Chapter 4

Cytokines in Inflamed Mucosa of IBD Patients

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Additional information is available at the end of the chapter

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Abstract

Cells of the innate and the adaptive immune system have been identified as the key players in inflammatory bowel disease (IBD) pathogenesis, and the cytokines are central components of the inflammatory pathways that take place in the gut mucosa during the active and chronic phases of IBD. The effector cell response is largely determined by the type of cytokines that predominate in the intestinal mucosa. Here we describe the main cytokine players in intestinal inflammation during IBD—related to innate immune responses (tumor necrosis factor α—TNFα), TNF-like cytokine 1A, IL-8), and related to adaptive immune responses—Th1 (IL-1β, IL-18, IFNγ, IL-12), Th2 (IL-4, IL-5, IL-13, IL-11, IL-33), Th17 (IL-17A, IL-17F, IL-21, IL-22, IL-25, IL-27), cytokines required for Th17 development (IL-6, TGFβ, IL-23), anti-inflammatory cytokine IL-10 and Tregs along with IL-2. Recently described innate lymphoid cells (ILCs) could also be potential sources of IFN-γ, TNF, IL-5, IL-13, IL-17, and IL-22. The effects of cytokines in the gut are described in conjunction with the clinical implication and available biologic therapy. The data in the literature and our own results make us believe that in order to achieve immune homeostasis in the gut, pro-inflammatory and anti-inflammatory responses that define the mucosal cell immunophenotype should achieve balance.

Keywords: IBD, cytokines, mucosal inflammation, Th17, Tregs

1. Introduction

Both ulcerative colitis (UC) and Crohn's disease (CD), usually referred to as inflammatory bowel disease (IBD), are examples of complex disorders, which include inflammatory and

autoimmune features with prominent intestinal immune dysregulation. Cells of the innate and the adaptive immune system have been identified as the key players of IBD. Cytokines are central components of the inflammatory pathways that take place during the active and chronic phases of IBD. However, a clear picture of these processes is still missing. Since the inflammation is located in the intestinal mucosa, the latter is the main source of biomarkers in IBD allowing various immunological pathways to be explored in the gut. Thus, the determination of cytokine expression profile could help to elucidate the local immune responses during intestinal inflammation. Expression of IBD-related proteins such as cytokines, chemokines, adhesion molecules, and their corresponding cellular and soluble receptors has revealed their significant role in the pro- and anti-inflammatory processes in the inflamed gut mucosa. Indeed, the implication of some cytokines in the immunopathogenesis of IBD is investigated intensively and proved in experimental models of intestinal inflammation. Lack of enough investigation in humans, however, predetermines the need for further studies since it is proved that the common clinical phenotype of colitis may result from largely diverse genetic or immunological backgrounds.

2. Intestinal inflammation and cytokines

Since the pathogenesis of IBD is related to both dysregulated innate and adaptive immune pathways, which contribute to the aberrant intestinal inflammatory response in genetically susceptible individuals, the main focus of research attempts is directed to the initiation, perpetuation, and cessation of gut inflammation associated with IBD [1].

Cytokines are abundantly produced by the cells of the gut-associated immune system maintaining lymphocyte homeostasis under both steady-state and inflammatory conditions. These small, cell-signaling protein molecules act in a paracrine, autocrine, or endocrine manner, coordinate the communication between immune and non-immune cells of the intestinal compartment, and modify acute and chronic inflammatory responses at both local and systemic levels [2]. Moreover, elevation of pro-inflammatory cytokines is considered to be associated with the severity of gut inflammation [3]. Therefore, it is no surprise that cytokines have been a major therapeutic management of IBD [4].

It is believed that dysregulated immune mechanisms are related to T cells in the gut in IBD pathogenesis. Unregulated T lymphocytes activities can lead to autoimmunity, especially during inflammation when they can cause excessive tissue damage [5]. The ability of CD4+ T helper (Th) cells to alter the magnitude and outcome of the intestinal tissue-damaging inflammatory responses is mostly dependent on the production of distinct profiles of cytokines. Traditionally, the lesions in CD patients have been associated with a predominant activation of Th1 cells and production of large quantities of IFNγ under the stimulus of IL-12 through STAT4 signaling. By contrast, the lesions in UC patients were believed to be driven by Th2 cytokines, such as IL-4 and IL-13, through STAT6 activation. In the mouse model of IBD, CD3+ (T cell) depletion results in dramatic reduction of the gross pathology, neutrophil influx, and expression of pro-inflammatory cytokines and chemokines [6]. The cytokine

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expression pattern that strictly follows the polarization model of Th1 versus Th2, however, does not appear to be fully applicable in IBD. Nearly 20 years ago, Mosmann and Coffman concluded their paradigm with the prediction: "… further divisions of helper T cells may have to be recognized before a complete picture of helper T cell function can be obtained" [7, 8]. Indeed, several recent studies had led to the identification of more complex networks of cytokine interaction in IBD tissue, thus shedding light on the role of a distinct subset of T cells in the pathogenesis of IBD—Th17 cells. On the other hand, another T cell subpopulation, namely T regulatory cells (Tregs), is implicated in gut homeostasis and tolerance induction, and it is believed that Th17 and Tregs are in a mutually polarizing relationship [9]. An overview of the main cells and cytokines involved in intestinal inflammation is presented in **Figure 1**.

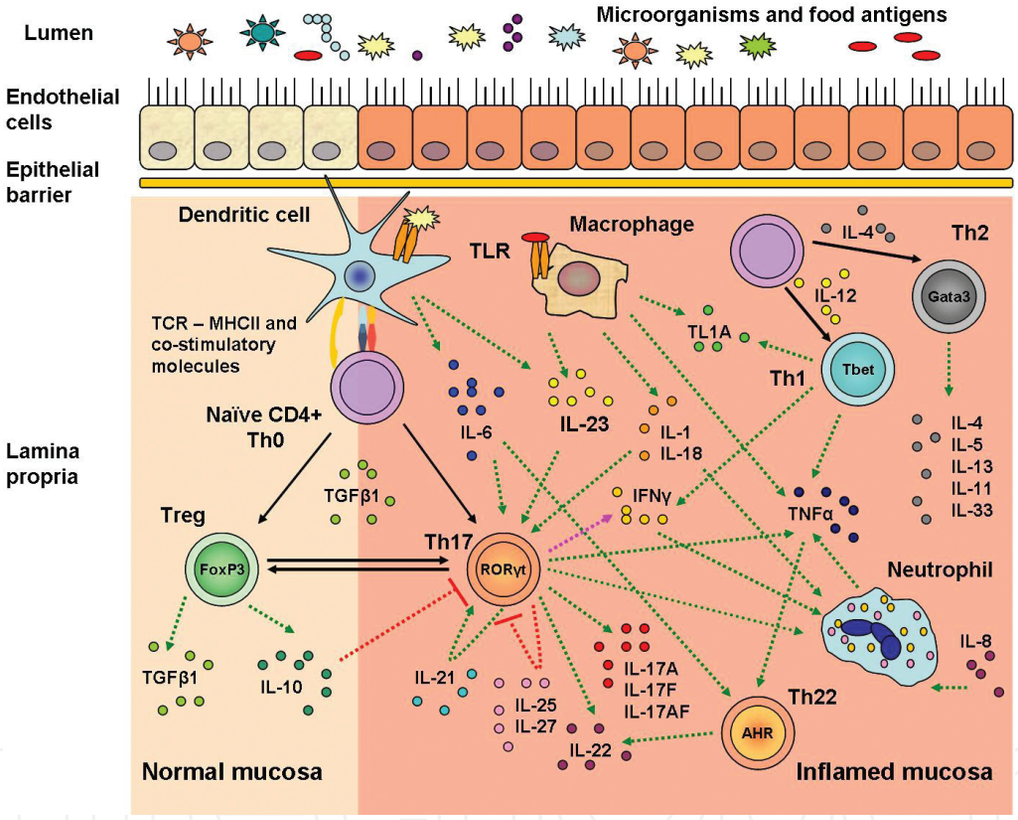

Figure 1. T-helper cells and cytokines interactions in normal and inflamed mucosa of IBD patients. The fate of naïve T cell depends on the interactions with the antigen-presenting cells (i.e. dendritic cells, macrophages) and the secreted cytokines. In normal mucosa, the abundant TGFβ1 directs naïve Th0 cells to Treg differentiation which secrete IL-10 and TGFβ1. "Danger" signals through TLR activation (on antigen-presenting cells), followed by secretion of IL-6, IL-23, IL-1, etc., with the simultaneous presence of TGFβ1, all favor the development of Th17 cells. The latter secrete many cytokines, for example, IL-21 acts as an autocrine positive regulator but IL-25 and IL-27 inhibit Th17 cells in autocrine manner. Th17 cells could also secrete IL-17 cytokines, TNFα, and in special circumstances—IFNγ; thus, Th17 cells play an intermediate role between innate and adaptive immune response, especially during inflammation in the intestinal mucosa. The balance between Th17 cells and Tregs is desired to maintain the immune homeostasis in the gut. However, Tregs and Th17 cells can convert into each other demonstrating same plasticity, depending on the cytokine milieu. Nevertheless, there are other players in the inflamed mucosa such as Th1, Th2, and Th22 cells. Legend: black arrow—cell differentiation; green arrow—secretion; pink arrow—possible secretion; red arrow—inhibition; TCR—T cell receptor; TLR—Toll-like receptor; MHCII—major histocompatibility complex—Class II; TL1A—TNFα-like 1 A.

Thus, the effector response is largely determined by the combination of cytokines that predominate in the intestinal mucosa, and it defines the mucosal T cell immunophenotype in each case [2].

2.1. Innate immune response and related cytokines

Dendritic cells (DCs), macrophages, epithelial cells, and myofibroblasts are able to recognize pathogen-associated molecular patterns (PAMPs) through their pattern-recognition receptors including Toll-like and NOD-like receptors. This recognition results in nuclear factor (NF)-kB activation with gene transcription and production of pro-inflammatory cytokines, such as IL-1 and TNFα, ensuring an effective innate response against microbial antigens. That also triggers antigen presentation, maturation, and up-regulation of co-stimulatory molecules which lead to efficient adaptive immunity involving T cell activation [10]. There is evidence for down-regulated protein level of TLR-3 in IBD, whereas TLR-2 and TLR-4 are up-regulated in intestinal mucosa of active IBD [11]. A specific mutation in NOD2 gene induces loss of NF-kB function during TLR-2 activation with a subsequent increased risk of infection with commensal bacteria and increased susceptibility to the ileal form of CD [12]. Recent studies suggest that increased mucosal permeability in the intestinal mucosa during IBD flare allows infiltration of a large number of granulocytes into the colonic mucosa. These leukocytes are activated, have a prolonged survival time, and release various pro-inflammatory cytokines (e.g. IL-1β, IL-6, TNFα, IL-18), which exacerbate and maintain the inflammation in the gut [13].

2.1.1. TNFα

TNFα links the innate and the adaptive immune responses and has crucial importance in the pathogenesis of IBD by inducing the differentiation of stromal cells into myofibroblasts and promoting their production of matrix metalloproteinases. The latter induce enterocyte apoptosis and digestion of gut basement membrane [10]. TNFα also exerts its pro-inflammatory effect through cytokines such as INFγ, IL-1β, and IL-6 [12].

TNFα is a well-established inflammatory mediator in CD whereas contradictory reports exist in UC [12]. There is a lack of studies on the mucosal expression of TNFα and the prediction of the clinical course, and only a few reports announced the predictive value of mucosal TNFα concentrations and the response to therapy in IBD patients. In fact, increased levels of TNFα and IL-15 have been previously reported in intestinal biopsies from IBD patients in remission without biopsy alterations [14]. Interestingly, the presence of TNFα in non-affected areas of IBD mucosa may not be sufficient to trigger mechanisms of mucosal damage. In preliminary reports, normalizing of mucosal TNFα seemed to predict a longstanding remission after stopping of anti-TNFα therapy in UC [12].

Certain TNFα polymorphisms (i.e. TNFα-308 A allele) are associated with increased serum levels of TNFα and therefore with higher susceptibility of IBD [15].

2.1.2. TNF-like cytokine 1A

TNF-like cytokine 1A (TL1A) is a novel member of TNF superfamily of proteins, produced by endothelial cells, macrophages, lamina propria T cells and plasma cells, monocytes, and monocyte-derived DCs [16]. Association with its functional receptor provides co-stimulatory signals for activation of T lymphocytes, leading to cell proliferation, cytokine secretion, and amplification of pro-inflammatory pathways, as well as induction of apoptosis in target cells [2]. Several studies have clearly demonstrated that TL1A and its receptor are up-regulated at mucosal protein and mRNA levels in IBD patients. TL1A is localized in the lamina propria and shows preferential expression on plasma cells and mucosal DCs. Of great importance is the fact that TL1A was shown to increase IL-13 secretion by natural killer T (NKT) cells, which are considered to be central to the mucosal injury that takes place in UC pathogenesis. Furthermore, TL1A induces IFNγ secretion in synergy with stimulation via TCR or IL-12/IL-18 [2]. TL1A expression is induced by TNFα and IL-1α as well and since the latter are abundantly expressed in the inflamed mucosa of UC patients, they may provide a strong stimulus for enhanced TL1A expression. On the other hand, several microorganisms were shown directly to stimulate TL1A secretion by DCs via TLR-signaling (TLR-4), LPS-induced and NFkB-dependent pathway [16]. Moreover, there is an inhibitory component of the TL1A receptor which could augment pro-inflammatory pathways at the intestinal mucosa by rendering activated lymphocytes resistant to apoptosis. Thus, increased expression of this inhibitory TL1A receptor may offer a survival advantage to effector lymphocytes, preventing their elimination and perpetuating tissue injury [2].

2.1.3. IL-8

IL-8, as a member of the CXC chemokines family, is not only a strong chemoattractant for neutrophils, monocytes, etc. but also triggers the secretion of superoxide anions and lysosomal enzymes in neutrophils, thus contributing to the tissue damage during inflammation. IL-8 mRNA expression in the inflamed mucosa is shown to be significantly higher than the level in non-inflamed mucosa of IBD patients or in the normal mucosa of non-IBD patients [13].

2.2. Th1 profile-related cytokines

Th1 cells are an essential part of the adaptive immune response, mainly against intracellular microorganisms and protozoa. The master transcription factors for Th1 definition are STAT4 and T-bet. Th1 cells in gut mucosa which are induced by increased levels of IL-12 and IL-18 are thought to cause intestinal inflammation in CD patients via production of high amounts of IFNγ. The latter induces enterocyte apoptosis and triggers the release of TNFα by activated mucosal macrophages. Th1 cells by themselves appear as an important source of TNFα [10].

2.2.1. IFNγ

IFNγ is a mediator of intestinal inflammation in CD, but contradictory reports exist for UC. However, increased levels of IFNγ have been observed in the inflamed mucosa from UC

patients too. IFNγ levels also correlated with the clinical activity but not with the endoscopic score in UC, whereas no correlation to the clinical activity was observed in CD patients [12].

2.2.2. IL-12

The role of IL-12 in intestinal inflammation will be discussed later along with IL-23.

2.2.3. IL-1

IL-1 exists in two forms, IL-1α and IL-1β, encoded by different genes but exhibit almost identical functions [16]. The major sources of IL-1 are activated myeloid cells and its production can be induced by bacterial lipopolysaccharide, TNFα, IFNα, IFN-β, IFN-γ, as well as IL-1. IL-1 was found to promote Th17 development in the presence of IL-6 and TGFβ, and also to potentiate their actions in humans but not in mice. Moreover, it has been reported that IL-1 can increase their effect on Th17 definition. However, the mechanism through which IL-1 influences Th17 differentiation is not fully determined yet [17]. Some suggestions include that IL-1β or IL-1α cooperates with IL-23 to enhance IL-17 production independent of TCR stimulation. Additionally, IL-1 may suppress the inhibitory effect which IL-2 exerts on Th17 cell production through induction of IL-1R, IL-23R, and transcription factor RORγt [16].

IL-1β was shown to be increased in CD and UC patients, whereas the IL-1-receptor/IL-1β ratio was negatively associated with the IBD activity. When comparing the IBD patients with controls, a significant variation in genotype frequency of the IL-1β promoter polymorphism was found. Higher levels of the pro-inflammatory cytokine IL-1β would be expected to increase the likelihood of developing IBD since higher levels of such cytokines occur in this disease [15].

2.2.4. IL-18

IL-18 is another member of the IL-1 pro-inflammatory cytokine family. IL-18 is an epithelial-derived cytokine that has been proposed to promote barrier function in the intestine, but its effects on intestinal T cells are poorly understood. Although IL-18 is mainly responsible for inducing IFNγ production and Th1 differentiation, this cytokine might be involved in Th17 cell definition as well. Antigen-presenting cells express IL-18R on their surface and its binding with the cytokine is required for generation of Th17 cells through an IL-23-dependent mechanism. Moreover, IL-18 synergizes with IL-23 in the induction of Th17 cell [16] However, there are more reliable proofs about the involvement of IL-18R in Th17 cell definition, but not for IL-18 itself. Probably this action might be fulfilled through binding of an unknown alternative ligand, distinct from IL-18, to the receptor [17]. In contrast, Maloy et al. [18] demonstrated that during steady state, intestinal epithelial cells constitutively secrete IL-18, which acts directly on IL-18R1-expressing CD4+ T cells to limit colonic Th17 cell differentiation. In addition, they found that IL-18R1 signaling was critical for Tregs-mediated control of intestinal inflammation, though IL-18R1 is not required for Tregs development [18]. Thus, since IL-18 may regulate differentially homeostatic and inflammatory subsets of T cells, this finding has potential for treatment of IBD and other chronic inflammatory disorders.

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IL-18 was found elevated in the inflamed colonic mucosa of UC and CD patients and polymorphisms in the IL-18R1-IL-18RAP locus are associated with IBD susceptibility [18]. Moreover, the local expression of IL-18 has been shown to be associated with the grade of inflammation [19].

2.3. Th2 profile-related cytokines

Th2 cells, another important part of the adaptive immune system, are mainly involved in the effector responses against extracellular parasites, including helminths, as well as in allergy pathogenesis. They are defined by the transcription factors STAT6 and GATA3 [7]. The importance of Th2 response in IBD is still under debate. In UC, the inflammatory response is less skewed along specific pathways, even though there is enhanced production of IL-4, IL-5, and IL-13, cytokines made by Th2 cells, unlike CD where Th1 activation has been mainly employed in pathogenesis [20].

2.3.1. IL-13

IL-13 exerts the potential to increase intestinal permeability and induce both enterocyte differentiation and apoptosis. IL-13 is released mainly by Th2 cells but another source of that cytokine is NKT cells. NKT cells express surface CD161 but not invariant T cell receptor, which is a well-established characteristic of this population. They produce IL-13 in response to stimulation of antigen-presenting cells expressing surface CD1d. Most probably, these atypical NKT cells are stimulated to produce IL-13 in the colonic mucosa by flora-derived microbial products [2]. This was observed in patients with UC, but not in CD patients. Further studies revealed that CD161-expressing NKT cells showed IL-13-dependent cytotoxic activity against colon epithelial cells [2]. Moreover, IL-13 independently exerts harmful effects on epithelial barrier function, such as derangement of tight junction integrity, decreased restitution velocity, etc. [2]. Therefore, blockade of IL-13 downstream signaling may be an effective anti-inflammatory approach in UC which requires further investigations.

2.3.2. IL-11

IL-11 is a member of the IL-6 cytokine family and exerts pleiotropic effects on various cell types as it acts synergistically with other cytokines such as IL-3 and IL-4, thus it has been implicated in Th2-mediated sensitization and inflammation. IL-11 also prevents cell death and inhibits inflammation at sites of tissue injury. IL-11 mediates anti-inflammatory effects by down-regulation of LPS-induced NFkB activation, thus preventing transcription of inflammatory genes [12]. This may be implemented in IBD therapy, but still needs additional verification.

2.3.3. IL-33

IL-33 is the latest identified member of the IL-1 family of cytokines. mRNA and protein expression of IL-33 was detected in normal colonic cells both at the surface epithelium and in crypts, as well as in inflamed bowel onto lamina propria mononuclear cells (CD11b+ monocytes/macrophages and CD19+ B cells), endothelial cells, and subepithelial myofibroblasts.

During active intestinal inflammation, IL-33 actively participates in the epithelial-immune cell crosstalk that takes place in IBD mucosa. IL-33 expression is augmented under stimulation with IL-1β and TNFα, two cytokines that are enriched at the inflamed mucosa and are of pathogenic relevance in UC, as well as after TLR-3 and TGFβ signals [2].

Regarding mucosal expression, up-regulation of IL-33 appears to be specific for UC, as it was not observed in CD colonic inflammation [2]. Moreover, IL-33-expressing myofibroblasts were absent in fissuring areas in patients with colonic CD. Therefore, these observations may provide information of distinctive pathway between the two forms of IBD [2].

IL-33 was shown also to induce particularly the expression of Th2 effector molecules IL-5 and IL-13. Given the central role of IL-13 in UC, IL-33 may be involved in UC pathogenesis through the induction of IL-13 secretion. It has been proposed that IL-33 may function as "alarmin" for the gut-associated immune system activating toward intestinal inflammation or perpetuating the ongoing one [2].

2.4. Prerequisite cytokines for Th17 development

To emphasize the importance of Th17 in intestinal inflammation, here we start with the description of the prerequisite cytokines for the development of Th17 cells from naïve T cells.

2.4.1. TGFβ1

Transforming growth factor β (TGFβ) is a potent cytokine with multi-faceted regulatory and inhibitory activities and has two forms—TGFβ1 and TGFβ2. TGFβ1 is a pleiotropic cytokine best known for its potential to induce peripheral tolerance in the absence of IL-6 [12]. One of the mechanisms by which TGFβ1 is able to maintain tolerance is to support the survival of naturally occurring FoxP3+ Tregs (nTregs) in thymus. In addition, along with IL-2 and retinoic acid, TGFβ1 promotes the differentiation of induced Tregs (iTregs). Another mechanism of TGFβ-induced tolerance is to suppress the innate immune cells such as DCs and NK cells [5].

TGFβ1 also regulates the development of resident macrophages in the normal intestine, which possess some unusual features such as constitutive production of IL-10 and TNFα, refractory to TLR stimulation, high expression of MHCII and CXCR1, and avid phagocytic activity. Thus, this is another mechanism through which TGFβ1 favours local homeostasis [21].

TGFβ1 plays an important role under inflammatory conditions. In the presence of IL-6, TGFβ1 drives the differentiation of Th17 cells which promotes further inflammation and augmentation of ongoing autoimmune conditions. In addition, TGFβ1 in combination with IL-4 promotes the differentiation of IL-9-producing and IL-10-producing T cells, which surprisingly lack suppressive function and also promote tissue inflammation [12]. Increased protein levels of TGFβ1 are found in the mucosa of both CD and UC patients, whose levels correlated with the severity of disease in CD but not in UC patients [5, 12]. We also found significantly higher gene and protein levels of TGFß1 in the inflamed mucosa of CD patients alone [22]. This is not surprising since the tissue remodeling properties of TGFβ1 are well-established. Interestingly, TGFβ1 orchestrates the differentiation of both Tregs and Th17 cells

in a concentration-dependent manner—low doses induce Th17 cell differentiation while higher doses inhibit Th17 cell development and promote Tregs [5, 11].

2.4.2. IL-6

IL-6 is a pleiotropic cytokine with regulatory effects on inflammation development. In addition to its stimulatory effects (i.e. induction of acute phase proteins), IL-6 also has inhibitory functions (i.e. cessation of the antiviral antibody response after certain immunizations). Recent studies have demonstrated that IL-6 has a crucial role in the regulation of the balance between Th17 cells and Tregs [23]. IL-6 activates a receptor complex consisting of IL-6R and the signal transducing subunit gp130 which activates downstream STAT1 and STAT3. STAT3 regulates IL-6-induced expression of RORγt and RORα, the crucial transcription factors for Th17 cells. In contrast to STAT3 activation, STAT1 inhibits the development of Th17 cells. Although IL-6 activates both STAT1 and STAT3, it has been demonstrated that in Th17 cell activation, they play two different roles—STAT3 maintains while STAT1 suppresses it [23]. Furthermore, STAT family members activated by various cytokines provide both positive and negative regulation for Th17 development (i.e. IL-27 inhibits Th17 differentiation through STAT1) [23]. TGFβ1 can induce gene activation of both FoxP3 and RORγt, but FoxP3 is able to associate with RORγt, thus inhibiting its transcriptional activation. Nevertheless, in the presence of IL-6 this inhibition is abrogated, so IL-6 could act as a potent promoter of Th17 instead of Tregs differentiation. All facts taken together, IL-6 appears as the main partner of TGFβ in priming naïve T cells to IL-17 production, playing a pivotal role in Th17 polarization and initiation of inflammatory immune response. Currently, it is also accepted that IL-6 is able to induce expression of IL-23R in T cells, making them responsive to IL-23 which sustains the Th17 phenotype [17].

Increased levels of IL-6 and its soluble receptor are up-regulated in active CD patients, and mucosal IL-6 levels were correlated with the degree of clinical activity in CD and UC [12]. In consent with these findings, in a group of 37 IBD patients, we also found both mRNA transcripts of TGFβ1 and IL-6 up-regulated in patients' mucosa compared to the mucosa of non-IBD persons, along with increased IL-17 mRNA in inflamed tissue [22, 24].

Several polymorphisms regarding the IL-6 gene are described to be also associated with susceptibility to IBD development, such as IL-6 174 [15].

Although anti-IL-6 antibodies therapy has become a novel therapeutic strategy for some inflammatory and autoimmune disease, including CD, IL-6 inhibitory treatment acts primarily on initial CD4+ T cells response including Th17 differentiation, rather than on the effector phase [23]. However, it still remains controversial whether this antibody can inhibit Th17 differentiation in a manner that is clinically meaningful.

2.4.3. IL-23

IL-12 and IL-23 share the common p40 subunit, but whereas IL-12 drives the classical Th1 response characterized by IFNγ production, IL-23 maintains an IL-17-secreting T cell population. Th1 responses may develop normally in the absence of IL-23, but in IBD patients, their manifestations require the presence of IL-23. The systemic inflammatory response and the

elevated concentrations of pro-inflammatory cytokines in the serum are driven by IL-12 while the local intestinal inflammation and production of IL-17 in the intestinal mucosa are controlled by IL-23 [11, 12, 25].

IL-23 is crucial in orchestrating the crosstalk between innate and adaptive immunity with a key role in driving early responses to microbes. In a recent study, Kamada et al. showed that IL-23 is secreted preferentially by a subset of sentinel mucosal cells expressing both macrophage (i.e. CD14, CD33, CD68) and DC markers (i.e. CD205, CD209) [26]. These cells are present in a large number in CD-involved tissue and produce IL-12 and IL-23 in response to environmental danger signals [8, 26]. The presence of pathogens or pathogen-related products (such as lipopolysaccharide) can strongly influence the production of IL-12 and/or IL-23 depending on the microbial agent. This happens within a few hours after exposure and these early events in pathogen encounter are likely to shape subsequent responses toward IL-12 or IL-23 expression [8]. It was shown that some of the pathogenic functions of IL-23 in the gut are mediated by atypical T cell populations, such as γδT cells, invariant NK cells, and innate lymphoid cells, inducing them to secrete Th17-related cytokines and contributing to intestinal inflammation [10]. IL-23 might be also closely associated with the neutrophil influx [12].

The precise function of IL-23 in Th17 regulation is still not entirely clear, although there are a lot of speculations. IL-23 failed to induce the differentiation of naïve T cells into Th17 cells due to lack of IL-23R on naïve T cells [16]. It was subsequently demonstrated that IL-23R is not expressed on naïve T cells. Instead, IL-23 acts as a survival signal for Th17 cells by the mechanism probably similar to TNFα [23, 27].

The synthesis of the common p40 subunit for both IL-12 and IL-23, and the functional heterodimeric IL-23 is enhanced in the gut of CD patients [11]. Along with other authors' findings, we detected up-regulated mRNA levels of IL-23 in inflamed mucosa, as well as significantly increased serum level of IL-23 among IBD patients in comparison with non-IBD persons [24], and we suggest that anti-IL-23 therapy could be beneficial for IBD patients.

Identification of multiple single nucleotide polymorphisms (SNPs) in the IL-23 receptor gene that has been associated with both UC and CD suggested that the IL-23 axis might play a central role in chronic inflammation. IL-23R SNPs that influence IBD susceptibility have provided a new picture of the way the local immune response can promote intestinal tissue damage [11]. Small differences in cytokine levels as a result of gene polymorphisms may have an important effect on the inflammatory response and thus, influence the pathophysiology of IBD [15]. Interestingly, one of these polymorphisms, Arg381Gln, confers protection against developing CD [20]. Nonetheless, the mechanism through which these SNPs confer either risk or protection from IBD remains unknown [15].

2.5. Th17 cells and produced cytokines

The discovery of an IL-23-dependent T cell population that produces IL-17 but not IFNγ or IL-4 suggested there is an additional Th cell subset. Th17 cells have derived their name from their ability to produce IL-17, also termed IL-17A. Th17 cells also produce other cytokines including IL-17F, IL-21, IL-22, TNFα, and IL-6 [17, 23]. However, analysis at the single cell level

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has revealed that not all Th17 cells secrete the whole spectrum of cytokines, probably reflecting the heterogeneity of this cell's subset [25]. The IL-17 cytokine family also includes IL-17B, IL-17C, IL-17D (IL-27), IL-17E (IL-25), and IL-17A/F (**Figure 1**). The cytokines IL-27 and IL-25 have lowest protein homology to IL-17A. They are not produced by Th17 cells but act as negative regulators on the Th17 subset development. IL-27 is structurally related to IL-6 and is able to attenuate chronic inflammation by promoting IL-10 production [17]. In line with this, IL-27 and IFNγ are responsible for the inhibition of Th17 development in a STAT1-dependent manner [23], as described above. Another negative regulator of Th17 cells is IL-25, identified as a genetic homologue of IL-17, produced by Th2 and mast cells. IL-25 is involved in the expression of the Th2 cytokines IL-5 and IL-13, thus, favors Th2 responses. IL-25 deficiency is involved in pathologic inflammation, associated with increased expression of IL-17 and IL-23 [17].

CCR6, presented not only on Th17 cells, but also on Tregs, B cells, neutrophils and immature DC, plays a critical role in the migration of these cells to the sites of inflammation. TGFβ1 was shown to be the main factor for induction of CCR6 mRNA expression in Th17 cells and DCs [19]. IL-17-producing Th memory cells selectively express both CCR6 and CCR4, unlike Th cells producing IFNγ or both IFNγ and IL-17 which express CCR6 and CXCR3 [16]. Indeed, CCR4 is important for homing to the gut, where most RORγt+IL-17+ T cells are found [16].

The relationship among Th1, Th2, and Th17 cells is complex and still not clear. Th1- and Th2-related cytokines inhibit Th17 cell differentiation while IL-17 is not able to suppress Th1 or Th2 cells, or does it weakly. The suppression of IFNγ and IL-4 or their absence represents a way by which TGFβ1 could promote Th17 cell development. TGFβ1-driven Th17 cell differentiation can also occur in the absence of IFNγ and IL-4 [11]. In parallel with these findings, it was reported that IL-17-producing cells could be generated independent of the specific cytokines and transcription factors required for Th1 and Th2 differentiation [17]. Moreover, Th17 cells could develop from naïve T cells only in the combined presence of IL-6 and TGFβ1 [12, 20]. Thus, TGFβ induction of Th17 cells and also of Tregs, which are usually contradictory acting, is dependent on the presence of IL-6. This explains the apparent discrepancy of TGFβ1 involvement in both anti- and pro-inflammatory events in the intestine mucosa [17].

2.5.1. IL-17

IL-17 is an effector cytokine in gut immunity, which may have either pro-inflammatory or tissue-protective effects in the mucosa depending on the experimental or clinical model used. On one hand, IL-17 contributes to the mucosal barrier function by several mechanisms which, upon activation, result in a mucosal immune response toward pathogens [6]. IL-17 also promotes tight junction formation and increases trans-epithelial resistance in polarized intestinal epithelial cells by stimulating the production of antimicrobial peptides such as lipocalin-2, β-defensins, and calprotectin. This suggests that the latter are involved in the maintenance of immunological homeostasis and/or in the control of specific inflammatory pathways [19]. Thus, the Th-17-related cytokines mediate protective effects in host gut against various bacteria and fungi, particularly at mucosal surfaces [10, 11]. Interestingly, pathogens that have evolved to take advantage of various aspects of the mucosal response gain an edge

over the resident commensal bacteria and colonize the gut with priority. Despite that Th17 responses appear to be detrimental by promoting pathogen colonization of the mucosa, in the end, they result in decrease in bacterial dissemination from the mucosa that protects the host by inducing slight inflammation [6]. In line with this, it was shown that Th17 cells are constitutively present in the human and mouse intestinal mucosa and that Th phenotype is driven by the commensal bacteria in the gut. Additionally, stimulation of DCs with TLR ligands (e.g. fungal Dectin-1) induces synthesis of IL-6, TNFα, and IL-23 that promotes the differentiation of Th17 cells [11]. From this point of view, blocking Th-17 cytokines could have more deleterious than beneficial effects for the host [25].

On the other hand, IL-17 might mediate tissue inflammation by triggering several inflammatory pathways and by inducing various pro-inflammatory cytokines (e.g. IL-1, IL-6, TNFα, G-CSF, GM-CSF), chemokines (e.g. IL-18, CXCL-1, CXCL8, MIP-1), and enzymes (COX-2, matrix metalloproteinases). Both IL-17 and IL-22 stimulate granulopoiesis by inducing expression of the granulocyte colony stimulating factor (G-CSF) and IL-17A which rapidly recruits neutrophils to the inflammatory site. This mechanism has important evolutionary significance [25]. The neutrophil response gains time for the induction of the following antimicrobial Th1-IFNγ response which takes several days to develop. Once the appropriate immune effector functions occur, the IL-12/IFNγ axis becomes the dominant pathway in host defence. This is important for initial control of the infection, but if the IL-23/IL-17 immune pathway becomes dysregulated, there is a danger of autoimmune pathology development, such as IBD. These observations, including the fact that T-bet is expressed at lower level in Th17 cells, led McKenzie et al. to favour the hypothesis of a common lineage precursor of Th1 and Th17 cells [8]. Furthermore, the tissue localization and timing of IL-12 versus IL-23 responses explain the idea that IL-12/IFNγ axis is involved in systemic inflammatory conditions (such as lupus), whereas the IL-23/IL-17 axis appears to regulate tissue-specific disorders (such as IBD) [8].

Another layer of complexity to the mucosal existence of Th17 cells is other cell types, which can secrete IL-17-related cytokines: $\gamma\delta$T cells (secreting IL-17 in response to IL-23), NK, NKT cells (able to produce IL-17 and IL-22), and DCs (can secrete IL-22 in response to bacterial infection). Paneth cells, which are common in the ileum, also secrete IL-17A [6, 19]. As all these cells express the IL-23 receptor, the secretion of IL-23 by DCs comprises a trigger which potentiates early T cell activation and adaptive immunity development [6]. Thus, it appears that early activation of both adaptive and atypical innate-like T cells can lead to the expression of IL-17 and IL-22. However, dysregulated production of IL-17, IL-22, and TNFα in local tissue can result in chronic immune-mediated tissue destruction [8].

Studies in murine models of IBD strongly suggest that Th17 cells and their related cytokines contribute to tissue-damaging immune responses in the gut [25]. Up-regulation of Th17-related cytokines, however, does not represent a specific hallmark of IBD in humans, as increased levels of IL-17A and other Th17-related-markers have been seen in patients with rheumatoid arthritis, multiple sclerosis, psoriasis, etc. [11]. Immunohistochemistry studies have shown that in active UC, the IL-17-expressing cells were located mainly within the lamina propria, while in active CD, these cells were scattered throughout the submucosa and muscularis propria of the gut. Corresponding with this, it was shown that RNA transcripts for IL-17A and IL-17F

were up-regulated in the inflamed mucosa of UC and CD patients [3, 11, 22, 28]. Both IL-17 and IL-23 are correlated to the severity of UC [12]. More recently, Annunziato et al. demonstrated that the number of IL-17-producing T cells is higher in CD than in normal gut mucosa, and some of these cells also produce IFNγ [29].

Th17 cells have shown possession of functional plasticity. Some of the IL-17A-producing cells simultaneously express IFNγ (**Figure 1**). Majority of IL-17/ IFNγ-producing cells express CD161, a well-known marker of NKT cells, also identified recently on IL-17-producing memory T cells [11]. Th17 cells can be converted into Th1 cells if they receive appropriate stimuli, such as IL-12 which enhances the expression of Th1-related markers (i.e. T-bet and IFNγ) and down-regulates RORγt and IL-17. Additionally, recent studies have shown that treatment of intestinal lymphocytes with IL-23 can facilitate the production of either IL-17A or IFNγ in UC or CD, respectively [11].

This is in consent with the demonstration that some of the pathogenic effects of IL-23 in the gut are linked to the ability of this cytokine to turn on IFNγ production. Switching from IL-17A to IFNγ production occurs if Th17 cells are activated by a lack of TGFβ1 [25]. Th17 cells and their possible conversion to Treg direction is going to be described later.

This very complex and non-equivocal relationship of both pro-inflammatory and tissue-protective effects of IL-17 in the gut may explain the unsuccessful anti-IL-17 therapy in CD patients [10].

2.5.2. IL-21

IL-21, an IL-2-related cytokine produced by Th17 cells in response to IL-6, increases the expansion of this cell subtype by a positive autoregulatory feedback loop. IL-21, which is up-regulated in inflamed IBD mucosa, induces Th1 and Th17 immune responses in the mucosa [10], but a mixture of both Th1 and Th17 cytokines is needed to promote full pathology in the gut. In this context, a promising inducer could be IL-21, whose activity seems to be necessary for expanding both Th1 and Th17 cell responses in the intestine. [25]. As we have already noticed, IL-21 is overproduced in the gut mucosa of IBD patients, but the vast majority of IL-21-producing CD4+ T cells co-express IFNγ but not IL-17A. This fact suggests that Th1 but not Th17 cells are the major sources of IL-21 in the human gut [11]. There is evidence that IL-21 also enhances the expression of Th1-related transcriptional factors and IFNγ production in NK cells [11].

IL-21, like IL-17, stimulates gut fibroblasts to produce tissue-degrading matrix-metalloproteinases and enhances the secretion of chemoattractants (i.e. MIP-3α) by epithelial cells [10, 11]. IL-21, like IL-6, could also initiate Th17 differentiation together with TGFβ1 [23], even in the absence of IL-6 [16, 17]. IL-21 enhances the expression of IL-23R in Th17 cells, through a process that is dependent on STAT3 and RORγt, making these cells responsive to IL-23. IL-21 as well exerts additional biological functions that could contribute to its pro-inflammatory effect in the gut like inhibition of the peripheral differentiation of Tregs and making CD4+ T cells resistant to Treg-mediated immune suppression [11].

2.5.3. IL-22

IL-22 is a member of the IL-10 cytokine family and a Th17-related cytokine but it appears to be differentially regulated. IL-22 provides signals through a heterodimer comprising IL-22R and IL-10Rβ. The IL-22 receptor is highly expressed in tissues such as epithelial cells of the gastrointestinal tract. Via STAT3 signaling pathway, the activation of proliferative and/or anti-apoptotic programs starts, and this allows maintenance of epithelial barriers of the gut [5]. Most of the Th17 cytokines are highly dependent on the transcription factor RORγt for their expression, unlike IL-22 whose expression is dependent on the transcription factor aryl hydrocarbon receptor [5]. Th22 cells are another Th subpopulation characterized by the expression of this transcription factor and secretion of mainly IL-22 [5].

IL-22 has a dual functional nature in modulating the responses during tissue remodeling. IL-22 promotes induction of acute inflammatory proteins, mucins, and antimicrobial peptides (i.e. β-defensins), which are important for tissue integrity during inflammation. This mechanism ensures proper organ function and escape of potentially harmful effects by restricting the passage of luminal commensal flora and food antigens to the lamina propria [5, 25, 30]. It is important to point out that this process depends on the inflammatory context (the overall cytokine milieu and the tissues involved). Thus, IL-22 is important for control of pathogenic bacteria that need to translocate through host epithelial barriers to disseminate, especially in the gastrointestinal tract [5]. IL-22 also enhances intestinal barrier integrity by stimulating epithelial cell growth, goblet cell restitution, and mucus production, thus contributing to the healing of damaged tissue.

On the other hand, IL-22 can cause further inflammation by stimulating colonic fibroblasts to secrete inflammatory cytokines (e.g. TNFα, IL-8, IL-11, and leukaemia inhibitory factor), IL-6, chemokines, and matrix metalloproteinases [11]. It is not surprising that IL-22 is highly expressed during chronic inflammation [5] in mucosal samples of patients with active CD, because of the known dysbacteriosis and expected pathological microbial agents, and to a lesser degree in patients with UC, where autoimmune phenomena are more common.

IL-22 is also expressed by innate immune cells such as CD11c+ and NK cells located in the colon. The latter cells do not secrete IFNγ and are not highly cytotoxic [30]. IL-23, a traditional activator of NK cells, induces IL-22 expression in NK cells. Unlike TGFβ and IL-10 that directly modulate the immune response, IL-22 does not have direct effects on immune cells since these cells lack the expression of IL-22R [30]. This way, TGFβ1 and IL-10 are involved in maintaining immune homeostasis under steady-state conditions instead.

IL-22 is an ideal therapeutic candidate since it specifically modulates tissue remodeling and does not have direct effects on the immune response. Treatment with recombinant cytokine or gene therapy delivery of IL-22 may alleviate tissue destruction during inflammation owing to its selective modulation of tissue responses [5].

2.6. Role of FoxP3+ Tregs and related cytokines in gut inflammation

The main function of Tregs is to modulate the adaptive immune responses, and forkhead/winged helix transcription factor forkhead box P3 (FoxP3) is the master transcription factor

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for Tregs [23]. Two main subpopulations of Tregs have been best described: naïve (nTregs) and inducible Tregs (iTregs). The latter is believed to be derived by peripheral transformation of naïve T cells stimulated by IL-19, vitamin D3, antigens, and TGFβ1. So far, Treg function in IBD is not completely characterized [12].

Tregs are crucially involved in the maintenance of gut mucosal homeostasis by suppressing abnormal immune responses against the commensal flora or dietary antigens. They exert their function by producing the anti-inflammatory cytokines IL-10 and TGFβ, thus preventing both the activation and the effector function of T cells. Additionally, the regulatory activities of the immune response through mediators such as IL-10 and TGFβ still need to be profiled, especially those that might take place in the unaffected areas of IBD patients [14]. A certain number of Th17 and CD4+CD25+FoxP3+ Tregs cell is presented in the intestine even in the healthy state, partly due to the presence of enteric bacteria which favor the production of both Th17 and Tregs. DCs in the intestine or mesenteric lymph nodes also actively promote the production of both cell types. However, there are points of divergence, for example, the retinoic acid produced by DCs in the intestine induces only Tregs. In spite of the essential function of IL-2 as a growth factor of effector T cells, including Tregs, IL-2 has an inhibitory effect on Th17 cell production. Furthermore, IL-2 deficiency leads to systemic autoimmune disease, partly because of its involvement in the differentiation and survival of Tregs [16]. Recent studies have revealed that IL-2 deficiency promotes differentiation of Th17 cell subset in a STAT5-dependent mechanism. At present, the recognized precise mechanism is exerted by suppression of IL-17 expression by directly binding to the IL-17 gene promoter of STAT5 [16].

The importance of Tregs in maintaining immune homeostasis was once again emphasized with the X-linked IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy), caused by mutation of FoxP3. IPEX patients quite often complain of gastrointestinal symptoms, suggesting that Tregs dysfunction may be involved in human IBD too [31].

A significant increase in production of Tregs in active-phase IBD mucosal lesions, as well as decreased numbers of Tregs in peripheral blood of IBD patients was described [9]. However, in active IBD a reduced number of peripheral Tregs have been reported to be reverted by anti-TNF treatment [12]. Indeed, Tregs are increased in the intestinal mucosa of IBD patients in comparison with the mucosa of healthy volunteers [22, 24, 27, 32]. Tregs isolated from inflamed tissue display no obvious defect in their suppressive function, at least in vitro [9]. However, Monteleone et al. found that Tregs obtained from the active-phase IBD mucosal lesions possess an ability to suppress T cell activation [11, 25]. Since Th17 cells appear to be resistant to the Tregs-mediated immunosuppression, it is likely that during chronic inflammatory process, such as in IBD, Tregs may be dysfunctional and might augment rather than suppress Th17-mediated immune responses [11]. At first, this phenomenon was explained as a feedback loop associated with an increase in the Treg cell attracted by IL-2 which is produced locally at sites of inflammation. On the other hand, however, up-regulated Th17 cells in response to increased production of pro-inflammatory cytokines were postulated [27]. Th17 cells, but not Tregs, are induced in the presence of pro-inflammatory cytokines, in addition to TGFβ1. Thus, Treg dysfunction may not be intrinsic but rather due to extrinsic milieu of activated cells that are resistant to suppression, and pro-inflammatory settings in the affected IBD mucosa [9, 33].

Plasticity of Tregs and Th17 is further demonstrated by the possibility of conversion between both subsets [27, 33]. Hu et al. have reported that Tregs express membrane-bound TGFβ and in the presence of IL-6, they convert to Th17 cells [34]. This could be an important warning regarding cell therapy with Tregs to treat chronic immune disease, including IBD, because the "homeostatic" Tregs may convert to pathogenic Th17 cells during inflammation where IL-6 is abundant [27]. Numerous studies have shown that in inflammatory cytokine environment, Tregs can lose FoxP3 expression and acquire expression of other transcription factors that define another lineage of CD4+ T cells as well as effector function. As we have already mentioned, exposure of Tregs to IL-6 results in a partial conversion to Th17 cells. Interestingly, although most IL-17-producing cells lost FoxP3 expression, some cells express both FoxP3 and IL-17. It is unclear, however, whether the resultant cells are suppressive [9]. So, once again it must be mentioned that the Th17/Tregs balance appears to play a very crucial role in IBD development [27].

2.6.1. IL-10

IL-10 is secreted by many types of immune cells including Th2, Tregs, Tr1 (IL-10-producing FoxP3-CD4+ T cells), Th3 (TGFβ and IL-10-producing CD4+ T cells induced in oral tolerance), NKT cells, B cells, macrophages, and DCs [5]. IL-10 binds to its heterodimeric receptor, composed of unique for IL-10 subunit (IL-10Rα) and shared with IL-22 subunit (IL-10Rβ). Although not completely sufficient, STAT3 is required for the inhibitory functions of IL-10. Importantly, STAT3 induces the expression of transcription factors that regulate various cytokine signaling pathways including IL-6. IL-10 down-regulates IL-12 production and expression of co-stimulatory molecules in macrophages and DCs, thereby reducing the Th1 response generation [5].

IL-10 is a key regulator of the immune system by limiting the inflammatory responses that could otherwise cause tissue damage. IL-10 is essential for homeostasis of the immune system, especially in the gastrointestinal tract where the tolerance is most needed. Evidence for that is the highly-susceptible-to-colitis IL-10-deficient mice which develop aberrant immune responses to commensal bacteria. This colitis is more severe when combined with a deficiency in TGFβ signaling [5].

Small intestine and colonic lamina propria showed the highest frequency of IL-10-expressing cells. Recent findings show that macrophages in the lamina propria preferentially induce IL-10-producing cells while DCs promote the generation of Th17 cells. On one hand, blocking IL-10 during infection can result in more severe pathology or even fatality of the host, but on the other hand, high production of IL-10 is associated with sustained chronic infections and its blockade promotes pathogen clearance. Thus, once again, the milieu of the intestines favors the generation of IL-10-producing T cells leading to tolerance against commensal bacteria, whereas the expression of IL-10 in peripheral tissues under infectious conditions leads to suppression of the immune response [5]. In line with this, when IL-10 was previously found to be abundantly expressed by macrophages in areas of dense inflammatory infiltrate, it had been directly related to the attenuation of the mucosal inflammation [14]. Knowing nowadays

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about the dual role of IL-10, it is not unexpected that IL-10 is presented at a higher level in the inflamed mucosa of IBD patients [13]. These findings were confirmed by us as well [24].

Some IL-10 gene polymorphisms have been associated with susceptibility to IBD (i.e. IL-10—1082) and more significantly with UC alone. Whether the polymorphisms are directly involved in regulating cytokine production, and consequently disease pathophysiology of IBD, or serve merely as markers that are in linkage disequilibrium with susceptibility genes, is still unclear [15].

The involvement of IL-10 in the regulation of the pathogenic function of Th17 cells has been definitively demonstrated in experiments where non-pathogenic Th17 subtype expressing IL-10 is generated by IL-6 and TGFβ1, even though in the absence of IL-23. These cells also prevent the induction of the disease in an IL-10-dependent manner [35]. Even though IL-10 effectively treats colitis in mouse models and suppresses inflammatory cytokine production in vitro in intestinal cells of patients with IBD, clinical trials using recombinant IL-10 to treat IBD in humans have been largely disappointing, irrespective of the acceptable side-effect profile of the therapy [36].

2.7. Role of innate lymphoid cells in gut inflammation

Innate lymphoid cells (ILCs) are recently described cells that have been involved in both maintenance and loss of gut homeostasis. ILCs are phenotypically and functionally distinct subsets of cells that inhabit the intestinal mucosa. However, they produce cytokines associated with effector T-cell responses early in inflammatory lesions of patients with IBD [37]. The novel family of cells comprises three subsets: ILC1, ILC2, and ILC3 [38]. ILC1 express the transcription factor T-bet resembling Th1 cells with production of IFN-γ and TNF; thus, they contribute to host resistance to intestinal pathogens. ILC2 produce Th2 cytokines, such as IL-5 and IL-13, and they are dependent on the transcription factor GATA-3. ILC3 which express the transcription factor RORγt produce IL-17A and IL-22 mirroring Th17 cells [37]. ILC3 is involved in gut homeostasis by secreting IL-22 and promoting IL-10 and antimicrobial peptide production. Epithelial stress-induced ligands and inflammatory conditions may switch ILC3 to ILC1 secreting TNF and IFN-γ under the influence of IL-12. The pro-inflammatory cytokines of ILC1 and ILC3 lead mainly to epithelial apoptosis and neutrophil recruitment. ILC2 are able to contribute to IBD complications by producing the fibrogenic cytokine IL-13 [37].

Since ILCs might be substantial drivers of mucosal inflammation, targeting ILC subsets may be a new exciting treatment option for IBD patients.

3. Conclusion

From a clinical perspective, IBD is a chronic persistent disease characterized by repeated relapses and remissions. One explanation could be that memory Th cells created during the disease development persist in the body, including during remission, in a manner that is dependent on the various cytokine presentations. Effector cytokines in the mucosa may induce

inflammation at the time of the initial episode and during relapses. However, the ambiguity and contradictory actions of given cytokines confound the understanding of their interactions in dynamics of the immune response, and that leads to lack of synonymous conclusions about them. There is still strong need for further investigation, particularly in the gut mucosa, to fully comprehend their roles in the complex dynamic network of the immune mediators.

Th17 cells have been shown to play a central role in murine and human IBD. Inhibition of the Th17 pathway may be a promising treatment for IBD, with respect to the role of other subsets of Th1 and Th2 cells. The data in the literature and our own experience make us believe that in order to achieve immune homeostasis in the gut, pro-inflammatory and anti-inflammatory responses that define the mucosal cell immunophenotype, should achieve balance. Thus, following the clinical periods of remissions and relapses, it is important to observe their immunological equivalents in the gut and possibly in whole blood, namely regulatory and pro-inflammatory cytokines secreted by different types of immunocompetent cells.

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