

IBD:

Immunologic Research at The Mount Sinai Hospital

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Abstract

An evolution in our understanding of the inflammatory bowel diseases (IBD), ulcerative colitis and Crohn's disease, correlates with increased knowledge of the function of the mucosal immune system. In the early 1960s and 1970s, IBD was considered to be an autoimmune disease in which there was a directed attack by humoral and cellular elements of the immune system against intestinal tissues. These studies did not withstand the test of time, and from the 1970s through the 1990s there was a growing appreciation that defects in cellular immunity, not auto-reactive in nature, played a larger role in disease pathogenesis. Research at Mount Sinai focused in on these cellular T cell defects and helped pave the way for the current model of disease pathogenesis. **Key Words:** IBD, Crohn's disease, ulcerative colitis, immunology, epithelial cells, T lymphocytes.

Introduction

CROHN'S DISEASE, as described by Dr. Burrill Crohn and colleagues at The Mount Sinai Hospital in 1932 (1), was initially thought to be an infectious disease, comparable to mycobacterial infections of the intestine, described in the past. The description of Crohn's disease came at a time when there was a limited understanding of the immune system and when genetic diseases were only recognized if they were autosomal dominant or recessive. The concept of a distinct mucosal immune system was decades away, and complex genetic disorders could not even be explored. Still, Crohn and his colleagues were not too far off. As is the case for mycobacterial diseases, the expression of disease relates to the host's response, which is genetically regulated and immunologically mediated. Tools to analyze these latter components of the host response only started to become available in the 1970s as lymphocytes, monoclonal antibodies and cytokines were described. It is only recently that we have had the ability to characterize specific genes, with high throughput technology to map genetic loci.

The end result of these advances is a new hypothesis relating to the pathogenesis of inflammatory bowel disease (IBD). It encompasses many of the components of host response which had previously evoked passionate arguments concerning their validity or regarding the roles of such areas as genetics, environment, immune system, specific pathogens, psychogenic causes, allergies, etc. The current consensus takes many of these factors into account and is built upon an interactive concept. Simply stated, the hypothesis is that in a genetically predisposed host, there is an abnormal immune response to both pathogens and non-pathogens in the gut. IBD is one of many chronic inflammatory diseases known to be multigenic.

Genome-wide screening analyses have identified loci which are IBD generic as well as ulcerative colitis (UC) and Crohn's disease (CD) specific (2–11). It may be that CD and UC involve 5–8 distinct genes and that the nature of the gene defect not only dictates the type of disease but also the subcategory of the disease (e.g., fistulizing vs. inflammatory CD, left sided vs. pancolitis, ileitis vs. segmental colitis). It is plausible that one or more of these genes codes for defects in host defense, as for example, abnormalities in barrier function or defects in mucosal immunoregulation. IBD can be viewed as two events: the trigger or initiating event, and the perpetuation of the immune/inflammatory

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response. Genetic abnormalities may account for one or both of these events.

In early descriptions of CD and UC, emphasis was placed on infectious etiologies. This was plausible, since as alluded to earlier, CD looked like intestinal tuberculosis and UC looked like many infectious types of colitis. However, attempts to define transmissible agents were fraught with poor reproducibility and misinterpreted data. Many of the studies, using stool filtrates from IBD patients to transmit disease into various animal models, reported the ability of an agent in IBD stool to induce colonic inflammation or foot pad granulomas in mice. These were largely the result of reactions to foreign bodies and not a true IBD picture (12, 13). Furthermore, there was the clinical observation that some patients developed IBD following different documented intestinal infections (e.g., post-turista, Salmonella, viral gastroenteritis, etc.). These observations suggested that there may not be a specific infectious cause of IBD, but rather that any infection (in a genetically predisposed host) may result in the initial inflammatory reaction in IBD. Either the persistence of the organism, or defects in regulating the inflammatory response, would result in the perpetuation of the disease. This scenario has recently been validated with studies in a variety of new animal models of IBD, where either barrier function or immune regulation has been altered (see below) (14–29). If these animals are reared in a germ-free environment, they fail to develop colitis (still exposed to dietary antigens — proteins, carbohydrates and lipids) (14, 16, 19, 22, 30–36). If their gut flora is replete with nonpathogenic normal flora (particularly anaerobic flora (*Bacteroides vulgatus*), the disease is expressed. This has been shown to be true in every animal model tested to date, suggesting a uniform role for normal flora in IBD. In fact, one German group has documented a loss of the normal tolerance for autologous flora in patients with IBD (16). These findings suggest that both the initiation and perpetuation of inflammation in IBD relate to an aberrant reaction to the normal constituents in the gut. It places the defect squarely on the shoulders of the host immune/inflammatory response.

Immunologic Research in IBD at Mount Sinai

Role of Cell-Mediated Immunity

How did Mount Sinai contribute to the current paradigm described above? Early studies at Mount Sinai helped switch the focus away from

autoimmunity and the role of antibody, to the current focus on cellular or T cell-mediated immune responses. These studies assessed the integrity of T cells in the systemic immune system. The concept at that time was that cells in the peripheral bloodstream might mirror what was actually happening in the intestine. Thus, Sachar and his colleagues (37, 38) assessed skin test reactivity (delayed type hypersensitivity) in patients with UC and CD. They found an increased incidence of anergy (i.e., limited or no skin test reactivity) in these patients, regardless of the site or extent of disease. These studies did not take into account the fact that many of these patients were taking immunosuppressive steroids and/or that their nutritional status (i.e., hypoalbuminemia) might alter the ability of their T cells to function. These questions were addressed in subsequent studies. Meyers et al. (39) asked whether the anergy seen related only to memory responses or whether the T cell defect was more proximal, i.e., the initial T cell response. Using dinitrochlorobenzene (DNCB), a contact skin-sensitizing agent which classically elicits a T cell-mediated delayed-type hypersensitivity reaction, they studied patients and their first-degree relatives for the integrity of their T cell response. As suggested from Sachar's studies, T cell reactivity to DNCB was significantly depressed in patients with both CD (87% reduction) and UC (53% reduction). However, follow-up studies in family members supported the absence of a genetic component. More important, a restoration of T cell reactivity after surgery to remove the affected bowel (although this was more evident in UC patients than in CD) was described. These findings suggested that the defects in T cell reactivity reported by several groups was likely to be secondary to factors reversible by surgery (i.e., reduction in medication, restoration of nutritional status, elimination of inflammation) (40). Other studies by Heimann et al. (41) and Gelernt et al. (42) supported the concept that some restoration of immunological defects in peripheral blood could be achieved following surgical intervention. These studies helped to underscore the need to develop technology to assess immune function in the affected organ, the small bowel and colon.

The Role of the Intestinal Epithelium in Mucosal Immunoregulation

Moving into the gut meant that the normal mechanisms of mucosal immunoregulation needed to be defined. Several groups had already reported that the lymphoid populations in the gut

were quite different from those of the systemic immune system. It was also clear that the stimulus for an immune response (antigen [Ag]) was going to be distinct from Ag given intramuscularly or subcutaneously, given the fact that antigens in the gut lumen were subject to extremes of pH, proteolytic enzymes, etc. In addition, sites of Ag uptake in the gut were clearly distinct from those in lymph nodes and spleen. With this as a background, Mayer and Shlien (43) first described a novel role for human intestinal epithelial cells (IEC), that of antigen-presenting cells (APC) which could process and present Ag to T lymphocytes. However, unlike conventional APC (macrophages/dendritic cells/B cells), T cells proliferating in response to Ag presented by isolated IEC were found to be CD8⁺ Ag-nonspecific suppressor T cells. This discrepancy was quite unusual, given the fact that IEC express restriction elements (major histocompatibility complex [MHC] class II molecules) which typically interact with and activate CD4⁺ T cells. Over the past decade, the mechanisms involved in this selective activation have been defined. IEC express nonclassical class I molecules such as CD1d, which are capable of activating suppressor T cells (44). However, CD1d, cannot perform this function alone. Yio and Mayer (45) described an epithelial surface glycoprotein, gp180, which is expressed on normal IEC, binds to CD8 and promotes the activation of CD8⁺ T cells. gp 180 binds to CD8 at sites which are distinct from those used by classical class I molecules (human leukocyte antigen [HLA] — A, B, C) and, therefore, does not interfere with the function of CD8⁺ cytolytic T cells. This glycoprotein is, however, a critical component in the activation of CD8⁺ suppressor T cells. While an *in vivo* model supporting this role for IEC has not been clearly defined, there are a large number of *in vivo* and *ex vivo* studies which support such a role for IEC. The activation of CD8⁺ T cells locally could explain the local, controlled, physiologic inflammation seen in the gut. Thus, Ag sampled via the IEC would result in a suppression of local responses.

Defects in IEC Function in IBD

How does this relate to IBD? In 1990, Mayer and Eisenhardt (46), using the IEC co-culture system, reported that IEC derived from patients with UC and CD failed to activate CD8⁺ suppressor T cells. Importantly, this defect was noted regardless of where the tissue used to isolate the IEC was derived (i.e., both inflamed and noninflamed

tissue). This suggested that the defect in CD8⁺ T cell activation might be more generic. In 1997, Toy et al. (47) provided an explanation for these *in vivo* studies. Using an antibody against gp180, derived in the laboratory, they noted that defects in the expression of this molecule existed for both CD and UC. However, the defects were different. In UC there was an alteration in the form of gp180 expressed (apical and not basolateral), and in CD there was a marked decrease in total expression. The end result would be the same. If there was no interaction of gp180 with T cells, no CD8⁺ suppressor T cells would become activated. In fact, the enhanced class II Ag expression on the cells, described by Mayer et al. (48) and others, helped to promote proliferation of CD4⁺ T cells in these co-cultures. The failure of suppressor mechanisms in IBD is not a new concept. Several groups have suggested this from studies in peripheral blood, including Godin et al. (49), who documented an increase in the percentage of CD4⁺ T cells in the peripheral blood. Without regulation, CD4⁺ T cell activation by luminal antigen might continue to go on unabated, with perpetuation of inflammation.

The aberrant activation of CD4⁺ T cells can have several consequences, the first being increased cytokine production. Mount Sinai has approached this prospect in two ways: direct measurement of cytokine in diseased tissue and the use of therapies which alter cytokine production or function. In the first case, Salomon et al. (50) measured γ -interferon (γ -IFN) production by lamina propria lymphocytes from normal patients and disease controls. In both UC and CD, there was a marked increase in γ -IFN production. Given the marked inflammatory nature of this cytokine, its presence clearly helped to explain many of the reported phenomena, including macrophage activation, enhanced class II expression on IEC, etc. (48). Therapies directed against cytokines are also a useful tool to define mechanisms. Lichtiger et al. (51) published the results of a trial of IV cyclosporin in patients with presurgical UC. The initial results were outstandingly successful, eliminating the need for colectomies in more than 80% of patients. Cyclosporin is a potent inhibitor of cytokine synthesis, particularly IL-2. The prototype anti-cytokine therapy is the newly approved anti-tumor necrosis factor (TNF) mAb (infliximab). TNF, produced by activated macrophages and T cells, may also explain several of the histopathologic features of CD. Neutralization of TNF or alteration in the cells producing TNF can have prolonged salient effects on these patients (52–54). Thus, targeting the

effects of aberrantly activated T cells may be quite effective in modulating the disease. The identification of these cells may be one way of accomplishing this therapeutic effect. In a series of studies by Shalon et al. (55) and Posnett et al. (56), it became clear that the population of CD4⁺ T cells present in the gastrointestinal tract of patients with CD was clearly altered compared to normal controls. While the function of these expanded populations was not addressed, it was postulated that these may represent the aberrantly activated T cells described above. Studies to define the true nature of these populations are in progress.

Cytokines produced by these activated T cells may also alter the function of other cells in the mucosal environment. Focusing on the effects of cytokines on the epithelium, Panja et al. (57) defined receptors for inflammatory cytokines expressed by IEC derived from normal controls as well as from patients with IBD. While there is an increase in the number of these cytokines in the mucosa, there is no increase in receptor expression. This suggests that the epithelium in IBD patients is not more sensitive to inflammatory cytokines but may be reacting to the altered cytokine environment. The effects of the increased concentration of cytokines are evident. mRNA for a variety of growth factor receptors was reduced in IEC derived from IBD patients (EGF-R, IGF1-R, CSF1-R, and PDGF-R-beta) (58), and the steady-state levels of transcripts of H-ras and five nuclear proto-oncogenes (c-myc, c-fos, c-jun, junB, and N-myc — involved in cell cycle) were lower in epithelial cells from involved or uninvolved IBD samples than in normal epithelial cells from patients with either sporadic colon cancer or diverticulitis (59). These findings might be of significance with regard to the increased incidence of adenocarcinomas reported in both CD and UC. These inflammatory cytokines may also play a role in inducing or suppressing other mediators important for mucosal integrity. Sperber et al. (60, 61) described a novel mucin secretagogue, MMS-68, which stimulates mucin secretion by intestinal and airway epithelial cells. When studying patients with IBD, they found that there was a decrease in the level of expression of MMS-68 in their tissues. Altered mucin production has been described in IBD and may be an important contributor to the defects in barrier function described in these diseases. Such a defect could result in the enhanced transmigration of bacteria from the lumen into the mucosa-associated lymphoid tissue, allowing for persistent inflammation.

When viewed in composite, the studies from Mount Sinai have suggested defects in the initial activation of important regulatory cells following antigen challenge. These defects are then magnified by the establishment of a cytokine environment which promotes further inflammation and tissue destruction. The IEC may be a focal point for many of these pathways. Correcting these defects and/or reducing their effects on the epithelium may be a viable approach to novel therapies.

Summary

The concept that IBD represents an aberrantly stimulated or poorly regulated CD4⁺ T cell response is growing in strength. *In vivo* and *in vitro* data attest to its validity. Animal models of either T cell dysregulation or altered barrier function develop IBD. Those with B cell defects or defects in nonregulatory T cell subpopulations do not. The *in vitro* and *in vivo* data are starting to coalesce. What is likely to emerge is that adaptive immune, nonimmune and innate immune responses play a role in concert in the development of IBD. These types of studies are ongoing.

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