Immune and non-immune components orchestrate the pathogenesis of inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) pathogenesis is driven by the interactions between the innate and the adaptive immune system. Both systems are actually expressed not only by immune cells, but also by essentially all types of non-immune cells. Non-immune cells have classically been considered as simple targets of the aberrant inflammatory process occurring in IBD. However, the discovery that many of the functions traditionally attributed to immune cells are also performed by non-immune cells has caused a shift to a multidirectional hypothesis in which non-immune cells and even acellular elements are considered active players of IBD pathogenesis. The aim of this review is to summarize the current role played by each cell type in IBD pathogenesis.
Introduction

The human inflammatory bowel diseases (IBD), Crohn’s disease (CD) and ulcerative colitis (UC), result from an inappropriate immune response to the microbiota of the gut, catalyzed by the genetic susceptibility of the individual. Genome-wide association studies (GWAS) have been used to identify susceptibility genes in patients with IBD, and have highlighted the multifaceted nature of disease pathogenesis (56). This approach has both confirmed the involvement of disease pathways identified through other techniques, and identified a role for other pathways that had not previously been recognized. Genes for barrier function as well as the innate and adaptive immune responses all play a role, but now new roles are being identified for pathways not classically considered in the study of IBD.

The gastrointestinal tract represents a particular challenge to the innate immune system, as it is both an important entry site for external pathogens and home to a wide variety of beneficial commensal bacteria. The intestinal immune system must therefore protect against infection, while averting a pathogenic response to the normal microbiota. It is hoped that uncovering the pathways used by the intestinal immune system to prevent immune pathology may uncover therapeutic approaches to a broad range of autoimmune and inflammatory conditions.

The importance of the traditional immune components of the innate immunity has received a great deal of attention in the study of IBD. The innate immune system response is mobilized by recognition of carbohydrate and lipid motifs on pathogens, known as pathogen-associated molecular patterns (PAMPs). These are recognized by pattern-recognition receptors, such as Toll-like receptors (TLRs) and Nod-like receptors (NLRs) (58). Unlike elsewhere in the body, the innate immune system in the mucosa of the gut defaults to a state of hyporesponsiveness, with an attenuated response to pathogens, and tolerance to commensal bacteria and food antigens. However, this attenuation and/or tolerance appears to be overturned in patients with IBD, who exhibit hyper-responsiveness to stimuli that would normally not induce an inflammatory response. This is supported by GWAS that have identified variants in the innate immune system genes NOD2, NCX5, IRGM and ATG16L1 as risk factors in patients with CD (10).

Although dysfunction in the innate immune system is essential to chronic inflammation in
patients with IBD, it is also linked with dysfunction in the adaptive immune system (25).

Indeed, many of the overt manifestations of IBD are associated with the adaptive immune system like, for example, the consistent presence of heavy infiltration of activated CD4+ lymphocytes in the inflamed mucosa of patients with CD and UC. Abnormalities in regulatory pathways control both the innate and the adaptive pathways, and components of the innate immune system, including dendritic cells (DCs) and various cytokines, determine the type of adaptive T cell response that predominates during the inflammatory process (10).

Finally, a more recent body of evidence has implicated non-immune components in the pathogenesis of IBD. Studies of environmental factors, the intestinal microbial flora, the tissue response and the underlying genetics have identified a significant contribution of non-immune cells to the pathogenesis of IBD. Epithelial, endothelial, mesenchymal, nerve and vascular cells, platelets and the extracellular matrix all play a role (13), and can perform many of the functions traditionally attributed to classical immune cells. In particular, they secrete or express on their surface molecules involved in the immune response, particularly those of innate immunity (13).

Given the involvement of so many different cellular systems in what is clearly a highly integrated pathophysiological network, it will be necessary for us to understand how they all interact if we are to fully understand the mechanisms underlying the pathogenesis of IBD (Figure 1).
Cell types involved in innate immunity

Dendritic Cells

In the intestine and associated lymphoid tissues, dendritic cells (DCs) have been implicated in both the maintenance of tolerance towards the commensal microflora and dietary antigens, and in the generation of protective immune responses against pathogens. DCs are able to perform these dual and apparently contrasting roles by sensing and then responding to the local environment, allowing it to shape the ensuing immune response, and thereby responding to the normal microbiota without causing pathology (43). As our understanding of the mechanisms underlying this duality of function improves, we are beginning to learn the types of signals that mediate the different roles both in the steady state and under conditions of chronic inflammation, such as those present in IBD.

DCs sense the intestinal luminal environment both by recognizing luminal antigens that are transported into the lamina propria, and by directly sensing the luminal content. Antigens reach DCs in the mucosa through several mechanisms, including apoptosis of epithelial cells, and through specialized epithelial cell present in the follicles of Peyer’s patches, microfold (M) cells, which transcytose antigens from the lumen to the mucosa. In addition, DCs directly sample the intestinal lumen by extending dendrites between intestinal epithelial cells and into the intestinal lumen (44). Such extensions are visible under steady state conditions (8), but their frequency greatly increases in the presence of invasive bacterial species (8; 43; 44).

Migration of DCs from the lamina propria to the draining mesenteric lymph nodes (MLNs) occurs at a low level but in a constitutive manner. These DCs deliver antigens from commensal bacteria and apoptotic epithelial cells to the mesenteric lymph nodes (MLNs) (23; 34), where they interact with B and T cells to initiate a tolerogenic response. In particular, intestinal DCs promote the generation of T regulatory cells (Tregs) in the periphery, which suppress the function of effector T cells. Generation of Tregs could also reflect the diversion of naive T cells that are strongly reactive to innocuous antigen to the Treg-cell lineage, which would prevent them from subsequently inducing pathology (43).

When pathogens are present, the trafficking of DCs to the MLNs is enhanced. The activated
DCs orchestrate a protective immune response, inducing effector cells and determining whether a T helper (Th) 1, Th2 or a Th17 response will predominate (9). This behavior can also be induced by a pro-inflammatory milieu, where they encounter cytokines such as IL-1 or TNF-α (9).

In patients with IBD there is a failure to tolerize to the commensal microflora, leading to an exaggerated immune response (17). Accumulation of DCs is found at sites of inflammation, attracted by the upregulation of chemokines or addressins, for which DCs possess receptors (3). As a correlate of this high level of recruitment of DCs into the gut, DCs are relatively depleted in the peripheral blood of patients with active IBD (3).

In addition to reacting inappropriately to phagocytosed antigens, intestinal DCs may also receive inappropriate signals from intestinal epithelial cells (IECs) during gut inflammation. IECs isolated from patients with CD do not express thymic stromal lymphopoietin, and fail to control the DC-mediated proinflammatory response (45), resulting in abnormal release of IL-12 by DCs, which then drives Th1-type inflammatory responses (45). NOD2 expression on the surface of DCs may also play an important role in their response to bacteria, as DCs derived from patients who have a mutated form of the NOD2 gene have an impaired ability to induce expression of IL-17 in response to bacterial muramyl dipeptide (MDP) (54).

Evidence from animal models also supports an important role for DCs in chronic intestinal inflammation. Similarly to observations in humans, in murine models of colitis there is accumulation of activated DC throughout the lamina propria and MLNs (51). Several lines of evidence support the hypothesis that interactions between these activated DC and the intestinal microbiota are important in the pathogenesis of colitis in these animals. Animal studies focusing on early events of inflammation have identified DC and T cell aggregates, and associated T cell proliferation, with the degree of expansion proportional to the severity of intestinal inflammation, supporting a role for DC priming or restimulating pathogenic T cell responses (33). These data suggest that dysfunctional DCs may prime effector T cell responses to commensal antigens.

Macrophages
In the intestine, macrophages are found directly underneath the epithelium in the lamina propria, where they interact with and phagocytose luminal microbes immediately after they breach the epithelial cell layer (48); for example, they are highly effective at killing phagocytosed enteric bacteria such as *S. typhimurium* and *E. coli* (50). However, although intestinal macrophages efficiently eradicate bacteria that have entered the lamina propria, unlike macrophages from other body compartments, they do not necessarily initiate an immune response. In addition, the lamina propria macrophages have a unique pattern of surface marker expression that reflects the fact that they do not function as professional antigen-presenting cells (APCs), with low expression of co-stimulatory molecules (49), the Fc receptors for IgA and IgG, the complement receptors, and the integrin α2β1 (49; 50). They also do not produce inflammatory cytokines in response to PAMPs or cytokines, or following phagocytosis of necrotic cells (48).

The functional properties of intestinal macrophages are determined by soluble factors, for example TGF-β and IL-10, produced by a wide variety of cell types, including epithelial cells, subepithelial myofibroblasts, fibroblasts, lamina propria lymphocytes, and intraepithelial lymphocytes (35). Macrophages are also involved in tolerance, by inducing T cells to become anergic or differentiate into Tregs, and can influence whether the adaptive immune response will be of a Th1, Th2 or Th17 nature (36).

In contrast to the physiological conditions above described, in patients with IBD, there is a full rather than an attenuated response by macrophages to phagocytosed antigens. This is accompanied by increased numbers of macrophage in the inflamed mucosa, which appears to be at least partially the result of the arrival of circulating monocytes that migrate into the intestinal mucosa and, unlike resident macrophages, mount a rapid response to luminal microbial antigens. At this stage, many of the phenotypic and functional characteristics of the macrophages in the inflamed intestinal mucosa differ from those under physiological conditions. For example, these macrophages express T cell co-stimulatory molecules such as CD40, CD80, and CD86 (46), and the PAMP receptors TLR2, TLR4, CD89, TREM1, and CD14 (49). In particular, aberrant CD14 expressing macrophages from the mucosa of patients with IBD overexpress IL-12 and IL-23 *in vitro* in response to microbial simulation (27), and become an important source of TNF.
Studies in humans and animal models of IBD support a role for dysregulated macrophage-induced immune responses to the intestinal microbiota in the pathogenesis of IBD (28). Mutations in autophagy genes are associated with CD, and macrophages from patients with such mutations have an impaired ability to eradicate intracellular bacteria (31). Associated mutations include single nucleotide polymorphisms in the autophagocytic genes ATG16L1 and IRGM, and the phagosomal gene NCF4 (58).

The IL-10 pathway is an important controlling element for the role of macrophages in colitis, as mice in which Stat3 is selectively disrupted have impaired IL-10 signaling in macrophages, and spontaneously develop colitis (53). Similarly, mice lacking expression of IL-10 spontaneously develop colitis, which is a consequence of preferential differentiation of macrophages into proinflammatory subsets that produce large amounts of IL-12 and IL-23 (28). Depletion of macrophages in IL-10−/− mice prevents the development of colitis (28).

**Cell types involved in adaptive immunity**

Dysfunction of the innate immune system induces functional abnormalities of the adaptive immune system, and this underlies many of the characteristics of the chronic inflammatory processes in IBD. In patients with IBD, there is heavy infiltration of activated CD4+ T lymphocytes into the inflamed mucosa, in response to an inflammatory environment characterized by enhanced production of chemoattractants. Moreover, in murine models of colitis as well as in patients with IBD, blockade of the activation or activity of CD4+ T cells can stem the ongoing mucosal inflammation. There is also evidence that these mucosal CD4+ T lymphocytes exhibit both enhanced cell cycling (52) and resistance to apoptosis. In this context, studies in mouse models of IBD and humans have shown that blocking IL-6, which enhances T-cell apoptosis, has the downstream effect of inhibiting mucosal inflammation (24).

The CD4+ T cells are composed of four main subgroups: three sets of helper T cells, i.e., Th1, Th2, and Th17; and Tregs, and each secrete characteristic types of cytokines. These subgroups appear to be differentially involved in IBD, with increased production of the Th17 cytokine IL-17 and the Th1 cytokines IFN-γ and TNF-α in the intestinal mucosa of patients
with CD (30), while UC is also generally marked by an increase in IL-17, but Th2 cytokines predominate instead of Th1 (22; 30). In particular, a role for the Th17 subgroup in both forms of IBD has been indicated by the involvement of polymorphic variants of Th17 cell function in both CD and UC (2).

The important role of DCs in differentiating between UC and CD is underlined by the fact that DC cytokines influence the predominance of the different subgroups of CD4+ lymphocytes. IL-27 appears to be essential in the Th2-associated immune-inflammatory response observed in UC (41), and IL-27-driven inflammation is mediated by direct stimulation of invariant NKT cells, a cell type that has recently been suggested to be involved in the epithelial cell damage seen in both UC. Importantly, deficiency of IL-27 in animal models of colitis results in a Th1 response characteristic of CD (40).

On the other hand, IL-12 and IL-23 are highly related heterodimeric cytokines that share one subunit, but while IL-12 (p35 and p40 heterodimer) supports Th1, IL-23 (p19 and p40) supports Th17 (32). In the gut of patients with CD high levels of IL-12 are produced, likely driving the Th1-cell polarization observed in these patients (37). Further support for a role for Th17 in both UC and CD comes from GWAS, which have detected a strong association of IL-23R polymorphisms with CD and UC (18), and polymorphisms of the shared IL-12/IL-23 subunit have also been associated with both forms of IBD (20; 21; 42).

In addition to subgroup-polarizing stimuli, cytokines appear to drive and maintain ongoing T helper cell responses (38). IL-21 augments the proliferation of CD4+ T lymphocytes and regulates the profile of cytokines secreted by these cells (29). Analysis of IL-21 protein in biopsies of patients with IBD and controls showed that IL-21 is overproduced in the inflamed intestine of patients with CD, with CD4+ T cells infiltrating the mucosa as the main cellular source of IL-21. IL-21 may therefore be part of a positive feedback loop that expands and maintains the ongoing Th1 cell response (38). Enhanced IL-21 production is also seen in mucosal samples taken from patients with UC, and although it has not been demonstrated to enhance Th2 cytokine production, nonetheless this may indicate that it maintains T cell activation regardless of the polarized pathway used.

**Involvement of non-immune cells in IBD pathogenesis**
**Intestinal epithelial cells**

Serving as the barrier between the luminal contents of the gut and the mucosa, the epithelium prevents the majority of the food antigens and bacteria from coming into contact with components of the intestinal immune system. However, it is becoming clear that the epithelium also plays a more active role in innate immunity. Involvement of epithelial cells in sampling of the luminal contents, and subsequent tolerance or removal of pathogens has been indicated by their surface expression of pattern recognition receptors, including TLR and NOD family members. The importance of this to disease pathogenesis is gradually being elucidated. In addition, M cells are a specialized type of epithelial cell that are found adjacent to Peyer's patches and lymphoid follicles. They are structurally different from normal intestinal epithelial cells, with several adaptations that facilitate luminal sampling, including reduced mucin secretion, and modified apical and basolateral surfaces that promote uptake and transport of luminal contents to professional APCs.

Gut epithelial cells also function as APCs, constitutively expressing class I, II and non-classical MHC molecules on their surface. In particular, surface expression of these MHC molecules is upregulated in response to pro-inflammatory stimuli, and greater expression can clearly be observed in inflamed areas of IBD intestine compared with in healthy areas. This is also the case in patients with CD, in whom MHC class II molecules are elevated at the basolateral surface of the intestinal epithelium, and in the late endosome (7). This is particularly strategic as the late endosome is the site where they would intersect with antigens, and the basolateral surface is where they would be presented. Epithelial cells also express co-stimulatory molecules that, along with the MHC class II molecules, can stimulate or inhibit T cell responses (12).

A bidirectional interaction between epithelial cells and immune cells has been demonstrated, as Th2 cytokines reduce the expression of TLRs by epithelial cells (39). In addition, turnover of epithelial cells is increased in patients with UC, while the regenerated epithelium is composed of less differentiated cells. This may be a demonstration of the importance of interaction between the epithelium and cells of the immune system under steady state conditions, as a study has demonstrated that direct cell-cell contact with lamina propria
lymphocytes promoted differentiation and maturation of epithelial cells (11).

The epithelium therefore clearly functions as far more than just a barrier, but rather plays an active role in tolerance and immune responses in the epithelium. Alterations to its physiological functions might therefore lead to the development of IBD.

**Intestinal endothelial cells**

The intestinal microvasculature is central to inflammation, playing many key roles in both the initiation and perpetuation phases. Leukocytes enter the interstitial spaces of the gut by migrating through the endothelium, and in turn the endothelium is key in governing the number and type of leukocytes that can infiltrate into the gut (15). During active inflammation, the leakiness of the endothelium increases, with increases in adhesion of leukocytes, promotion of coagulation, and concomitant angiogenesis (14; 16). Under these conditions, cytokines and other inflammatory mediators induce changes in the pattern of expression of molecules on the surface of the vascular endothelium that enhance interactions with leukocytes, and their subsequent recruitment (14; 16).

In patients with IBD, leukocyte adhesion to the microvascular endothelium of the gut is significantly enhanced. Indeed, when cultured vascular endothelial cells are isolated from chronically inflamed areas of the intestine of patients with IBD, leukocyte adhesion is much greater compared with uninflamed areas from the same patients, or from control individuals (4; 5). This correlates with enhanced expression of leukocyte homing molecules on the surface of microvascular endothelial cells in the gut (6).

Although the importance of the vascular endothelium to inflammation has been well characterized, on the other side of this coin is the lymphatic endothelium, through which leukocytes exit the interstitial spaces and enter the lymphatics as an important step in the resolution of inflammation (47). The lymphatic endothelium therefore plays a distinct but equally important role as the vascular endothelium, and almost certainly warrants the same level of attention by investigators. Given the essential role played by the lymphatics in the resolution of inflammation, it is possible to envision a critical role for dysfunction of the lymphatic vasculature in the development and/or maintenance of diseases of chronic inflammation such as IBD. However, investigation of the role for the lymphatics at a molecular and cellular level has only recently gained attention, and such a role has yet to be
clearly elucidated. It is therefore noteworthy that, since as early as the 1930s, pathologists
have reported that the fundamental alteration in the mucosa of patients with CD is consistent
with chronic lymphangitis (55). Then in the 1970s it was demonstrated that obstruction of the
lymphatics of the small intestine in rats and pigs could generate fistulizing intestinal disease
that shared many characteristics with CD (26; 55); indeed these models are felt to more
closely resemble CD in humans than any one generated subsequently.

The decoy receptor D6, expressed on the surface of the lymphatics, is a critical
controller of experimental colitis. In addition, D6 is upregulated on the lymphatic endothelium
and intestinal leukocytes of patients with IBD (57). D6 expressed on the lymphatic
endothelium of mice appears to exert a protective effect, again supporting a role for the
lymphatic system in the control of intestinal inflammation through the decoy receptor D6.

**Fibroblasts**

In patients with CD the prolonged and chronic nature of the mucosal injury is also a function
of dysregulation of the mechanisms that mediate repair of the mucosa under physiological
conditions (19). As a result of this, there is excessive deposition of collagen, leading to the
formation of fibrotic strictures. Collagen and fibrogenic factors are produced by myofibroblasts
and fibroblasts in the lamina propria, which also produced increased levels of matrix
metalloproteinases (MMPs) in response to pro-inflammatory stimuli. These MMPs cleave
multiple components of the extracellular matrix and are under the control off tissue-specific
inhibitors. However, the excessive production that occurs in patients with IBD likely upsets
this equilibrium, contributing to the tissue damage and mucosal remodeling in these patients.

Due to their close juxtapositioning to epithelial cells, colonic sub-epithelial myofibroblasts
(SEMFs) are important in the regulation of the proliferation and differentiation of epithelial
cells, in addition to influencing the extracellular matrix composition around the epithelial cells
(1). Importantly, IL-17 regulates various immunologic functions of SEMFs, which may
therefore be involved in the pathogenesis of IBD. This includes the secretion of a wide
spectrum of pro-inflammatory and pro-fibrotic factors. SEMFs also express TLRs,
upregulation of which has been demonstrated in response to pro-inflammatory triggers, and
stimulation of which again triggers expression of inflammatory factors.
Conclusions

As it becomes increasingly clear that chronic inflammation is a highly integrated process, a sea change in the way we consider and study the underlying mechanisms is necessary. Indeed, it is now apparent that all cell types can exert immune-like properties, supplementing, interacting with and controlling the professional immune-specialized cells and mediators. Our perception of the division between innate and adaptive immunity is changing in a similar manner, since virtually all cell types can be involved in innate immunity and directly or indirectly modulate adaptive immune responses. In addition to the cell types discussed above other elements are also involved, including neutrophils, NK cells, platelets and the extracellular matrix. When all these elements are taken together, it appears that in both the normal and inflamed mucosa there might be a relative dominance of innate compared to adaptive immune responses (Figure 1). This speculative conclusion needs additional supporting evidence, as our understanding of the relative regulatory and effector function of each cell type is yet to be fully reached. Part of the problem in reaching a more full understanding of mucosal responses in health and disease is how it has been approached until now. In fact, thus far investigators have studied single cell type types in isolation, or even one system at a time. However, if we are truly to understand the full complexity of a mucosal biological response, particularly in inflammatory conditions like IBD, it will be essential to perform studies in a far more integrated fashion, and in particular analyze the cross-talk between different cells and systems (“integration biology”). Understanding the ways in which the cells interact with each other, and which molecules mediate the cross-talk between them, will likely result in new targets for therapeutic intervention in patients with IBD.

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**Figure legend**

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Immune-non-immune interactions in the gut. Several locally resident cell types orchestrate IBD pathogenesis through complex reciprocal cross-talks mediated by both the innate and the adaptive immune system.
Epithelial cells
Non-immune cells

Dendritic cells
Macrophages
Neutrophils
NK cells

Innate immunity
IBD
Adaptive immunity

T cells
B cells

Platelets
Endothelial cells
Mesenchymal cells
Extracellular matrix

Non-immune cells