

Association among genetic predisposition, gut microbiota, and host immune response in the etiopathogenesis of inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), is a chronic disorder that affects thousands of people around the world. These diseases are characterized by exacerbated uncontrolled intestinal inflammation that leads to poor quality of life in affected patients. Although the exact cause of IBD still remains unknown, compelling evidence suggests that the interplay among immune deregulation, environmental factors, and genetic polymorphisms contributes to the multifactorial nature of the disease. Therefore, in this review we present classical and novel findings regarding IBD etiopathogenesis. Considering the genetic causes of the diseases, alterations in about 100 genes or allelic variants, most of them in components of the immune system, have been related to IBD susceptibility. Dysbiosis of the intestinal microbiota also plays a role in the initiation or perpetuation of gut inflammation, which develops under altered or impaired immune responses. In this context, unbalanced innate and especially adaptive immunity has been considered one of the major contributing factors to IBD development, with the involvement of the Th1, Th2, and Th17 effector population in addition to impaired regulatory responses in CD or UC. Finally, an understanding of the interplay among pathogenic triggers of IBD will improve knowledge about the immunological mechanisms of gut inflammation, thus providing novel tools for IBD control.

Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Immune response; Etiology; Microbiota

Introduction

Inflammatory bowel disease (IBD), which comprises Crohn's disease (CD) and ulcerative colitis (UC), is an uncontrolled inflammatory disorder of the gastrointestinal tract. These diseases affect mostly people between 30 and 40 years of age and, currently, the number of registered cases is increasing around the world (1). Since each country or continent establishes an appropriate methodology to collect their own data, it becomes difficult to accurately compare the distribution of IBD around the globe. Even so, most studies agree that Western countries, which include mainly North America and Western Europe, are the main contributors to the burden of IBD, although other regions also present a growing number of cases (1).

In Brazil, Victoria et al. (2) conducted a study in the midwest of São Paulo State, in which incidence rates were 4.48 and 3.5/100,000, whereas the prevalence rates were 14.81 and 5.65/100,000 for UC and CD, respectively. Thus, IBD requires constant monitoring of public health agencies, and more accurate studies are necessary in developing countries in order to determine the need for intervention, since the global burden is imminent.

The specific trigger for the onset of inflammatory responses in IBD is not completely established, but the interplay among immune deregulation, environmental factors, and genetic polymorphisms contributes to the complex and multifactorial nature of this pathology.

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Genetic predisposition in IBD

Regarding the genetic influence on CD and UC, numerous polymorphisms have been identified and linked to the disease pathogenesis. Alterations in Toll-like receptors (TLRs) were related to IBD susceptibility (*TLR4* and *TLR9*) and extensive colonic disease (*TLR1*, *TLR2*, and *TLR6*), while genetic variations in nucleotide binding oligomerization domain (NOD)-like receptors (NLRs) led to an increased predisposition to CD (3-5). Indeed, polymorphisms in *NOD2*/caspase recruitment domain-containing protein 15 (*CARD15*) were the first genetic modifications linked to IBD onset (5). Thereafter, genome-wide association studies have shown about 100 allelic variants related to different susceptibilities to CD or UC (6,7). These include the NOD-like receptor family, pyrin domain containing 3 (*NLRP3*) inflammasome, autophagy-related 16-like 1 (*ATG16L1*), immunity-related GTPase family M protein (*IRGM*), and others, which are summarized in Table 1.

Intestinal microbiota and IBD

Along with genetic predisposition and environmental factors, microbiota is a key element in IBD pathogenesis, either as a mechanism that predisposes to or that protects against the development of intestinal inflammation. The direct relationship between altered microbiota and patients with CD or UC points to the microbial composition of communities resident in the gastrointestinal tract as one of the factors that could play a relevant role in the development and progression of IBD.

Accordingly, several studies have shown the role of specific and combined microorganisms in the severity of

gut inflammation. For example, a reduction in anaerobic bacteria commonly found in intestinal microbiota such as *Bacteroides* (*Bacteroidetes* phylum), *Eubacterium*, and *Lactobacillus* species (*Firmicutes* phylum) has been reported in IBD patients (14). In CD patients, there are fewer *Firmicutes* bacteria and a simultaneous increase in *Proteobacteria* (15). These patients also present a reduction in the *Clostridium leptum* group, particularly *Faecalibacterium prausnitzii* (16).

Some of these reports about microbiota alterations in IBD are related to genetic factors, as observed in the risk alleles of *NOD2* and *ATG16L1* genes that have been linked to ileum CD (17). The disease phenotype in patients with these risk alleles is intimately associated with shifts in microbial composition. Moreover, the genetic changes were also associated with abnormalities in Paneth cell function (18), localized mainly in the ileum. These cells secrete antimicrobial peptides and thus function as potential controllers of lumen bacteria. Recently, variations in the *FUT2* genotype were linked to substantial differences in the microbiota community composition and diversity (13). *FUT2* is a gene responsible for the presence of ABO histo-blood group antigens in body fluids as well as in the gastrointestinal mucosa, and its deficiency is linked to susceptibility to infection and disease, including CD (13). All of these studies point to a substantial loss of bacterial diversity as a dominant factor in IBD pathogenesis. This reduction is characterized as dysbiosis, which may be responsible for a shift in the homeostatic healthy flora to detrimental proinflammatory microbial species, which can later predispose to intestinal inflammation (19,20). The use of antibiotics also causes dysbiosis, providing further evidence for the role of microbiota in IBD pathogenesis. Indeed, the use of

Table 1. Examples of polymorphisms associated with inflammatory bowel diseases (IBD).

Gene	Putative changes in IBD	Polymorphism associated to	References
<i>NOD2/CARD15</i>	Impairment of pathogen recognition and α -defensin secretion	Susceptibility to CD	5, 8
<i>NLRP3</i>	Deregulation of IL-1 β synthesis	Susceptibility to CD	9
<i>ATG16L1</i>	Impairment of autophagosome formation	Susceptibility to CD and UC	10
<i>IRGM</i>	Impairment of the process of autophagy	Susceptibility to CD and UC	10
<i>PTPN2</i>	Impairment of the process of autophagy in IEC	Susceptibility to CD and UC	11, 12
<i>FUT2</i>	Impairment of the secretion of ABO antigens and alterations in gut microbiota	Susceptibility to CD	13
<i>JAK-2/STAT3</i>	Alteration in T cell activation?	Susceptibility to CD	11
<i>ICOSLG</i>	Alteration in T cell activation?	Susceptibility to CD	11
<i>CCR6</i>	Alteration in leukocyte migration and activation at the inflammatory site?	Susceptibility to CD	11

ATG16L1: autophagy-related 16-like 1; *CARD15*: caspase recruitment domain-containing protein 15; *CCR6*: chemokine (CC motif) receptor type 6; CD: Crohn's disease; *FUT2*: fucosyltransferase 2; *ICOSLG*: inducible T-cell costimulator ligand; IEC: intestinal epithelial cells; *IRGM*: immunity-related GTPase family M protein; *JAK-2*: janus kinase-2; *NLRP3*: NOD-like receptor family, pyrin domain containing 3; *NOD2*: nucleotide-binding oligomerization domain 2; *PTPN2*: protein tyrosine phosphate non-receptor type 2; *STAT3*: signal transducer and activator of transcription 3; UC: ulcerative colitis.

5-aminosalicylic acid or some antibiotics can suppress, but not eliminate, bacterial adherent biofilm in patients with IBD (21). However, some opportunistic bacteria like *C. difficile* can proliferate more than others during the use of antibiotics, causing disease that can range from mild diarrhea to colitis or toxic megacolon, and occasionally death, in patients who have received previous antimicrobial treatment (22).

Because commensal microbes produce numerous active metabolites, the microbiome is essential for several aspects of host health, such as metabolism, immune response, and physiology. Members of the Firmicutes phylum, which, as described earlier, are reduced in IBD (15), are known producers of short-chain fatty acid (SCFA) metabolites such as acetate and butyrate, which play an anti-inflammatory role in the gut (23). These are a preferred nutrient source for epithelial cells in the gastrointestinal tract, and their deficiency can induce a dysfunction, with subsequent disruption of the epithelial barrier. The commensal microbe-derived nutrient, butyrate, also plays an immune regulatory function, because it can drive the differentiation of regulatory T (Treg) cells *in vivo* and *in vitro* by inducing the acetylation of histone H3 in the promoter of Foxp3 locus (23). As described in detail later, the balance between regulatory and effector responses is crucial to the outcome of inflammatory diseases like IBD, and Treg cells expressing the transcription factor Foxp3 are one of the main regulatory mechanisms required for disease control. In fact, by inducing Treg cells, butyrate can ameliorate colitis in mice, caused by adoptive transference of CD4⁺ CD45RB^{hi} T cells into Rag1^{-/-} mice (23). Another bacterial intestinal product that potentiates Treg cell development in the periphery is propionate, a SCFA with epigenetic effects similar to butyrate, i.e., the inhibition of histone deacetylase (24).

Recently, a specific member of gastrointestinal microbiota was identified, the segmented filamentous bacteria (SFB), that is a critical inducer of Th17 cell proliferation in the intestine (25). It is known that this population of effector CD4 T helper lymphocytes is one of the major contributors to gut inflammation in IBD. In fact, mice expressing a human-derived antimicrobial peptide gene (*DEFA5*), showed a significant decrease in SFB, and, as a consequence, fewer interleukin (IL)-17-expressing T cells (26). Apart from the studies pointing to SFB in protection or inflammation, no definitive concern about their effect in IBD is totally clear. Indicative evidence for the role of this bacterial group during IBD is that mice deficient in aryl hydrocarbon receptor (AHR), known to develop a Th17 response, present a high prevalence of SFB, and, after administration of an antibiotic cocktail that targets these bacteria, the production of intestinal IL-17 is decreased (27). Altogether, these studies confirm the key importance of gut microbiota in the immunological balance that underlies the development of intestinal inflammation,

and they point to future and on-going novel approaches for IBD treatment based on manipulation of the host microbiome.

Immune aspects

Innate immune response in IBD

Since IBD is a chronic and relapsing inflammation of the bowel mucosa, it is traditionally established that both innate and adaptive immune responses play an important role in the disease pathogenesis, together with the predisposing factors described earlier.

The innate immune system is the host's primary response against foreign aggressor agents. The customary innate response comprises physical barriers (e.g., the epithelium that covers the intestinal mucosa), phagocytic cells, dendritic cells (DCs), natural killer (NK) cells, cytokines, inflammation-related proteins (the complement system, C-reactive protein), antimicrobial peptides (defensins, cathelicidins), and pattern recognition receptors (PRRs). Most, if not all of these innate immune system components are modified in IBD, leading to an uncontrolled inflammation of the intestinal mucosa.

The epithelial barrier is a physical defense that comprises intestinal epithelial cells (IECs), tight junctions, and a layer of mucus (Figure 1). The IECs are responsible for absorption of nutrients or ions in the gut and for protection against penetration of resident microbiota, which can activate specific receptors, the PRRs, first in the epithelial cells (28). The subsequent downstream signaling in IECs leads to proinflammatory protein synthesis that engages in cross-talk with the immune system. Moreover, the epithelial barrier is more sensitive and permeable in patients with IBD (29), and IECs may be susceptible to an increased rate of apoptosis or shedding that contributes to the decrease in intestinal defenses (30).

Regarding tight junctions, the reduction of occludins and other related proteins, along with the decrease in structural complexity induced by proinflammatory cytokines [e.g., tumor necrosis factor (TNF)], also increase the permeability of the epithelium and subsequently impair its function (31). The proinflammatory cytokine IL-6 also influences IEC permeability by enhancing expression of claudin-2 (32). In addition, patients with UC and CD showed different patterns of mucin expression, which may be increased or decreased depending on the affected intestinal region, thus leading to defective mucus production (33). At first glance, therefore, most components of the epithelial barrier are altered in some way during IBD. Further studies need to be conducted in order to investigate whether a single trigger can lead to these changes or whether the sum of the changes acts together in the development of the disease.

Once these barrier alterations increase the gateway to pathogens in the intestine, IBD patients become more

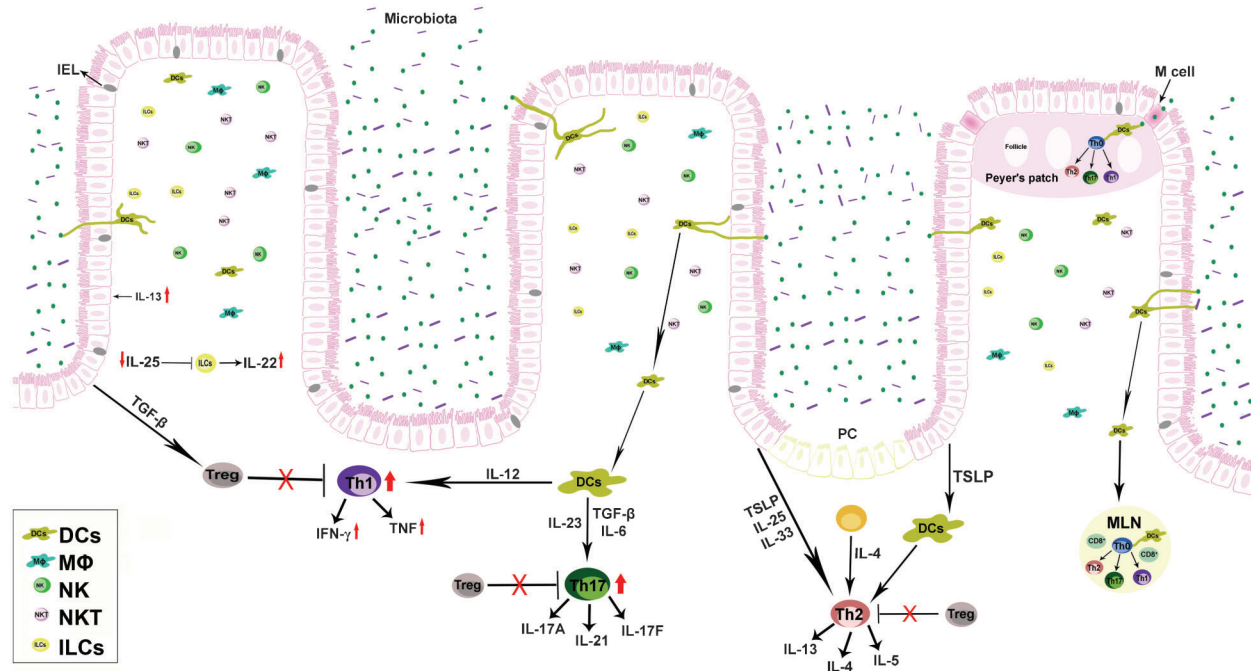


Figure 1. Intestinal immune response in the development of inflammatory bowel disease (IBD). The immune surveillance of the intestine is composed of several cell types, including dendritic cells (DCs), macrophages (M Φ), natural killer (NK) cells, natural killer T (NKT) cells, innate lymphoid cells (ILCs) and intraepithelial lymphocytes (IEL). All these components are altered in some way during IBD. The breakdown of immunological tolerance causes recognition of the intestinal microbiota by the local immune system, activating the inflammatory cascade. The physical/chemical barriers composed by IEL, Paneth cells (PC), microfold cells (M cells) or their products are impaired during the disease and fail to restrain bacterial invasion. In lamina propria (LP), DCs capture and recognize bacterial antigens by projecting their dendrites into the intestinal lumen between intestinal epithelial cells (IEC), while in the Peyer's patches (PP) the antigens become available to DCs through M cells. Afterwards, the LP and PP DCs migrate to the mesenteric lymph nodes (MLN) and present the antigens to naive T cells (Th0). Meanwhile, the PP DCs may also present antigens to Th0 cells located in the follicles. The antigen presentation in the presence of the cytokines interleukin (IL)-12 or IL-23/IL-6/transforming growth factor-beta (TGF- β) leads to polarization of Th0 cells into a pro-inflammatory Th1 or Th17 profile, respectively, which are increased due to low number or function of regulatory T cells (Treg). Th1 and Th17 cells secrete cytokines such as tumor necrosis factor (TNF), interferon γ (IFN- γ), IL17A, IL-17F and IL-21, which in turn contribute to tissue injury and excessive inflammatory response, especially in Crohn's disease. In addition, cytokines such as IL-4, IL-33, IL-25 and thymic stromal lymphopoietin (TSLP) directly or indirectly contribute to the differentiation of Th0 cells into Th2 profile in ulcerative colitis, which may also be under low control of Tregs. The red arrows indicate components that are altered in IBD. The red crosses indicate the absence or reduced function in IBD. It is noteworthy that cytokines and immune mechanisms depicted in the figure are shown according to the scope and information presented in this review. Thus, the mediators described here can also be synthesized from other cell sources as well as other immune pathways may contribute to IBD pathogenesis.

susceptible to bacteria translocation. Thus, antigens coming from the intestinal microbiota are available to gut resident cells [like antigen-presenting cells (APCs)] and constantly stimulate the immune system, which in turn enhances and extends local inflammatory activity (Figure 1). Then, because of the large amount of antigens supplied to APCs in the absence of intestinal homeostasis, the PRRs located in these cells, which may include IECs, are constantly activated, inducing the production of additional inflammatory mediators.

In the gut, excessive stimulation of TLRs located in different cell types like DCs, macrophages, neutrophils, NK cells, B cells, and others may lead to chronic inflammation. IBD patients have a different pattern of

TLR expression in IECs compared to healthy individuals, with increasing expression of TLR4 in UC and CD patients and decreasing expression of TLR3 in CD patients (34). Although presenting normal expression in IECs, TLR9 is unresponsive in IBD patients if challenged with cytidine-phosphate-guanosine oligodeoxynucleotides (CpG), a specific ligand for TLR9 (35), and this finding seems to be very relevant, because signaling by this receptor may be important to disease control. In addition, overexpression of TLR4 and TLR2 in intestinal macrophages during inflammatory conditions contributes to the hyperresponsiveness of the immune system (36). These data corroborate studies that observed the improvement of disease using the TLR4-specific antagonist in murine

models of IBD (37). Furthermore, polymorphisms in *TLR1*, *TLR2*, *TLR4*, *TLR6*, and *TLR9* were identified in CD or UC. While *TLR4* and *TLR9* are associated with increased susceptibility to IBD development (3,38), some of these changes (*TLR1* and *TLR2*) seem to be related to the disease magnitude or to IBD-associated comorbidities such as pancolitis onset (4).

As stated earlier, cytoplasmic NLRs are intracellular PRRs expressed in many cell types, including IECs or APCs. These receptors recognize peptidoglycans from the bacterial wall, activating intracellular signaling pathways such as NF- κ B (via kinase receptor-interacting protein 2) or inflammasome. Besides reducing the expression of α -defensins and other antimicrobial peptides by Paneth cells, polymorphisms in *NOD2* increase the susceptibility to luminal bacterial infection, while mutations in this molecule also interfere in autophagy and subsequent antigen presentation by DCs during IBD (5,8,39). Furthermore, the decrease in both number and proliferation rate of intestinal epithelial lymphocytes is observed in *Nod2*^{-/-} mice as well as dysbiosis, which contributes to increased risk and poor outcome of disease in experimental models of colitis (40,41). However, other studies showed augmented frequency of CD4⁺ Treg cells in the lamina propria of naive *Nod2*^{-/-} mice, thus partially explaining why polymorphisms in *NOD2* (in humans) or the absence of this molecule in *Nod2*^{-/-} mice are not sufficient, alone, to induce spontaneous intestinal inflammation (42).

DCs are essential to the control of immunity against pathogens and tolerance. They have a wide range of PRRs, including TLRs and NLRs, allowing them to distinguish between intestinal microbiota and pathogens. Thus, DCs may activate or silence T cell responses (Figure 1). In healthy individuals, DCs exhibit an immature phenotype and induce nonresponsiveness of T cells. Typically, DCs reach the intestinal lumen by projecting their dendrites out of tight junctions between epithelial cells (43). With the large amount of antigens supplied to DCs in the inflamed intestinal epithelium, these leukocytes acquire a mature and activated phenotype, modifying the pattern of surface molecules (e.g., chemokine receptors, adhesion molecules) and thus inducing effective immune responses (44). There are also several subtypes of DCs that can be subdivided according to their different functions, location, or surface markers. Besides CD11b and CD11c, other integrins such as CD103 and CX₃C-chemokine receptor 1 (CX₃CR1) are also used in conjunction, to identify and to sort different DC subtypes (44). In the gut, there are CD103⁺ DCs that are essential for the steady state of the intestinal mucosa and that finely regulate the number of effector and Treg cells in this tissue (45). However, DC subsets expressing E-cadherin are present in the intestine during IBD and are related to the inflammatory profile found in the disease (46). The number of DCs and other APCs also appear to be

increased in experimental colitis and in patients with IBD (44). Furthermore, DCs are more responsive to stimuli such as lipopolysaccharide in IBD and contribute to the exacerbation of the disease (47). In addition, monocyte-derived E-cadherin⁺ DCs play an important role in experimental colitis by producing cytokines such as IL-6 and IL-23, which in turn contribute to T helper (Th)17 cell development and colon damage (46). Apparently, TGF- β 1 may increase the inhibitory function of immature DCs in experimental colitis, probably by inducing CD4⁺Foxp3⁺ Treg cells (48).

Macrophages are another subset of APCs present in large quantities in body tissues, including the intestine (Figure 1). The function of these cells is to eliminate and present antigens to T lymphocytes, besides producing cytokines to recruit other immune cells to the inflammation site (28). Two major subtypes of macrophages have been described and they are commonly divided into inflammatory (M1) and anti-inflammatory (M2). In the gut, during intestinal homeostasis, the main population of macrophages displays a CX3CR1^{high} phenotype and is non-responsive to inflammatory stimuli, although their phagocytic capacity is preserved (49). However, in IBD, another subpopulation of macrophages, CX3CR1^{low/int}, expands due to the migration and differentiation of monocytes into the inflammatory site (49). This M1 macrophage overexpresses receptors [such as triggering receptor expressed on myeloid cells-1 (TREM-1) and PRRs] that amplify signaling for the synthesis of inflammatory cytokines (e.g., TNF, IL-1, and IL-6), which in turn exacerbates the local immune response (50).

The NK population can recognize injured cells and promote their death through absence of recognition for major histocompatibility complex class I. They may also produce cytokines, especially interferon gamma (IFN- γ), and influence the maturation of DCs. In IBD, NK cells have increased IL-21R expression, which results in greater action of IL-21 and proinflammatory cytokine production. This condition seems to polarize T helper cells to a Th17 profile (51). Recent studies have shown that there are two types of NK cells expressing the markers NKp44 or NKp46, and the ratio of these cells in the intestinal mucosa is altered in CD, thus contributing to disease progression (52).

Other leukocytes recently involved in IBD pathogenesis are the innate lymphoid cells (ILCs). They play a key role in tissue remodeling, combating microbes, lymphoid tissue development, and tissue homeostasis, including intestine (53,54). Similar to their lymphoid progenitors, ILCs are divided into subtypes based on the pattern of cytokines synthesized and on the expression of specific transcription factors (53-55). It is known that these cells play an important role in gut homeostasis as well as in the pathogenesis of IBD (Figure 1). Cytokines synthesized by both ILCs and lymphocytes (IL-17, IFN- γ) may contribute to disease development or control. Studies have shown

that IL-22-producing ILCs, which express AHR, are essential to restrain bacterial infection in the gut and are decreased in the intestine of AHR knockout mice, thus affecting mucosal immunity (55). Corroborating this finding, IL-22-producing ILCs are the main producers of IL-22 in the intestine, and this production is counterbalanced by IL-25 synthesis from IECs, which, in turn, is regulated by intestinal flora (Figure 1). Thus, when the intestinal barrier is disrupted, the fine interplay between commensal microbiota and the host is broken, with a subsequent increased IL-22 production (56). In addition, IL-22 protects the gut during inflammation in experimental colitis models (57). However, IL-23 causes colitis in mice by inducing ILCs to synthesize IL-17 and IFN- γ and to promote the infiltration of these cells into the colon (54).

Adaptive immune response in CD and UC

Besides the important alterations in the innate immune response, the predominant cytokine profile in IBD will also depend on which T helper profile develops and thus the adaptive immune response that arises during the course of disease (Figure 1). Initially, around the 1990s, it was recognized that CD and UC differed from one another mainly because UC appeared to be mediated by Th2 cytokines, with elevated production of IL-5, while CD was mediated by a Th1 profile, characterized by increased IFN- γ and IL-12 production, which activates the transcription factors STAT1 and STAT4, respectively (58). In fact, patients with CD have increased production of IL-12 by lamina propria cells compared to controls (59), and, to better understand IBD pathogenesis, different animal models are used, most of them with immune responses driven against antigens of the normal mucosal microbiota with IL-12 and IFN- γ production (60). In this context, the findings regarding participation of the Th1 response in IBD were reinforced by the fact that colitis induced by trinitrobenzene sulfonic acid (TNBS) or cell transfer in mice mimics CD and generates an exacerbated Th1 inflammation, with high levels of IL-12, IFN- γ , and TNF (57,60,61). In fact, colitis induced by TNBS is reversed by treatment with anti-IL-12p40 (60).

However, the concept that CD is mediated only by Th1 cells was recently reviewed after the description of the Th17 population that produces several proinflammatory cytokines such as IL-17A, IL-17F, IL-21, IL-22, and IL-26, which exert an important role in the development of IBD (62). Mice deficient in IL-17 or IL-21 have a milder colitis when compared to wild-type animals (63,64). Moreover, in the absence of IL-21, naive T cells from these knockout mice do not differentiate into Th17 cells (64). These data suggested that the immune response orchestrated by Th17 cells may amplify the inflammatory pathways during the development of intestinal inflammation (Figure 1). On the other hand, mice lacking IL-22 have severe colitis, with destruction of their gut architecture (57). The association of Th17 cells with IBD pathogenesis was also

characterized in cell-transfer colitis models. RAG knockout mice that were deficient in IL-23p19 (subunit present in IL-23 and absent in IL-12) did not develop colitis when transferred with naive T cells and had a significant decrease in the production of TNF and IFN- γ . Yet, when RAG^{-/-} animals were deficient in IL-12p35 (subunit present in IL-12 and absent in IL-23) and received naive T cells, they developed colitis and had a moderate increase in TNF, IL-17, and IFN- γ (65). Therefore, since IL-12 and IL-23 are essential for Th1 and Th17 differentiation, respectively, these results demonstrate the crucial involvement of Th17 cells in IBD pathogenesis. In addition, when SCID mice received antigen-specific T cells from wild-type mice, five times more IL-17 compared to IFN- γ was observed in the lamina propria of these animals (66), perhaps because these activated cells are more responsive to IL-23 than to IL-12.

Recently, it became clear that Th17 cells have certain plasticity and in an IBD context are not different, since these cells, when present in the inflamed gut of CD patients, can be characterized by IL-17⁺IFN- γ ⁻, IL-17⁻IFN- γ ⁺, or IL-17⁺IFN- γ ⁺. In addition, double IL-17 and IFN- γ producing T cells have different features from Th1 cells; they are able to produce IL-22 and CCL20 and lead to the expression, in epithelial cells, of antimicrobial genes resembling Th17 cells (67). However, IL-17⁺IFN- γ ⁺ cells are not stable, and under stimuli *in vitro* with IL-12 they may become IFN- γ single-producing cells with properties of the Th1 lineage (67). It should be reemphasized that Th1 cells are also highly pathogenic in IBD, especially CD.

As stated earlier, cytokines involved in UC are from the Th2 pattern of immune response (Figure 1). The main model to study UC is through intrarectal administration of the hapten oxazolone, which causes a mild colitis associated with superficial inflammation in the gut, resembling human UC (68). In this model, there are cells producing IL-4 involved in Th2 conditions, and treatment of colitis with anti-IL4 prevents disease, whereas administration of anti-IL-12p40 exacerbates the intestinal inflammation or has no effect on the disease (68). In addition, in the oxazolone-induced experimental model of UC, there is an initial production of IL-4 followed by a more prolonged response mediated by IL-13, which is responsible for the induction of the disease and is mainly produced by NK T cells (69). These observations prompted a search for CD1d-restricted NK T lymphocytes in the context of IBD and, surprisingly, a high frequency of type II NK T cells, which express a lot of different T cell receptors and cannot be identified because they do not respond to α -galactosylceramide, was found in the inflamed tissue of a patient with colitis. Furthermore, these nonclassical NK T cells were responsible for the elevated amount of IL-13 in the gut of patients, besides being cytotoxic for intestinal epithelial cells (70,71).

As mentioned previously, inflammation caused by

proinflammatory cytokines produced by CD4⁺ effector T lymphocytes can be regulated by Treg lymphocytes. These cells are responsible for suppressing the uncontrolled immune responses against commensal microbiota or dietary antigens, thus being of great importance for gut homeostasis (72). Defects in Treg function or in their ability to contain effector cells are related to IBD pathogenesis (Figure 1).

The main findings regarding colitis protection by Treg cells emerged from cell transfer colitis models to mice with impaired immune systems. When immune-deficient mice receive CD4⁺CD45RB^{high} naive T cells, colitis develops (73). The disease is counterbalanced by the transfer of CD4⁺CD25⁺ Treg cells, which protect against intestinal inflammation and result in the resolution of experimental IBD (73). In addition, the depletion of Treg cells *in vivo*, using an anti-CD25 antibody treatment, leads to worsening of intestinal inflammation in SAMP1/YitFc (SAMP) mice. Furthermore, there is proliferation of CD25⁻Foxp3⁺ cells, which do not have a regulatory function and presented inflammatory features (74). These observations provide evidence that the marker Foxp3 is not enough to indicate suppressor function of Treg cells.

The ability of regulatory T cells to control colitis is dependent on immunosuppressive cytokines such as TGF- β and IL-10 (75,76). Blockade of the TGF- β signaling pathway in murine T cells results in spontaneous colitis (77). In fact, some IBD patients present disorders in TGF- β signaling, caused by upregulation of the inhibitory molecule Smad7 (78), and in the lamina propria of these patients the Treg cell functions against effector T cells can be restored by a Smad7 antisense oligonucleotide (78). On the other hand, mice deficient in IL-10 develop spontaneous colitis driven by microbiota (79), while children with mutations that result in loss of function of IL-10 receptor developed enteritis (80).

The main evidence that defects in Treg cells leads to the development of IBD in humans came from studies in patients with X-linked syndrome (IPEX). In this syndrome, a primary immunodeficiency disorder is caused by a mutation in Foxp3, and these individuals have significant intestinal inflammation (81). In addition, patients with IBD have decreased Treg cells in the peripheral blood compared to healthy controls (82), or these cells have less ability to suppress autologous T cell proliferation (83). There is evidence that Treg cells from peripheral blood and intestine of IBD patients are more susceptible to apoptosis than those from non-inflamed colon tissues, and in patients treated with anti-TNF there is a decrease in Treg cell apoptosis together with an increase in the number of these cells and reduction in disease activity (84). Once again, there is no complete evidence that Treg cells are functional or not, because these studies did not take into consideration all cell types capable of being suppressed by Treg cells. In addition, *in vitro* suppression

assays usually do not contain all cytokines and immune mediators present in the original environment of the disease. Furthermore, it is noteworthy that several effector cells express Foxp3 and some Treg cells do not present this marker (85), so multiple studies could be reassessed to make sure that Foxp3⁺ cells have indeed a regulatory capacity that is lost during IBD.

Besides natural Foxp3⁺ Treg (nTreg) cells, which are derived from the thymus, there are many different types of regulatory lymphocytes that have been described, especially in the gut. Induced Treg (iTreg) cells are converted from naive CD4⁺ T cells in the periphery, in the presence of TGF- β (86). However, until now there have been no fully reliable markers that can distinguish iTreg cells from nTreg cells, and their mechanisms of action are very similar (86,87). Beyond that, in the presence of IL-10 or after repeated antigen presentation, naive CD4⁺ T cells can be differentiated into IL-10-producing type 1 regulatory T cells (Tr1). These CD4⁺Foxp3⁻IL-10⁺ cells (Tr1) are able to suppress both IL-17⁺IFN- γ ⁻ and IL-17⁺IFN- γ ⁺, producing T cells via cytokine-dependent mechanisms (mainly IL-10) in a colitis model (88). Moreover, it is known that the probiotic *Bifidobacterium breve* induced development of Tr1 cells that express IL-21, AHR, and cMaf in the gut, thus preventing intestinal inflammation (89). On the other hand, some bacteria like *Bacteroides fragilis* produce polysaccharide A that can prevent experimental colitis by suppression of IL-17 production by intestinal immune cells, and this effect is related to IL-10-producing CD4⁺Foxp3⁺ T cells (90).

Altogether, although T cells are seen as key drivers of intestinal inflammation, there must be an association of knowledge between innate and adaptive immunity for a better understanding of the complex and differentiated intestinal mucosal immune system, especially during IBD.

Concluding remarks

Despite efforts employed to clarify the etiology and pathogenesis of IBD, the multifactorial aspects of the disease make this a difficult task. As far as we know, genetic factors and intestinal microbiota alterations lead to increased predisposition to CD or UC, with a large impact on the immune system. On the contrary, the vulnerability of some immune compartments and hyperactivity of others in susceptible patients may also facilitate gut dysbiosis and later contribute to damage observed in the disease. Hence, it is reasonable to speculate whether there is a single or predominant trigger in IBD development or whether these several alterations are required, together and simultaneously, to promote the disease. Furthermore, the sequence of these occurring events is also not yet totally clear and thus, to fill this gap, future studies are necessary in order to achieve a deeper and more complete understanding of IBD pathogenesis.

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