found that the risk of T-cell non-Hodgkin's lymphoma in IBD patients is increased with the use of combination anti-TNF and thiopurine therapy but not with the use of anti-TNF monotherapy [43]. However, recent cohort study of 189,289 patients with IBD reported that the use of thiopurine monotherapy or anti-TNF monotherapy in patients with IBD was associated with a small but statistically significant increased risk of lymphoma, and this risk was higher with combination therapy than with each of these treatments used alone [44].

4.2 Anti-TNF drugs and paradoxical side effects

Knowledge about immune diseases secondary to TNF target therapy is relatively new. Until 2007, altogether 233 cases of immune diseases secondary to TNF targeted therapy were reported [45]. Nowadays, increasing number of various paradoxical reactions is published such as psoriasiform skin lesions, uveitis, ileitis or colitis, joint manifestations, vasculitis and autoimmune disease (lupus and myositis), and sarcoidosis-like lesions. There are currently no predictors of their occurrence, and the optimal clinical management is still a matter of debate. Mostly paradoxical reactions are poorly described, and their prevalence and pathogenesis are not known. Therefore, it is important to be aware of all possible side effects of TNF therapy to properly inform the patient about potential side effects of anti-TNF therapy before the treatment.

Psoriasis or psoriasiform skin lesions are one of the most frequently reported paradoxical reactions. Until November 2008, altogether 120 cases of psoriasis in patients treated with anti-TNF drugs were published. Among them 18 cases were found in patients with IBD (15%) [46]. Nowadays, increasing number of studies has shown that psoriasis can develop in IBD patients (adults or children) without any history of psoriasis and independent of the type of anti-TNF drugs [46–48]. However, in IBD patients with a history of psoriasis, anti-TNF treatment may trigger reappearance (3/21) [47] or exacerbation of the psoriasis (2/18) [46, 48].

Retrospective cohort (917) reported that 29% patients undergoing anti-TNF therapy (infliximab) developed skin lesions such as psoriasiform eczema, xerosis cutis, palmoplantar pustulosis, and psoriasis. The average time from the start of TNF therapy to the onset of skin lesions varied from 14.3 weeks [46] to 2 years [46–48]. In most patients psoriatic lesions were effectively treated with topical steroids, and in patients with severe psoriasis or patients without response to topical therapy, anti-TNF therapy was discontinued [47]. In another study in almost half of patients changed their initial anti-TNF agent despite conventional skin-directed therapies, and one-third of patients discontinued all anti-TNF therapy [48].

Lichenoid drug reaction in association with anti-TNF therapy was also reported. Until 2015, only seven cases were reported in association with anti-TNF drugs. Oral lichen planus occurred between 8 weeks and 6 months after anti-TNF therapy. Outcome was mainly favorable with improvement or recovery with or without cessation of the TNF blocker. Authors recommend a careful monitoring for oral manifestations in IBD patients treated with TNF inhibitors. OLP is thought to be mediated by dendritic cells and T cells [49].

Patients treated with anti-TNF therapy (i.e., etanercept, adalimumab, and infliximab) can develop sarcoidosis-like lesions. Until 2017, altogether 90 cases were reported, 6 cases in IBD patients. Median duration between initiation of anti-TNF therapy and diagnosis was 22.5 months (range 1–84 months). Most frequently affected organs were lungs, skin, and eyes [50].

Patients with IBD developed new onset arthritis or synovitis after 2.5 ± 1.6 years of successful anti-TNF treatment. The onset of paradoxical arthritis appeared when IBD patients were in clinical and endoscopic remission but with signs of histologically diagnosed subclinical inflammation. The inhibition of inflammatory pathways alternative to TNF (IL12/1L23) may be an effective therapeutic option for severe paradoxical articular manifestations [51].

The lupus-like syndrome can be observed in 0.5–1% of patients treated with anti-TNF drugs and appears independent of the type of anti-TNF drugs. Most patients develop fatigue or fever, musculoskeletal or skin symptoms, or serositis, a rarely major organ disease. The symptoms resolve after discontinuation of TNF therapy [52, 53].

5. The beneficial role of TNF

Soon after the identification of TNF and production of recombinant TNF, it was recognized that the biological effects of TNF may be both injurious and beneficial. TNF can have a direct cytostatic and cytotoxic effect on human tumor cells, as well as a variety of immunomodulatory effects on various immune effector cells, including neutrophils, macrophages, and T cells. It can have a number of anti-infective and metabolic effects [54].

Today, in the era of anti-TNF drugs, the beneficial role of TNF is often in the shadow and is highlighted only after the appearance of a new adverse effect of anti-TNF drugs in clinical use.

Experimental studies have shown that TNF has important role in maintaining intestinal integrity [55]. If infection or injury occurs, TNF is rapidly released to promote the acute-phase inflammatory response (i.e., IL1, IL6-production of pro-inflammatory cytokine cascade) and to trigger the localized accumulation of leukocytes. Endothelial cells respond to TNF by releasing chemokines (IL-8, MCP-1, IP-10) and adhesion molecules (E-selectin, ICAM-1, VCAM-1). Collectively, these solubles and cell surface molecules lead to the recruitment of distinct populations of leukocytes to sites of infection/injury to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair. Indirectly, TNF also contribute to increased local blood flow and vascular permeability and regulation of coagulation. TNF increases mediators such as prostaglandins and platelet-activating factor [56].

However, in case of chronic TNF deprivation, intestinal barrier is more sensitive to infection and injury. Mice with TNF deprivation (caused by anti-TNF drugs or target mutations) failed to resist *L. monocytogenes* infections and died few days after the infection [57]. Mice deficient in TNF or TNFR1 are highly susceptible to *Mycobacterium* and *Staphylococcus* infection as well [54, 59]. It was found that TNF

deprivation caused delayed elimination of bacterium from the spleens and livers. However the effect was dose and time dependent. The worst results were observed when anti-TNF drug was given between days 0 and 2 of infection [57].

TNF has also important role in maintaining and protecting epithelial cells from toxic injury. For instance, DSS, a toxic agent that damages the intestinal epithelia, induce development of an acute inflammation in mice, which usually resolves in a few weeks. However, when mice have blocked production of TNF (induced by deletion of TNF gene or anti-TNF drugs), the inflammation in the intestine becomes devastating and life-threatening [58].

All these studies demonstrate that homeostatic concentrations of TNF have important protective role against intestinal injury. However, homeostatic concentrations of TNF are also important for effective innate and adaptive immune responses. It was found that mice genetically deficient in TNF completely lack splenic primary B-cell follicles and cannot form organized follicular dendritic cell networks and germinal centers [59]. Thus, chronic TNF deprivation may cause disturbances in innate and adaptive immunity. TNF is an important regulator of macrophage function required to control infection and can also contribute to containment of the disease by promoting migration of immune cells and granuloma formation at sites of infection. In case of tuberculosis, an intracellular pathogen, formation of granulomas and walling off the bacteria by macrophages and T cell (central memory T cells ($CCR7^+CD27^+$) and effector memory T cells ($CCR7^-CD27^-$)), is thus one of the protective mechanisms to control tuberculosis infection. In latency, infection is contained in a nondividing state within macrophages. However, anti-TNF therapy disturbs the physiological TNF-mediated immunoinflammatory responses and causes disease reactivation or dissemination seen in patients receiving TNF blockade [38].

It is interesting that increased susceptibility to infection and a slightly increased risk for malignancy have been expected side effects of anti-TNF drugs and have been confirmed in clinical practice. However, the observation that anti-TNF drug could lead to aggravation of preexisiting autoimmune diseases or onset of a new inflammatory diseases was not expected. Although numerous experimental studies have shown complex role of TNF in the innate and adaptive immunity [60], only paradoxical side effects of anti-TNF drugs clearly demonstrated that the maintenance of homeostatic TNF concentrations is important for normal function of organism. Recently, it was confirmed that paradoxical psoriasis is caused due to the TNF deprivation. Namely, in normal condition a production of type I IFN by plasmacytoid dendritic cells (pDC) is downregulated by TNF. In case of TNF deprivation (caused by anti-TNF drugs), production of IFN by pDC is not regulated anymore. The resulting type I interferon overexpression is responsible for the skin phenotype of paradoxical psoriasis, which, unlike classical psoriasis, is independent of T cells [61].

6. Conclusions

Although our understanding of TNF has increased considerably over the past two decades, novel finding is well in line with what had been predicted from previous mouse studies. However, the observation that anti-TNF drugs could lead to aggravation of preexisiting diseases or onset of a new inflammatory diseases was not expected. Nevertheless, paradoxical reaction appears independently of the underlying disease or the type of anti-TNF drugs used and regresses upon discontinuation of therapy, which suggests that paradoxical reactions really are a side effect of TNF blockade and not de novo disease. Thus, paradoxical reactions can

once again remind us that TNF physiologically possess various beneficial roles, and thus the maintenance of homeostatic TNF concentrations is important for normal function of an organism.

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Conflict of interest

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Section 2

Immunosuppressor and Corticosteroid Therapy

Chapter 3

Traditional Drugs: Mechanisms of Immunosuppressor and Corticosteroid Therapies for Inflammatory Bowel Diseases

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Abstract

The inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis are immunological dysfunctions of the gastrointestinal tract that develop because of multifactorial processes, including genetic predisposition, gut dysbiosis, and excessive inflammation in susceptible subjects. These pathologies affect millions of people worldwide, with substantial impact on healthcare systems and patients' quality of life. Considering the chronic inflammation that underlies the IBD presentation, the main treatment options are related to the control of patients' inflammatory response, through immunosuppressor and modulatory therapies. Therefore, in this chapter we reviewed the main mechanisms associated with the treatments that are aimed at suppressing mucosal immunity and the effects of corticosteroid therapies in Crohn's disease and ulcerative colitis.

Keywords: inflammatory bowel disease, Crohn's disease, ulcerative colitis, immunosuppressor, corticosteroid, therapy

1. Introduction

The treatment of Crohn's disease and ulcerative colitis has central purposes such as to induce and maintain the patients' remission, while restraining the disease's secondary effects and improving the quality of life of the affected subjects. Pharmacological therapy against these pathologies converges on controlling the exacerbation of immune response, either with systemic agents, such as corticosteroids, azathioprine (AZA), aminosalicylates, and methotrexate, or topical anti-inflammatory drugs. Traditionally, the treatment for CD and UC follows a "step-up" approach. However, in the last years, a "top-down" strategy was implemented in IBD therapy, beginning to treat patients with biological agents, especially for more aggressive diseases [1]. After the main control of the inflammation, biologicals can be withdrawn, and weaker immunosuppressor medicines can be used, such as AZA,

aminosalicylates, or other drug alternatives for maintenance of disease remission [2], with different mechanisms of action, as discussed in the following section.

2. Mechanisms of action of IBD's therapies: from corticosteroids to immunosuppressor drugs

2.1 Glucocorticoids

Corticosteroids, a type of steroid hormones, are lipophilic molecules derived from cholesterol. Glucocorticoids, whose major representative is cortisol, play a role in the metabolism of lipids and carbohydrates and in the immune response, through immunosuppressive mechanisms. These hormones are synthesized by the adrenal glands in response to psychological or physiological stressful stimuli, such as excessive inflammation. The synthesis of glucocorticoids occurs after hypothalamic production of corticotropin-releasing hormone (CRH), which activates the pituitary secretion of corticotropin (ACTH) that, in turn, leads to adrenal release of cortisol, in a fine-tuned circadian rhythm [3].

Many of the immunosuppressive and anti-inflammatory functions of glucocorticoids occur after the binding of this hormone to the glucocorticoid receptor (GR). This molecule was described in the 1970s [4] and presents two isoforms of GR, GRα and GRβ, which differ in the C-terminal domain, being that the α forms the most prevalent in many human cells [5].

Glucocorticoids may exert their effects by non-genomic and mainly by the genomic signaling pathways [6]. One of the first evidences on the formation of a glucocorticoid-GR complex dated from 1972 in a study, which showed that free glucocorticoids penetrate hepatoma cells and bind to a cytoplasmic receptor, forming a complex which migrates to the nucleus shortly thereafter [7]. In the nucleus, the glucocorticoid/receptor complex binds to specific DNA sequences, named *glucocorticoid responsive elements* (GRE) [8]. Such binding to GREs may lead to repression and downregulation of target genes, especially those related to inflammatory response such as IFN-γ [9], TNF [10], and adhesion molecules [11], but may also lead to transcriptional activation of genes such as IL-10 [12], which plays an important anti-inflammatory activity. Another mechanism for gene transcription regulation by the glucocorticoid/receptor complex is the interference with other transcription factors, such as NF-κB, NFAT, and AP-1 [13], which also results in the inhibition of inflammatory responses.

Cortisol was first synthesized around 1937/1938 by Tadeusz Reichstein, who won the Nobel Prize about 10 years later for his work [14]. The first use of corticosteroids as an immunosuppressive and anti-inflammatory treatment occurred in the 1940s for rheumatoid arthritis in a study by Hench et al., who showed a decrease in symptoms when patients were treated with these hormones, besides disease relapse when treatment was stopped [15]. Since then, corticosteroids have been effective in treating other diseases, including intestinal inflammation [16].

Today, corticosteroid therapy is one of the most widely used and most effective drugs in the treatment of IBD, especially in acute inflammation, to induce disease remission [17]. However, there are important limitations regarding their long-term use, because of the drug's side effects. In line with that, despite the anti-inflammatory role in experimental colitis, budesonide worsens the general status of the mice, leading to endotoxemia and impaired epithelial repair in the gut, which are findings that could partially explain the fails in long-term glucocorticoid therapy for intestinal inflammation [18]. In contrast, mice exposed to dextran sodium sulfate for colitis development and treated for short term with the glucocorticoid

dexamethasone had decreased intestinal inflammation, with reduced expression of pro-inflammatory cytokines such as IFN-γ and IL-1, diminishment of IFN- γ-producing $CD4^+$ T cells and augmented frequency of anti-inflammatory cytokine-producing cells such as IL-10. Moreover, the increase in the frequency of regulatory markers such as GITR, CTLA-4, PD-1, CD73, and FoxP3 in treated mice pointed to a relevant role for this short-term therapy in the induction of immune regulation [19], despite the long-term adverse effects of these drugs. These findings corroborate the relevance of this hormone in the regulation of mucosal immunity. In fact, regulatory T cells deficient for glucocorticoid receptor fail to control intestinal inflammatory diseases, in vivo. In addition, these knockout regulatory T cells acquire Th1 phenotype and secrete IFN-γ, with a consequent failure to inhibit the proliferation of $CD4^+$ T cells. Then, not only the synthetic glucocorticoid is important to inflammation control, but the glucocorticoid receptor is critical for regulatory T cell functions neither [20].

Regarding the pivotal role of microbiota in the development of gut inflammation [21], it is known that the commensal intestinal bacteria may be involved in the mechanisms of action of glucocorticoid and mediate the anti-inflammatory effects of dexamethasone in the colon [22]. Indeed, the evaluation of mucosa transcriptomics of ulcerative colitis patients pointed to a corticosteroid-response gene signature that could predict response to this therapy, together with notable changes in gut microbiota [23]. In Crohn's disease or ulcerative colitis, the bacteria translocation in the gut is originally restrained by local phagocytic cells such as neutrophils, which in turn may contribute to tissue damage due to their excessive inflammation triggered in an attempt to control microbial invasion. Then, the mechanisms and efficacy of corticosteroids in IBD also involve the reduction in the chemokines responsible for the recruitment of neutrophils, besides natural killer cells and activated T lymphocytes to the gut, during ulcerative colitis [24]. There is also a decrease in adhesion and chemotaxis of these cells to the intestinal mucosa [25].

Although the efficacy of corticosteroid for the treatment of autoimmune and inflammatory diseases has been demonstrated, prolonged utilization of these drugs is associated with an increased risk of developing eye diseases such as glaucoma or cataract, hyperglycemia or insulin resistance, dermatological affections, and purpura [26]. Moreover, there is an increased risk of gastrointestinal problems such as peptic ulcer with perforations, bleeding, and acute pancreatitis [27]. The use of corticosteroids can also cause psychiatric and cognitive disorders [28], psychosis, and also sleep-related disorders [29]. Moreover, because of its immunosuppressive and anti-inflammatory effects, many patients who use corticosteroids may suffer from reduced effectiveness of the immune system and are at risk for opportunistic infections [30].

2.2 Aminosalicylates

The aminosalicylates (5-aminosalicylic acid, 5-ASA, or mesalazine) are one of the most used therapeutic choices to control mild to moderate inflammatory bowel diseases (IBD). Sulfasalazine (SASP), balsalazide, and olsalazine are prodrugs in which an azo bond is added to the structure to connect the 5-ASA moiety to carrier molecules. Sulfasalazine was the first aminosalicylate used for IBD and provided the basis for this class of medications. It was developed in the late 1930s, by the Swedish physician Nanna Svartz for the treatment of patients with rheumatic polyarthritis. Interestingly, some of the patients who were treated with SASP had ulcerative colitis too, and, surprisingly, their condition became more stable [31]. Therefore, SASP was soon being chosen as a treatment option for patients with IBD. Later, metabolic studies revealed that when this drug reaches the colon, the azo bond is cleaved by

bacterial azoreductase, liberating 5-ASA and sulfapyridine, which is responsible for most of the usual adverse effects related to sulfasalazine [32]. In fact, in earlier elegant studies from the 70–80 decades, 5-ASA was shown to be the therapeutically active compound in sulfasalazine, while sulfapyridine plays a role as a carrier molecule, not required for clinical efficacy of the drug. These works were very important to drive the development of pure 5-ASA preparations useful for the treatment of IBD. Therefore, since aminosalicylates are among the most common therapeutic agents for these diseases, many studies have been performed in an attempt to discover the mechanisms of action of these drugs in the gut inflammation.

When the initial triggers break the mucosal tolerance in IBD, there is a vast infiltration of leukocytes in the intestine, with consequent production of soluble mediators of inflammation such as cytokines, chemokines, and eicosanoids. Some of these mediators are significantly elevated in the inflamed mucosa of IBD individuals, corroborating the pathogenesis of the disease, due to their pro-inflammatory impacts upon the bowel. In fact, the increased levels of seven eicosanoids, including prostaglandin (PG)E2, PGD2, thromboxane (TBX)B2, 5-HETE, 11-HETE, 12-HETE, and 15-HETE are found on mucosal biopsies from patients with ulcerative colitis, being correlated with the severity of inflammation [33]. Similarly, prostacyclin I2, PGE2, and TBXA2 are increased in cultured gut biopsies of active colitis patients, and, notably, the levels of these inflammatory mediators are reduced in the presence of 5-ASA. In fact, the activated leucocytes in patients' mucosa release toxic reactive oxygen metabolites and harmful eicosanoids such as LTB4, which seems to be an essential chemotactic agent in these diseases [34]. Therefore, considering the therapy mechanisms, sulfasalazine can effectively repress LTB4 and 5-HETE production by human polymorphonuclear leukocytes [35], while sulfasalazine, 5-ASA, and olsalazine (a 5-ASA dimer) potently inhibit colonic macrophage chemotaxis toward LTB4 [36]. These data suggested that one of the mechanisms of action of these drugs could be the inhibition of eicosanoids and then it is plausible to infer that the therapeutic inhibition of LOX or COX pathways could be useful in both ulcerative colitis and Crohn's disease.

Platelet-activating factor (PAF) is another phospholipid mediator released early in inflammation by a diversity of cell types, playing important roles in inflammatory conditions, including IBD. In active Crohn's disease, PAF levels are significantly higher and more elevated in inflamed than in noninflamed areas [37]. In parallel, PAF is increased in the colon and ileum from Crohn's disease patients [38], while biopsies of inflamed areas taken from ulcerative colitis subjects produce PAF spontaneously [39]. In this context, sulfasalazine and 5-ASA greatly reduce the synthesis of this mediator when incubated with mucosal biopsy specimens, indicating that these drugs exert beneficial effects in the inhibition of inflammation induced by PAF [40].

Chronic gut inflammation is also related to enhanced production of reactive metabolites of oxygen and nitrogen, since both reactive oxygen species (ROS) and nitric oxide (NO) deeply modulate the inflammatory responses. The generation of these reactive species can be attenuated by sulfasalazine, as it inhibits the binding of N-formyl-methionyl-leucyl-phenyl-alanine (fMLP) to its receptor on neutrophils [41] and also the superoxide production [42]. Interestingly, olsalazine and sulfasalazine are both potent inhibitors of superoxide production and degranulation of human neutrophils stimulated with fMLP, in contrast to 5-ASA and sulfapyridine, which do not have this ability [43]. On the other hand, 5-ASA can be converted to the oxidation products salicylate and gentisate, when the drug is incubated with activated human mononuclear cells and neutrophils, indicating that 5-ASA may scavenge toxic oxygen and nitrogen metabolites [44]. Similarly, evidences from an

in vivo study pointed once more to a scavenge role of sulfasalazine as a mechanism of action, thus reducing experimental intestinal inflammation induced by acetic acid [45]. In humans, 5-ASA oxidation products can be found in the stools of IBD patients using sulfasalazine, suggesting that this drug indeed plays a role as scavenger for ROS and NO in these diseases [46].

A series of studies have demonstrated that sulfasalazine and its metabolites, at clinically relevant concentrations, also inhibit the release of cytokines produced by multiple cell types, including T cell mediators such as interleukin (IL)-2 [47] and those produced by monocytes or macrophages, like IL-12 [48], IL-1β, and tumor necrosis factor (TNF) [49]. Precisely, how sulfasalazine represses the release of cytokines has not been fully elucidated yet, but some studies have shown, for example, that sulfasalazine inhibits TNF expression in macrophages by inducing apoptosis [49] or inhibiting nuclear factor kappa B (NF-KB), a transcription factor crucial to the production of inflammatory mediators [50]. In the last years, the effects of sulfasalazine have been extensively studied in experimental models of intestinal inflammation. The chemically treated animals develop inflammation signs similar to those of human IBD, such as severe bloody diarrhea, body weight loss, colon length shortening, and gut pathological changes. In general, sulfasalazine treatment is able to reduce these signs and the colitis severity. Moreover, the drug significantly decreases the levels of inflammatory markers such as ROS [51], NF-KB, COX-2 [52], IL-6, TNF, IL-1 [53], NO [53], inducible nitric oxide synthase (iNOS) [52], myeloperoxidase (MPO) [54], monocyte chemoattractant protein-1 (MCP-1) [51], intercellular adhesion molecule-1 (ICAM-1) [51], and LTB4 [55], which are frequently overexpressed in IBD and widely known to be involved in chronic inflammatory disorders. Taken together, these experimental findings pointed to different mechanisms of action of sulfasalazine in the control of innate inflammatory reactions in gut mucosa, with outstanding relevance to the disease outcome.

Regarding adaptive and regulatory responses, it is known that a close relationship exists between colonic inflammation and T helper 1 (Th1) or Th17 immune reactions, which are related to the severity of inflammation in both human and experimental IBD [56]. In accordance, in a colitis model, mesalazine is able to inhibit Th1 and Th17 responses in contrast to an induction of regulatory immune profile, as observed by the disease amelioration, reduced expression neutrophil activity, IL-1β, TNF, IL-12, IFNγ, IL-17, IL-6, and RORγt, along with an augment in the suppressive cytokines IL-10 and TGF-β and in the transcription factor Foxp3 [57]. These data indicate that another mechanism of action of aminosalicylate drugs could be by decreasing pathogenic while increasing regulatory responses in intestinal inflammation.

The peroxisome proliferator-activated receptor ligand-γ (PPARγ) plays a significant role in the immune control through its capacity to repress the expression of inflammatory cytokines and induce the differentiation of leukocytes toward anti-inflammatory phenotypes. Importantly, by using experimental approaches with epithelial colon cell lines and human biopsies, Rousseaux et al. showed that 5-ASA activates PPARγ, pointing to the receptor as an important drug's target for the control of intestinal inflammation [58]. In line with that, regulatory T cells (Tregs) play an indispensable role in suppressing exacerbated inflammatory immune responses that can be harmful to the host, such as in IBD [59]. Recently, Oh-Oka et al. proposed a new anti-inflammatory mechanism for mesalamine (5-ASA) in colitis, involving colonic Tregs. The oral treatment with this drug leads to the accumulation of Tregs in the colon lamina propria associated with increased levels of the active form of the anti-inflammatory cytokine TGF-β. These alterations attributed to mesalamine are dependent on the activation of aryl hydrocarbon

receptor (AhR), a transcription factor that regulates several immune processes, including Treg activation and differentiation [60].

Altogether, these studies show that aminosalicylates play an important role in the regulation of IBD responses.

2.3 Thiopurines

One of the most prescribed strategies for IBD therapy is the use of thiopurines, mainly azathioprine (AZA) and 6-mercaptopurine (6-MP). AZA is a prodrug that is metabolized by nonenzymatic mechanisms to be converted to 6-MP and other metabolites. Therefore, patients could be treated with AZA or directly with 6-MP, but the final metabolites produced from the thiopurines are the same. Also, both drugs generate endogenously active products able to interfere on DNA and RNA synthesis [61].

The discovery of AZA and 6-MP yielded a Nobel Prize in Medicine in 1988 for Gertrude B. Elion and George Hitchings. At first, the thiopurines were used in cancer therapy, in order to stop cell proliferation. Nonetheless, the immunosuppressive effect of thiopurines was evident as well as their efficiency in prolonging renal allograft transplant survival [62]. Thereafter, AZA and 6-MP began to be used in the clinics for inflammatory and rheumatic diseases. Since then, many mechanisms of action of thiopurines were proposed, mainly involving immunological axis in an attempt to unravel their immunosuppressive effects.

Some thiopurine metabolites, such as deoxyguanosine triphosphate (dGTP) and 6-thioguanine (6-TG), can be incorporated to DNA, replacing the natural purines adenine (A) and guanine (G). Then, during the DNA replication, a high level of substitution 6-TG could be particularly cytotoxic [63]. These DNA modifications are not restricted to cancer cells, and lymphocytes can be affected by the purine analogue 6-TG as well [64]. Besides that, some evidences point to the inhibition of de novo synthesis, which produce purines, by the thiopurine therapy. Then, the lack of abundant nitrogenous bases impairs the lymphocyte replication either, which contributes to the immunosuppression [65].

The thiopurines have the capacity to downregulate the expression of inflammatory genes in activated T lymphocytes [66]. One of these genes is the TNF-related apoptosis-inducing ligand (TRAIL), which is important to induce apoptosis and is upregulated in activated T lymphocytes. Despite being apparently contradictory, TRAIL could increase T cell proliferation and IFN-γ production [67], a phenomenon that is pathogenic for Crohn's disease patients. It is important to state that IFN-γ is a cytokine that accompanies the Th1 response, which increases gut inflammation. Also, CD27, which is a member of TNF superfamily, is downregulated by AZA [66]. This receptor is required to T cell maintenance and for B cell activation. Consequently, a low expression of CD27 could facilitate the lymphocyte death [68]. Besides, CD27 is involved in the NF-κB activation and IFN-γ production [69]. In fact, the 6-TG incorporation into T cell DNA is correlated to the decreased IFN-γ production in CD patients [70]. Lastly, the thiopurines could reduce the expression of the α4-integrin as well [66]. This integrin is mandatory to the lymphocyte accumulation in the gut and the chronic inflammation [71].

It is clear that the accumulation of T lymphocytes in the gut mucosa is one of the main hallmarks for the exacerbated inflammation and disease worsening. Accordingly, thiopurines also reduce T cell proliferation and the consequent excessive inflammatory mediators produced by this population. Indeed, 6-MP that impairs the A and T purine integration into the replicant DNA and replaces them for mimetic purines compromises the cell cycle and T cell proliferation. 6-MP interferes in the G1 to S phase transition and progression through S phase in cell cycle, with

consequent increase in lymphocyte death [72]. Thereby, it is unquestionable that the thiopurine metabolites incorporate into the genetic material and negatively influence the DNA integrity or stability, which causes cellular death. In the last decade, the first conclusive and detailed studies about the thiopurines' molecular mechanism of action in T lymphocytes explained better the delayed effects of these drugs, besides the incorporation of mimetic purines, as described above.

The Ras-related C3 botulinum toxin substrate 1 (Rac1) is a GTPase protein that activates MEKK/IκB/NF-κB (mitogen-activated protein kinase kinase/IKK/nuclear factor kappa-light-chain-enhancer of activated B cells) and signal transducer and activator of transcripition-3 (STAT-3) pathways, both of which lead to the accumulation of B-cell lymphoma-extra large (Bcl-xL) in the mitochondria. The enhancement of this protein results in an anti-apoptotic effect to cell survival. However, AZA and the 6-MP metabolite 6-thioguanine triphosphate (6-Thio-GTP) bind to Rac1, which impairs MEKK and STAT-3 phosphorylation, and consequently the anti-apoptotic effect by Bcl-xL is lost. Instead of that, there is an enhancement of Caspase-9, an apoptotic pathway of human cells involving mitochondria [73]. Interestingly, these mechanisms require the co-stimulation by CD28 in T cells.

The bind of CD28 by costimulatory molecules leads to lymphocyte's lamellipodia formations, which are projections of the cytoskeletal protein actin, necessary for T cell movement and membrane readjustment to make contact with antigen-presenting cells (APC). GTPase Rac1 also mediates this process [74]. Later, it was observed that thiopurines also bind to and block Rac2 activation, while the treatment with these drugs impairs the lamellipodia formation. Additionally, upon binding to Rac proteins, AZA and its metabolites reduce ezrin-radixin-moesin protein (ERM) desphosphorylation and subsequently the formation of APC-T cell conjugates, necessary for an effective immune adaptive response. Likewise, that was dependent on CD28 activation too [74]. Taken together, these results suggested that AZA and its metabolites binding Rac1 promote T cell apoptosis, by decreasing Bcl-xL and increasing caspase-9, but also interfere in T cell function or activation. Recently, a Bcl-2 inhibitor was suggested as a novel therapy to patients refractory to AZA treatment, despite Bcl-2, as a biomarker, cannot predict AZA treatment response in IBD patients [75].

In 2009 a study confirmed that 6-MP and 6-TG decrease the lymphoproliferative capacity of T cells, but in a physiological concentration (5 μM) [76]. The thiopurine therapy causes, in vivo, specifically depletion of T CD4 memory cells, thus reducing the capacity of response to a recurrent antigen. Considering that in IBD there is continuous microbial translocation and antigen presentation [77], this should explain, at least in part, the delayed onset of the drug's effect on the disease.

Thiopurine metabolites are also capable to inhibit the inflammatory response of macrophages and epithelial cells. These drugs significantly reduce the activity of c-Jun N-terminal kinase (JNK) and STAT3, as well IL-6, IL-8, CCL2, and CCL5 and inducible nitric oxide synthase (iNOS) expression. However, only iNOS in macrophages and IL-8 in epithelial cells are decreased dependent on Rac1 [78]. In fact, AZA restores the paracellular permeability after TNF-induced apoptosis. The treatment improves the expression of tight junctions and adherens junctions, such as occludin and E-cadherin [79]. Thus, the reduction of Rac1 is proposed as a biomarker for effectiveness of thiopurine treatment in patients with IBD [80].

It seems that the use of thiopurines can modulate the frequency of diverse immune cell populations, even by an indirect pathway. For example, patients treated with AZA have increased CCR5 expression in circulating monocytes. These $CCR5^{+}$ cells are considered to have an anti-inflammatory profile, with increased CD163 and diminished TLR4-induced TNF and IL-6 secretion, probably in an attempt to achieve immunoregulation under AZA treatment [81]. Moreover,

thiopurine therapy decreases CD160 expression [82], as well as natural killer (NK) cells and the population of B lymphocytes in the peripheral blood of IBD patients [83]. Indeed, the reduction in B cells is one of the reasons for using combo therapy with AZA plus infliximab (IFX), instead of IFX alone. AZA diminishes the antibody formation against IFX and then improves the patients' responsiveness to the biological treatment [84].

The presence of variant Tγδ cells, specifically the TCR Vδ2, in the gut mucosa of Crohn's disease patients is associated with worse clinical prognosis and inflammation [85]. However, AZA is able to ablate this population in the blood and mucosa of patients treated with this drug, suggesting other potential mechanisms of action of AZA in the control of intestinal inflammation [86].

Besides the cellular changes, thiopurines are also capable of modulating soluble mediators, by decreasing IL-1β, TNF, and IFN-γ or increasing IL-10 *production* in vivo [87]. Likewise, the higher expression of inflammatory cytokines in detrimental to anti-inflammatory mediators may dictate the augmented production of matrix metalloproteinases (MMPs) in contrast to inhibitors of metalloproteinases (TIMPs), which are correlated to the control of the disease and improvement of intestinal barrier [88]. In line with that, the treatment with thiopurines reduced the pro-inflammatory effects, with decreased neutrophil MMP-9 and MMP-26 production, besides increased TIMP-3 expression by enterocytes [89].

Finally, a last mechanism of immune regulation was recently described involving AZA's use. This drug can induce autophagy, which is a natural mechanism to recycle cellular components and to promote cell survival, depending on PERK sensor and mTORC1 in lymphocytes. Hence, modulation of autophagy could represent an additional mechanism of inflammation control through AZA treatment in IBD [90].

2.4 Methotrexate

Methotrexate (MTX), originally known as amethopterin, is a folate antagonist. Its history and clinical use refers to Faber and Diamond [91], who reported the utilization of aminopterin, the first folic acid antagonist, as a treatment for acute leukemia in children. MTX, which is a derivative of aminopterin and is distinguished by having an additional methyl in its structure, subsequently replaced aminopterin after a study reported its lower toxicity in an experimental model of acute leukemia in rats [92]. The idea behind the use of antifolates for the treatment of neoplasias was based on the knowledge that folates function as cofactors for DNA biosynthesis. Subsequently, the ability of MTX to interfere in DNA synthesis was proven experimentally [93], and years later lower doses of MTX also began to be studied for other conditions such as psoriasis [94] and rheumatoid arthritis [95].

For IBD, Kozarek et al. [96] were the first to report the ability of this drug to induce clinical and histological remission in patients with Crohn's disease, but it was only after two randomized controlled trials (RCTs) of the North American Crohn's Study Group (NACSG) that MTX was formally established as a possible therapy for this disease [97]. On the other hand, there is no strong scientific basis for recommending the use of MTX as a monotherapy for UC. Nevertheless, the utilization of high or low doses of MTX in combination with anti-TNF has been shown to be effective in disease control at the same extent in both Crohn's disease and ulcerative colitis patients [98]. In summary, because of these and other results, MTX is usually recommended in specific conditions, especially depending on disease outcome and response to other therapies [99].

MTX acts as an antineoplastic drug when used at high doses and as immunosuppressive at low doses [100]. This led to the investigation of other possible

mechanisms capable of inducing immunosuppression, in addition to interfering in cell proliferation. In line with that, there is a lack of specific investigation unraveling the exact mechanisms of action of MTX in IBD, but this drug is capable of inducing apoptosis in activated T cells [101], inhibiting IL-8 production by peripheral blood mononuclear cells [102], and increasing extracellular adenosine levels. This metabolite has potent anti-inflammatory properties [103] in patients with rheumatoid arthritis [104] and potentially in IBD [105]. Clearly, more experimental studies are needed to better understand the action of MTX in IBD, but those mentioned above represent possible mechanisms that could explain the relative success of MTX as an immunomodulatory therapy, especially for Crohn's disease.

2.5 Cyclosporine

The cyclosporine A (CsA) is an immunosuppressor drug initially used for organ transplantation on the late 70 and 80 decades [106]. Some years later, it was utilized as an alternative treatment for ulcerative colitis (UC) patients refractory to glucocorticoids, because of its strong immune regulatory effects [107].

CsA is a lipophilic cyclic peptide that is metabolized by hepatic enzymes of cytochrome P450 pathway [108]. Its immunosuppressor activity depends on the intracellular binding to cyclophilins with further inhibition of the calcium-calcineurin pathway and the resulting blockage of the nuclear activated T cell factor (NFAT) translocation to the nucleus [109], thus avoiding cellular activation. Consequently, there is reduction in the transcription of genes related to cytokine production such as IL-2, IL-4, and IFN-γ [110], inhibition of CD4 expression, cell proliferation [111], and activation of CD8 lymphocytes [112]. Therefore, the blockage of NFAT is considered one of the main effects of this immunosuppressor drug [113].

Upon in vitro treatment of peripheral blood mononuclear cells (PBMCs), from ulcerative colitis or Crohn's disease patients with CsA, there is reduction of TNF, IL-17, and IL-10 in samples from all donors, besides an exclusive significant IL-13 decrease in subjects with UC. Also, CsA stimulates the cellular apoptosis of PBMC from patients with UC, though not by the mitochondrial route [114]. In an experimental colitis model, the treatment with CsA reduces the clinical activity of the disease and mRNA expression of several inflammatory cytokines such as IL-1β, IL-6, and TNF [115].

Hence, though the therapy with CsA has shown to be beneficial, the systemic treatment can be limited due to its side effects such as nephrotoxicity, hypertension, seizures, production of ROS or hydrogen peroxide, and opportunistic infections [116].

2.6 Tacrolimus

Tacrolimus (Tac) was isolated in 1984 from the fungus strain *Streptomyces tsukubaensis*. It was initially used in the treatment of transplants and later in therapies for inflammatory or autoimmune diseases [117]. This drug is a substrate for cytochrome P450 isoenzymes (CYP3A), and the expression or activity of these enzymes in liver and intestinal cells may vary between individuals, thus contributing to different pharmacokinetic profile of Tac therapy [118].

The Tac, compared to CsA, has a more potent inhibitory action against T cell activation, leading to immunosuppression. It binds to FKBP-12, with further inhibition of the calmodulin-dependent phosphatase activity of calcineurin [119]. Thus, it inhibits the action of activated nuclear T cell factor (NFAT), reducing the production of IL-2. In line with that, Tac can also decrease the activity of NF-κB [120]. Therefore, besides IL-2, Tac is a calcineurin inhibitor that leads to reduced

production of IL-3, TNF, IFN-γ, and IL-17, as well as the release of histamine from mast cells and proliferation of $CD4^+$ or $CD8^+$ T cells in a variety of inflammatory processes [121]. Tac treatment in bone marrow-derived macrophages also leads to reduced IL-12p40, IL-12p70, and IL-23 during LPS stimuli [122].

As described, in vitro treatment with Tac inhibits the activity of leukocytes such as T lymphocytes, NKT, and antigen-presenting cells, usually present on colon tissue. Moreover, the administration of Tac in trinitrobenzene sulfonic acid (TNBS) colitis results in the reduction of neutrophil infiltrate in the intestinal mucosa associated with inhibition of T cell activation, as well as decreased expression of CXCL1 and CXCL2 chemokines [123]. Most interestingly, Tac is able to inhibit the expression of IL-17 and TNF [124], suggesting that this drug could assume therapeutic effect on diseases mediated by Th17 responses, such as IBD. Furthermore, the rectal treatment in mice leads to better results than oral administration of the drug [125].

In experimental granulomatous colitis, treatment with Tac results in the reduction of intestinal permeability, neutrophil activity, as well as extra-intestinal manifestations of the disease, such as hepatic and splenic granulomas, caused by the colitis-inducing agent [126]. On the other scenario, myofibroblasts isolated from normal gut tissues and stimulated in vitro with TNF show increased phosphorylation of the p38 subunit of MAP kinase, leading to augmented CCL2 and CXCL10 expression. However, in vitro treatment with Tac suppresses the expression of CCL2 and CXCL10 mRNA by inhibiting phosphorylation of MAP kinase, indicating that these effects could be one of the mechanisms of therapeutic action of Tac on intestinal inflammation [127].

Hence, although this therapy may result in satisfactory IBD outcome, research has pointed that after mucosal healing, it is desirable to change this therapeutic intervention to other immunosuppressor drugs, in order to reduce the long-term adverse effects caused by Tac, such as nephrotoxicity [128].

3. Conclusions

The introduction of pharmacological therapies for IBD is of high importance to achieve remission and maintenance of quiescent disease in affected patients. Nonetheless, although these drugs act by diverse mechanisms, all of them are relevant in constraining the activation and perpetuation of the exacerbated immune-inflammatory responses that underline the gut inflammation in Crohn's disease and ulcerative colitis. Then, the balance between adequate control of inflammatory responses and drugs' adverse effects dictates the efficiency of corticosteroid and suppressor treatments in IBD.

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Section 3

Anti-TNF Therapy

Chapter 4

Inhibitors of Tumoral Necrosis Factor Alpha in Inflammatory Bowel Disease

Carlos Walter Sobrado, Natália Sousa Freitas Queiroz and Caio Almeida Perez

Abstract

The treatment of inflammatory bowel disease (IBD) has undergone a major paradigm shift in the last two decades with the introduction of biological drugs. Tumoral necrosis factor (TNF) antagonists were the first monoclonal antibodies available for treatment of IBD. New emerging concepts as early initiation of treatment during the "opportunity window," and "treat to target" with a tight control strategy have contributed to optimum utilization of these drugs allowing better long-term outcomes for treated patients. This chapter aims to review all current pivotal data regarding efficacy and safety of infliximab, adalimumab, certolizumab pegol, and golimumab, as long as real life experience with these agents. Comparative efficacy among anti-TNF agents and the role of therapeutic drug monitoring in the management of IBD will also be discussed. Last, the authors present future perspectives with the drugs and position anti-TNF agents as viable therapeutic options in the current IBD therapeutic armamentarium.

Keywords: biologics, TNF inhibitors, therapy, Crohn's disease, ulcerative colitis

1. Introduction

The treatment of inflammatory bowel disease (IBD) has remained a challenge for physicians involved in disease care because of its chronic nature and the impact on patient's quality of life. Traditionally, the pharmacological arsenal for the treatment of Crohn's Disease (CD) includes the aminosalicylates (sulfasalazine, mesalazine), immunosuppressants (e.g., azathioprine, 6-mercaptopurine, and methotrexate), corticosteroids (e.g., prednisone, hydrocortisone, methylprednisolone, and budesonide), and antibiotics. This therapeutic armamentarium, regarded as "conventional," does not seem to interfere with the natural history of the disease, while improving the symptoms of many patients [1–3].

In the last two decades, there has been a major paradigm shift in the treatment of IBD, with the introduction of biological drugs (monoclonal antibodies) [4, 5]. Biological drugs were the only class of drugs that alter the natural course of the disease, reducing the risk of hospitalizations, and surgeries [6]. However, insights into the importance of early and optimized therapy have prompted interest in a 'treat to target' approach to achieve good disease control. This strategy involves treating to a pre-defined target that is associated with optimal long-term outcomes. Regular

monitoring of objective measures of disease is required, and treatment is optimized based on these findings to ensure the target is achieved and maintained.

The natural course of inflammatory bowel disease is characterized by periods of remission and exacerbation and, over time, patients can develop irreversible damage such as stenosis and fistulas in Crohn's disease shortening and lead pipe appearance of colon in UC. However, it has been shown that early diagnosis with identification of severity predictors factors [7] and the early initiation of treatment with biological drugs during the "opportunity window," where symptoms are mainly derived from the diseases inflammatory activity in its initial phase, significantly reduces the rate of surgical complications, such as fistula stenosis in CD, as well as the need for colectomy in patients with UC who present severe acute colitis or chronic colitis refractory to corticoid, aminosalicylates, and immunosuppressive therapy [8–10].

The current IBD treatment goals include not only symptoms control, mainly but also sustained control of inflammation, through the mucosal healing and complication prevention (fistulae, abscesses, stenoses, dysmotility, and dysplasia), which may lead to hospitalization, surgery and substantial impact in quality of life [1, 11, 12]. In 2015, the International Organization for the Study of Inflammatory Bowel Diseases for the Study of Inflammatory Bowel Diseases (IOIBD) published the

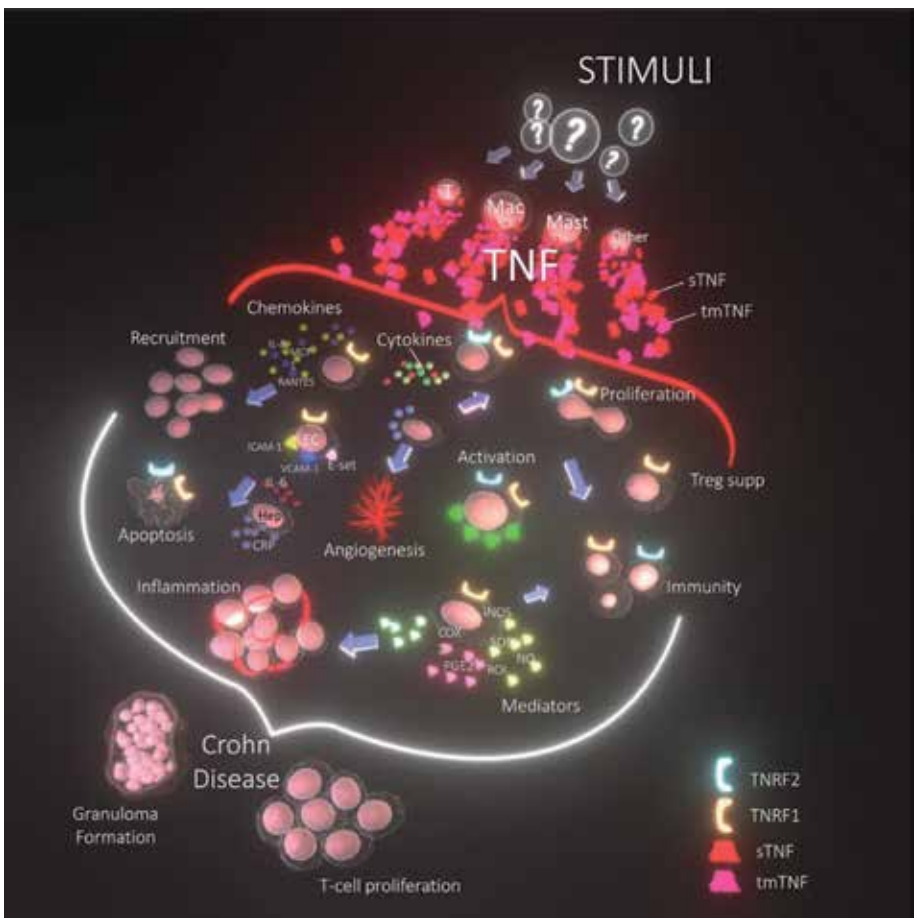

Figure 1.
TNF's mechanisms of action - In the pathophysiology of Crohn's disease, TNF is produced at high concentrations by a variety of cell types, presumably induced by endogenous or microbial stimuli. A cascade and network of cellular responses mediated by TNF are shown in the the diagram.

selecting therapeutic targets in inflammatory bowel disease (STRIDE), where 28 experts in IBD developed recommendations based on a systematic literature review and expert opinion proposing the strategy "treat to target" in IBD. In this publication, the recommended therapeutic targets were clinical remission defined by improvement in bowel movements and the resolution of the associated rectal bleeding for UC or abdominal pain for Crohn's disease. Furthermore, endoscopic remission with no ulcerations in CD and an endoscopic Mayo score 0-1 in UC should be confirmed [11].

The "treat to target" strategy defines the therapeutic goals that professionals should aim for, although it is important to emphasize which treatment strategy should be adopted in order to achieve the desired outcome. With that purpose, in 2017 the effect of tight control management on Crohn's disease (CALM) trial was published, a multicenter phase 3, randomized controlled trial designed to evaluate the safety and effectiveness of two treatment strategies in patients with CD scaling to biological therapy based on predefined criteria of treatment failure. The primary endpoint was the mucosal healing, defined by a CDEIS <4 score and the absence of deep ulcers at the end of 48 weeks, patients were randomized into two groups: the "tight control," where therapy was scaled based on clinical evaluation and biomarkers (fecal calprotectin and CRP), and the clinical management group, in which only the symptom assessment was considered. It was observed that a significantly higher proportion of patients in the "tight control" group reached the primary endpoint compared to the clinical management group, showing that the escalation of biological therapy guided by targets in patients with early CD is associated with better clinical and endoscopic outcomes when there is an association of clinical evaluation and biomarkers for decision-making [13].

The first group of biological medicines was composed by tumoral necrosis factor (TNF) antagonists, approved for use in Crohn's disease patients in 1998. The currently available anti-TNFs for treatment of CD are infliximab, adalimumab, and certolizumab pegol (**Figure 1**).

2. Infliximab (Remicade®)

The infliximab (IFX), a chimeric monoclonal IgG1 antibody, was the first biological used in CD. In 1997, Targan et al. published a randomized controlled trial demonstrating the superiority of the drug in inducing clinical remission in moderate to severe CD compared to placebo. Four groups were defined, involving 108 patients, to receive doses of 5, 10, 20 mg/kg, or placebo. The primary outcome was clinical response after 4 weeks, defined by a decrease of 70 points, or more in CDAI score after a single infusion. It was observed that 81% of patients receiving 5 mg/kg, 50% of those who received 10 mg/kg, and 64% receiving 20 mg/kg achieved the goal, compared to only 17% in the placebo group ($p < 0.001$). This was the first comparative, randomized, placebo-controlled trial involving IFX in the treatment of CD. It is a landmark in biological therapy because it has demonstrated superiority of this drug over placebo in a single infusion and guided the currently adopted dose of 5 mg/kg [14]. Subsequently, Present et al. published a longer lasting study with 18 weeks of follow-up involving patients with penetrating CD that had active fistulas. In addition of the initial dose at week 0, IFX was administered at weeks 2 and 6 in two groups with 5 or 10 mg/kg compared to the placebo group. The primary endpoint was the 50% reduction in the drainage of fistula, which occurred in 68, 56, and 26% in the groups 5, 10 mg/kg, and placebo, respectively, with statistical significance [15].

With the efficacy of IFX in inducing clinical remission established, in order to evaluate its efficacy in maintain clinical response in CD, in 2002 the ACCENT I study was published, the most relevant publication related to IFX in CD; a multicenter study (US, Europe, and Israel), controlled trial involving 573 patients with moderate to severe Crohn's disease (CDAI between 220 and 400). All patients received a dose of IFX 5 mg/kg and were assessed after 2 weeks. Of these, 325 (58%) had clinical response (CDAI decrease of 70 points or more at baseline) and were randomized at week 2 into 03 groups: 5, 10 mg/kg, and placebo. Following treatment regimen suggested by Present et al., doses were administered at weeks 0, 2, and 6, and subsequently administered every 8 weeks. The primary endpoint was clinical remission (CDAI <150 points) maintained after 30 and 54 weeks of initiating therapy. It was observed that those who responded to induction dose had higher remission rate at weeks 30 and 54. The maintenance of clinical remission rates at 54 weeks were significantly higher in the groups that received IFX 5 and 10 mg/kg (28.3 and 38.4%, respectively) compared with placebo (13.6%), showing the effectiveness of maintenance therapy with IFX. No statistical significance was observed in the difference between 5 and 10 mg/kg groups. In addition, in the placebo group, there was no mucosal healing at week 10, whereas patients receiving IFX in doses of 5 and 10 mg/kg, healing was observed in 31% of cases [16]. Following this line of reasoning, ACCENT II was published in 2004, a phase III randomized, double-blind, placebo-controlled study that included 306 patients with penetrating CD (enterocutaneous and perianal fistula), of which 282 were randomized at week 14 after the induction therapy (weeks 0, 2, and 6) for receiving infusions of 5 mg/kg or placebo every 8 weeks, aiming to evaluate the loss of IFX response in both groups after 54 weeks of treatment. It was observed that the time to loss of response was significantly higher in the IFX group over placebo (>40 weeks vs. 14, $p < 0.001$), and after 54 weeks, only 19% of the patients in the placebo group did not have fistulas in compared to 36% in the IFX group ($p = 0.009$) [17].

In order to assess the IFX therapy effectiveness in induction and maintenance of clinical response in moderate to severe UC two phase III placebo-controlled studies were subsequently published: the ACT I and II. With a total of 364 patients involved in each study, they were randomized to receive placebo, 5 or 10 mg/kg at weeks 0, 2, and 6, followed by infusions every 8 weeks through weeks 46 (ACT I) and 22 (ACT II). The primary endpoint was to evaluate clinical response (defined as decrease of three points in Mayo score and, at least, one point in the sub-item for rectal bleeding) at week 8, having as secondary endpoints the clinical response or remission after corticosteroid withdrawal and mucosal healing at weeks 8, 30 in both studies, and at week 54 in ACT I. In this last study, only 37% of patients in placebo group had clinical response at week 8 versus 69% ($p < 0.001$) in the 5 mg/kg group and 62% ($p < 0.002$) in the 10 mg/kg group. In ACT II, 64% of patients receiving IFX 5 mg/kg and 69% of those who received 10 mg/kg had clinical response at week 8 compared to 29% of those receiving placebo ($p < 0.001$ for both comparisons). In both studies, clinical response was more frequently observed at week 30 among patients who received IFX ($p < 0.002$ for all comparisons). In ACT I, after 54 weeks, more patients receiving IFX 5 or 10 mg/kg (45 and 44%, respectively) showed clinical response compared to placebo (20%, $p < 0.001$) [18].

The pivotal studies mentioned consolidated IFX use as induction and maintenance therapy in CD and UC. However, in general, the clinical trials inclusion criteria are too restrictive, restricting the participation of most patients in daily clinical practice. One of the biggest real-life studies evaluating the effectiveness of treatment of CD with IFX was published in 2009 by Schnitzler et al. from Leuven group. Six hundred fourteen patients were evaluated with a median of

55 months follow-up, in which approximately 11% were primary non-responders. Of the 547 remaining, 63.3% of patients had sustained clinical benefit. Treatment was discontinued in 31.7% of cases due to complete remission, 12.8% due to adverse events, and 21.6% due to loss of response to the drug. This study demonstrated that good results can be obtained with IFX treatment in the real world, when the requirements of controlled studies are often not attained [19].

In order to evaluate the safety profile and long-term repercussions of IFX treatment based on real life clinical experience, Sandborn et al. published in 2012 a study involving 492 CD patients treated between 1998 and 2002 at the Mayo Clinic and followed until 2009. It was shown that approximately 80% of patients showed clinical response to induction therapy, of which 25% with partial and 75% with complete response, in agreement with previously reported data [16, 17]. Dose escalation or shortening of the interval between infusions occurred in approximately 57% of patients who received maintenance dose with a cumulative probability of a therapeutic adjustment of 19% in the first year, 57% in 5 years, and 74% in 10 years of follow-up, reflecting that there is a loss of response over time. Note that 10% of the 182 patients who received maintenance therapy, discontinued its use because of loss of response. The cumulative probability of adverse events was around 35% in the first year, increasing to 86% after 10 years of therapy. Approximately 5% of patients developed cancer, with a cumulative probability of 9.1% in 10 years, though it was unclear if this increased incidence of cancer was related to the CD itself, the use of IFX or because this study was performed at a reference center with a specific profile of patients. The most common infectious complications were bacterial infection (intra-abdominal abscesses and pneumonia) and viral [20].

Long-term studies have demonstrated that, despite its effectiveness, IFX shows loss of response over time, with frequent need for dose escalation due to their immunogenicity. Then was raised the possibility of association of anti-TNF with immunosuppressive agents such as azathioprine (AZA) and 6-mercaptopurine, as synergists agents. In this context, the SONIC study was published in 2010 evaluating 508 patients with CD randomized to three different treatment strategies: IFX monotherapy, AZA monotherapy, or combination therapy with the two drugs. After 30 weeks of treatment, approximately 57% of patients treated with the combination therapy achieved corticosteroid free clinical remission (primary endpoint), compared to 44.4% in IFX monotherapy group ($p = 0.02$) and 30% in AZA monotherapy group ($p < 0.001$ for combination therapy; $p = 0.006$ for IFX). The mucosal healing rate was also higher in the combination therapy and IFX monotherapy groups compared to isolated AZA ($p < 0.001$ and $p = 0.02$, respectively). The difference between the IFX monotherapy and combination therapy groups in this outcome was not statistically significant ($p = 0.06$) [21]. With a similar study design, the SUCCESS was published in 2014, analyzing 239 patients with moderate to severe UC who were randomized to treatment with the combination therapy (IFX + AZA), IFX monotherapy, or AZA alone. Steroid-free clinical remission at week 16 was achieved by 39.7% of patients treated with the combination therapy compared to 22.1% in the IFX group ($p = 0.017$) and 23.7% in the AZA group ($p = 0.032$). Similarly, the difference in mucosal healing was only statistically significant when the combination therapy was compared to AZA monotherapy (62.8 and 36.8%, respectively, $p = 0.001$) [22].

The data presented above have reassured that IFX, marketed for over 20 years, are efficient and have a satisfactory safety profile, being considered as a first-line biological treatment of IBD, especially in the management of perianal Crohn's disease and severe acute colitis. Moreover, it plays an important

role in the management of extra intestinal manifestations and the prevention of postoperative recurrence [23].

3. Adalimumab (Humira®)

Adalimumab, a fully humanized monoclonal antibody IgG1, was the second anti-TNF antibody released for treating IBD. The first paper published on the efficacy of ADA in induction of remission in CD was the CLASSIC I trial in 2006. Aiming the assessment of clinical response after 4 weeks of treatment (CDAI <150 points), 299 patients naïve to anti-TNF therapy were randomized to receive, respectively, at weeks 0 and 2, a dose of ADA 40/20, 80/40, 80/160 mg, or placebo. The results showed major clinical remission rate at a dose of 160/80 mg (36%) compared to placebo (12%, $p < 0.001$). Secondary endpoints were to evaluate the partial clinical improvement, defined by a decrease of 70 or 100 points in the CDAI. The first one was obtained with the three therapeutic regimens and the last only by the 160/80 mg dose, which has defined this regimen as the best option for ADA induction therapy [24].

In order to establish the efficacy of ADA in maintaining clinical response, CLASSIC II was subsequently published evaluating 55 patients from the CLASSIC I who were in clinical that were further randomized to three different treatment regimens: ADA 40 mg every other week, 40 mg weekly, or placebo until completing 56 weeks. In addition, 204 patients from CLASSIC I who were not in clinical remission were enrolled in an open label arm to use ADA 40 mg every other week. The primary endpoint was to evaluate the clinical remission (CDAI <150 points) among randomized patients and it was observed that 79% of patients receiving ADA every other week and 83% of those who received ADA weekly were in clinical remission against 44% in the placebo group ($p < 0.05$) Among the 204 patients assigned to treatment with ADA 40 mg every other week, 46% achieved clinical remission at the end of the 56 weeks. It is noteworthy that this is a study with a low randomized sample [25].

In order to emphasize the sustained efficacy of ADA in CD therapy, in 2007 Colombel et al. published the CHARM trial, a phase III study involving 854 patients who initially were subjected to induction with ADA, of which 499 (58%) had initial clinical response (CDAI decrease in ≥70 basal line) and were randomized to maintenance therapy with ADA 40 mg every other week, 40 mg weekly, or placebo with assess of clinical remission (CDAI <150) after 26 and 56 weeks of therapy. Analyzing the randomized groups, it was noted that clinical remission was significantly greater in the groups using ADA than to placebo at week 56, with 41% in the group receiving the drug weekly, 36% in the group receiving every other week, and 12% in the placebo group ($p < 0.001$). There was no statistically significance in the difference observed between the groups treated with ADA, confirming that the best initial regimen therapy with ADA is 40 mg every other week. It was noted that the superior results observed in CLASSIC II may be due to the fact that patients randomized in this study were in clinical remission, while in CHARM patients with a partial clinical response were included, giving a difference in population of the two studies, preventing direct comparison between them [26]. Analyzing the subgroup of patients who had been previous treated with IFX and discontinued therapy due to loss of response or intolerance it was also observed a higher remission rate compared to placebo, confirming that ADA therapy is a plausible alternative in this group of patients.

In order to properly evaluate the effectiveness of ADA as a rescue therapy in patients with CD who have intolerance or loss of response to IFX, GAIN study was

further published in 2006. Similarly to CLASSIC I, clinical remission was assessed at the end of 4 weeks after the randomization of 325 patients to receive induction therapy (160 and 80 mg at weeks 0 and 2) or placebo. It was observed that 21% of patients with ADA therapy reached the primary endpoint compared to only 7% in the placebo group ($p < 0.001$). This study has demonstrated that ADA is indeed an alternative for patients with refractory CD or is intolerant to IFX [27].

The therapy with ADA in UC was described later, when, in 2010, ULTRA 1 was published evaluating the drug efficacy in induction of clinical remission in patients naive for biological drugs. The study included 390 patients randomized into three groups to receive ADA in induction regimen with 160/80 mg at weeks 0 and 2, followed by 40 mg at weeks 4 and 6; 80/40 mg at weeks 0 and 2, followed by 40 mg every other week and the placebo group. At the end of 8 weeks, approximately 19% of patients in group 160/80 mg showed clinical remission compared with 9.2% of patients in the placebo group ($p = 0.031$), showing modest efficacy of this therapeutic regimen in UC patients who failed therapy with corticosteroids and/or immunosuppressant. The induction regimen with ADA 80/40 mg compared to placebo did not present statistical significance [28].

To analyze the efficiency in the induction of remission and also the maintenance of clinical response ULTRA 2 was sequentially published, studying 494 patients with UC who were initially stratified by prior use or not of anti-TNF alpha and randomized for induction therapy with ADA 160/80 mg at weeks 0 and 2 followed by ADA 40 mg every other week or placebo. The primary endpoint was clinical remission at weeks 8 and 52. Analyzing the group as a whole, there was no statistically significant difference at week 8, however, at week 52, 17.3% of patients with ADA achieved clinical remission superior to placebo group (8.5%, $p = 0.004$). The superiority was also observed at the end of 52 weeks (12.4 vs. 22%, respectively; $p = 0.029$). In the subgroup previously experienced with anti-TNF alpha, a statistically significant superiority was observed at the end of 52 weeks (10.2% in the ADA group vs. 3% in the placebo group, $p = 0.039$) [29].

Even though data in pivotal studies for ADA in UC are not as robust, Tursi et al. published in 2018 the results of a real-life study involving 102 UC patients demonstrating drug efficacy and safety more consistently. The primary outcome was the induction and maintenance of remission, defined by a Mayo score ≤ 2. At 3 months, 54.9% of patients achieved clinical remission and during an average follow-up of 18 months, 56.6% of the patients were in this same situation. Secondarily, clinical response and mucosal healing was achieved by 89.2 and 76.7% of the patients, respectively. Only three patients underwent colectomy (two because of primary therapeutic failure and one for secondary loss) and one patient discontinued treatment due to leukopenia [30].

In relation to real life experience in CD, Loftus et al. recently published the results of PYRAMID registry, evaluating the efficacy and safety of ADA in patients naive to biological therapy followed for 6 years. Taking into consideration the Physician's Global Assessment (PGA) and clinical remission (Harvey Bradshaw index <5), 2057 patients were analyzed with an improvement baseline PGA from 7.5 to 3.9 in the first year and 3.3 in the sixth year. The rate of patients in clinical remission increased from 29 to 68% and 75% after 1 and 6 years, respectively. As related to adverse events, 11.1% of patients had severe infections and the incidence of malignancy was relatively low (1.9%) [31].

ADA has demonstrated superiority to placebo for induction and maintenance of remission in patients with CD and UC. Its subcutaneous administration seems to be a more convenient approach to patients who prefer to self-administer. It is also considered a first-line agent in the management of moderate to severe CD and UC patients refractory to conventional therapy with a satisfactory safety profile.

4. Certolizumab pegol (Cimzia®)

Certolizumab pegol (CZP), a pegylated humanized Fab fragment of IgG1 was also studied in CD. Although the initial induction trial did not demonstrate statistically significant difference in clinical remission after 6 weeks of treatment compared to placebo, PRECISE 2 study was further published assessing maintenance of clinical response in 213 patients that responded to induction phase with 400 mg at weeks 0, 2, and 6 and had values of CRP ≥ 10 mg/L (50% of 428 patients with a reduction in CDAI >100 points after induction phase). These patients were randomized into two groups to receive either 400 mg of CZP or placebo. At the end of 26 weeks of follow-up, 62% of patients treated with the drug maintained clinical response, showing superiority over placebo (34%, $p < 0.001$). Second, it was observed that this superiority was maintained even for patients with CRP < 10 mg/L after the induction phase [32]. Subsequently, analyzing CD patients treated with CZP and followed for 7 years, it was seen that it showed a comparable safety profile to the others anti-TNF drugs [33].

Since chronic inflammatory diseases usually have a higher incidence and prevalence in females, there is much discussion about what would be the best therapeutic strategy to be adopted during pregnancy, once treatment suspension may be associated with "flares" of the underlying disease with deleterious effects for both the mother and fetus, in addition to the fact that anti-TNF alpha present variables degrees of placental transfer that can influence the immune response of the newborn. Due to its molecular conformation devoid of the Fc region, which prevents recognition by the FcRn receptor and consequently the active placental transfer, certolizumab pegol was evaluated as a safe treatment option during pregnancy [34].

In 2017, a prospective pharmacokinetic study (CRIB study) was published evaluating 16 patients with at least 30 weeks pregnancy who were treated with CZP (three of them with CD) to assess the degree of placental transfer to the fetus via the dosage of the serum level of the drug in the newborn plasma. Patients were required to receive the last dose of CZP within a maximum of 35 days before delivery to be included. It was observed that even with maternal plasma levels within the therapeutic range of CZP, 13 of the 16 neonates had no detectable levels of CZP in plasma and one shows minimum levels (0.09% concentration in maternal plasma), which hardly had any clinical consequences [34]. In accordance with previous studies, it was shown that CZP presents minimal to no placental transfer even when used in the third trimester of pregnancy, unlike IFX, or ADA [35]. In the same year, CRADLE study analyzed breast milk from 17 mothers who were treated with the CZP (five of them with CD), showing that the drug concentration in breast milk is minimal, with a relative dose transferred to the newborn well below the 10% limit considered safe. Besides that, adverse events in patients exposed to CZP were consistent with the known safety profile and newborns had an adverse event profile that could be expected in an untreated population of similar age [36].

The CZP presents itself as another subcutaneously administered anti-TNF option for CD with a suitable safety profile, especially in women in the reproductive phase.

5. Golimumab (Simponi®)

Golimumab (GOLI), a fully humanized antibody anti-TNF alpha administered subcutaneously, has been described as effective in induction of clinical response and remission in ulcerative colitis in 2014, with the publication of PURSUIT-SC. This

study combined the analysis of a phase 2 study (used to evaluate the appropriate dose of induction therapy) and phase 3, demonstrating the superiority of the drug over placebo. After determining the doses of 200/100 and 400/200 mg at the weeks 2 and 0 as the most appropriate induction regimen, 761 patients were randomized 1:1:1 to receive said regimens or placebo. At the end of 6 weeks, it was observed that the groups randomized to receive the golimumab 200/100 and 400/200 mg had better clinical response (51 and 54.9%, respectively) than placebo (30.3%; $p < 0.0001$ for both comparisons), with no statistically significant differences between the dosing schedules. Second, GOLI also demonstrated superiority to placebo regarding clinical remission and mucosal healing [36].

Having 464 patients who responded to induction therapy with GOLI in previous studies (PURSUIT-SC and PURSUIT-IV), PURSUIT-M evaluated the efficacy of the drug in maintaining clinical response. Patients were randomized to receive 50, 100 mg, or placebo every 4 weeks and evaluated after 52 weeks of treatment at week 54. As a result, 47% of patients receiving 50 mg and 49.7% of those who received 100 mg had sustained clinical response, while 31.2% of those receiving placebo had the same result ($p = 0.01$ and $p < 0.001$, respectively). Second, it was observed that about 28% of the patients who had received 100 mg of golimumab were in clinical remission and 42.4% in endoscopic remission, reinforcing its superiority over placebo, in which 15.6% were in clinical remission ($p = 0.004$) and 26.4% achieved mucosal healing ($p = 0.002$) [37].

Thus, GOLI is presented as another subcutaneous anti-TNF therapy option for ulcerative colitis. Due to its recent approval, more data on its long-term safety and real life experience are needed (**Table 1**).

Main studies	Objective	Primary end point	Results	Conclusion
Infliximab				
Cohort de Targan et al.	Assess the efficacy of IFX in inducing clinical response in patients with moderate to severe CD	Reduction of CDAI ≥ 70 points after 4 weeks of single induction dose	Placebo: 17% had clinical response IFX 5 mg/kg: 81% had clinical response IFX 10 mg/kg: 50% had clinical response IFX 20 mg/kg: 64% had clinical response	A single induction dose is superior to placebo to induce clinical response in patients with moderate to severe CD
ACCENT I	Assess the benefit of maintenance therapy with infliximab in patients with active CD who responded to a single initial infusion of infliximab	Clinical remission at week 30 (CDAI < 150) and time to loss of clinical response by week 54	Placebo: 21% in remission at week 30; mean time to loss of response of 19 weeks IFX 5 mg/kg at weeks 2 and 6, followed by 5 mg/kg every 8 weeks: 39% in remission at week 30; mean time to loss of response of 38 weeks IFX 5 mg/kg at weeks 2 and 6, followed by 10 mg/kg every 8 weeks: 45% in remission at week 30; mean time to loss of response >54 weeks	Patients who initially responded to IFX are most commonly in remission at week 30 and 54, when a dose of IFX is maintained every 8 weeks

Main studies	Objective	Primary end point	Results	Conclusion
ACCENT II	Assess the efficacy of maintenance treatment with IFX in the closure of fistulas in patients with CD having one or more fistulas who have responded to the induction therapy with IFX	Time to loss of response during 54 weeks of follow-up among patients who had a response at week 14 and were randomized	Placebo: mean time of 14 weeks to loss of response IFX: mean time to loss of response of over 40 weeks	Patients with penetrating CD responding to induction therapy are more likely to have a sustained clinical response to maintenance therapy over a 54-week period
ACT I	Assess the efficacy of IFX in induction and maintenance therapy in patients with moderate to severe UC	Clinical response at week 8 and secondarily, clinical remission and mucosal healing at weeks 8, 30, and 54 (among other secondary end points)	Clinical response at week 8: • Placebo: 37.2% • IFX 5 mg/kg: 69.4% • IFX 10 mg/kg: 61.5% Clinical remission at weeks 8, 30, and 54: • Placebo: 14.9, 15.7, and 16.5% • IFX 5 mg/kg: 38.8, 33.9, and 34.7% • IFX 10 mg/kg: 32, 36.9, and 34.4% Mucosal healing at weeks 8, 30, and 54: • Placebo: 33.9, 24.8, and 18.2% • IFX 5 mg/kg: 62, 50.4, 45.5% • IFX 10 mg/kg: 59%, 49.2, 46.7%	Patients with moderate to severe UC treated with IFX at weeks 0, 2, and 6, followed by maintenance every 8 weeks, more commonly have a clinical response at weeks 8, 30, and 54 than those who received placebo
ACT II	Assess the efficacy of IFX in induction and maintenance therapy in patients with moderate to severe UC	Clinical response at week 8 and secondarily, clinical remission and mucosal healing at weeks 8 and 30 (within other secondary end points)	Clinical response at week 8: • Placebo: 29.3% • IFX 5 mg/kg: 64.5% • IFX 10 mg/kg: 69.2% Clinical remission at weeks 8 and 30: • Placebo: 5.7 and 10.6% • IFX 5 mg/kg: 33.9 and 25.6% • IFX 10 mg/kg: 27.5 and 35.8% Mucosal healing at weeks 8 and 30: • Placebo 30.9 and 30.1% • IFX 5 mg/kg: 60.3 and 46.3% • IFX 10 mg/kg: 61.7 and 56.7%	Patients with moderate to severe UC treated with IFX at weeks 0, 2, and 6, followed by maintenance every 8 weeks, more commonly have a clinical response at weeks 8 and 30 than those receiving placebo

Main studies	Objective	Primary end point	Results	Conclusion
SONIC	Comparatively assess the efficacy of IFX monotherapy, AZA monotherapy or combined therapy in patients with moderate to severe CD naïve for biological therapy	Clinical remission free of corticoid and, secondarily, mucosal healing at week 26	Clinical remission at week 26: • AZA: 30% • IFX: 44.4% • IFX + AZA: 56.8% Mucosal healing at week 26: • AZA: 16.5% • IFX: 30.1% • IFX + AZA: 43.9% Note: the observed difference in mucosal healing between the IFX and IFX + AZA groups was not statistically significant	Patients with moderate to severe CD treated with IFX or IFX + AZA are more likely to achieve clinical remission free of corticosteroids than those treated with AZA alone
SUCCESS	Comparatively evaluate the efficacy of IFX monotherapy, AZA monotherapy, or combined therapy in patients with moderate to severe UC naïve for biological therapy	Clinical remission free of corticoid and secondarily mucosal healing at week 16	Clinical remission at week 16: • AZA: 23.7% • IFX: 22.1% • IFX + AZA: 39.7% Mucosal healing at week 16: • AZA: 36.8% • IFX: 54.6% • IFX + AZA: 62.8% Note: the difference in mucosal healing observed in IFX and IFX + AZA groups was not statistically significant	Patients naive for biological drugs with UC treated with combined therapy are more likely to achieve clinical remission than those treated with monotherapy drugs. Combined therapy is associated with better mucosal healing rates when compared to AZA monotherapy
Adalimumab				
CLASSIC I	Assess ADA's efficacy in inducing clinical remission in patients with moderate to severe CD naive for biological therapy	Clinical remission at week 4 after initial induction therapy	Placebo: 12% of the patients achieved remission ADA 40/20 mg: 18% of the patients achieved remission 18% (p = 0.36) ADA 80/40 mg: 24% of the patients achieved remission (p = 0.06) ADA 160/80 mg: 36% of the patients achieved remission (p = 0.001)	The ADA was superior to placebo in clinical remission induction in patients naive for biological therapy with moderate to severe CD, with a dose of 160 mg at week 0 followed by 80 mg at week 2 as the recommended regimen
CLASSIC II	Assess the efficacy and safety of ADA in maintenance therapy in	Maintenance of clinical remission at week 56 in the group of patients randomized after	Placebo: 44% of the patients had clinical remission ADA 40 mg every other week: 79% of	ADA was more effective than placebo in maintain remission after 56 follow-up

Main studies	Objective	Primary end point	Results	Conclusion
	patients with moderate to severe CD	responding to induction therapy	the patients maintained clinical remission ADA 40 mg weekly: 83% of the patients maintained clinical remission	
ULTRA I	Assess the effectiveness of ADA in clinical remission induction in patients with moderate to severe UC naive for biological therapy	Clinical remission at week 8 after initial induction therapy	Placebo: 9.2% of the patients achieved remission ADA 80/40 mg: 10% of the patients achieved remission ($p = 0.833$) ADA 160/80 mg: 18.5% of the patients reached remission ($p = 0.031$)	The 160/80 mg dose of ADA was effective and safe in inducing clinical remission in patients with moderate to severe UC who failed to corticoid or immunosuppressive therapy
ULTRA II	Assess the efficacy and safety of ADA in maintenance therapy of patients with moderate to severe UC	Maintenance of clinical remission at week 8 and week 52 after induction therapy	Placebo: 9.3% at week 8 and 8.5% at week 52 ADA: 16.5% at week 8 and 17.3% at week 52	ADA was effective and safe in maintaining clinical remission in patients with moderate to severe UC who failed to corticoid or immunosuppressive therapy
CHARM	Assess the efficacy and safety of ADA in maintenance therapy in patients with moderate to severe CD who responded to induction therapy	Percentage of patients who responded to induction and achieved clinical remission at weeks 26 and 56	Placebo: 17% at week 26 and 12% at week 56 ADA 40 mg every other week: 40% at week 26 and 36% at week 56 ADA 40 mg weekly: 47% at week 26 and 41% at week 56	ADA maintenance therapy in patients with moderate to severe CD who responded to induction therapy was more effective than placebo in maintaining clinical remission after 56 weeks of follow-up
GAIN	Assess the efficacy of ADA in inducing clinical remission in patients with moderate to severe CD who lost response or were intolerant to IFX	Clinical remission at week 4 after ADA induction therapy	Placebo: 7% achieved clinical remission at week 4 ADA: 21% achieved clinical remission in week 4	ADA was more effective than placebo in inducing clinical remission in patients who lost or were intolerant to IFX
Certolizumab pegol				
PRECISE 2	Assess the efficacy and safety of CTZ in inducing and maintaining	Clinical response rates in patients with baseline CRP ≥10 mg/L at week 26	Placebo: 36% of patients maintained clinical response CTZ: 62% of patients	Among patients who responded to the initial induction dose, maintenance of CTZ was more

Main studies	Objective	Primary end point	Results	Conclusion
	response and clinical remission in patients with moderate to severe CD who have responded to induction therapy		maintained clinical response	effective in maintaining clinical response than placebo
Golimumab				
PURSUIT	Assess the efficacy of golimumab in maintaining clinical response in patients with moderate to severe UC who responded to induction therapy	Maintenance of clinical response at week 54	Placebo: 31.2% of the patients maintained clinical response Golimumab 50 mg: 47% of the patients maintained clinical response Golimumabe 100 mg: 49.7% of the patients maintained clinical response	Golimumab maintenance therapy was more effective than placebo in maintaining clinical response after 54 weeks of follow-up

Table 1.
Main studies with Anti-TNF in inflammatory bowel disease.

6. Comparative efficacy among anti-TNF agents

As stated above, the treatment of IBD with the advent of anti-TNF alpha and more recently, other classes of biological drugs (anti-integrin, anti-IL 12/23 etc.) has dramatically changed the natural history of the disease and the incidence of complications. However, no head to head studies directly compared the efficacy of different drugs. Lacking such data, the decision on which treatment regimen to be used is mainly based on reported clinical experience, proposed algorithms by clinical trials, patient preference and safety profile [38].

Although imperfect, indirect comparative analyses, such as network meta analyses are available evidence to assess efficacy of different drugs. In 2018, Singh et al., through a systematic review and network meta-analysis, compared the efficacy and safety of treatment with various biological drugs in CD in naive patients for biological therapy (first-line therapy) and in patients previously tested with some anti-TNF (second-line therapy). Comparing direct and indirect evidence from 18 randomized controlled trials (RCT's) involving patients with moderate to severe CD, it was observed that anti-TNF alpha, particularly IFX and ADA, were the options with strongest evidence in the induction of clinical remission and response as well as maintenance therapy. Ustekinumab and vedolizumab appear to have similar efficacy in the first-line therapy and were not higher when compared to IFX or ADA. The CZP at the standardized dose has been reported as inferior to the other agents.

As second-line therapy (non-RCT using IFX or CZP as a second biological drug was identified), in the specific subgroup of patients who lost response or were intolerant to IFX, ADA seems to be superior compared to other agents. It is noteworthy that, for patients with primary nonresponse to IFX, the effectiveness of the ADA is uncertain, scenario in which ustekinumab seems to gain prominence. The safety profile and the incidence of major adverse events were assessed in

maintenance studies, not being seen clear superiority of one agent over the other, although the risk of adverse events appears to be low to IFX and ustekinumab. However, RCT's involved in the analysis were not powered to determine this difference, so this result should be evaluated with caution. Vedolizumab, a gut-selective anti-integrin, has not been clearly associated with an increased risk of serious infections in RCT's analysis and longitudinal cohorts. It is noteworthy that the risk factors most associated with severe infections were concomitant use of corticosteroids, narcotics and severe disease activity [38].

The same group published a meta-analysis evaluating the therapy in UC, where, besides the efficacy of induction/maintenance of clinical remission and safety profile of the drug, mucosal healing was also assessed. Combining direct and indirect evidence of 14 RCT's including 4212 patients with moderate to severe disease, the group concluded that, as first-line therapy, all evaluated agents (IFX, ADA, golimumab, and vedolizumab tofacitinibe) were superior to placebo, with IFX and vedolizumab considered the most effective in the inducing of clinical remission and mucosal healing. In general, ADA was considered the least effective agent for both outcomes. Comparing IFX to ADA, data obtained favor IFX for induction of remission, however, as maintenance therapy, it appears to be no significant difference between the two drugs [39]. Superiority of IFX can be associated with pharmacokinetics and bioavailability of the drug since its dosage is variable according to the weight of the patient, unlike ADA with a fixed dose.

As second line therapy, tofacitinib (JAK-2 inhibitor) seems to be the best choice for induction of remission and mucosal healing. A direct meta-analysis further demonstrated that vedolizumab and ADA were not superior to placebo, conferring a low level of evidence to indicate these drugs as a therapeutic alternative in this scenario. Importantly however, the studies that assessed ADA included only patients who lost response or were intolerant to IFX as part of the patients treated with vedolizumab were not primary responders to IFX, which may be linked to a specific population with a more aggressive form of the disease, disadvantaging vedolizumab in this analysis. This information was not clear in studies with tofacitinib and no study using IFX or golimumab as a second biological drug was identified [39].

As a maintenance therapy, because of differences in the design of studies, RCT's involving IFX and ADA were considered separately from those involving golimumab, vedolizumab, and tofacitinib. As stated earlier, IFX and ADA appear to be equally effective in maintaining remission in naive treatment patients. The other drugs were also superior to placebo in patients who responded to induction therapy and did not seem to differ from each other. Regarding the safety profile, none of the options was significantly worse compared to placebo in the incidence of adverse events. Taking into account the incidence of serious infections, vedolizumab seems to be the safer drug, since there was no difference compared to placebo, while golimumab and tofacitinib were associated to higher risk of infection [39].

In an innovative way, the preliminary results of VARSITY, the first head to head trial in IBD were presented in a specific event. It is a phase 3b double-dummy, controlled and randomized trial, comparing ADA and vedolizumab in the treatment of moderate to severe UC. With a total of 769 patients who had failed conventional therapy (25% had been exposed to any anti-TNF), which were randomized into four groups to receive vedolizumab vs. placebo or adalimumab vs. placebo, clinical remission (primary endpoint), and mucosal healing were assessed after 52 weeks. It has been observed that patients treated with vedolizumab achieved clinical remission rates of 31.3% and mucosal healing of 39.7%, significantly better than patients treated with ADA (22.5% $p = 0.0061$ and 27.7% $p = 0.0005$, respectively), with no statistically significant difference in the incidence of infections and adverse events [40].

7. Safety of anti-TNF agents

The use of TNF-alpha inhibitors and their combination with thiopurines has proved to be more effective in controlling severe forms of CD and UC compared to monotherapy [21, 22]. However the use of these drugs is associated to a higher risk of adverse events, particularly infections and malignancies [41, 42].

The analysis of a cohort study involving a large number of patients [43], showed a higher risk of serious and opportunistic infections in combination therapy than with the use of anti-TNF or thiopurines alone. Comparing anti-TNF and thiopurines in monotherapy, there was a higher incidence of serious infections and mycobacterial infections associated with anti-TNF, however, there is no difference in the incidence of opportunistic infections in general, since thiopurines were associated with higher chance of viral opportunistic infections and anti-TNF to bacterial infections. It is noteworthy that the results of a previous meta-analysis showed an increased incidence of opportunistic infections by bacteria and mycobacteria in patients treated with the combination therapy compared to monotherapy with anti-TNF, inferring that the use of thiopurines adds an extra risk for developing infections [44]. There was a higher incidence of viral opportunistic infections when combination therapy was compared to monotherapy with anti-TNF, but it did not differ when compared to monotherapy with the thiopurines, suggesting that the risk of this complication in the combination therapy is due to the use of thiopurines [45].

It should be considered that not only therapeutic option is linked with a higher risk of infectious complications, but also the patient's age, disease severity, and concomitant use of corticosteroids, all those associated with a worse outcome [46].

Classically, therapy with thiopurines is associated with an increased risk of malignancy in patients with IBD, particularly non-Hodgkin's lymphoma, hepatosplenic lymphoma associated with EBV, cervical cancer associated with HPV, urinary tract cancer, and non-melanoma skin cancer, both as monotherapy and in combination therapy with an anti-TNF agent [42]. However, the association between malignancy and anti-TNF alpha use remains uncertain. In prior meta-analysis involving 21 placebo-controlled trials including more than 5000 patients with CD, treatment with anti-TNF was not associated with an increased risk of cancer development [47].

Through the analysis of the TREAT™ Registry database, a prospective cohort study that evaluated the outcomes of long-term treatment regimens in DC involving 6237 patients in with more than half used the IFX sometime in the follow-up, it was found that, in general, the incidence of cancers (benign or malignant) was similar between the group treated with IFX and with the other therapeutic options [48]. In this study, age, disease duration and smoking were associated with increased risk of cancer. In a more recent meta-analysis including 44 RCT's and more than 14,000 patients, and the incidence of malignancy as a secondary outcome, it was not possible to conclude that the use of anti-TNF significantly affect the risk of cancer. However, the data were scarce and periods of exposure and follow-up were too short to allow conclusions [41]. The incidence of melanoma is described as higher in patients with IBD in general, however, some studies suggest a possible association with the use of anti-TNF [49] while others do not [50].

A recent French cohort gathered data from nearly 190,000 patients to assess risk of lymphoma in patients with IBD that used azathioprine and/or anti-TNF agents. Surprisingly, not only the use of thiopurines but also the use of anti-TNF monotherapy was associated with a small but statistically significant increased risk of lymphoma among patients exposed. The risk was greater in the combination therapy than either drug alone [51].

Other adverse events associated with anti-TNF therapy are described and should also be remembered. Since there are reported the reactivation of tuberculosis and hepatitis B virus after initiation of therapy, the pretreatment screening, in order to guide the treatment of latent tuberculosis and prophylaxis with antiretroviral, is indicated [52, 53]. In those patients who are in triple immunosuppression, *Pneumocystis jirovecii* prophylaxis may be considered [54]. Infusion reactions (relatively frequent), angioedema, anaphylaxis, lupus-like syndrome, psoriasis induced by anti-TNF, eczematous lesions, demyelinating syndromes, and heart failure are also described [54].

8. The role of therapeutic drug monitoring in the management of IBD with anti-TNF agents

Although effective in the induction and maintenance of clinical remission and mucosal healing, the therapeutic fail of anti-TNF is not uncommon in IBD, occurring in patients which are considered as primary non-responders (10–40% approximately) or lose the response in the first year of treatment (24–46%), and those who have some adverse effect that lead to treatment interruption [24, 26]. An understanding of the factors involved in therapeutic failure, as the patient's profile, the presentation of the disease and the relationship between the concentration of the drug and its interaction with the anti-drug antibodies (ADAbs), are useful tools to guide the best strategy to be followed [55].

The concentration of drug in the site of action is directly linked to the magnitude of the expected pharmacological response and its monitoring in specific scenarios can assist in therapeutic decision. For example, the patient may experience an inadequate response due to the low concentration of the drug secondary to increased clearance, differing completely from one that has inadequate response with therapeutic trough levels, suggesting mechanistic failure. In the first situation, dose escalation can be effective, while the second would most benefit from exchange to a medication with a distinct mechanism of action (**Figure 2**) [56].

Based on these assumptions, in 2017, the American Gastroenterological Association has published a technical review on the role of therapeutic drug monitoring (TDM) as an auxiliary tool in decision-making regarding treatment of IBD. In the absence of adequate response, the dosage of the trough level of the drug has been suggested as a first step (reactive approach): if the serum levels of anti-TNF are within the therapeutic range, it is characterized the failure to the mechanism of action and class exchange is possibly the best option. However, if serum levels are below the appropriate, dosage of ADAbs can bring additional information: if they are high, it is likely that the clearance of the drug is being immune-mediated, and it is plausible the exchange of medication for a drug of the same class, besides the association with immunomodulator. If the ADAbs level is undetectable or low, it is likely that the clearance is increased due to mechanisms not immune-mediated, such as severe inflammatory burden leading to rapid use of anti-TNF and/or excessive loss in feces (indicated by hypoalbuminemia, CRP, and high fecal calprotectin), which would allow the optimization of the dosage instead of changing the biological agent. This strategy seems to be more effective than making decisions empirically, despite the low level of evidence further described [56]. In patients with the disease in remission, the dosage of the trough level and ADAbs as an auxiliary tool in decision-making (proactive proposal) is still uncertain, with few studies that corroborate its effectiveness, mainly regarding cost savings [57, 58].

In order to examine predictors of therapeutic failure to anti-TNF, PANTS study has been recently published, a randomized clinical trial involving patients with luminal CD naive for biological therapy who started treatment with IFX or ADA.

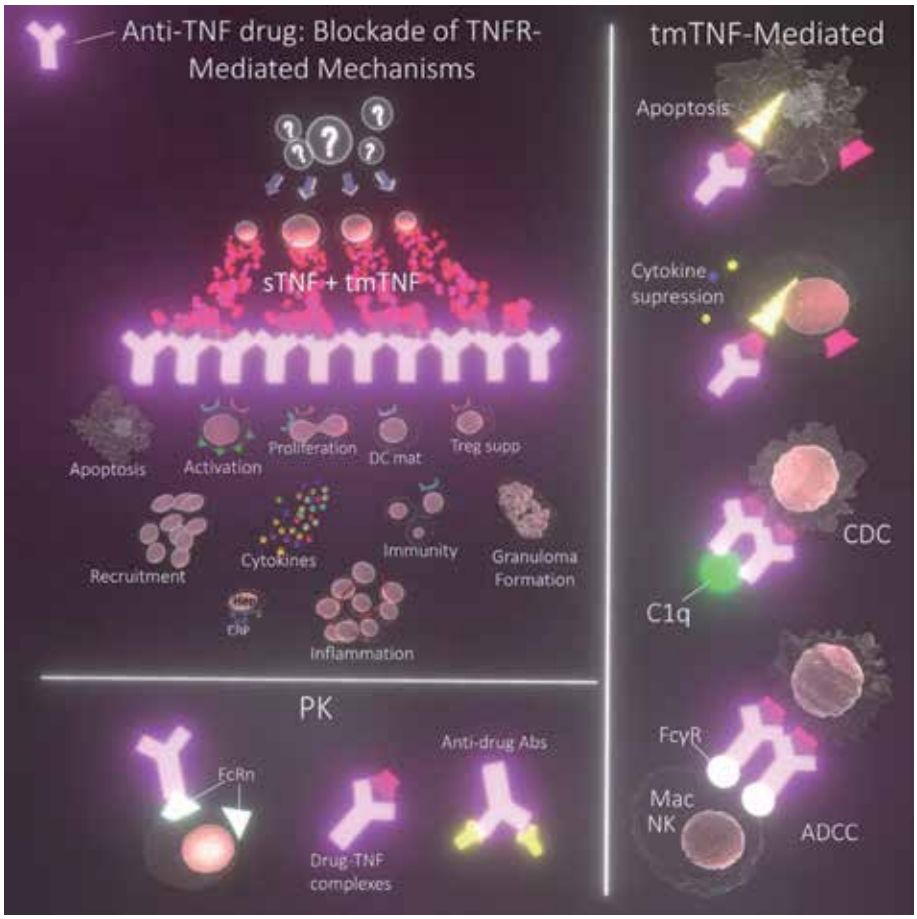

Figure 2.
anti TNF's mechanisms of action are illustrated above. The inflammatory cascade triggered by TNFR is disrupted by anti TNF-mediated direct blockade, which prevents binding of sTNF and tmTNF to specific receptors. On the right are the results of tmTNF antagonization by the drug, which include cytotoxicity of the CDC (complement – dependent cytotoxicity) or ADCC (antibody-dependent cellular cytotoxicity), as well as reverse signaling via tmTNF. The pharmacokinetics-related are illustrated at the bottom of the image.

Through regression logistic, it was identified that only low trough level in week 14 (IFX < 7 mg/L and ADA < 12 mg/L) was associated to the absence of primary response. Obesity, smoking, hypoalbuminemia, high levels of inflammatory markers, and the development of immunogenicity were associated with lower serum levels of the drug. It was also observed that low levels at week 14 were independently associated to non-clinical remission at week 54, and were associated with increased formation of ADAbs. The combination with immunomodulators (azathioprine or methotrexate) was associated with lower immunogenicity for both IFX and ADA, and in the group of patients with IFX, combination therapy was associated with higher clinical remission rate at week 54 compared to monotherapy with IFX, unlike ADA, which was not more effective in maintaining remission when associated with immunomodulators [55].

9. Final considerations

The initiation of therapy with tumor necrosis factor inhibitors certainly was a milestone in the treatment of inflammatory bowel disease, drastically changing the natural course of the disease and offering better quality of life to treated patients.

With an acceptable safety profile, anti-TNF agents are excellent therapeutic options in severe forms of the disease, with proven efficacy in both Crohn's disease and ulcerative colitis. The association with immunomodulators, particularly to infliximab is associated with better outcomes. A lack of head to head trials that compares the biological drugs limits the assessment of superiority among them to indirect comparisons, making it crucial that such evidence come to light. Therapeutic drug monitoring seems to be useful tools in decision-making and can increase the therapeutic success rates obtained. However, in the face of current evidence, it has not yet been consolidated as a cost effective strategy.

Future perspectives involving anti-TNF agents include the development of new molecules of this class. Currently, several new TNF-alpha inhibitors have been studied in patients with CD. The DLX 105 (esbat Tech) is an anti-TNF antibody that has been studied specifically in patients with fistulizing CD, trough a local injection in a phase II trial (ClinicalTrials.gov NCT01624376), but no results are available to date. Other two anti-TNF-alpha oral therapies, V565 (VHsquared) and OPRX-106 (Bio Protalix) are in the pipeline. The V565 is currently recruiting patients with moderate to severely active CD to a phase II study (NCT02976129) after favorable results in a phase Ib (NCT03010787). The OPRX-106 demonstrated efficacy in clinical improvement of biomarkers in a phase II study of patients with mild to moderate UC. It is worth to wait for these promising therapies, since the oral mode of administration may be more convenient for some patients [59, 60].

In the era of the new mechanisms of action, this critical analysis consolidates the anti-TNF agents as viable therapeutic options in the current IBD therapeutic armamentarium.

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Chapter 5

Biological Therapy in the Prevention of Complications of Crohn

Dolores Ortiz-Masià

Abstract

In recent years, the advent of biological agents has revolutionized the treatment of inflammatory bowel disease (IBD). TNF is a cytokine with a very important role in the pathogenesis of Crohn's disease (CD), so it is a therapeutic target to highlight. The efficacy and safety of the anti-TNF, IFX and ADA is widely established, becoming two of the therapeutic pillars of CD. Today the experience with other more recent antibodies and stem cells therapy is more limited. Various limitations such as lack of studies, heterogeneity in inclusion criteria, and achievement of objectives make it difficult to establish which treatment is more appropriate in each case and even the superiority between drugs and/or cellular therapy. This chapter will compare the different currently available therapies with special interest in new therapies and their relationship in the prevention of complications of Crohn's disease.

Keywords: Crohn's disease, fibrosis, inflammation, biological therapy, inflammatory bowel disease, stem cells

1. Introduction

Acute inflammatory processes affecting the intestine are relatively frequent and self-limiting, such that the intestinal mucosa is able to regenerate and regain homeostasis in a matter of days. However, chronic inflammation can cause irreversible structural changes and severe complications in the gastrointestinal tract [1].

The term inflammatory bowel disease (IBD) is used to refer generically to chronic inflammatory diseases, with recurrent course and unknown origin that affect the gastrointestinal tract and are distinguished mainly on the basis of histological findings. Basically in this group we find Crohn's disease (CD) and ulcerative colitis (UC), processes that have their peak incidence in young people and that constitute the most relevant pathologies within this classification. These pathologies are identified and diagnosed thanks to clinical, endoscopic, and histological characteristics, although on certain occasions it is not possible to distinguish which form of IBD is present, being classified as indeterminate colitis. Microscopic colitis is a term designated for a group of colitis in which we find microscopic but not macroscopic alterations when endoscopy or surgery is performed. Unlike the previous ones, this process mainly affects elderly people and includes collagenous colitis and lymphocytic colitis. Despite having similar epidemiological, clinical, and

even therapeutic characteristics, there are a series of peculiarities that help to define the existing process [2]. CD is characterized by the existence of transmural inflammation, cryptic abscesses, and the formation of granulomas, being able to affect any part of the intestine and may reappear after surgical resection of the affected segment [3]. UC, on the other hand, is typically associated with inflammation and ulceration limited to the mucosa and submucosa, only of the colon and rectum. In this way, and unlike CD, UC has a definitive treatment of the pathology in proctocolectomy. The main differential characteristics between CD and UC are shown in **Table 1**. The incidence of both diseases is increasing in the last decades and in the case of CD at younger ages [4].

Current CD therapies are solely targeting inflammation by administration of immunosuppressive therapies, corticosteroids, or biologicals. While these therapies in some—but not all—cases lead to symptomatic disease remission, recurrent flares interspaced with periods of remission will still result in cumulative gut wall remodeling. The evolution towards organ failure and surgical resection occurs in 70% of cases, with a subsequent need of second surgery in up to 30% of cases [5]. The postsurgical recurrence can occur very early, even a few weeks after surgical

Characteristics	**Crohn's disease**	**Ulcerative colitis**
Macroscopic		
Intestinal region	Ileum ± colon	Colon
Distribution	Sautéed lesions	Diffuse lesions
Stenosis	Yes	No
Wall appearance	Thick	Thin
Microscopic		
Inflammation	Transmural	Limited to *mucosa*
Pseudopolyps	Moderate	Important
Ulcers	Depths	Superficial
Lymphoid reaction	Important	Moderate
Fibrosis	Important	Mild or absent
Serositis	Important	Mild or absent
Granulomas	Yes	No
Fistulas	Yes	No
Clinics		
Rectal involvement	Frequent	Almost always
Small bowel involvement	Frequent	Rare
Perianal fistula	Yes	No
Malabsorption of fats and vitamins	Yes	No
Malignant potential	Affecting the colon	Yes
Relapses after surgery	Frequent	No
Toxic megacolon	No	Yes
pANCA	+	++
ASCA	++	+

The table shows the main macroscopic, microscopic, and clinical characteristics between CD and UC.

Table 1.
Differential characteristics between CD and UC.

resection, because the drugs currently available for the prevention of postsurgical recurrence have limited efficacy; up to 50% of cases return to CD activity despite preventive treatment, which may lead to further surgery with consequent loss of bowel function which may eventually lead to the development of short bowel syndrome as an irreversible complication in some patients. Therefore, management of CD patients undergoing bowel resection should be oriented towards prevention, early detection, and, in the worst case, treatment of postsurgical recurrence [6].

Given the great evolution experienced in IBD therapy, there is a need to compare the effectiveness of different treatments in the achievement of objectives as well as a clear definition of the objectives. The symptoms, although an indicator of quality of life, have a very poor correlation with the severity of inflammation. On the other hand, endoscopic activity, serological markers, and fecal calprotectin have greater correlation with the future need for surgery and occurrence of complications.

Among the objectives of the therapeutics of IBD, we highlight the induction of remission, the reduction of hospitalizations and surgeries, and the effectiveness of cellular therapy in fistulizing and luminal disease.

The general objective of this chapter is to address this gap in literature by reviewing bibliography comparing the different biological therapies available and their influence on the prevention of complications.

2. Crohn's disease

CD is a chronic, recurrent inflammatory disease that belongs to the spectrum of IBD. It predominantly affects the gastrointestinal tract, being able to find lesions in any part of it, from the mouth to the anus. In it we also find important extraintestinal manifestations and association with other autoimmune diseases [7]. CD is an entity whose incidence increases as the development of society advances, being very prevalent in developed countries and rare in less developed countries.

The maximum incidence is observed between the second and fourth decade of life, and a second peak is observed between the seventh and ninth, although it is increasingly diagnosed at earlier ages [4].

2.1 Etiopathogenesis

Although several factors have been described that may be related to the development of CD, the exact causes of this process remain unknown (see **Table 2**).

Genetics	Seventy-one susceptibility locus for CD have been identified on 17 chromosomes
Environmental factors	Non-breastfeeding, improved hygiene conditions, sedentary lifestyles, western diet and fast food, tobacco, contraceptives, environmental pollution
Microbiota	Reduction of commensal microbiota: *Bacteroidetes* and *Firmicutes* Increase in potentially pathogenic flora: *Mycobacterium avium paratuberculosis*, *Campylobacter*, *Salmonella*, and *E. coli*
Alteration of the immune system	Deregulation in the immune system that initiates, mediates, and perpetuates inflammation. Rapid recruitment and inappropriate accumulation of leukocytes in the affected intestinal wall

The table shows the main causes of CD.

Table 2.
Etiopathogenesis of CD.

Several studies [8, 9] have found different genetic alterations that increase susceptibility to this disease along with certain environmental triggers, resulting in an altered immune response, both innate and adaptive, and epithelial bowel dysfunction. An alteration in the commensal microbiota has also been described, with a decrease in the potentially beneficial flora and an increase in that which is potentially pathogenic [8]. Genetic alterations, the immune system, microbiota, environmental factors, and their combined effects occupy a large number of pages in the scientific literature, and their description surpasses the objectives of this study.

2.2 Symptoms, diagnosis, and classification

CD is a heterogeneous entity comprising different phenotypes, so the symptoms are and change with the course of the disease. It usually has an insidious onset, the most common symptom being chronic diarrhea (80% of patients), followed by abdominal pain (70%), primarily in the right iliac fossa.

Other symptoms are weight loss (50%), malnutrition, fatigue, malaise, and the presence of rectorrhagia (more common in UC). Perianal disease (4–10% debut with it), nausea, vomiting, asthenia, anorexia, fever, and night sweats may also occur.

Diagnosis is currently established by combining clinical presentation and laboratory findings (such as anemia, elevation of globular sedimentation velocity and serum C-reactive protein, elevation of calprotectin and/or lactoferrin in stool, endoscopic appearance, histology, and radiological and/or biochemical findings). Serological and genetic tests are not recommended as routine diagnostic methods. However, despite advances in diagnostic methods, in the first year, up to 5% of cases with the diagnosis of CD has to be changed to UC or indeterminate colitis [10].

Once the diagnosis of CD is established, it is necessary to categorize patients based on the Montreal classification [11] and investigate the possible existence of extraintestinal manifestations and other autoimmune diseases (see **Table 3**). This stratification of patients makes it easier for them to receive the best follow-up and treatment in an individualized manner as well as to identify early possible complications [12]. However, it is important to bear in mind that the patient's stratification is not stable. It has been seen that 19% of patients progress to more aggressive forms of the disease 90 days after being staged and up to 51% of patients did so 20 years after the initial diagnosis [12]. These patients progressed developing complications that were not present at the time of diagnosis.

Age at diagnosis	A1:<16 years
	A2:17–40 years
	A3:>40 years
Location	L1:terminal ileum
	L2:colonic
	L3:ileocolon
	L4:upper gastrointestinal tract
Behavior	B1:without stricture formation, non-penetrating
	B2:stenosant
	B3:penetrating
	P:perianal disease

Table 3.
CD Montreal classification [11].

Complications depend on the clinical course and control of the disease. Some may appear in any phenotype, such as massive hemorrhage, toxic megacolon, and neoplasia of the colon (the IBD favors the presence of multiple tumors with a higher degree of malignancy), while other complications are encompassed in different phenotypes of the disease. Thus, in the obstructive fibro-stenotic pattern, we find stenosis, intestinal obstruction, and perianal disease; and in the penetrating, fistulas and abscesses.

Most complications require a surgical approach; in fact, 70–80% of patients with CD will need some surgery throughout their lives. Even so, there are recurrences in 88% of the cases, being very frequent the surgical reintervention.

2.3 Treatment

There are currently multiple drugs available for the treatment of IBD; however, there are no predictive response factors that allow us to select the most appropriate drug for a patient at any given time. In general, the choice of treatment is made on an individual basis according to the activity, location, and phenotype of the affectation.

The objectives include symptomatic control, remission of the outbreak and maintenance of long-term remission, as well as endoscopic healing, as there is no curative treatment. The drugs used are:

- *Aminosalicylates*: indicated as maintenance treatment in mild to moderate CD. They include those molecules with aminosalicylic acid or 5-ASA in their molecular structure (also known as mesalazine in Europe and mesalamine in the USA). They have been shown to reduce the incidence of relapses (28 versus 55% with placebo) and have a higher percentage of remissions versus placebo (43 versus 18%, respectively). However, the efficacy in postoperative patients is greater. In general, they are well tolerated, and adverse effects such as gastrointestinal disorders, headache, arthralgias, and cutaneous eruptions may appear. The nephrotoxicity and hematological toxicity are the more serious, but infrequent, effects [13, 14].

- *Glucocorticoids*: indicated for the induction of remission in an outbreak. They intervene on the vascular [decreasing permeability] and cellular [inhibiting tissue migration and phagocytosis of macrophages] phases. Prednisone is usually used at a dose of 40–60 mg/day orally or intravenously in severe outbreaks (with remission rates of 66–73%). Budesonide has shown similar efficacy to prednisone for mild to moderate ileocolonic CD (55% remissions). In addition, its topical action confers fewer adverse effects. However, they have not shown efficacy as a therapy for maintenance. In addition, this would be inadvisable given the risk of dependence and adverse effects: fluid retention, stretch marks, redistribution of body fat, subcapsular cataracts, myopathy, osteonecrosis, emotional disturbances, withdrawal symptoms, etc., many of which are related to the duration of treatment [15, 16].

- *Antibiotics*: have no role in the treatment of CD, except metronidazole in perianal disease.

- *Thiopurines*: azathioprine (AZA) and 6-mercaptopurine (6MP). They are used in the management of corticosteroid-dependent CD, in the prevention of postsurgical recurrence, and in combination with biologics. The efficacy is appreciated from 3 to 4 weeks both as maintenance therapy and in perianal

disease. They present a great interindividual variability, due to the genetic polymorphisms of TPMT (thiopurine methyltransferase), an enzyme that activates them. In general they are well tolerated, being hepatotoxicity, myelotoxicity and pancreatitis acute, the adverse effects to highlight, able to justify abandonment of the treatment. Other effects are nausea, fever, skin rash, hepatitis, and the development of lymphomas.

- *Methotrexate*: inhibitor of dihydrofolate reductase (folic acid antagonist). It is effective in maintenance of remission (65 versus 39% with placebo), with an evaluation necessary of hepatic enzymes. Hypersensitivity pneumonitis is a very rare but very serious adverse effect. Supplementation with folic acid reduces adverse effects. It is a teratogenic drug, so it is contraindicated during pregnancy and lactation [17].

- *Calcineurinics*: cyclosporine (CyA) and tacrolimus. Its usefulness is limited, although tacrolimus seems useful in perianal EC. CyA has a series of cases that support it in luminal and perianal EC, but the evidence is not robust.

- *Hematopoietic stem cell transplantation (HSCT)*: might be useful in some treatment-resistant cases. Mesenchymal stem cells (MSCs) have regenerative and immunomodulatory properties which lead to reduction of inflammation and healing of affected intestinal tissue in CD. Meta-analysis studies show that 23–40.5% of patients achieved remission after systemic infusion of MSCs [18, 19].

- *Monoclonal antibodies*: include the anti-TNF and anti-integrin α4β7, which we will discuss with more depth below.

3. Biological therapy in CD

Biologic therapy was introduced as a treatment for CD 20 years ago, revolutionizing the handling of it. So far, infliximab (IFX), adalimumab (ADA), vedolizumab (VDZ), and ustekinumab have been approved in Europe for this purpose. In general they have a good safety profile, although the experience is limited in new drugs.

They have been shown to be effective in decreasing intestinal damage from inflammation, surgeries, and admissions, improving the quality of life of patients. Its benefits, specially their early administracion as well as their favorable safety profile, have meant that they are being used more and more frequently.

It should be noted that before starting treatment with biological therapy, it is necessary to rule out an active infection (mainly tuberculosis or hepatitis B). In addition, the appearance of hypersensitivity reactions, cutaneous reactions, cytopenias, heart failure, and autoimmune hepatitis forces to rule them out and assess a possible interruption of treatment. Its paradoxical inflammatory reactions have been described with psoriasis and dermatitis, which can affect even 10% of patients. Treatment with biologics contraindicates attenuated vaccines.

Its potential adverse effects make it necessary to stratify the patients, so that only those with severe or complicated illness receive early intensive therapy. Although there is no established definition of serious or complicated disease, greater complications are seen in patients who start the disease young (<40 years), perianal disease and/or ileocolic localization, with need to administer corticosteroids in the treatment of the first outbreak, in these cases. When two or more factors are present, it is indicated to start the treatment of the first outbreak with immunomodulators or biologicals. Various studies support that, although the monoclonal

antibodies are more expensive than other treatments, the decrease in the number of hospitalizations and surgeries contributes to increase the cost/benefit ration of the therapy, especially as a therapy of the maintenance.

3.1 Anti-TNF

Anti-TNFs are so far the most effective agents in the treatment of moderate-to-severe luminal disease (induction of remission and maintenance) and Crohn's fistulizer, and they are the first-line treatment in complex perianal disease. In Europe, IFX and ADA are approved in EC and CU and golimumab in CU. The results obtained have raised treatment expectations, with healing of the mucosa being the main objective, associated with a lower rate of hospitalizations and surgery and with a higher percentage of long-term remission. Difficulty in selecting patients that are going to benefit from these treatments lies in safety problems (risk of infections, infections, etc.) and its high cost.

Anti-TNFs have demonstrated a good safety profile, the main drawback being the risk of infections, such as tuberculosis, pneumocystis, and nocardiosis. More than half of infections occur in the first 6 months of treatment and in guidelines combined with immunosuppressants. All of these risks justify the recommendation to update the vaccination schedule before starting treatment, as well as screening for latent infections [20].

The increased risk of cancer is controversial in the literature. A meta-analysis that included 12 cohort studies concluded that although the risk of melanoma is increased by 37% in patients with IBD, treatment with anti-TNF did not influence it [21].

Less frequently, they have also been associated with optic neuritis, seizures, and demyelinating disorders, including multiple sclerosis and exacerbation of heart failure symptoms grade III/IV. Adverse effects make it necessary to discontinue treatment in 20.6% of patients with IFX and 14.4% with ADA [22–25].

Another aspect to mention is the lack of effect (30%) and the loss of therapeutic efficacy, which occurs in 23–26% of patients in the first 12 months of treatment. The causes are varied: in some patients there is a pharmacodynamic failure, when the main pathway of inflammation is not dependent on TNF. Others do not get a good pharmacokinetics, when the concentrations in plasma are insufficient, due to increased clearance or appearance of anti-drug antibodies.

There is evidence that good plasma levels of anti-TNF are associated with greater clinical efficacy, so monitoring of antibody levels has become a tool to optimize the treatment. They appear more frequently in patients treated sporadically than those treated every 8 weeks. In these situations, it is possible to add immunosuppressants (AZA, 6-MP, or methotrexate).

3.2 Anti-integrin α4β7

Until 2015, anti-TNFs were the only biologicals approved for the treatment of IBD in Europe. This year anti-integrin α4β7 antibodies were incorporated: vedolizumab (VDZ) and ustekinumab. In general, they present an acceptable safety profile, as no case of leukoencephalopathy has been recorded to be progressive multifocal, its most fearsome adverse effect. As for the rest of the adverse effects, specific monitoring is not required.

Vedolizumab is a recombinant humanized IgG1 AcM that specifically blocks the integrin α4β7 by joining MadCAM-1. It has recently been approved for EC and moderate-to-severe CU that have failed conventional treatment but also as a first-line drug. It is administered via IV, for which it has demonstrated efficacy in inducing remission and maintaining disease, the maintenance in postoperatives

being its main indication. It has been postulated that its answer is slower because it does not block the pre-existing inflammation; it simply avoids recruiting more inflammatory cells. In addition, transmural involvement of CD may explain its action to be slower than CU.

The induction dose is 300 mg IV in weeks 0, 2, and 6, followed by 300 mg every 8 weeks as maintenance. A long-term loss of response has been noted, although usually in patients who have already failed other biologics. VDZ is a well-tolerated drug with a good security profile in IBD. The risk of infections increases but no cases of progressive multifocal leukoencephalopathy (PML), and the frequency of transfusion reactions is lower than that of the 5%. The development of anti-VZD antibodies occurred in less than 4% of patients, being a cause of therapeutic failure [26].

Natalizumab is a humanized IgG4 against the subunit α4, so it blocks both the integrin α4β7 and integrin α4β1; it therefore, has a non-specific action. It has shown promising results as maintenance therapy but has not been approved by its association with cases of progressive multifocal leukoencephalopathy. It is approved for CD in the USA under very severe conditions (concomitance with multiple sclerosis).

3.3 IL-12 and/or IL-23 inhibitors

IL-12 and IL-23 have been shown to be key cytokines in the adaptive immunity, which is found in IBD and intervenes in its chronification. Both ILs have in common the subunit p40, whose blocking inhibits the intracellular signaling cascade. The Crohn's immune response is influenced by resident lymphocytes and those recruited into the lymphoid organs. Antibodies from this group, such as the ustekinumab, prevent the binding of soluble IL-12 and IL-23 to their specific receptors, although they do not intervene on cytokines that are already attached to their membrane receptor. The blockage of IL-12 prevents the activation of Th1 lymphocytes, and IL-23 prevents the production of IFNγ, TNFα, IL-1β, and IL-6.

3.4 Sphingosine receptor modulators

Sphingosine-1 is a phospholipid that binds to specific receptors (S1P1–5) expressed in lymphocytes, dendritic cells, cardiomyocytes, and endothelial cells, regulating multiple cellular activities such as growth and survival, vascular integrity, and lymphocytic migration.

Sphingosine modulators behave like agonists producing functional antagonism, sequestering lymphocytes in peripheral lymphoid organs, and reinforcing the endothelial barrier (which makes intestinal migration difficult) [27].

3.5 JAK kinase inhibitors

Protein kinases are enzymes capable of modifying other proteins or enzymes, altering their function depending on the target. Certain polymorphisms of the same ones have been related with greater susceptibility to IBD. The signaling of this group of drugs is very complex, but it is a promising research in the field of IBD therapy (currently in phase 3 for both UC and CD).

In its mechanism of action, B lymphocytes and T effectors decrease without affecting the T regulators.

3.6 Biological therapy in the induction of remission

We speak of partial or total remission to refer to the reduction or disappearance of symptoms and signs of disease.

The effectiveness of biological therapy in the induction of remission is indisputable. However, the percentages vary considerably between different molecules. At week 4, remission rates reach 75% with IFX [28] and 59% with ADA [29].

The study PRECISE 1 investigated the effects of CTZ at week 6 and shows remission rates of 37% with CTZ and 31.4% for VDZ [30]. Clearly higher percentages are noticeable with IFX and ADA.

Considering luminal disease, remission rates have been described as 63.8 and 54.1% for IFX and ADA, respectively, and remission in cortico-dependent patients as 76.3 and 44.7% for IFX and ADA at 12 months. Combination with immunosuppressants led to higher remission rates in patients with IFX (81 versus 52%), but not in ADA [31].

In general, IFX is given to patients with a more severe phenotype of the disease, as it is believed to have faster action and more clinical experience. However, the results were similar in patients who received IFX and ADA, without finding significant differences in Crohn naïve patients except in the safety profile (adverse effects were more frequent with IFX than with ADA, 36.1 versus 15.5%, respectively), including transfusion reactions, skin rashes, arthralgias, and hypersensitivity [31]. This information is contradicted by other studies, such as the meta-analysis of Singh and collaborators, whose results support the superiority of IFX over the rest of the biologics for induction of clinical remission in naïve anti-TNF patients [32].

3.7 Impact of biological therapy in the prevention of complications of Crohn's disease

The effectiveness of biological therapy in the prevention of hospitalizations and surgeries has not yet been clearly demonstrated. We know from previous studies that in the prebiological era, approximately 50% of patients required surgery 10 years after diagnosis, with a risk of recurrence of 50% at 10 years, and 80% of patients required surgery at some point in their lives. Recent studies indicate that surgery rates since the introduction of biologics (2001–2008) are lower than those of 1988. In addition, a very relevant characteristic of biologics is their high cost, and it is here that the reduction of the overall cost through the prevention of complications becomes especially important [33, 34].

The anti-TNF therapy reduces significantly the hospitalizations and surgeries in patients with CD. No differences were observed between IFX and ADA, with a reduction of 46% (36–60%) of the hospitalizations and 13–42% of surgery with IFX. The onset of treatment may also be relevant in modifying the natural history of the disease. In this line, it has noted that early use of biological therapy (less than 2 years after diagnosis) improves the course of the disease. However, no significant reduction in the number of surgeries has been found in hospitalizations with patients treated with VDZ or AZA in similar follow-up periods [33].

4. Hematopoietic stem cell transplantation

Human stem cell therapy for the treatment of CD is still in its infancy, and whether SCT is associated with improved outcomes is unclear.

Preliminary studies have shown that allogeneic HSCT may restore, at a genetic level, the immune system [35, 36], and autologous HSCT could remove atypical clones by immunoablation and replacement with not committed stem cells (SCs), allowing for the de novo generation of an altered T-cell repertoire [37]. Some studies describe that autologous and allogeneic HSCT produce a long-term treatment-free disease regression in some patients with CD [19]. Nevertheless, the Autologous

Stem Cell Transplantation International Crohn's Disease Trial [38] did not validate a statistically significant improvement in continued disease remission at 1 year of autologous HSCT compared with orthodox therapy, suggesting that further studies are needed in order to know the feasibility of using HSCT in patients with refractory CD [19].

4.1 Luminal disease

The number of patients requiring surgical resection for the stenosing and uncontrolled inflammatory complications of CD has not declined significantly, despite advances in biological therapy. Moreover, following a surgical resection, many patients will require a second operation. Currently, the use of systemically infused mesenchymal stem cell to reduce the altered inflammatory response and to repair impaired tissue has a promising future for avoiding surgery and its potentially serious complications. Conversely, since biological therapies are not always useful in some patients, the development of all-purpose anti-inflammatory therapies for patients with inflammatory luminal disease is still needed.

In luminal disease, the mechanism of the intravenous transplantation of MSCs is not understood yet. Animal studies and graft-versus-host disease treated by bone marrow MSC studies suggest, on the one hand, that the MSCs are able to transmigrate from the circulation into the inflamed tissues as a response to cytokine stimulus; on the other hand, MSC can release anti-inflammatory cytokines, which can modify the phenotype of macrophages towards repairing phenotype and can mediate the activation and proliferation of regulatory T and B cells.

One study that demonstrated the safety and viability of MSC in luminal disease was evaluated in nine patients with refractory CD, where the patients received two infusions of autologous bone marrow-derived MSC (days 0 and 7). At 6 weeks, endoscopic improvement was reported in two patients and clinical improvement in three, while three patients required surgery due to worsening disease [39]. In the same line, similar results were also seen in 15 CD patients with moderate-to-severe active disease who were refractory to anti-TNFα therapy [40]. In that study, at 6 weeks, a clinical response was observed in 80% of patients, clinical remission in 53% of patients, and endoscopic improvement in 47% of patients [40].

Evidence that MSC therapy contributes to neoplastic development is currently lacking. However, this view is based on a systematic review in which not all patients were assessed by repeated endoscopy during the 10-year follow-up, so the presence of dysplastic lesions cannot be excluded [19]. New and better studies are needed to test the safety of MSC therapy in luminal disease.

4.2 Fistulizing disease

Fistulae commonly complicate CD. There has been more research on the efficacy of MSC therapy in perianal fistulizing CD than on luminal CD. In all cases, the reduction of fistula frequency and the improved rate of complete fistula closure are the most important therapeutic goals. Administration of the therapeutic agent is performed locally under general anesthesia during perianal surgery. In the intervention, the surgeon initially scans the fistula tracts to remove setons and residual inflamed tissues. Once the internal opening is sealed with absorbing suture, the submucosa surrounding the internal orifices of fistulas and parallel to the lumen of tracts receives an injection of MSCs. The difference between results depends on different parameters like used dosage, origin and type of MSCs, therapeutic schedules, definition of end points, and therapeutic efficacy.

The safety and therapeutic potential of MSCs in treating perianal CD was first demonstrated in 2005 when autologous adipose-derived MSC was injected into nine perianal fistulae from four patients. After 8 weeks, complete healing was observed in six fistulae [41]. Fistula tract healing has been observed in 71% of patients treated with MSC and fibrin glue as compared to 16% of patients treated with fibrin glue alone. In patients receiving MSCs, closure was observed in 46% of patients after a single treatment and in a further 25% after a second rescue treatment [42, 43].

The currently available largest randomized, double-blind placebo-controlled study summarizes the clinical data of fistulizing CD patients which show that a greater proportion of patients in the treated group than the placebo group achieved the combined remission at week 24 in the intent-to-treat population (53 of 103 (51%) vs. 36 of 101 (34%)) [44].

5. Conclusions

- The evidence places IFX over the rest of the biologics in the induction of remission in patients with naïve CD. It has shown higher remission percentages in numerous quality studies and in direct meta-analysis comparisons. While this information is contradicted by other articles, IFX seems to be more effective and faster acting, so it is the preferred biological therapy in patients with severe disease. In addition, it is the only one that has proven to be more effective in combination with immunosuppressants.

- The biological treatments are the only ones that have shown effectiveness in the reduction of hospitalizations and surgeries. A number of studies have highlighted the superiority of IFX over other biologicals, as well as the equivalence between ADA and CTZ.

- Clinical trials demonstrated that MSC transplantation has an outstanding, durable efficacy with low fistula recurrence in biological therapy-refractory fistulizing CD; however, further clinical trials are required to confirm its effectiveness in luminal CD.

Conflict of interest

The author declares no conflict of interest.

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Chapter 6

Biological Therapy for Inflammatory Bowel Diseases: Screening Prior to Initiating and How to Proceed When Surgery Is Necessary

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Abstract

Biological therapy has revolutionized the management of inflammatory bowel disease (IBD) in the last 30 years. However, these drugs have side effects and adverse events. Before starting this therapy, it is necessary to screen for specific infectious diseases and monitoring protocols. Screening for human immunodeficiency virus, hepatitis C, hepatitis B, and *Mycobacterium tuberculosis* infections must be included. In addition, vaccination should be checked and updated if necessary. Despite the advent of biological therapy, a significant number of patients with IBD will need surgery in their lifetime due to either clinical intractability or disease complications. Many of them will be on biological therapy, and there is a considerable controversy about adverse effects of biologics on surgical outcomes. In this chapter, we will approach the screening required to start this therapy and how to proceed when surgery is necessary in these patients.

Keywords: inflammatory bowel disease, surgery, biologic agents, complications, infectious diseases, vaccines

1. Introduction

Biological therapy brought a better control of inflammatory bowel diseases (IBD). However, its use requires specific care before the beginning and during the treatment. Some essential points in its management have raised discussions.

We address the needs before starting the biological therapy and how to proceed when surgery is required. A brief review of what is necessary before the use of these drugs is also provided.

The management of other immunosuppressive agents such as corticosteroids, azathioprine, 6-mercaptopurine, and methotrexate is not covered in this chapter.

2. Pre-exposure biological therapy evaluations

The general recommendations include screening patients for risk factors of infection [1]:

- Comorbidities (e.g., transplant history, malignancy, renal or liver failure, diabetes mellitus)
- Age
- Occupation
- History of travel to areas of endemic diseases
- High-risk sexual activity, drug abuse
- Exposure to tuberculosis
- Blood transfusion

Patients receiving treatment with therapeutic monoclonal antibodies (specifically the tumor-necrosis-factor alpha inhibitors) are considered immunodeficient [2]. Therefore, before the onset of biological therapy, we should screen for some diseases, and the patients should be properly immunized [3–5].

Screening for human immunodeficiency virus infection (HIV), hepatitis C (HVC), hepatitis B (HVB), and *Mycobacterium tuberculosis* infection for all patients must be performed prior to starting biological therapy [1, 4–6].

Because of the risk and the severity of infections, which are increased in HIV-infected patients receiving biological therapy, they should be closely monitored. Biological therapy is not contraindicated in HIV-infected patients [4].

Screening of HVC in some European countries is not recommended because of its low prevalence and the fact that patients with HVC can be treated with biological therapy [5]. However, immunomodulators may influence active chronic HVC infection and may worsen liver function when concomitant infection with hepatitis B (concomitant HVB and HVC infection is common in some regions of the world) [4].

Every patient with hepatitis B negative tested (HBsAg, anti-HAbs, and anti-HBcAb negatives) should be vaccinated before starting biological therapy. One to two months after the last dose of vaccine, patients should have their serological response evaluated. If infection is present by testing before vaccination, other specific tests should be performed, and the patient should be evaluated by a specialist for the need of treatment. The importance of care in relation to HVB infection consists in the fact that reactivation of HBV is a well-described complication of immunosuppression [4, 5, 7].

One infectious agents which should get more attention before the beginning of the biological therapy is *Mycobacterium tuberculosis*, because the reactivation of latent tuberculosis is increased and more severe in patients who follow this therapy. Proper latent tuberculosis search is performed by an assessment of an exposure history, skin test (PPD-tuberculosis skin test), interferon gamma release assay (IGRA), and chest X-ray, according to local prevalence and national

recommendations. Complete therapeutic regimen for latent tuberculosis must be initiated if identified after the screening examination and the biological therapy should be delayed [4, 5]. This full latent tuberculosis investigation can be modified or even indicated only if some exposure is suspected depending on where the patient lives. In this case, regional guidelines for prophylaxis must be followed. Only 22 countries worldwide represent 80% of the world's incidence. Therefore, local variations in the tuberculosis screening are accepted [4–6, 8].

If results of IGRA test or PPD are negative, they should be repeated, but there is no consensus as to how long [5].

Despite the variations in relation to screening for latent tuberculosis, when an alteration in the PPD test (≥5 mm) is found, the prophylaxis with isoniazid or appropriated antituberculous therapy must be initiated and maintained for 6 months. After at least 4 weeks with the use of the medication, we can initiate biological therapy [6].

3. Vaccination

It is worth noting that the patient's entire history of vaccination must be checked. If they did not receive the vaccines, they must be updated. Attention should be given, also to the recommended waiting period between each vaccine and the initiation of therapy.

In addition to the hepatitis B vaccine, the patient should also receive the following vaccines before starting the treatment with biological therapy [4–7, 9]:

- Varicella zoster virus (VZV)—if lacking clear history of chickenpox, shingles or if past vaccination history is uncertain
- Tetanus and diphtheria toxoid—every 10 years
- Human papilloma virus—according to national guidelines
- Zoster vaccine—for immunocompetent individuals over 60 years old
- Influenza virus—annual vaccination for all patients
- Hepatitis A—in endemic areas
- Pneumococcal (PPSV 23 and PCV13 vaccines)—every 3–5 years
- Meningococcal—for certain at-risk individuals (college students living in residential housing, military recruits, and immunosuppressed patients like asplenia, HIV, and complement deficiency)

It is important to emphasize that immunosuppressed patients do not respond properly to immunization. In addition, patients receiving biological therapy cannot be vaccinated with live attenuated virus (varicella zoster, yellow fever, measles, mumps, and rubella) [9].

A brief algorithm for preparing the patient for biological therapy is outlined in **Figure 1**.

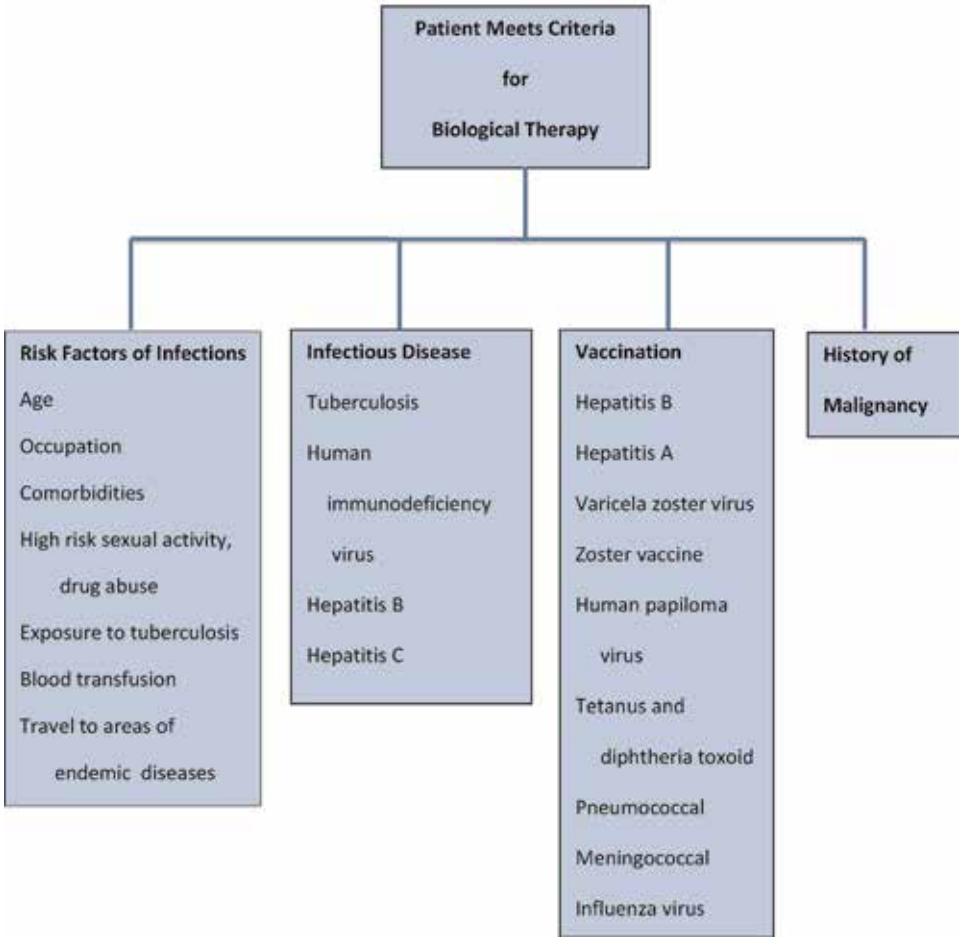

Figure 1.
Algorithm for preparing the patient for biological therapy.

4. Biological therapy and surgery

When the screening and prophylaxis prior to initiating biological therapy involve numerous details regarding each disease that should be treated or prevented, the issue regarding biological therapy use and performing surgery can be come even more complex. This complexity is due to the difficult analysis of patients since the groups submitted to surgery are extremely heterogeneous.

Despite the increasing number of available biological agents available, many patients will require operation due to intractability or complications of IBD. In a systematic review and meta-analysis of population-based studies, Frolkis et al. [10] showed that the risk of intestinal surgery among patients with IBD has decrease over the past six decades. They concluded that the risk of surgery in Crohn's disease after 1, 5, and 10 years of diagnosis was 16.3, 33.3, and 46.6%, respectively, and in ulcerative colitis was 4.9, 11.6, and 15.6%, respectively.

In this way, many IBD patients will be on biological therapy when surgery is indicated. Literature data are conflicted with regard to the preoperative management of biological therapy in IBD surgery. Several large single-center studies and systematic reviews have found an increased risk of infectious complications with the use of anti-TNF preoperatively [11–14], whereas others have not [15–20].

These inconsistent results may be due to single institution experience, different duration of biological therapy, different periods between the last biologic dose and surgery, and concomitance with use of immunosuppressive agents and besides, may be a reflection of the severity of the disease and not the biological itself [16, 18, 21, 22].

Most studies use a 3-month cutoff to include patients in the anti-TNF group, but the serum level of the drug should also be taken into account [22]. Lau et al. [23] observed in their study that 53% of the IBD patients using preoperative anti-TNF had no detectable drug level at the time of surgery and it was more frequent in the ulcerative colitis group.

Regarding ulcerative colitis, the results are also conflicting with some studies showing increase in postoperative complications [24], while others show no association in preoperative anti-TNF therapy and increased risk of infectious and noninfectious complications after surgery [25].

Lightner et al. [26], in a retrospective multicenter cohort study, observed that IBD patients (Crohn's disease and ulcerative colitis) treated with vedolizumab had increased risk of postoperative surgical site infection and mucocutaneous separation of the diverting stoma as compared with anti-TNF-treated patients. They studied 146 patients who received vedolizumab 12 weeks before abdominal surgery and 289 patients who received anti-TNF therapy. However, two systematic reviews and meta-analysis [27, 28] did not find increased risks of postoperative complications with the use of vedolizumab when compared to either preoperative anti-TNF therapy or no biological therapy. Studies regarding the use of ustekinumab comparing with anti-TNF therapy also demonstrated no increase in the risk of postoperative complications [29, 30].

The occurrence of infectious and noninfectious complications after surgery in patients with IBD depends on several factors besides biological therapy. Among them one can mention the concomitance of the use of other medications, especially corticosteroids; the very severity of the disease; anemia, marked malnutrition in these patients, and smoking which greatly influence the occurrence of these complications [31]. Literature dates are conflicting, and in most studies, the patients and disease are heterogeneous. In addition, the time of exposure to the biological, the

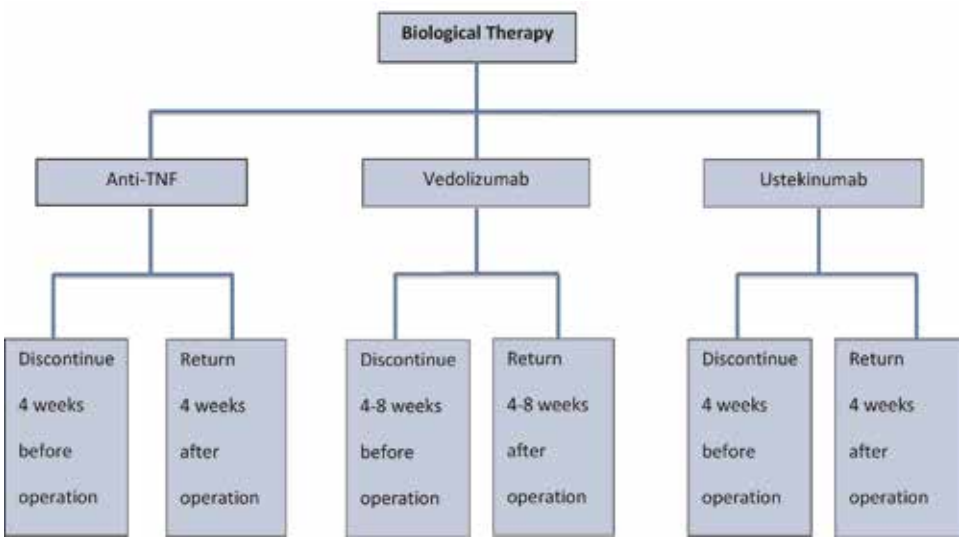

Figure 2.
Biological therapy and surgery. Source: Adapted from Lightner AL. Perioperative management of biologic and immunosuppressive medications in patients with Crohn's disease. Dis Colon Rectum 2018;61:428-31.

interval between the last dose and the surgery, the serum level of the medication, and drug pharmacokinetic should be considered.

For patients who are receiving biological therapy and will undergo abdominal surgery, we should consider [32] the following:

- Discontinue the medication 4 weeks before operation for anti-TNF-α and ustekinumab and 4–8 weeks before for vedolizumab.
- The medication should be reintroduced after 4 weeks, if necessary.
- For urgent situation, there is no need to delay the operation. The increased risks of infectious complications do not outweigh the risk of delaying surgery.
- Consider derivative ileostomy in emergency surgery and severely malnourished patients (serum albumin <3 g/L, body weight loss >10%) and/or concomitant use of corticosteroids (**Figure 2**).

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Section 4

Anti-Integrin, Anti-p40 Subunit and JAK Inhibitor Therapies

Chapter 7

Anti-Integrins, Anti-Interleukin 12/23p40, and JAK Inhibitors for the Inflammatory Bowel Disease Treatment

Karine Mariane Steigleder, Fernando Lopes Ponte Neto, Cristiane Kibune Nagasako and Raquel Franco Leal

Abstract

Inflammatory bowel diseases (IBD) present a broad inflammatory cascade that is sometimes difficult to control. Patients with ulcerative colitis (UC) and Crohn's disease (CD) are exposed to intense and harmful effects that compromise their quality of life. There is a constant need for new classes of drugs that act on different fronts of inflammation control. Initially, biologics revolutionized inflammatory bowel disease treatment. Anti-tumor necrosis factor (anti-TNF) agents and infliximab, followed by adalimumab and certolizumab pegol, have been proven to induce clinical and endoscopic remission. However, some patients are primary nonresponders, and a significant proportion of initial responders lose response throughout the treatment. The emergence of new therapies, such as anti-integrins, anti-interleukins, and inhibitors of Janus kinase (JAK), can become an alternative option for patients with previous therapeutic failures, besides offering greater safety than other biological therapies up to now. Among anti-integrins, vedolizumab is the drug with proven efficacy in both induction and maintenance of remission and has local and selective action in the intestine. Ustekinumab represents the group of anti-interleukins, acting to control interleukin-12 (IL12) and interleukin-23 (IL23). JAK inhibitors (tofacitinib) act on intracellular inflammatory mediators and have the advantage of being orally administered.

Keywords: ulcerative colitis, Crohn's disease, vedolizumab, ustekinumab, tofacitinib

1. Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are the main inflammatory bowel diseases (IBD) [1]. Inappropriate inflammatory response is multifactorial and involves environmental, genetic, immune-mediated, and gut microbial factors [2].

IBDs were previously more prevalent in North America, Europe, and Oceania, but since 1990 the incidence rate is stable or decreasing in those areas. In contrast, increasing incidence was observed in developing regions, such as Latin and South America, Asia, and Africa, making it a rising global disease [3].

For years, the therapeutic management of IBD has been restricted to local action medications with mild anti-inflammatory power, such as amino salicylates and corticosteroids. Adverse effects of prolonged use of corticosteroids include infections, diabetes, osteoporosis, cataracts, metabolic syndrome, and esthetic changes that further raise morbidity and mortality [4].

Immunomodulators initially used in rheumatologic conditions have also been applied in IBD treatment. Thiopurines (azathioprine and 6-mercaptopurines) and methotrexate were widely used against both UC and CD, but these medications alone failed to induce and maintain clinical and endoscopic remission in a significant percentage of cases. Failure to control the disease increases the risk of complications, like strictures, abscesses, fistulas, and the need for surgical approaches. Additionally, worse quality of life and an increase in clinical complications like anemia and malnutrition [5] may occur.

The therapeutic revolution of IBDs began with biological therapy containing anti-tumor necrosis factor (anti-TNF) agents such as infliximab, which was widely used in the management of rheumatologic, dermatological, and inflammatory bowel diseases. Subsequently, other drugs of the same class emerged, such as adalimumab, a fully human monoclonal antibody, and certolizumab, which does not have the Fc portion, making it less immunogenic [5].

Anti-TNF treatment (alone or in combination with immunomodulators) can induce clinical and endoscopic remission. However, only 10–30% will have a primary no-response, and over 50% will, after an initial response, have a secondary loss [6].

New classes of immunobiological therapies are available to treat patients with loss of response to anti-TNF treatment, since the response to a second anti-TNF is low [4, 5]. Integrins and interleukins are the main targets of the available drugs to treat IBD. They act on receptors of cells involved in the inflammatory process and on proinflammatory cytokines, respectively. Furthermore, the intracellular inhibition of kinases by JAK inhibitors acts on intracellular inflammatory mediators. Each of these action pathways will be detailed in this chapter.

2. Anti-integrins and anti-interleukin 12/23p40

2.1 Anti-integrins

Integrins are cell surface glycoprotein receptors that play a role in leukocyte adhesion, signaling, proliferation, and migration [7]. Migration of circulating leucocytes from blood to intestinal tissue is a key step for intestinal inflammation. α4β7 integrin expressed on the surface of the leukocyte binds to mucosal addressin cell adhesion molecule-1 (MAdCAM-1) expressed on endothelial cells. Anti-integrin blocks the action of integrins, inhibiting leukocyte trafficking from the systemic circulation to the gastrointestinal endothelial cells [8].

Natalizumab is a chimeric recombinant human IgG4 antibody that blocks the α4 subunits in α4β7 and α4β1 integrins on leukocytes, inhibits binding to vascular cell adhesion molecule 1 (VCAM-1), and decreases inflammatory cells in affected gastrointestinal tissue, contributing to induction and maintenance of remission in CD [9]. Natalizumab also blocks lymphocyte infiltration in the central nervous system and is also approved for multiple sclerosis treatment [10]. However, association with progressive multifocal leukoencephalopathy (PML), a rare disabling and potentially fatal neurological syndrome caused by reactivation of the John Cunningham virus (JCV), has limited its use in treating CD patients [11].

Etrolizumab is a humanized IgG1 monoclonal antibody against the β7 subunit of the α4β7 and αEβ7 integrins that blocks binding to MAdCAM-1 and E-cadherin, respectively [12]. Still under study, it is not part of the IBD therapeutic arsenal, and studies failed to demonstrate an advantage when compared to placebo [13].

Vedolizumab is an anti-integrin currently used to treat IBD. It is a humanized monoclonal antibody composed of two light chains of kappa subclass and two heavy chains linked by two disulfide bridges, which form an immunoglobulin that targets α4β7 integrin, selectively blocking gut lymphocyte trafficking [14]. Its inhibitory effect on T-lymphocyte recruitment is reversible with its suspension. Renal elimination occurs after drug degradation into peptides and amino acids in the liver. The drug half-life was estimated at 25.5 days, being the potential predictors of poor response to therapy: albumin <3.2 g/dl and weight > 120 kg [15].

The currently recommended dosage is 300 mg vedolizumab with intravenous administration at weeks 0, 2, and 6 and then every 8 weeks. The interval can be shortened to every 4 weeks when the patient's response is not satisfactory [15].

Studies showed no difference in serious adverse events resulting in death, life-threatening conditions, hospitalization, or disability, comparing vedolizumab and placebo [14]. However, possible adverse events can occur such as nasopharyngitis, headache, arthralgia, and other less uncommon events. Among the contraindications, we can highlight the presence of active infections, such as tuberculosis, sepsis, cytomegalovirus, listerioses, and opportunistic infections such as PML [16, 17].

There are no controlled studies of vedolizumab during pregnancy and breastfeeding, and current data are based on observational cases. FDA classified this drug as category B, being safe for use in pregnancy. During breastfeeding, caution is required because it is not known if the medication is transferred to the newborn [4].

In 2013, the randomized, double-blind, placebo-controlled phase 3, GEMINI I study showed the efficacy of vedolizumab in induction and maintenance of remission in patients with UC. For induction, a 300 mg-day intravenous dose repeated at 15 days was used. For maintenance, both groups received the medication after 4 or 8 weeks; therapeutic serum levels were obtained with 95% saturation of the α4β7 receptor and proven clinical remission for 52 weeks. The intestinal selectivity of vedolizumab gives the drug greater safety, especially in countries with a marked presence of mycobacteria. No cases of PML were documented during the GEMINI I study [14].

Also, in 2013, the GEMINI II placebo-controlled, randomized, double-blind, phase 3 study evaluated induction and maintenance remission in patients with moderately to severely active CD for 4 years in 39 countries. The study analyzed patients aged 18–80 years, diagnosed for at least 3 years, with active CD. Compared to placebo, patients treated with vedolizumab had better response in both induction and remission maintenance at week 52. The rates were discrete and may be justified by patient selection bias, since a significant part of the group had severe disease, difficult to control and refractory to anti-TNF treatment [18].

Of the 1434 patients who used vedolizumab for 52 weeks evaluated in the GEMINI I and GEMINI II studies, 56 (4%) had anti-drug antibodies, of which only 9 (0.6%) had persistent positivity after two or more consecutive dosages. Immunogenicity increases with exposure time reaching 10% at week 66. However, it is believed that the presence of antibodies in low to moderate-titer does not affect drug response, as therapeutic failure occurred in only nine patients and elevated antibody levels were maintained for a prolonged period [15].

In 2014, Sands et al. (GEMINI III) evaluated the response of vedolizumab in patients with previous anti-TNF treatment. The study showed an advantage of

anti-integrin when compared to placebo only after 10 weeks, concluding that in patients who fail anti-TNF treatment, longer time is required to achieve clinical remission with vedolizumab [16].

The VARSITY study presented in 2019 was the first study that compared two biological drugs (vedolizumab and adalimumab). The randomized, double-blind phase 3 study evaluated clinical and endoscopic response at week 52 in patients with moderate-to-severe active UC treated with standard drug doses. Vedolizumab was more effective in inducing clinical remission and mucosal healing. There was no statistically significant difference between the drugs when the outcome evaluated was steroid-free remission. Both drugs were safe and well tolerated for treatment of moderate-to-severe UC [19].

No studies compared the efficacy of different biological agents in patients with CD.

2.2 Anti-interleukin 12/23p40

IBD presents a large infiltration of leukocytes, especially T lymphocytes. When activated, these cells produce a high concentration of cytokines that have an important role in the inflammatory process of the disease [20]. However, there seems to be a distinction in the profile of cytokines produced in CD and UC. While in CD there is a predominant synthesis of type 1 helper T-cell (Th1) cytokines, such as interferon-γ (IFN-γ) and TNF-α; in UC, Th2 cytokines, such as interleukin-5 and interleukin-13, are more relevant [20, 21].

CD mucosa has an increased production of interleukin-12 (IL-12), a pro-inflammatory cytokine that induces IFN-γ production and promotes Th1 cell differentiation [22, 23]. IL-12 is a heterodimeric cytokine produced by macrophages, monocytes, and dendritic cells, with two covalently linked subunits: p40 and p35 [23].

The IL-12p40 subunit can be combined with another cytokine, derived of IL-6 subfamily structures, the p19 protein, to form the p19p40 complex, also nowed like interleukin-23 cytokine (IL-23) [24]. The natural function of IL-23 is to coordinate inflammatory responses within peripheral tissues. However, unregulated expression of IL-23 may promote detrimental immune pathology at these sites [25]. In IBD, IL-23 may play the role of initiating and perpetuating innate T cell-mediated intestinal inflammation [26], thus leaving the place to IL-12/IFN-γ/T-cell pathway in the late phase [20].

A systematic review published by MacDonald et al. evaluated the use of ustekinumab (CNTO 1275) and briakinumab (ABT-874), monoclonal antibodies that target the standard p40 subunit of the cytokines IL-12 and IL-23 (IL-12/23p40), in patients with CD [27]. In this review two studies that compared briakinumab to placebo and four studies that compared ustekinumab to placebo were analyzed.

In 2004, Mannon et al. investigated two different doses of briakinumab. This was a multisite, randomized, double-blind, placebo-controlled study where 79 CD patients received 1 or 3 mg of anti-IL-12p40 monoclonal antibody subcutaneous injections versus placebo. The results showed that the use of anti-IL-12p40 might induce clinical responses and remissions in patients with active CD, with responses in 75% of CD patients compared with 25% in the placebo group. These results were associated with decreases in Th1-mediated inflammatory cytokines (IFN-γ and TNF-α) at the site of disease. There were no significant differences in the rate of adverse effects between placebo and anti-IL-12, except a higher rate of local reactions at injection sites in the former group [28].

The other study testing the use of briakinumab was published in 2015 and evaluated the efficacy and safety of this drug [29]. This was a phase 2b, multicenter,

double-blind, parallel group study, conducted with 246 patients with moderate-to-severe CD stratified by prior TNF-α antagonist use and response to anti-TNF-α therapy, who were randomly given placebo and 200, 400, or 700 mg briakinumab over the period of 0, 4, and 8 weeks. On week 12, patients who got clinical response in the placebo or 400-mg induction groups proceeded to the maintenance phase with the same protocol. Those who responded clinically with 700 mg were randomized to receive placebo and 200 or 700 mg briakinumab at weeks 12, 16, and 20 during the maintenance phase. Patients in remission stopped receiving the study drug at week 24. During the induction and maintenance phase of this study, briakinumab was well tolerated and had a safety profile similar to placebo. However, the authors pointed out that infusion reactions were observed in a higher percentage of patients treated with briakinumab than placebo during the induction phase (up to week 12). After week 12, adverse events and severe reactions occurred at a higher rate, mainly due to the increase in serious infusion reactions. The sponsor stopped the study during the open-label phase due to poor induction of remission results.

These investigations did not find severe side effects comparing briakinumab and placebo. However, there were common reactions to the site of injection and some secondary infections produced by briakinumab therapy. Due to these results, the production of briakinumab was interrupted [27].

Sandborn et al. conducted a phase IIa study of ustekinumab comparing clinical effects to placebo [30]. They made a double-blind, crossover design with 104 patients with moderate-to-severe CD, including both TNF-α antagonist naive patients and those who had previously failed one or more of these agents. These patients were divided into four groups: two groups received subcutaneous treatment doses, of which one group received placebo at weeks 0–3 and then 90 mg ustekinumab at weeks 8–11, while the other group received 90 mg ustekinumab at weeks 0–3 and then placebo at weeks 8–11. The other two groups followed the same weekly protocol, but the pathway was intravenous, and the dose was 4.5 mg/kg ustekinumab. Furthermore, a sub study like open-label trial evaluated the effects of four weekly subcutaneous injections of 90 mg or one intravenous infusion of 4.5 mg/kg ustekinumab in 27 patients who were primary or secondary nonresponders to infliximab, but it was not placebo-controlled. They showed that ustekinumab induced a clinical response in CD patients, who were previously treated infliximab, with the best effect in weeks 4–6 [30].

In 2012, Sandborn et al. published another study that evaluated ustekinumab therapy in patients with moderate-to-severe CD which was resistant to anti-TNF-α. This was a double-blind, placebo-controlled phase 2b trial with 526 patients who were randomized to receive intravenous ustekinumab (1, 3, or 6 mg/kg) or placebo at week 0. After 6 weeks, the clinical response was measured, and 145 patients who responded to ustekinumab were randomized to receive subcutaneous injections of ustekinumab (90 mg) or placebo at weeks 8 and 16 in the maintenance phase. Patients who used ustekinumab as an induction therapy had a higher response than the placebo group but did not differ in remission. These patients, during the maintenance phase with ustekinumab administration, had a significant increase in response and remission rates when compared to placebo. It is noteworthy that some serious infections occurred during the study, which affected 7 patients (6 receiving ustekinumab) in the induction phase and 11 (4 receiving ustekinumab) in the maintenance phase [31].

According to MacDonald et al., strong evidence indicates that ustekinumab is efficient for remission induction and that it improves symptoms in patients with moderate-to-severe CD. Moderate- to high-quality evidence implies that the optimal dose of ustekinumab is 6 mg/kg [27].

In addition to the improvement in patients' clinical condition and symptomatology, positive responses were also observed in histological examinations of patients who used maintenance therapy with ustekinumab every 8 weeks [32]. When analyzing histological data from participants in phase 3 induction and maintenance studies, significant histological improvement was observed in patients receiving ustekinumab compared to placebo [32].

Indeed, in 2016, with phase III UNITI trial program's positive results [33], the US Food and Drug Administration (FDA) approved ustekinumab for the treatment of moderate-to-severe CD [34]. Although there is no increased risk of serious adverse events, further studies are needed to assess the long-term benefit of their use in patients with CD [27].

Recently a study was published evaluating the use of ustekinumab to treat patients with moderate-to-severe active UC who do not respond well or were unable to tolerate conventional treatment or biological therapies [35]. It was a randomized, double-blind, placebo-controlled phase 3 study. Patients receiving a single intravenous ustekinumab dose of 130 mg or 6 mg/kg body weight (320 and 322 patients respectively) achieved clinical remission, endoscopic healing, clinical response, and mucosal healing at week 8, significantly better than placebo. It has been shown to be effective not only for the treatment of CD but also for the induction and maintenance of remission in patients with moderate-to-severe UC [35].

Serious infections were the most common side effects in the ustekinumab studies [27]. The therapeutic target of this drug is the p40 subunit, and it is not selective for IL-12 or IL-23. IL-12 is known to mediate protective systemic antimicrobial immunity, so this immune suppression may be responsible for these secondary opportunistic infections [23].

Studies support the specific blockade of IL-23, for its blockade may be as effective as the blockade of both cytokines but may result in fewer infectious problems [26]. A recently published review study evaluated the use of two drugs as a specific antagonist of the p19 subunit [34]. Risankizumab and brazikumab are the first anti-IL23p19 whose results were positive in randomized placebo-controlled phase II study to induction and maintenance therapy for moderate-to-severe CD patients. This review showed that both adverse events and serious adverse events did not differ between the treated groups and placebo. These results were observed in phase II studies with risankizumab and brazikumab, to treat not only IBD but also psoriasis. Based on symptomatic, endoscopic, and positive biomarker results, as well as treatment safety and efficacy during phase II trials, phase III studies are ongoing. These studies will help answer questions about the optimal dosage of drugs and their action at other levels of CD involvement [34].

3. JAK inhibitors

Despite advances in the therapeutic arsenal of IBDs, significant numbers of patients do not achieve mucosal healing. Janus kinase (JAK) inhibitors already used in oncological, rheumatological, and dermatological disease treatment are being studied as a new therapeutic resource against IBDs.

Many cytokines involved in IBD act on the JAK/signal transducer and activator of transcription (STAT) cell signaling pathway, generating cellular responses through gene expression [36]. By binding to specific membrane receptors, cytokines activate JAK, which catalyzes the phosphorylation of the complex enabling STAT binding [37]. After phosphorylation, STATs dimerize, leave the receptor, and go to the cell nucleus to activate the transcription of the target gene [38].

Some JAKs, like JAK1, JAK2, and JAK3, play an important role in the growth, differentiation, and survival of immune system cells in general. Unlike the others, JAK3 is present in hematopoietic cells, acting mediated signaling pathways by IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 [38]. According to Lovato et al., patients with CD have an overactivation of STAT3 and STAT4 in intestinal T cells [39]. Therefore, despite the importance in diverse cellular activities, changes in the JAK/STAT signaling pathways have been related to various immune disorders [38].

JAK inhibitors have been developed and are under clinical investigation to assess their ability to attenuate the inflammation process in UC [40]. Tofacitinib (CP-690,550), a first-class JAK360 inhibitor, works by inhibiting JAK1/JAK3 and has a lower side effect on JAK2 and TYK2 [41]. This JAK inhibitor was tested in clinical trials to verify its potential treatment for some immune system disorders, including CD and UC [42].

To test the efficacy of tofacitinib in UC, a double-blind, placebo-controlled, phase 2 trial study was conducted [40]. Patients with moderately to severely active UC (n = 194) randomly received placebo or tofacitinib at a dose of 0.5, 3, 10, or 15 mg twice daily for 8 weeks. Significant clinical remission (Mayo score ≤ 2, with no subscore >1) at 8 weeks occurred in patients who received 10 mg (48%, $p < 0.001$) and 15 mg (41%, $p < 0.001$), compared with 10% in placebo group. Endoscopic remission at 8 weeks occurred in patients receiving 10 mg (30%, $p < 0.001$) and 15 mg (27%, $p < 0.001$), compared with 2% in placebo group.

OCTAVE Induction 1 and 2 were phase 3, randomized, double-blind, placebo-controlled studies in patients with moderately to severely active UC [43]. Patients randomly received tofacitinib (10 mg twice daily) or placebo for 8 weeks. In the OCTAVE Induction 1 (n = 598 patients), remission occurred in 18.5% of the patients in tofacitinib group and in 8.2% in placebo group ($p = 0.007$). In OCTAVE Induction 2 (n = 541 patients), remission occurred in 16.6 versus 3.6%, respectively ($p < 0.001$). According to the results, tofacitinib use showed remission induction after 8 weeks of use in patients with moderate-to-severe UC compared with placebo [43].

In OCTAVE sustain study, the rate of maintenance of clinical remission was evaluated. The patients with clinical response to induction therapy in OCTAVE Induction 1 and 2 were followed for 52 weeks. The patients were randomized into three groups (placebo, 10 mg and 5 mg, 2 times daily). The clinical remission at 52 weeks occurred in 34.3% (n = 68/ 198) of patients taking 5 mg; 40.6% (n = 80/197) with 10 mg; and 11.1% (n = 22/198) in the placebo group. The mucosal healing rate at 52 weeks was 37.4% (n = 74/198) in patients on 5 mg; 45.7% (n = 90/197) in those who used 10 mg; and 13.1% in the placebo group (26/198) [43].

In patients with CD, initial studies with JAK inhibitors have shown unsatisfactory results in inducing clinical and endoscopic remission of the disease. In a, multicenter, randomized, double-blind, placebo-controlled phase 2 study, patients with severe CD (CDAI between 220 and 450) were randomized to receive placebo or 1 mg, 5 mg, and 15 mg tofacitinib, twice daily for 4 weeks. Clinical response and remission were similar between both groups. However, this outcome could be associated with a selection bias in the control group [44].

Another multicenter phase IIb, randomized, double-blind, placebo-controlled study evaluated patients with moderate-to-severe CD. Patients were assigned randomly to receive placebo or tofacitinib 5 or 10 mg twice daily for 8 and 26 weeks. The rates of clinical response (decrease in CDAI $\geq$100 from baseline) and clinical remission (CDAI <150) at week 8 and 26 were not significantly different from the placebo [45].

In 2018, tofacitinib was the first JAK inhibitor to be approved by the US FDA and the European Medicines Agency's Committee for Medicinal Products for Human Use (CHMP) to treat moderate-to-severe active UC. According to EMA it should be used in patients who have tried conventional therapy or biological agents and failed or did not progress positively.

There is another small molecule, still in the testing phase, which selectively acts on important specific pathways in UC and CD, thus limiting some of the side effects such as bacterial and viral infections [41].

Indeed, tofacitinib therapy in rheumatoid arthritis showed an increased risk of infection, including herpes zoster [46]. Herpes zoster infection, among others, was also observed in the OCTAVE study comparing the use of 10 mg tofacitinib with placebo [43]. Vaccination against herpes zoster is indicated 3–4 weeks before starting tofacitinib treatment as a preventive strategy [47].

The other most common adverse effects of using JAK inhibitors are influenza, rhinopharyngitis, arthralgia, and headache. Studies in patients with rheumatologic diseases and psoriasis have not shown increased cardiovascular risk in patients treated with tofacitinib [43], although there may be an increase in HDL and LDL cholesterol serum levels [40].

In a cohort analysis, including OCTAVE I and II and Sustain, with UC patients exposed to tofacitinib, 25 cases of pregnancy occurred, but no definitive conclusions about maternal and fetal risks, due to methodological limitations (absence of control group, retrospective study, and small number of cases) [48]. Further studies are needed to assess medication safety in pregnant women. It is not currently approved for pregnant and breastfeeding women [47, 48]. In addition, information provided by the manufacturer itself showed preclinical trials with rabbits and rats that showed a risk of fetal malformations with the use of tofacitinib but at doses 10 times higher than recommended for humans [Pfizer Inc. Xeljanz prescribes information, http://labeling.pfizer.com/ShowLabeling.aspx?id=959 (2014, accessed July 13, 2019)] [47].

Vermeire et al. evaluated the efficacy of filgotinib, a kind of selective JAK1 inhibitor [49]. This search was a randomized, double-blinded, placebo-controlled phase II FITZROY study, with CD patients with moderate-to-severe activity. The patients received 200 mg filgotinib once daily or placebo for 10 weeks. As a result, the number of patients who received the drug and went into remission was much larger than that of those who received placebo after 10 weeks of treatment. This study showed the first evidence for potential clinical efficacy and safety of a selective JAK1 inhibitor for the treatment of active CD [49]. Filgotinib might represent a new oral treatment to induce remission in patients with CD, but a phase III study will still be necessary [42]. According to Soendergaard et al., a combined phase IIb/III randomized, placebo-controlled study with filgotinib for the treatment of moderate-to-severe UC (the SELECTION1 study) is ongoing.

4. Conclusion

Inflammatory bowel diseases have very complex pathophysiological mechanisms, which makes treatment difficult. Advances in research presented here show new possibilities for alternative treatments, some already approved by the FDA (ustekinumab and tofacitinib) and others still under investigation.

The study of these alternative biological therapies is very important to help treat severe CD and UC patients with previous therapeutic failures, who no longer respond to or have not adapted to conventional treatments.

Conflict of interest

The authors declare no conflict of interest.

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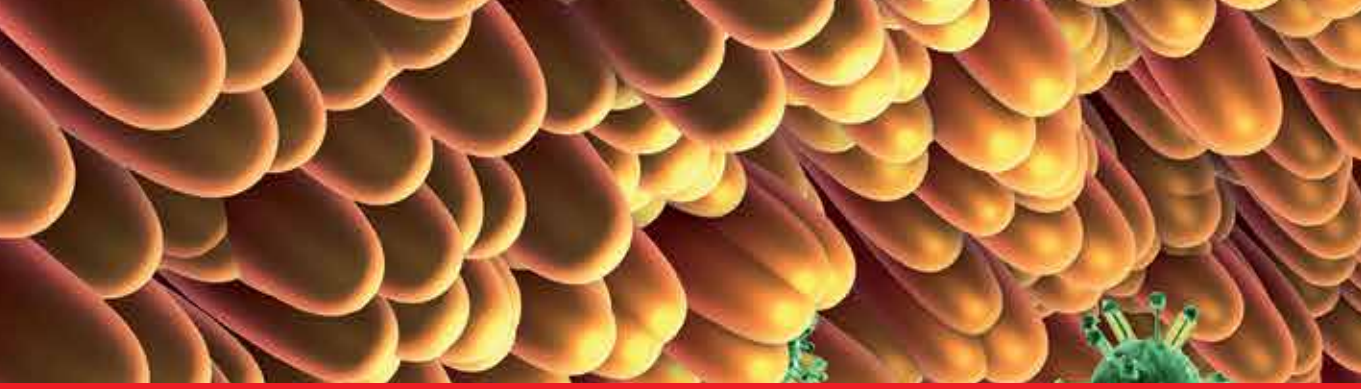

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The treatment of inflammatory bowel disease (IBD) has posed a major challenge since its appearance. Biomedical researchers, physicians, gastroenterologists, and surgeons have struggled to improve the quality of life of their patients and have sought, above all else, to keep the disease under remission for as long as possible. Blockers for tumoral necrosis factor alpha (TNF-α) were the first biological drugs to be discovered and for this reason they played a crucial role in the subsequent evolution of IBD treatment. The aim of this book is to provide an overview of such drugs and the latest developments in IBD immunopathology. Our contributors discuss the main indications, efficacy, and possible side effects of the different types of drugs available today for IBD treatment.

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