Dysregulation of Intestinal Mucosal Immunity: Implications in Inflammatory Bowel Disease

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The mucosal interstitia of the intestine and colon are continuously exposed to large amounts of dietary and microbial antigens. Fortunately, the mucosal immune system has evolved efficient mechanisms to distinguish potentially pathogenic from nonpathological antigens. There are, however, situations in which this immune regulation fails, resulting in chronic gut inflammation.

The intestinal mucosal immune system is an important location where decisions regarding host responses to enteric antigens are made. For example, the mucosal immune compartment must be able to choose the appropriate effector function (e.g., tolerance vs. clearance) necessary to deal with each encountered antigen whether it be innocuous or pathogenic in nature. This decision-making process is a complex interplay between several cell types present in the mucosa (e.g., T cells, B cells, dendritic cells, etc.). Specific subsets of CD4+ T lymphocytes play a major role in mediating and regulating these effector functions in vivo and are called T helper (Th) cells. On the basis of their patterns of cytokine production, murine and human Th cells may be divided into several additional subsets termed Th1, Th2, Th0, Th3, Thp, and Tr1 cells. Activated Th1 cells secrete interleukin (IL)-2, interferon (IFN)-γ, and lymphotoxin-α, whereas Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. Th0 cells synthesize and release both Th1 and Th2 cytokines, whereas Th3 cells produce large amounts of transforming growth factor (TGF)-β. Evidence is accumulating to suggest that both Th1 and Th2 cells are derived from a common IL-2-secreting precursor T cell termed Thp. There is also good evidence demonstrating that macrophage- or antigen-presenting cell (APC)-derived IL-12 is crucial for inducing Th1 differentiation, whereas IL-4 is required to promote the differentiation of Thp cells into Th2 cells. A number of different studies have shown that Th1 cells are involved in an immune-mediated inflammatory response termed delayed-type hypersensitivity (DTH) or type IV hypersensitivity. This type of cell-mediated immunity is the primary defense against certain infectious agents such as intracellular bacteria, fungi, and protozoa. This protective immune response involves not only activation of Th1 cells and the subsequent release of their cytokines but also involves Th1 cytokine-mediated activation of macrophages and other phagocytic leukocytes to release additional proinflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-1β, IL-6, IL-8, IL-12, and IL-18. On the other hand, a Th2-type response is required for humoral immune responses (i.e., antibody production) and offers effective protection against extracellular pathogens such as helminths.

A recent study by Groux and co-workers (5) has characterized a new subset of CD4+ T cells that differ substantially from the classic Th1 and Th2 cells. These cells are generated by repetitive antigenic stimulation of mouse or human CD4+ T cells in the presence of IL-10 and are termed T regulatory-1 (Tr1) cells. These Tr cells are immunosuppressive in different models of immune-mediated inflammation. Indeed, Tr1 cells are capable of attenuating established colitis. Whether Tr1 cells act to inhibit the activation, recruitment, and/or effector functions of pathogenic leukocytes remains to be established.

As stated above, DTH is characterized by the production of several T cell- and macrophage-derived proinflammatory cytokines. Because many of these mediators may have devastating effects on surrounding tissue, DTH is tightly regulated to ensure efficient destruction of invading pathogens while minimizing injury to the surrounding tissue. There are, however, situations in which this immune regulation fails and severe inflammatory tissue injury may develop. This regulatory failure is thought to precipitate the onset of inflammatory bowel disease (IBD). To fully appreciate the molecular and cellular events that contribute to chronic gut inflammation, a brief overview of the mechanisms involved in immune control of intestinal inflammatory responses is provided.

Mounting an effective immune response against antigens within the intestinal and/or colonic interstitium involves the selective migration (i.e., homing) of naive T cells from the peripheral circulation into the Peyer’s patches or mesenteric lymph nodes draining the gut. This selective and very efficient extravasation process is accomplished by the specific interaction between T cell-associated L-selectin and its ligand (glycosylation-dependent cell adhesion molecule-1) located on specialized endothelial cells in lymphoid tissue called high endothelial venules. In addition, naive T cells may home specifically to the gut mucosa via the interaction of T cell-associated α4β7-integrin and endothelial cell-associated mucosal addressin cell adhesion molecule-1 (MAdCAM-1). If the naive T cell encounters its specific antigen presented on the surface of APCs in association with the major histocompatibility complex class II (MHC II), the T cell will bind the MHC II-antigen complex via its T cell receptor (TCR) complex and become activated. Activated T cells proliferate, shed their L-selectin, and increase surface expression of additional adhesion molecules such as LFA-1 (CD11a/CD18), very late antigen (VLA)-4 (α4β7-integrin), VLA-5, VLA-6, and CD44. The progeny of these activated T cells may then differentiate into effec-
tor (e.g., Th cells) or memory cells. Unlike effector cells, memory cells may exist for long periods of time (20 yr) in the absence of antigen stimulation (1). These lymphocytes do not produce effector molecules, such as cytokines, unless they again encounter antigen. Following the initial activation of T cells within the lymphoid tissue, effector or memory cells reenter the circulation via the efferent lymphatics and the thoracic duct.

On return to the systemic circulation, activated T cells no longer efficiently home to lymphoid tissue due to loss of surface L-selectin. Instead, these cells preferentially home to sites of infection/inflammation where the offending antigen originally gained access to the tissue (i.e., the gut). This new pattern of homing is mediated by the interaction of lymphocyte-associated VLA-4 (α4β1-integrin), LFA-1, and CD44 with venular vascular cell adhesion molecule (VCAM)-1, intracellular adhesion molecule (ICAM)-1 and -2, and hyaluronate, respectively (1). Thus effector and memory T cells possess very different homing patterns from those of naïve cells. Once extravasated, an effector or memory T cell may reencounter its specific antigen via the specific binding of its TCR to antigen bound by the MHC II complex expressed on APCs such as dendritic cells, macrophages, and possibly endothelial cells (Fig. 1). This second antigen-specific interaction activates lymphocytes to synthesize and release IFN-γ and IL-2. IL-2 promotes the clonal expansion of T cells and enhances the function of Th cells and B cells, whereas IFN-γ interacts with and activates APCs and macrophages to produce IL-12 (1). IL-12 then feeds back onto the T cells to further enhance production of IFN-γ and promote the differentiation of these T cells to Th1 cells capable of producing even larger amounts of IFN-γ, IL-2, and lymphotxin-α. IFN-γ can then activate endothelial cells and enhance endothelial cell adhesion molecule (ECAM) expression on the postcapillary venule. In addition, IFN-γ-activated macrophages produce large amounts of proinflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8, IL-12, and IL-18 as well as reactive oxygen and nitrogen metabolites (e.g., superoxide, hydrogen peroxide, nitric oxide). All of the above-mentioned mediators are thought to be important in recruiting and activating phagocytic leukocytes such as polymorphonuclear neutrophils (PMNs) and monocytes/macrophages for the efficient destruction of the invading pathogens. Failure to downregulate production of these proinflammatory cytokines leads to severe inflammatory tissue injury (Fig. 1).

**Regulation of intestinal immune responses**

The potential for the mucosal immune system to produce local tissue injury suggests that healthy individuals possess mechanisms to limit the immune response and in some cases completely suppress it. It is thought that this is accomplished by additional subsets of CD4+ T cells such as Tr1 and possibly Th3 cells (5). Tr1 cells have been suggested to suppress or limit intestinal DTH-induced injury by direct antigen-driven suppression of Th1 cells via the synthesis and release of large amounts of the regulatory cytokines IL-10 and TGF-β (Fig. 2). IL-10 is a Th2-type cytokine with a spectrum of biological activities. It is produced by several different cell types includ-
ing subsets of T cells, macrophages, and CD5+ B cells and serves to inhibit both the differentiation of Thp to Th1 cells as well as the synthesis of Th1 and/or macrophage-derived proinflammatory cytokines (12). In addition, IL-10 promotes the maturation of TGF-β-producing Th3 cells in vitro and attenuates the formation of macrophage-derived reactive oxygen and nitrogen metabolites (12). Finally, IL-10 has been shown to inhibit cell surface expression of MHC II molecules and ICAM-1. A second subset of CD4+ T cells that is thought to suppress Th1-type immune responses are the Th3 cells. These cells have been shown to produce large amounts of TGF-β and varying amounts of other Th2-type cytokines such as IL-4 and IL-10. TGF-β is a pleiotropic cytokine that has been shown to be important in downregulating the excessive inflammation observed in a variety of experimental mouse and human immune-based diseases, including IBD (15, 17). Although the biological activity of TGF-β is complex, in general it appears to inhibit the generation of Th1 cells, IL-12 production, and cell-mediated immunity in vivo (1, 17). Recent evidence suggests that both Th1 and Th3 cells possess TCRs that are specific for the same antigens recognized by the effector T cells they regulate. These observations suggest that the presence of regulatory cells within the intestinal and/or colonic interstitium provides a controlled immune response to luminal antigens, thereby limiting inflammatory tissue injury and dysfunction (Fig. 2). Not surprisingly, animals rendered deficient in certain regulatory cytokines such as IL-10 or TGF-β lack the appropriate regulatory cell responses and subsequently develop chronic gut inflammation (19).

IBD: breakdown in the regulation of intestinal immune responses

The IBDs (Crohn’s disease and ulcerative colitis) are chronic inflammatory disorders of the intestinal tract characterized by rectal bleeding, severe diarrhea, exudation, fever, abdominal pain, and weight loss. Histopathological inspection of tissue obtained from the involved segment of the small and/or large bowel reveals vasodilation, venocongestion, edema, infiltration of large numbers of inflammatory cells, erosions, and ulcerations (10). Despite several decades of extensive investigation, the etiology and specific pathogenetic mechanisms responsible for IBD remain poorly defined. Recent experimental and clinical studies suggest that the initiation and pathogenesis of these diseases are multifactorial, involving interactions among genetic, environmental, and immune factors (3). Regardless of how exactly these interactions ultimately promote chronic gut inflammation, it is becoming increasingly apparent that the immune system plays a crucial role in disease pathogenesis. Because the inflammation is localized primarily to the intestinal tract in IBD, investigators have focused on the intestinal lumen as the site for the antigenic trigger. Indeed, the chronic relapsing nature of IBD, coupled with the fact that a large percentage of patients with Crohn’s disease will experience recurrence of the disease following surgical resection of the involved bowel segment, suggests that the antigen or antigens that initiate and perpetuate this disease are part of the normal gut flora. Although several different etiologic theories have been proposed to account for this apparent immune activation,
data obtained from several experimental studies suggest that the chronic gut inflammation may result from a dysregulated immune response toward components of the normal gut flora.

One of the important conceptual advances in IBD research has been the proposal that failure to regulate normally protective cell-mediated immune responses in the intestinal and/ or colonic mucosa results in sustained activation of the mucosal immune system and the uncontrolled overproduction of proinflammatory cytokines and mediators (Fig. 1). This hypothesis has arisen from data obtained from several different studies using genetically engineered or immune-manipulated rodent models of IBD. These different models of chronic gut inflammation appear to stratify into three different groups. The first group consists of mice with alterations in T cell subsets and T cell selection, including the TCR-α chain- or TCR-β chain-deficient mice, MHC II-deficient mice, SCID mice reconstituted with CD4+CD45RBhi T cells, human CD3ε transgenic mice reconstituted with T cell-depleted F1 bone marrow cells, and HLA-B27 transgenic rats. The second group involves mice that develop spontaneous and chronic colitis as a result of targeted gene deletion of IL-2, IL-10, or TGF-β. Finally, genetically engineered mice deficient in certain cellular signaling proteins, such as Gαi2, develop spontaneous and chronic colitis (19). It has been suggested that most, if not all, of the genetically engineered or immune-manipulated mouse models of IBD develop chronic colitis due to a lack of appropriate downregulation of normal DTH responses toward luminal antigens. Furthermore, Powrie and co-workers (13) have proposed that a dysregulation of macrophage activation due to the absence of regulatory T cells is a key event in the pathogenesis of chronic gut inflammation. Resident macrophages are long-lived phagocytic and secretory cells found in the interstitium of a variety of different tissues including the gut, liver, lung, skin, nervous system, and lymphoid tissue. These tissue-associated leukocytes function as a first line of defense against a variety of infectious microorganisms and particulate matter. By virtue of their tissue distribution and their ability to produce or respond to local and circulating mediators, tissue macrophages occupy a central position in the inflammatory cascade. For example, activated macrophages will release a plethora of proinflammatory mediators including cytokines (TNF-α, IL-1β, IL-6, IL-8, IL-12, IL-18), metalloproteinases (collagenase, gelatinase), arachidonate metabolites (prostaglandins, leukotrienes), lipid mediators (platelet-activating factor), and reactive oxygen and nitrogen metabolites (superoxide, hydrogen peroxide, nitric oxide) (1) (Fig. 1). Many of these macrophage-derived mediators activate the venular endothelium to express ECAMs facilitating the recruitment of additional inflammatory cells such as neutrophils and monocytes, which in turn contribute to the inflammatory cascade by releasing copious amounts of TNF-α, IL-8, reactive oxygen and nitrogen species, and metalloproteinases (Fig. 1). This type of dysregulated DTH would ultimately result in a dramatic shift or imbalance in the cytokine production profile within the intestinal interstitium such that a Th1

FIGURE 3. All segments of the microcirculation contribute to the pathophysiology of chronic gut inflammation. A variety of inflammatory mediators (e.g., histamine, bradykinin, NO, prostaglandins) produced by the affected tissue dilates arterioles, leading to increased blood flow (hyperemia) and hydrostatic pressure in the downstream capillaries. The increased capillary hydrostatic pressure enhances the capillary filtration rate and contributes to the interstitial edema associated with gut inflammation. Proinflammatory cytokines [TNF, IFN-γ, IL-12] released by activated mast cells, macrophages, and lymphocytes activate venular endothelial cells and increase expression of endothelial cell adhesion molecules that mediate leukocyte-endothelial cell adhesion and eventual emigration (tissue infiltration) of leukocytes. Leukocyte emigration contributes to vascular protein leakage (extravasation). Consequently, inflammation results in a diminished endothelial barrier function in venules that promotes the accumulation of albumin and other plasma proteins in the interstitium. The resultant increase in interstitial oncotic pressure further promotes fluid filtration across capillaries and accelerates the development of interstitial edema (reproduced with permission from Ref. 11).
response would predominate. Indeed, this is exactly what is observed in IBD (18).

Intestinal microcirculation: target of the inflammatory response

One of the major functions of cell-mediated immunity (i.e., DTH) is to enhance recruitment, phagocytic function, and microbicidal activity of phagocytic leukocytes (PMNs, monocytes, macrophages) via the production of Th1 and macrophage-derived cytokines (Fig. 1) (reviewed in Ref. 11). A consequence of unregulated proinflammatory cytokine production is the sustained activation of the microvascular endothelium and the recruitment of large numbers of potentially injurious leukocytes into the intestine and/or colon. Proinflammatory cytokines and mediators released by activated mast cells, macrophages, PMNs, and lymphocytes activate venular endothelial cells and increase expression of ECAMs that mediate leukocyte-endothelial cell adhesion and the consequent emigration (infiltration) of leukocytes, a common feature in active episodes of IBD. Recently, we have shown large and significant increases in a variety of different ECAMs in different models of experimental and human IBD. For example, we have shown (8, 9) that the onset of chronic colitis is associated with large and significant increases in surface expression of E-selectin, ICAM-1, VCAM-1, and MAAdCAM-1. A recent series of experimental and clinical studies have demonstrated that infusion of an antisense oligonucleotide directed against ICAM-1 message produces clinical improvement in a mouse model of colitis as well as in steroid-resistant Crohn's disease patients (2, 20). Similarly, recent studies using small-molecular-weight antagonists or immunoneutralizing monoclonal antibodies to either $\alpha_4\beta_7$-integrin or MAAdCAM-1 demonstrate that the $\alpha_4\beta_7$-integrin/MAAdCAM-1 interaction plays an important role in the pathophysiology of different experimental models of colitis (6, 14). These findings underscore the importance of adhesion molecule expression in the progression of IBD and may represent potentially important therapeutic targets for the treatment of IBD.

Vascular endothelial cells normally serve as a barrier that minimizes the movement of fluid and proteins from the blood to the interstitium. However, the uncontrolled inflammatory response associated with IBD produces dramatic alterations in gut microvascular function that contribute greatly to the pathophysiology observed in both experimental and human IBD (Fig. 3). For example, many of the inflammatory mediators (e.g., prostaglandins, nitric oxide, bradykinin) produced during this unrestrained inflammatory response will increase blood flow (hyperemia), thereby producing the characteristic erythema observed in patients with IBD. Indeed, Hulten and co-workers (7) demonstrated that blood flow to the colon of patients with IBD increases two- to sixfold. A consequence of this enhanced arteriolar blood flow is increased hydrostatic pressure in the downstream capillaries, resulting in increased fluid movement across intestinal capillaries and subsequent interstitial edema (Fig. 3). Similarly, the fluid filtration that results from such a large increase in capillary pressure would
lead to disruption of the mucosal barrier and exudation of interstitial fluid into the lumen (4). Accumulation of plasma proteins within the mucosal interstitium results in an increase in interstitial oncotic pressure, which in turn alters the balance of forces governing capillary fluid exchange to favor enhanced filtration. Therefore, the combined effects of increased vascular pressures, resulting from arteriolar dilation, and an increased hydraulic conductance, which reflects the endothelial barrier dysfunction, leads to a profound enhancement of vascular fluid filtration in the inflamed bowel.

Since leukocyte infiltration is a hallmark histopathological feature of IBD, these cells may also contribute to the increased vascular permeability. There is increasing evidence to suggest that adhesion and emigration of leukocytes from inflamed postcapillary venules results in an increased plasma protein extravasation at the sites of leukocyte transendothelial migration (4). The resultant increase in interstitial oncotic pressure further promotes fluid filtration across capillaries and accelerates the development of interstitial edema (Fig. 3). Whether this relationship between vascular permeability and leukocyte trafficking represents physical disruption of endothelial junctions by transmigrating leukocytes or results from the production of reactive oxygen metabolites and/or proteases from activated, adherent leukocytes remains to be determined (4).

The formation of crypt abscesses represents the terminal step in PMN migration out of the circulation (Fig. 4). For PMNs to be present within the lumen of the bowel, these inflammatory cells must extravasate from the circulation and move through the interstitial matrix as well as interact with the basement membrane and basolateral aspect of the crypt epithelium before emigration into the lumen. The driving force for this directed migration out of the tissue into the lumen is provided by the bacterial gradient present in the distal bowel. Transendothelial as well as transepithelial migration of PMNs and other leukocytes may account for some of the fluid accumulation (edema) and enhanced mucosal (i.e., epithelial) permeability observed in patients with active IBD.

Conclusion
A major conceptual advancement that has been made in recent years in our understanding of the etiology of idiopathic IBD has been that the failure to regulate the normally protective mucosal immune response toward enteric bacteria results in the uncontrolled overproduction of proinflammatory mediators. Many of these mediators affect gut function by virtue of their effects on the microcirculation, resulting in a chronic inflammatory response. Mechanisms to limit proinflammatory cytokine and mediator expression as well as ways to block their effects on the microvasculature may prove useful in the treatment of IBD.

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