Chapter

Mucosal Immunology in the Inflammatory Bowel Diseases

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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

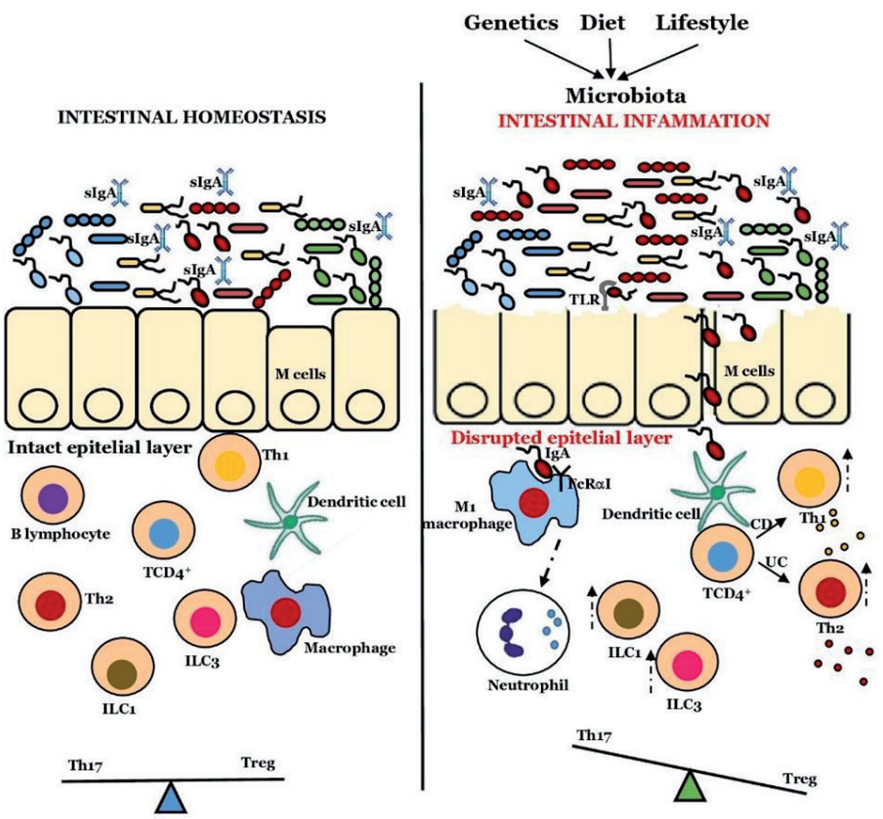

Figure 1.
During intestinal inflammation, such as IBD, barrier permeability is impaired, allowing the passage of luminal antigens into the lamina propria. These antigens can be recognize by TLR or captured by M cells. The exposure of immune cells to the luminal content induces TCD4$^+$ activation, differentiation 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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