

# Evolving paradigms in the pathogenesis of IBD

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**Abstract** The pathogenesis of all immune-mediated inflammatory diseases has been carefully studied over the past several decades, but it is only recently that we have come to appreciate common pathways and genes. This is especially true for the inflammatory bowel diseases (IBD) Crohn's disease and ulcerative colitis, where a keener appreciation of the contributions of genetics, environment, and immune response have been dissected. In fact, in many ways, IBD has become the model for studying such disorders. The complex nature of interactions is continuing to be defined, and novel therapies targeting defects in these interactions have been developed and are being tested in the clinic. The era of bench to bedside has finally matured, and cures for debilitating diseases are now in sight. This review describes our current state of knowledge of each component of IBD pathogenesis. What has evolved is a clearer picture and novel targets for therapy.

**Keywords** Inflammatory bowel diseases · Crohn's disease · Ulcerative colitis · Pathogenesis

## Introduction

Significant advances in the understanding of disease pathogenesis have led to a therapeutic revolution in inflammatory bowel disease (IBD). Importantly, a number of different factors have contributed to this unprecedented leap in our knowledge base (animal models, direct analysis of human diseased tissue, identification of disease

susceptibility genes, and the recognition of the role of environmental factors). The advances have come from a variety of sources: geneticists, microbiologists, epidemiologists, and mucosal immunologists reflecting the important input of various components to disease pathogenesis. These have contributed to the identification of major pathways and therapeutic targets and to the development of novel disease-modifying drugs. The results of the clinical studies represent, for the first time, a true proof of concept approach, where one can ask whether pathways or molecules identified via animal models or human tissues are truly important in the disease process *in vivo*.

When one looks at the disease process it can be divided into three phases—initiation, augmentation, and perpetuation/regulation. Both the innate and adaptive immune system contribute to the first two phases, while defects in regulation reflect alterations in adaptive immunity (Table 1). Agents that target the late phases of the initiation process (T-cell activation) and all phases of immunoregulation have a high likelihood of controlling disease activity. When one evaluates a target's potential for efficacy, one needs to characterize whether it is able to prevent a cell from getting to the site of inflammation, prevent the cell's activation once it gets there, or prevent the effects of cellular activation if the other two processes fail. Most therapies have been directed at the third component, since it is easier to target products of the immune system (anti-cytokines, anti-cytokine receptors). The results of these interventions have added further insights into disease pathogenesis (i.e., does intervention work or not, and if not, why not?).

Like all other immune-mediated inflammatory diseases (IMIDs), the actual development of disease reflects the contributions of host genetics, an environmental trigger, and the consequent immune response. Crohn's disease has been the poster child for the study of complex genetic

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**Table 1** Forms of Immunity: Implications for IBD

Forms of immunity	
Innate immunity	Adaptive immunity
Hard-wired	Requires priming
Immediate	Delayed or immediate
Cell bound receptors	Soluble and cellular
Pattern-molecular recognition	Antigen-specific
Macrophages, natural killer (NK) cells, non-professional antigen-presenting cells (APCs)	B and T-cells
Relationship to IBD: disease elements	
Initiation	Perpetuation
Augmentation	Augmentation
Effector cells	Regulation

Two forms of immunity exist: a more primitive innate form, recognizing bacterial and viral molecular patterns, resulting in a rapid but limited response and the adaptive form that is highly specific, demonstrates memory for previous exposures to pathogens but is slower to evolve. Each component of the immune system plays a key role in the pathogenesis of IBD. Here we propose where each might fit into the scheme of disease development

disorders, being the first disease in which a replicated genetic mutation was identified (NOD2/CARD15) [1, 2]. This success was followed by the identification of a number of additional genetic associations, including OCTN1/2 [3], MDR1 $\alpha$  [4], and most recently, ATG16L [5, 6] and IL23R [7]. Interestingly many of these genes encode molecules involved in innate immunity (see below) or epithelial barrier function (defective function). This is particularly important because the majority of the current biologic agents target effector molecules of the adaptive and not innate immune system or the epithelial barrier. Thus, these genetic links not only provide new clues to pathogenesis but also suggest novel therapeutic targets.

To understand these targets one needs to have a basic understanding of disease mechanisms. This is not an easy task as hypotheses of disease pathogenesis change and evolve. There are, however, basic concepts that have remained constant. It is clear that after we account for a genetic predisposition and environmental trigger, the culprit responsible for mediating the disease process is an altered immune response [8, 9]. In the normal intestine the immunologic tone is one of tolerance or suppression of immune responses against non-pathogens in the gut lumen. These non-pathogens include both commensal flora and dietary antigens. In IBD there is growing evidence that this tolerant state, at least to specific components of the flora, is lost (the presence of ANCA, ASCA, OmpC, and CBir serum antibodies is evidence for loss of tolerance) [10, 11]. Tolerance is mediated by cells that regulate immune

responses (regulatory cells) [12]. These may be ineffectively activated or maintained in IBD [13–15]. Hence, in the absence of regulation, an inflammatory response elicited by a (probably harmless) trigger is able to persist, resulting in an aggressive inflammatory reaction. While cellular constituents are responsible for the inflammation, it is more the products of the cells and the effects that they have on other local cell populations that give rise to what we see as the actual disease. Depending on the type of disease (Crohn's disease versus ulcerative colitis [UC]), the inflammatory response may be quite different, and even within Crohn's disease there may be multiple distinct pathways operating in different patients (the simplest example is fistulizing vs. inflammatory vs. fibrostenotic Crohn's [16]). Furthermore, there is increasing evidence that the inflammatory mediators may change with time, either as a result of therapy (anti-tumor necrosis factor [TNF]) or as part of the natural course of disease [17, 18]. This would suggest that a patient might lose response to a given agent with time, requiring them to move out of class for the next therapy. With these possibilities in mind let us look at the pathogenesis of IBD in its current state.

## Current paradigms for disease pathogenesis

### Genetics

As mentioned above, similar to all immune-mediated inflammatory diseases, the development of IBD reflects the interaction of at least three components; a genetic predisposition, an environmental trigger, and an unregulated or dysregulated immune response. Given the enormous advances in technology and understanding of the human genome, new genetic associations for IBD (more so for Crohn's disease) are being identified monthly. What we have learned is that Crohn's disease is more of a genetically regulated disease than UC [19–21], that environmental factors remain key (e.g., smoking) [22], that these diseases are multigenic likely accounting for disease heterogeneity [23], and that many of the genes identified to date support defects in innate immunity and epithelial barrier function [1–3, 5–7]. Just having a mutation in one susceptibility gene (e.g., CARD15/NOD2) is not a sine qua non for the development of disease. Indeed, in some populations the carriage rate in healthy controls for CARD15 mutations associated with Crohn's disease is 20% [24]. Thus, one cannot use the findings of a gene mutation for the purposes of genetic counseling or disease predictability. Interestingly, and as would be expected, having these mutations is associated with younger age of onset of disease and a more aggressive disease phenotype [25, 26] (Table 2).

**Table 2** Genetic mutations associated with IBD (partial list)

NOD 2/CARD15
Innate immunity
Enteric microbes
Intestinal barrier function
ATG16L1/IRGM1
Cell death
Autophagy
Bacterial clearance
IL-23 receptor
Bridging innate and adaptive immunity
IL-17/IL-22
IL-23 signaling pathway genes

IBD inflammatory bowel disease, IL interleukin

The same holds for some of the newer gene associations described; specifically the IL23R mutations and the ATG16L [6] mutation. The former is involved in the IL12/23 pathway of inflammation thought to be important in the pathogenesis of Crohn's disease (see below). The latter is associated with control of a process called autophagy, where cells under stress initiate intracellular programs that result in cell death. This process is thought to prevent the inflammation associated with necrotic cell death, and while we do not fully understand the functional consequence of the ATG16L gene mutation, failure to stimulate autophagy may promote inflammation [6].

Lastly, the defect in NOD2/CARD15 or OCTN1/II may affect the host's ability to localize and eradicate bacteria that gain entry to the host [3]. Failure to eliminate such triggers could result in aberrant inflammatory responses and, possibly more importantly, allow for persistence of antigens that are capable of triggering an adaptive immune response.

#### Environmental factors (initiation)

As alluded to above, it has become quite clear from genetic studies that genetics alone is insufficient to explain the development of IBD. Monozygotic twin studies show the concordance rate for disease being 40–60% [19, 20, 27, 28]. Since this number is not 100%, the environment must play a key role in the development of disease. Several triggers have been identified and there is strong evidence that no single factor or agent is responsible for the development of disease (e.g., *Mycobacterium paratuberculosis* [29]). Clinical observation has shown that agents that break the mucosal barrier are common triggers of disease. These include nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, and viral and bacterial infections. While smoking is a widely replicated risk factor for the

**Table 3** Environmental factors in IBD

#### Many distinct environmental triggers

“Barrier breakers”

Cessation of cigarette smoking (UC)

Initiation of cigarette smoking (CD)

Bacterial and viral infections

Drugs: NSAIDs, antibiotics

#### Bacteria clearly play a role in IBD

Gut flora indispensable to development of animal models of IBD

Treatment with metronidazole effective in fistulizing CD

Patients with IBD fail to show tolerance to their own flora

No evidence that a specific infection (e.g. *Mycobacterium paratuberculosis*) leads to the disease

Dietary contents may influence the type and function of the flora (e.g. aluminum, iron)

UC ulcerative colitis, NSAIDs nonsteroidal anti-inflammatory drugs, CD Crohn's disease

development of Crohn's disease, it has a protective effect for UC. The basis for this difference is unclear. Lastly, while diet and stress are not clearly linked to the development of IBD, there is a growing appreciation of the fact that diet alters flora or that components of the diet (e.g., metals) can alter the functional properties of the flora. Stress reactions have been shown to alter immune responses but the lack of reproducible quantitative data has left this potential trigger largely unexplored [30]. Importantly, different patient subpopulations express different genetic mutations. It is appealing to speculate that different environmental factors trigger disease in these different patient populations. Thus, “Crohn's disease type 1” may be the result of a bacterial infection in a patient with a NOD2/CARD15 mutation, while “Crohn's disease type 2” results from the effect of smoking in a patient with an ATG16L variant, etc. Understanding these connections will enable appropriate counseling in specific patient subpopulations (Table 3).

#### Immune response (augmentation)

There are two basic components to the immune response; the innate and adaptive response. Innate immunity is the more primitive form of immunity that lacks specificity and memory for pathogens that it has seen. It is responsible for the early initial immune response where localization and eradication of invading pathogens is critical to the host's survival [30, 31]. Adaptive immunity evolved because of the inability of the innate immune system to augment responses upon reinfection and the fact that larger pathogen loads easily overwhelm the innate response. Adaptive immunity is characterized by specificity and memory and is largely mediated by lymphocytes, T and B cells, that express antigen receptors on their surface [32]. Cytokines

produced by T cells in response to infection orchestrate an organized directed immune response that not only results in the eradication of an infectious agent but also gives rise to memory cells that prevent disease upon re-exposure to the offending microbe.

### Innate immunity

While the innate response was largely ignored for years because of its limited repertoire and lack of specificity, newer studies have strongly supported the fact that defects in innate immunity have significant consequences to the host and can alter adaptive immunity as well [33]. In fact, the innate immune response is largely responsible for paving the way for the adaptive response. In the intestine, innate immunity includes the epithelial barrier and phagocytic cells within the lamina propria (macrophages, dendritic cells [DCs], and neutrophils). Interestingly, patients exhibiting genetic defects in innate immunity (e.g., chronic granulomatous disease, Hermansky Pudlak syndrome) [34, 35] have an increased incidence of IBD. This correlation has led to the development of agents used to boost innate immunity (e.g., granulocyte macrophage colony-stimulating factor [GM-CSF]) as therapeutic agents in IBD [36].

### Adaptive immunity

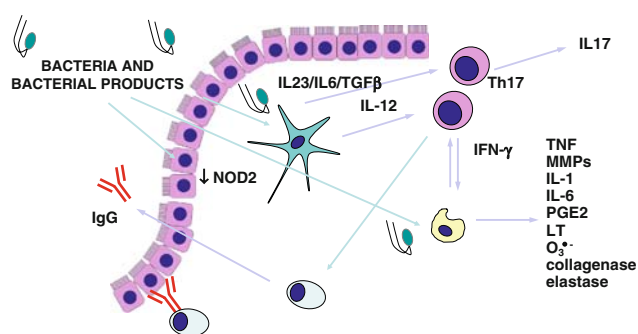
Despite the growing appreciation for the role of innate immunity in regulating immune responses in the gut, the adaptive immune system, specifically T lymphocytes, has been most tightly correlated with disease pathogenesis [37, 38]. T cells become activated, secrete cytokines and affect all other cell types within a local environment (macrophages, DCs, neutrophils, epithelium, endothelial cells, stromal elements). Both human and murine studies have led to the recognition that different T-cell populations are aberrantly activated in Crohn's disease versus UC [39, 40] and that even within Crohn's disease there may be distant subsets that drive the inflammatory response. Lastly, as alluded to above, there may be multiple pathways interacting simultaneously or changes in pathways as the disease evolves.

### Th1 versus Th2 versus Th17

Much to the chagrin of gastroenterologists, immunologists feel most comfortable categorizing populations of T cells as capable of secreting specific profiles of cytokines. The list of cytokines grows every year and many exhibit overlapping functions. What must be kept in mind is the fact that the immune response is plastic, changing constantly with environmental cues and that modest adjustments in either the timing, amount, or composition of cytokines in the

microenvironment can have profound effects on the visible immune response or immunopathology. This may be why some cytokines that were thought to play key roles in IBD pathogenesis were shown to be less relevant when targeted in human trials. Successful neutralization with specific agents (e.g., fontilizumab—anti-interferon [IFN] $\gamma$ ) had limited effects on disease activity [41] (Fig. 1).

Th1-mediated immune responses are typically evoked in response to an intracellular pathogen (e.g., virus, mycobacteria, histoplasma). The coordinated immune response elicited is meant to localize the infectious agent (e.g., form a granuloma) and secrete factors that either promote intracellular killing (IFN $\gamma$ , TNF $\alpha$ ) or induce the differentiation of cells that mediate killing (cytotoxic T lymphocytes). The hallmark of a Th1 response is the granuloma seen in purified protein derivative (PPD) skin responses (delayed type hypersensitivity reaction) or the granulomas seen in either mycobacterial infections (caseating) or Crohn's disease or sarcoid (non-caseating) [42]. T-cell activation occurs in the setting of an antigen presented by an antigen-presenting cell (APC; DC, macrophage, B cell). In the presence of interleukin (IL) 12 secreted by the APC, the T cell is now programmed to produce pro-inflammatory cytokines: IFN $\gamma$ , TNF $\alpha$ , and IL2 (under the control of a master transcriptional regulator Tbet [43]). These cytokines act on local cell populations to promote intracellular killing (superoxides, peroxides), enhance recruitment of other inflammatory cells (TNF increases vascular adhesion molecules such as Mad-CAM the target for  $\alpha 4\beta 7$ -expressing lymphocytes [44]), enhance secretion of chemoattractant cytokines, (chemokines), and promote local tissue destruction (induction of the secretion of collagenases, elastases, matrix metalloproteinases [45]). In the setting of an infectious disease, this



### 2 pathways (IL12 and IL23) can mediate inflammation

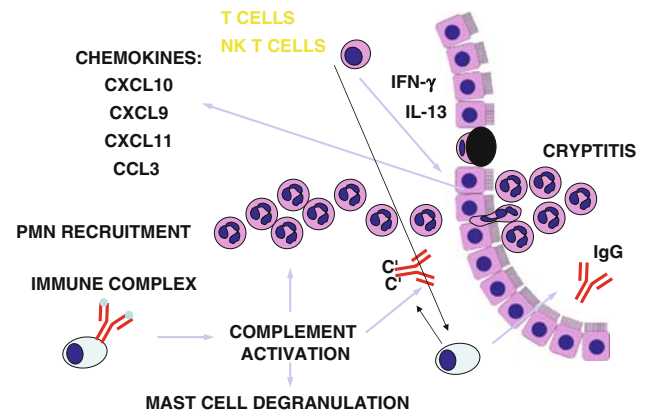
**Fig. 1** The pathogenesis of Crohn's disease relates to a genetic predisposition, an environmental trigger and an aberrant immune response. This latter response reflects defects in both innate and adaptive immunity. Innate immune defects account for altered responses to commensal flora including the production of pro-inflammatory cytokines IL12 or IL23 resulting in either IFN $\gamma$ /TNF $\alpha$  or IL17 production by T cells. These cytokines act on tissue macrophages to release tissue-altering enzymes that account for the disease expression. LT Lymphotoxin

response is meant to localize the infection and make the local microenvironment inhospitable for the pathogen. In the setting of a Th1-driven immune-mediated disease, the activation of this pathway is aberrant. IL12, composed of two chains—p40 and p35, is part of a larger family of diverse cytokines [46, 47]. IL12p40 is also used by another pro-inflammatory cytokine, IL23 (p40/p19 heterodimer) that affects a different T-cell population (Th<sub>17</sub>, see below). Blocking IL12 inhibits the generation of a Th1 response and blocks the production of the downstream cytokines (T-cell derived TNF $\alpha$ , IFN $\gamma$ , and IL2) although other cell types (natural killer [NK] cells and macrophages) can produce TNF $\alpha$  and IFN $\gamma$  and are not governed by IL12. Inhibition of IL12 is not without potential consequence. IL12 receptor and IL12-deficient families have been described and are prone to mycobacterial infections [48, 49].

Recently an additional Th subset has been described, Th<sub>17</sub> cells [50]. These cells produce IL17 and IL22, both pro-inflammatory cytokines capable of promoting local tissue destruction. TH<sub>17</sub> cells are activated by the combination of IL6 and transforming growth factor (TGF)  $\beta$  (stimulating the transcription factor ROR $\gamma$ t [51]) and are induced to survive and further differentiate to mature IL17-secreting cells by IL23. As mentioned above, IL23 shares the p40 subunit with IL12, so antibody against p40 results in the inhibition of both IL12 and IL23. IL17 and IL22 are found at increased levels in inflamed Crohn's mucosa suggesting that these cytokines may play a role in disease pathogenesis [52, 53]. This has clearly been shown to be the case in some animal models where selective inhibition of IL23 and not IL12 prevents mucosal inflammation. This shift in paradigm from Th1 to Th<sub>IL17</sub> is supported by the lack of effect of anti-IFN $\gamma$  in the treatment of Crohn's.

Another Th subset is the TH2 cell. These cells secrete IL4, IL5, and IL13 [54]. TH2 cells promote atopy with induction of IgE responses and eosinophil and mast cell activation. Initially, UC was thought to be a TH2-cell-mediated disease, but the absence of IL4 in UC tissues and the observation that both IL13 and IFN $\gamma$  are found at elevated levels in UC mucosa changed the dogma [55]. Recently one group [39] has suggested that the IL13 found in UC tissues comes from a non-T cell, an NK T cell, and that IL13 targets the epithelial cell to become dysfunctional [56]. Thus, UC may be more of a superficial epithelial injury disorder, while Crohn's disease may be more of a submucosal disease reflecting immune activation deeper in the tissue. The fact that infliximab has some salutary effect in UC further suggests that the aberrant immune response in this disease is more of a mixed picture [57] (Fig. 2).

Having made the point that there are specific T-cell subpopulations, the evidence for the sovereignty of these cells in humans is less clear-cut. Rarely is only one cell type activated in an immune response and there is always



**Fig. 2** The pathogenesis of ulcerative colitis relates to a genetic predisposition, an environmental trigger and an aberrant immune response. In contrast to the changes seen in CD, a different T cell subset is activated in UC. This subset produces IL13 as well as IFN $\gamma$  resulting in epithelial dysfunction, antibody production, and immune complex formation resulting in complement activation and mast cell degranulation. The effects of these activating events account for distinct pathologic findings and specific disease expression

plasticity in the response. For example, there are reports in humans of T cells producing both IL17 and IFN $\gamma$  [58], as well as others producing, IL10 and IFN $\gamma$  [59]. Equally as important, under certain conditions and microenvironmental cues, different effector cells may be recruited to the inflamed tissue. For example, CCR9+ CD4+ T cells are attracted to the inflamed small intestinal epithelium expressing the chemokine CCL25 (TECK) [60], while CXCR3+ memory T cells migrate towards inflamed colonic epithelium expressing IFN- $\gamma$ -inducible chemokines [61]. The classifications of T-cell subpopulations on the basis of cytokine secretion profiles are therefore helpful but not absolute. Furthermore, the findings described above underscore the point that an unregulated immune response of any type is poorly tolerated in the gastrointestinal (GI) tract.

#### Amplification of the immune response

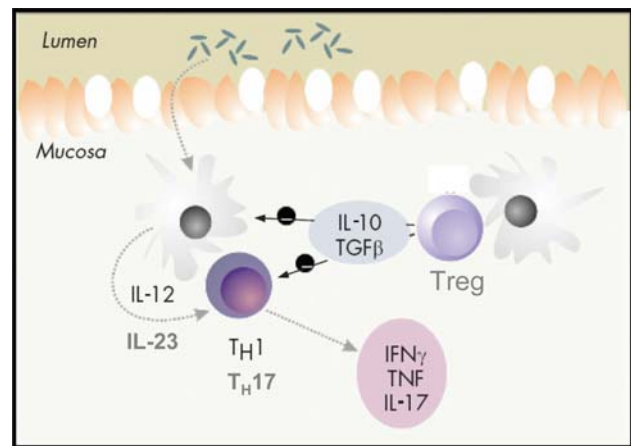
If the trigger/initiation of the inflammatory disorder is activation of the T cell, the amplification process reflects the downstream effects of this activation. T-cell derived cytokines act on cells in the local environment to produce chemokines that attract additional inflammatory cells into the local environment. These cells are regulated differently than cells that have grown up in the suppressed environment of the gut. They may be more easily activated and induced to secrete pro-inflammatory factors and tissue destructive enzymes. Since the variety of chemokines is great and redundancy overwhelming, inhibiting one chemokine is not likely to have a significant effect on the inflammation in IBD. A global chemokine inhibitor may, however, prove to be more effective.

Amplification is furthered by the enhancement of the expression of mucosal adhesion molecules (e.g., Mad-CAM1) [44] on the endothelium of vessels in the gut. Enhanced expression of addressins promotes the egress of cells from the peripheral blood. These too are less vulnerable to the rules and regulations guiding the activation of lymphocytes that are native inhabitants of the gut. Agents that block entry of cells into the gut would be predicted to have a beneficial long-term effect on inflammation. Since mucosal T cells from patients with IBD stay alive longer than normal mucosal T cells [62] and do not traffic to other sites, anti-adhesion molecule antibodies would not be expected to have an immediate effect on mucosal inflammation. In contrast, they would be expected to have a beneficial effect on maintaining remission. This appears to be the case in the clinical trials of anti-adhesion molecules performed to date [63].

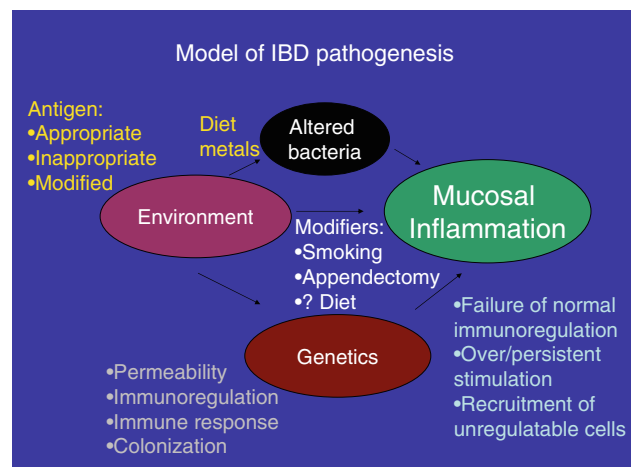
#### Perpetuation and regulation

##### Regulatory cells

The last and probably most critical pathway in the gut mucosa is the one involved in immune regulation. The immunologic tone of the GI tract, in contrast to the systemic immune system, is one of suppression. Normal individuals generally do not generate systemic immune responses against commensal flora or dietary antigens. Regulatory cells are responsible for the immunologically suppressed tone in the gut mucosa. Regulatory cells come in many flavors (T cells, B cells, DCs, macrophages, NK T cells), but the cell type that has received the greatest attention is the regulatory T cell [64, 65]. These too come in many varieties and are abundant and possibly redundant in the GI tract. Various CD4+ Tregs have been described as being involved in suppressing mucosal responses. Tr1 cells secrete IL10 [66], a potent immunosuppressive cytokine that may be responsible for suppressing responses to commensal flora. IL10-deficient mice develop a Crohn's-like inflammatory disease [67]. TH3 cells produce TGF $\beta$ , another potent immunosuppressant cytokine that promotes IgA production while suppressing T- and B-cell activation [68]. One of the most popular regulatory T cells is the CD4+25+ Treg [64, 65]. These are more systemic regulatory cells that require the transcription factor FoxP3 for their activation. The absence of FoxP3 in humans (IPEX syndrome) [69, 70] and mouse (Scurfy) results in autoimmune endocrine disease, immunodeficiency, and an autoimmune enteropathy (not colitis but small bowel pathology in a small subset of patients). FoxP3+ T cells are present in normal to higher numbers in IBD patients, suggesting that these diseases are not a result of defects in this population. Lastly, CD8+ T cells are regulatory in the gut as



**Fig. 3** Regulatory T cells are thought to be responsible for the controlled physiologic inflammation seen in the normal state. Several different Tregs exist in the GI tract including IL10 secreting CD4+ T cells (Tr1). TGF $\beta$  secreting T cells (TH3) and the contact dependent type, CD4+ CD25+ FoxP3+ Tregs



**Fig. 4** Model of IBD pathogenesis identifying the key elements (genetics, environment and immunity) along with subtle alterations that can occur in response to modifiers (cigarettes, metals, diet, etc.)

well. A number of different CD8+ Tregs have been described. Some of these (TrE cells) [15, 71] have been shown to be lacking in patients with IBD and may therefore be relevant to disease pathogenesis (Fig. 3).

A popular concept is that defects in Tregs allow for the perpetuation of active inflammation. While there are no direct studies supporting this concept, papers such as those cited above, as well as a documented defect in oral tolerance, in both IBD patients and a significant number of first-degree relatives, support this hypothesis [72, 73]. Thus, therapies directed at increasing the activation of Tregs would be of value in disease therapy.

### Tertiary inflammatory pathways

What has been focused on above relates to the activation and dysregulation of the immune response in IBD. However, what we actually see as the disease is the result of downstream events. These events are multiple and redundant and include tissue-altering enzymes such as collagenase, elastase, matrix metalloproteinases, superoxides, hydrogen peroxide, leukotrienes, and prostaglandins. Inhibiting any of these pathways can have a salutary effect on the inflammatory process, but if the upstream stimulus is not controlled, alternative tertiary inflammatory pathways can be activated and inflammation persists.

From the various sections described above it is clear that we have come to a point where we are able to dissect disease pathways (Fig. 4). Understanding each and every component of the inflammatory response has clearly aided our efforts in developing new approaches to disease management. Whether these translate into useful therapeutic tools can be determined only in direct human clinical trials.

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