Immunological aspects of inflammatory bowel disease

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Abstract

The etiologic agents for ulcerative colitis and Crohn's disease have not been identified. Although a large number of microbial and dietary agents have been proposed as candidates for roles in these diseases, none have been proven as causative agents. The mechanisms for the spontaneous exacerbations and remissions, characteristics of these diseases, are also undefined. Nevertheless, recent studies have provided new insight into the mechanisms involved in the initiation, production, and amplification of the immunologic and inflammatory responses that lead to the histologic findings and pathologic changes characteristic of inflammatory bowel disease (IBD). Inflammatory mediators and cytokines have been viewed as particularly promising for understanding both chronic inflammation of the intestine as well as providing important pathophysiologic mechanisms to target for therapeutic intervention. Although the cellular and molecular mechanisms inducing intestinal inflammation may not be unique to IBD, recent insight into the role of the mucosal immune system in the pathophysiology of IBD are beginning to lead to promising new therapeutic approaches. Of great future interest will be the delineation of the mechanisms involved in the downregulation of inflammation, a process that normally leads to the prompt resolution of specific, acute intestinal inflammation. Although little is known about the normal physiologic downregulation of inflammatory responses, this area is of great potential importance for future therapeutic approaches in IBD.

Key words: Inflammatory bowel disease, Ulcerative colitis, Crohn's disease, Inflammatory mediator, Cytokine, Mucosal immunology

Introduction

Inflammatory bowel disease (IBD) consists of two major illnesses, ulcerative colitis and Crohn's disease, which are chronic inflammatory disorders of the intestine of unknown origin. Recent studies have provided valuable insight into the role of the mucosal immune system in the amplification of chronic inflammatory and immunologic effector mechanisms that results in the histologic and clinical feature characteristic of IBD.

IBD is particularly common in young people. The peak incidence occurs in the second decade, although there is a small peak in the fifth to seventh decade. IBD occurs less frequently in infants and young children. At the children's hospital of Philadelphia, of our 325 pediatric IBD patients, 8% were diagnosed before 5 years of age and 2% were diagnosed before 1 year of age.

The immunopathology of IBD consists of a sequence of immunological steps that begins with initial antigen processing even-
ts. Ulcerative colitis involves only the colon; Crohn’s disease can involve either the colon or the small bowel, or both. In ulcerative colitis, there are mucosal ulcers and infiltration of the mucosa and submucosa with neutrophils, macrophages, and lymphocytes. In Crohn’s disease, the inflammatory infiltrate frequently contains granulomas and extends through all layers of the bowel wall rather than being confined to the mucosa and submucosa as in ulcerative colitis.

By the use of clinical manifestations and histologic differences, clear distinctions between ulcerative colitis and Crohn’s disease can be made. Such evidence includes disease distribution, histopathology, and spectrum of complications. Additional evidence pointing to a different pathogenic processes includes the presence of vasculitis in Crohn’s disease, the strikingly differences in angiotensin content of the mucosa, and the many specific abnormalities of colonic epithelium in ulcerative colitis. Despite the differences, however, ulcerative colitis and Crohn’s disease have one future in common—chronic mucosal inflammation. Most mucosal inflammatory events are, however, self limited. In contrast, spontaneous resolution of inflammation dose not usually occur in ulcerative colitis and Crohn’s disease. The underlying mechanisms for this failure to heal may well represent the primary pathogenic abnormalities in both ulcerative colitis and Crohn’s disease. Both diseases have been viewed as “chronic” inflammatory diseases because of their prolonged clinical courses and because their inflammatory infiltrates contain lymphocytes and macrophages, a histologic picture that is characteristic of chronic inflammation.

However, both diseases also have a less well recognized, but equally prominent “acute” component marked by a constant flux of neutrophils out of the circulation into the inflamed mucosa, and then through the epithelium and into the intestinal lumen. The etiologic agents for ulcerative colitis and Crohn’s disease have not been identified. A large number of microbial and dietary agents have been put forth as candidates for pathogenetic roles in these diseases but none have been proved to be correct. Nonetheless, a major focus has recently been placed on bacterial cell wall products, including peptidoglycans (PG-PS), formylmethionyl peptides (FMLP), and lipopolysaccharide (LPS). These products nonspecifically induce an intense activation of macrophages, granulocytes, and lymphocytes. Thus, bacteria, through the effects of their cell wall products, and/or toxins, are very important regarding initiating and/or perpetuating IBD (Fig. 1).

Currently therefore, the working hypothesis is that in IBD, ubiquitous and common bacterial cell wall products capable of initiating an inflammatory immune response, in a genetically predisposed individual, activate a sequence of immunologic processes, that are not appropriately down regulated. Initiating events then lead to macrophage activation, with the resultant production of a large amount of interleukin-1 (IL-1), IL-6, and TNF-α (Fig. 1). Recently, we demonstrated that involved IBD mucosa contains much more IL-8 mRNA than normal mucosa. Therefore, IL-8 also may play a central role in the initiation and perpetuation of inflammation in IBD patients.

Subsequent cell-mediated immunologic
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Fig. 1 Initiating events in IBD. A variety of potentially pathogenic organisms and antigens present in the gut lumen including mycobacteria, viral and bacterial agents have not been found to be involved in the etiology of IBD. Attention has therefore been focused on bacterial cell wall products and toxins which may be involved in the initiating events which lead to IBD. Bacterial cell wall products such as FMLP, LPS, and PG-PS are pro-inflammatory, can move across M cells and are then presented in increased amounts directly to underlying lymphocytes and macrophages. Furthermore, in IBD the epithelial cells express increased amounts of class II HLA antigens due to inflammation and could also therefore be involved in the presentation of antigens to mucosal lymphocytes and macrophages.

events involve the mucosal immune system, and lead to the chronic intestinal destruction associated in IBD. Although the nature of the etiologic agents in IBD are unknown, recent studies have given insight into the mechanisms for the amplification of the inflammatory response that results in the clinical and histologic changes characteristic of ulcerative colitis and Crohn's disease. A major factor in the functional and histologic changes seen in IBD appears to be soluble mediators of inflammation. Neutrophil and macrophage infiltration suggest the presence of soluble chemotactic agents that cause neutrophils and monocytes in the circulation to migrate into the mucosa. Mucosal edema and hyperemia reflect the presence of soluble mediators that enhance vascular permeability and vasodilation.

A number of the immunologic perturbations associated with IBD in the past have been found to be effects rather than causes, phenomena secondary to the sequel of the disease (inflammation and malnutrition for example) or to the immunosuppressive consequences of steroid therapy. Nevertheless, as advances have been made in our understanding of the immune system and as investigative techniques have become increasing sophisticated, we have been able to achieve growing confidence that recent studies are yielding new information on the immunopathogenesis of IBD; moreover, this information is beginning to result in promising therapeutic leads.
Gut Associated Lymphoid Tissue (GALT)

Although we may not always think of immunity as being a primary intestinal function, in actuality the mucosal immune system represents one of the largest collections of immune cells in the body. Furthermore, due to the multiple luminal bacteria and viruses that must be defended against, the mucosal immune system contains a number of important cell types, which participate in providing host defense mechanisms. Specialized epithelial cells, termed M cells, cover Peyer’s patches and allow the specific transport of viruses and large antigens into contact with the underlying lymphocytes and macrophages that comprise the cells of the mucosal immune system. After antigens are processed and presented to immature lymphocytes in the Peyer’s patch and lymphoid follicle areas, lymphoblasts mature outside of the intestine during migration through the lymphatic system, thoracic duct, and peripheral blood, after which they then “home” back as mature cells into the lamina propria. In addition to lamina propria mononuclear cells, the intestine also has a unique subcompartment of cells, intraepithelial lymphocytes (IELs), which are predominantly T cells residing in among intestinal epithelial cells. IELs are of the cytotoxic/suppressor cell phenotype (predominantly CD8-positive) and also bear a unique subcompartment of the T cell receptor, with the majority being gamma/delta bearing T cells. Interestingly, intraepithelial lymphocytes and gamma/delta T cells are markedly increased in the small intestine of patients with gluten sensitive enteropathy.

A number of infectious agents, such as reoviruses, adhere specifically to M cells but do not adhere to surrounding epithelial cells and can then be transported through the M cells across the epithelium into the underlying lymphoid follicles or Peyer’s patches, in order to allow antigen processing and presentation by macrophages and lymphocytes. Thus, although we usually think of the intestine as providing a very rigid barrier to a number of luminal antigens, M cells provide a way to sample the unique microbial and antigen-rich environment present in the intestinal lumen. This is a critical aspect of providing effective host defense mechanisms. However, it should be noted that M cells also allow a portal of entry for pathogenic viruses and bacteria that can adversely affect the host.

Peyer’s patch lymphocytes are immature after antigen sensitization, and one might expect that the simplest process would be for the cells to migrate into the nearby lamina propria and mature there. Instead, primed cells leave the intestine and thoracic duct during which time they begin to mature into lymphoblasts. They then circulate in the peripheral blood and subsequently “home” back to the gastrointestinal mucosa as well as other secretory sites. The unique intestinal antigen recognition system, therefore, allow large molecules in the gut to be transported across M cells, to then be presented by epithelial cells or underlying macrophages to the Peyer’s patch or follicle-associated lymphocytes, which then mature outside the gastrointestinal tract. While the cells undergo their “maturation journey”, they become immunocompetent and then home back to a number of other mucosal surfaces, in addition...
to the gut. The mechanisms of lymphoblast homing involve high endothelial venules that have specific antigens on their surface,\textsuperscript{17,20} termed addressins, which interact with organ-specific “homing” receptors on mature lymphoblasts.\textsuperscript{19} The cell surface interactions between lymphoblasts and high endothelial venules thus determine the site to which lymphoblasts will migrate. Specific receptors on the Peyer’s patch or intestinal lymphoid follicle endothelial cells direct lymphoblasts to “home” back to intestinal mucosal sites as opposed to peripheral node sites.\textsuperscript{17-20} Normal human lamina propria mononuclear cells bind poorly to peripheral lymph node high endothelial venules.\textsuperscript{20} Ultimately, as we learn more about the mucosal immune system with relationship to IBD, we may better understand the cell surface receptor related events that are involved in directing lymphoblasts to migrate specifically to sites of inflammation in IBD.\textsuperscript{20}

**Alterations of the Immune System in IBD**

Mechanisms of cell injury in IBD include both non-specific, “innocent bystander” target cell destruction by inflammatory mediators, as well as direct cell-mediated and antibody-mediated autoimmune injury to the intestine.\textsuperscript{1-3} Thus, the effector mechanisms of injury to the intestine in IBD involve both cell-mediated memory and nonspecific pro-inflammatory events.\textsuperscript{1-3} The effector cell-mediated events in IBD involve a sequence of carefully regulated steps (Fig. 2). After production of large amounts of IL-1 intestinal macrophages and dendritic cells, T-cell activation occurs, resulting in the clonal expansion of a variety of different T-cell subclasses (Fig. 2). Production of cytokines lead to the expansion of helper T-cells, and the resultant enhanced antibody synthesis and secretion results in the heightened activation of immune function. Interestingly, cytotoxic function by normal intestinal mononuclear cells quit reduced, yet in IBD, with the production of cytokines, the specific cytotoxic capabilities of subclasses of intestinal mononuclear cells may be increased.\textsuperscript{21,23}

Although findings regarding intestinal lymphocyte activation in ulcerative colitis and Crohn’s disease differ, we have used flow-cytometric analysis of isolated colonic lamina propria mononuclear cells to demonstrate that lymphocyte-activation antigens, including the IL-2 receptor, the transferrin receptor, and the 4F2 antigen, are expressed on increased percentages of intestinal B cells and T cells, as well as CD4+ and CD8+ T-lymphocyte sub-populations in both active ulcerative colitis and Crohn’s disease.

Normal IgA is the dominant and most important immunoglobulin in the gut, since it provides our initial protective mucosal defense mechanism.\textsuperscript{24,25} If IgA were to be playing a major role in the pathogenesis of IBD, one would expect to see markedly increased IgA production. However, the IgA response in IBD is at best unchanged and in some studies decreased.\textsuperscript{26,27} With regard to alterations in immunoglobulin production in IBD, we have found that there is an enormous increase in production of IgG by IBD intestinal lamina propria mononuclear cells.\textsuperscript{28-30} Interestingly, there is an important difference between the IgG subclasses secreted by ulcerative colitis and Crohn’s disease intestinal lamina propria mononuclear cells. IgG1 and IgG3 subclasses are greatly increased in ulcerative
colitis, while in contrast IgG1 and IgG2 subclasses are increased in Crohn's disease (Fig. 2). Using monoclonal antibodies and immunohistochemical studies, other workers have found similar results; that is, IgG1 is markedly increased in ulcerative colitis while IgG2 is increased in Crohn's disease. These observations are of interest because IgG subclasses have very different functional capabilities. IgG1 represents the dominant response to proteins and T cell dependent antibody responses. IgG2 and IgG4 subclass deficiencies can be found in pediatric patients who have meningitis, pneumonia, and other recurrent infectious processes. IgG1 and IgG3 are a much better complement pathway activator and are better at promoting opsonization than IgG2. Therefore, IgG1 and IgG3 may be particularly well poised to induce intestinal tract injury. Although in the normal intestine IgA is the major immunoglobulin of importance, IgG, with its ability to fix complement and activate mononuclear cells, may play a pivotal role in IBD by inducing intestinal injury and inflammation (Fig. 2).

There are a number of exciting research
areas toward which further investigations should clearly be directed. First, delineation of the mechanisms of activation of lymphocytes and priming of macrophages with subsequent production of cytokines will allow better understanding of the pathway that lead to cell-mediated recognition and destructive events in IBD. Second, with the availability of specific cytokine inhibitors and receptors antagonists, the role of this pathway in IBD can now be explored in clinical trials.

Third, the role of IgG and IgG subclasses in inducing intestinal injury and the nature of the antigens against which the IgG antibodies are directed will provide important information regarding initiating factors in IBD. Regulation of T cell function and granuloma formation by neuro-

peptides and cytokines secreted from the mucosal inflammatory cells will be particularly relevant for the development of medications targeted against Crohn’s disease.33,34

Ulcerative colitis and Crohn’s disease are obviously very different disease processes clinically, and we need to investigate further why there are granulomas in Crohn’s disease and not ulcerative colitis, why the differences between IgG subclasses occur in these two disorders, and what the processes are that lead to fibrosis and stricture formation in Crohn’s disease, but not ulcerative colitis.

Inflammatory Mediators and Immunopharmacology

One of the results of the highly activat-

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Fig. 3 Mechanisms of tissue injury in IBD. Inflammatory processes can be dramatically increased by a variety of mechanisms, including direct activation of granulocytes or neutrophils by bacterial products, priming of phagocytes directly by IgG, or specific immunologic effector events mediated by IgG activation of the complement cascade. After priming or activation of granulocytes and macrophages a host of destructive inflammatory mediators, can be related. These include leukotriens, prostaglandins, platelet activating factor, thromboxanes, oxygen radicals, and proteases. These inflammatory mediators and toxic oxygen radicals can lead to direct tissue damage by a variety of destructive mechanisms.
ed mucosal immune system seen in IBD is the triggering of nonspecific inflammatory processes (Fig. 3). It is in this area that most of occur current drug therapy is directed. Steroids and 5-aminosalicylic acid (5-ASA) clearly inhibit the nonspecific inflammatory processes seen in IBD.1–3 As a directed result of the constant and rapid influx of granulocytes and macrophages, which occurs in both ulcerative colitis and Crohn’s disease, these are large amounts of potent inflammatory mediators released, including prostaglandins, leukotrienes, platelet activating factor, and oxygen radicals (Fig. 3). The inflammatory mediators are then able nonspecifically to produce severe injury to numerous cell types in the gastrointestinal tract. The first inflammatory mediator examined was prostaglandin E2, which was found to be produced in markedly increased amounts in inflammatory bowel diseases.35

Interestingly, however, the use of nonsteroidal drugs and cyclooxygenase inhibitors has actually made IBD worsen in certain patients.1–3 The explanation for this was not entirely clear, until studies were carried out detailing the inhibition of the 5-lipoxygenase pathway by 5-ASA and the demonstration of markedly increased production of leukotriene B4 (LTB4) in IBD.36,37 LTB4 is a very potent chemoattractant and plays an important pro-inflammatory role in IBD. Nonsteroidal anti-inflammatory drugs (NSAID’s) inhibit the cyclooxygenase pathway and may thus lead to a decrease in protective prostaglandins and an increase in destructive leukotrienes. Interestingly, in studies from a number of groups looking at an animal model of inflammation, prostaglandins are protective to the mucosa. Thus, it may be important to discover which parts of the arachidonic acid metabolism pathway are blocked by pharmacologic agents. Drugs preferentially blocking the 5-lipoxygenase pathway may lead to the increased production of protective inflammatory mediators and the decreased production of destructive inflammatory mediators. Steroids work by many different mechanisms, one of which may be the decreased production of endogenous arachidonic acid from membrane phospholipids leading to a decrease in both prostaglandins and leukotrienes.37 We need to know much more about the cells of origin of inflammatory mediators in the intestine. Furthermore, studies are just beginning with regard to the effects of LTC4, LTD4, and LTE4 on vascular permeability and smooth muscle contraction.

5-ASA is an oxygen radical scavenger and leads to a decrease in the production of oxygen radicals. Oxygen radicals are made in large numbers by the tremendous influx of macrophages and granulocytes into inflammatory intestinal lesions (Fig. 3).38,39 Intriguingly, the levels of protective enzymes are very low in normal intestinal mucosa, including the levels of both superoxide dismutase and glutathione peroxidase.40 Normally oxygen radicals are degraded by a series of enzymes (superoxide dismutase, catalase, glutathione peroxidase, etc.) so that they do not damage the tissue. Thus, potentially, the gastrointestinal tract is an environment in which large numbers of oxygen radicals are likely to overpower normal protective mechanisms easily. The mechanism of action of corticosteroids in IBD is not yet completely understood, despite their use for many years. Corticosteroids have a number of important biologic
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and pharmacologic properties that may be related to their therapeutic effects in IBD. These include effects on lymphocyte differentiation, lymphokine synthesis, and interferon production. Corticosteroids also have effects on arachidonic acid metabolism that may relate to their therapeutic effects in IBD. Corticosteroids promote the synthesis of a protein, lipo-modulin, which inhibits phospholipase A₂ and thus, block the release of arachidonic acid from phospholipids. The inhibition of arachidonic acid release would block the synthesis of both cyclooxygenase and lipoxygenase products.

Summary

In the future, we need to better define the basis for and develop agents to inhibit the transition from normal host protective acute inflammatory events to chronic injurious cell-mediated events. Approaches to interfering with the continuous, unrelenting attraction of macrophages and granulocytes into inflammatory lesions in the intestine must be pursued. The continued examination of altering the production of leukotrienes and prostaglandins by phospholipase A₂ inhibition, the use of fish oils, lipoxygenase pathway inhibitors, and leukotriene receptor antagonists will provide new directions in treating IBD. A better understanding of oxygen radicals will lead to inhibition of production and the development of improved oxygen radical scavengers. Platelet activating factor and its antagonists should be examined in animal model and clinical studies.

In summarizing the sequence of events that lead to the inflammatory processes that occur in IBD, the cytokine and cell-mediated regulation of immunoglobulin production may be important because of the ability to trigger complement pathway activation, as well as activation of macrophages (Fig. 1, 2, 3). A number of processes may account for the large influx of macrophages and granulocytes, including cytokines (such as IL-8 and others), complement activation and LTB 4 (Fig. 1, 2, 3). Once the influx of macrophages and granulocytes occurs, a markedly increased production of oxygen radicals, proteases, LTB 4, platelet activating factor and other mediators, leads to what we see clinically as the final inflammatory processes in the intestine.

During the past decades, excellent progress has been made in elucidating the immunologic alterations that occur in inflammatory bowel disease. Furthermore, understanding the immunopharmacologic mechanisms of drug action has assisted us in allowing the development of new therapeutic approaches to IBD. As our knowledge of basic immunology and mechanisms of inflammation continues to exapand, exciting new treatment strategies will become available for use in our patients with ulcerative colitis and Crohn’s disease.

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