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TOPIC HIGHLIGHT

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Etiopathogenesis of inflammatory bowel diseases

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Abstract

Theories explaining the etiopathogenesis of inflammatory bowel disease (IBD) have been proposed ever since Crohn's disease (CD) and ulcerative colitis (UC) were recognized as the two major forms of the disease. Although the exact cause(s) and mechanisms of tissue damage in CD and UC have yet to be completely understood, enough progress has occurred to accept the following hypothesis as valid: IBD is an inappropriate immune response that occurs in genetically susceptible individuals as the result of a complex interaction among environmental factors, microbial factors, and the intestinal immune system. Among an almost endless list of environmental factors, smoking has been identified as a risk factor for CD and a protective factor for UC. Among microbial factors, no convincing evidence indicates that classical infectious agents cause IBD, while mounting evidence points to an abnormal immune response against the normal enteric flora as being of central importance. Gut inflammation is mediated by cells of the innate as well as adaptive immune systems, with the additional contribution of non-immune cells, such as epithelial, mesenchymal and endothelial cells, and platelets.

Key words: Inflammatory bowel disease; Chronic inflammation; Mucosal immunity; Innate immunity; Adaptive immunity; Environment; Commensal flora

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INTRODUCTION

It is fair to state most disease entities that still pose major clinical and therapeutic challenges are ones where the exact etiology remains obscure and the mechanisms of tissue injury appear to be exceedingly complex. This certainly seems to be the case for the two main forms of inflammatory bowel disease (IBD); i.e., Crohn's disease (CD) and ulcerative colitis (UC). It is now clear that CD and UC represent two distinct forms of chronic inflammation of the gastrointestinal tract and, as such, have different causes and different pathogenic mechanisms. Still, the factors underlying the appearance of both CD and UC are roughly the same, and include a temporal association with progressive changes in the environment, an intrinsic genetic predisposition, the existence of a rich enteric flora, and an abnormal immune reactivity which is ultimately responsible for damaging the gut and causing clinical manifestations. Even though the categories of underlying factors are roughly the same, there are variations in each category as well as differences in how the underlying factors interact. The end result is two related but distinct disorders named CD and UC. In this review, differences and similarities of the etiopathogenic factors in each form of IBD will be briefly illustrated and discussed.

ENVIRONMENTAL AND GENETIC FACTORS

A remarkable change in the types of diseases affecting humans has occurred during the last century, most remarkably so in the Western world. The most common illnesses responsible for morbidity and mortality have shifted from infectious to chronic inflammatory and neoplastic diseases. This shift has been best documented in Western countries[1], but the same phenomenon is now occurring in other parts of the world. The emergence of chronic autoimmune and inflammatory diseases, including IBD, throughout the world is closely linked to social and economical progress. This was initially noted in Northern Europe and North America but, after the Second World War, the same phenomenon occurred in the rest of Europe, Japan and South America. Most recently, the emergence of IBD is also being observed in the Asian Pacific Region[2].

The "hygiene hypothesis" has been proposed as the probable underlying reason for the switch from infectious to chronic inflammatory diseases, and it postulates that there has been a fundamental lifestyle change from one with high microbial exposure to one with low microbial

exposure[3]. A relative lack of microbial antigens early in life would lead to a less educated and weaker immune system, not equipped to properly handle new challenges later on in life and generating an ineffective immune response that is prolonged because it is powerless to eliminate the offending agent.

There are innumerable environmental modifications that can be ascribed to the hygiene hypothesis, including better housing, safer food and water, improved hygiene and sanitation, vaccines, the widespread use of antibiotics, lack of parasites, fewer infections, and better but selective nutrition. While contributing to the progressive decline of infectious diseases, at the same time these changes may have contributed to create a surge in allergic and autoimmune diseases[4]. A variety of environmental factors are considered risk factors for IBD, including smoking, diet, drugs, geography and social status, stress, the enteric flora, altered intestinal permeability and appendectomy[5]. Among them, cigarette smoking is the strongest example of the influence of the environment on IBD. Remarkably, smoking has a completely opposite effect on CD compared to UC, indicating that distinct pathogenic mechanisms underlie each form of IBD[6]. Smoking is a recognized risk factor for CD, increasing the frequency of disease relapse and need for surgery, and its discontinuation improves the disease course[7]. Cessation of smoking, however, increases the risk of UC, suggesting a protective role in this form of IBD[8]. Other environmental agents associated with IBD are oral contraceptives and nonsteroidal anti-inflammatory drugs (NSAIDs). These agents have also been investigated as having a cause-and-effect relationship with CD or UC. A direct causal relationship has not been found, but women taking oral contraceptives have twice the risk of developing CD than those not taking contraceptives[9]. In the case of NSAIDs there is a clear association with IBD, and patients in clinical remission have a higher risk of relapse if they use NSAIDs[10].

Although the epidemiological evidence linking environmental factors to IBD is fairly solid, it is widely believed that no environmental factor alone can directly cause CD or UC, and an intrinsic disease predisposition must also be present. Such predisposition depends on genetic susceptibility, and a number of established or potential susceptibility genetic loci have been identified in IBD. This topic will be discussed in greater depth in another chapter of this issue of World Journal of Gastroenterology.

MICROBIAL FACTORS

Pathogens

It is possible that classical infectious agents are the cause of IBD, but current evidence supporting this hypothesis is rather weak. Over the years, several microorganisms, such as *Listeria monocytogenes*, *Chlamydia tracomatis*, *Escherichia coli*, *Cytomegalovirus*, *Saccharomyces cerevisiae*, as well as others, have been proposed as having an etiologic role. In particular, Mycobacterium paratuberculosis as the agent of CD has received and continues to receive considerable attention. This bacterium is the cause of Johne's disease, a chronic granulomatous ileitis in ruminants that closely resembles CD. M. paratuberculosis was initially isolated from a few CD tissues[11], but follow up studies trying to confirm its presence by histological examination, attempts to culture it from tissue homogenates, search for its genome in intestinal tissues with highly specific probes, and assessment of serum antibodies have all yielded conflicting or inconclusive results. Moreover, controlled trials have failed to show a beneficial effect of antituberculous therapy in CD patients[12]. One of the last bacteria to be linked to CD is an adherent-invasive strain of *E coli* which is specifically associated with ileal CD[13], but its potential etiological role, if any, remains unclear.

The finding of paramixovirus-like particles in CD endothelial granulomas led to the suggestion that CD could be a form of chronic vasculitis caused by the persistence of the measles virus in the mucosa[14]. Based on epidemiological and serologic data, an association between perinatal measles and an increased probability to develop CD was hypothesized[15], but subsequent studies failed to confirm this association. Importantly, the overall decline of measles infection accompanied by the concomitant rise of CD during the last few decades speaks against an etiologic role of measles in CD.

Commensal bacteria

In contrast to the dwindling evidence that CD or UC are infectious diseases, evidence continues to mount that the indigenous commensal flora of the gut is the target of the immune response in IBD[16]. A large body of data from animal models of IBD indicates that the normal enteric flora is needed to develop experimental colitis. In fact, gut inflammation only arises in animals kept in a conventional but not a germ-free environment[17], supposedly because an immune response directed against enteric bacteria is essential to disease pathogenesis[18]. Thus, the paradigm "no bacteria, no colitis" was created to underscore the central role of the intestinal microbiota in IBD pathogenesis. This paradigm is supported by a variety of clinical observations in IBD patients. There is an increased number of bacteria in close contact with the mucosa in IBD patients[19]; IBD lesions occur preferentially in segments with the highest concentrations of bacteria (the ileo-cecal valve and the colon); surgical diversion of the fecal stream prevents reappearance of CD whereas restoration of the fecal flow induces disease recurrence[20]; modulation of the enteric flora with antibiotics and probiotics attenuates inflammation. In addition, pouchitis develops in a considerable proportion of UC patients, and is associated with a dysbiosis caused by the contact of the once near sterile small bowel mucosa with a rich colon-like flora repopulating the pouch soon after proctocolectomy[21].

Finally, most IBD patients show an enhanced systemic and mucosal immunological reactivity against gut bacterial antigens. Among these, based on serum antibody titers, bacterial flagellin has been recently reported as a dominant antigen in CD[22], apparently defining a population of patients with complicated CD[23]. It has been proposed that this immune reactivity is the consequence of a 'loss of tolerance' towards the autologous enteric flora, resulting in an inappropriate immune response in the mucosa which is manifested by the chronic inflammatory process typical

of CD and UC[24]. Under normal circumstances there is an intimate interaction between commensal intestinal bacteria and the immune system[25], and this complex crosstalk is under the control of immune tolerance[26]. Why tolerance is lost and an abnormal response to otherwise normal gut bacteria develops in IBD is still not entirely clear. However, the recent discovery that CD is genetically associated with mutations of the NOD2/CARD15 gene, whose product is a bacteria-recognizing cytoplasmic protein, points to defective mechanisms of bacterial sensing as the link between the gut flora and the altered immune response found in IBD[27].

CELLULAR FACTORS

The most common type of reaction that the body mounts against external or internal offending agents is inflammation. The gut is particularly susceptible to inflammation as indicated by the fact that, and even under normal circumstances, there is a baseline degree of "physiological inflammation" in the mucosa. This is caused by a tightly controlled immune response directed at an enormous array of dietary and microbial antigens, and it is translated by the presence of an abundant number of leukocytes in the lamina propria[28]. The ultimate goal of an effective inflammatory response is to eliminate the offending agent(s) and then disappear once the cause of inflammation has been eradicated. If inflammation persists and becomes chronic, it represents an inappropriate response that almost invariably leads to lingering injurious effects resulting in anatomical and functional abnormalities. Both CD and UC are typical chronic inflammatory processes of the gut which, by definition, are due to abnormalities of the intestinal immune system. Fortunately, major advances have occurred during the past three decades in our understanding of the cellular and molecular mechanisms mediating mucosal immunity and the alterations that lead to chronic gut inflammation[29].

Adaptive immunity

Abnormalities of intestinal immunity in IBD began to be described several decades ago in regard to the main effector cells of adaptive immunity; e.g., T-and B-cells. Initially, it was discovered that the production of antibodies, particularly IgG antibodies, in the systemic as well as mucosal compartments was drastically increased and that the relative proportions of immunoglobulin classes and subclasses were altered as a consequence of chronic gut inflammation[30-32]. In parallel with these studies, the possibility that some of these antibodies were true autoantibodies directed at self-components of the gut began to be explored. A series of studies suggested that IgG1 antibodies against a structural protein of colonocytes were selectively produced in UC, but not in CD, and could underlie the pathogenesis of this condition[33]. Until now, however, definitive proof for the existence of classical, tissue injury-inducing autoantibodies in UC is still missing. With the recognition of T-cells as central effector cells and their soluble mediators as key modulators of immunity, the focus of immune investigation in IBD shifted to T helper (Th) cell subsets and the soluble mediators they produce. A large number of cytokine abnormalities have been described, including pro-inflammatory and immunoregulatory molecules[34]. In CD, intestinal CD4+ T cells produce large amounts of INF-γ and display marked overexpression of the Th1-cell-specific transcription factor, T-bet[35], while mucosal macrophages produce large amounts of IL-12 and IL-18[36,37]. Additionally, CD mucosal T-cells are resistant to apoptosis and cycle faster than control cells[38,39]. In contrast, in UC nonclassical CD1d-restricted NK T-cells produce increased amounts of IL-13, and mucosal T-cells produce more IL-5, cycle slower and die more than control cells[39-41]. Based on these observations, it is now generally accepted that the two main forms of IBD are associated with distinct immune profiles which are classified as a fairly typical Th1 response in CD and an atypical Th2 response in UC.

More recently, the study of adaptive immune abnormalities in IBD has been focusing on possible defects of immunoregulation. Different types of immunoregulatory cells exist, the best defined being CD4 + CD25 high T-cells, which are critically important in preventing autoimmunity and suppressing excessive immune reactivity[42]. In IBD there is a contraction of this regulatory cell pool in the blood and only a moderate expansion in the inflamed intestine, suggesting the presence of insufficient regulation during active disease[43].

Innate immunity

With the discovery of an association of a group of CD patients (those with small bowel and stricturing disease) with mutations of the NOD2/CARD15 gene, whose product is found in cells mediating innate immunity (primarily macrophages and dendritic cells) and recognizes the bacteria-derived component muramyl dipetide (MDP)[44,45], a surge of interest in the role of innate immunity in IBD has occurred. Dendritic cells are scarce in the gut mucosa, but form a heterogeneous population of potent antigen-presenting cells pivotal to the balance between tolerance and active immunity and controlling the type of response - inflammatory or not - that follows detection of commensal bacteria[46]. In IBD, mucosal dendritic cells are activated, express increased levels of the toll-like receptors (TLR) 2 and TLR4- which mediate recognition of bacterial products - and CD40, and produce more IL-12 and IL-6[47]. All of these phenotypic and functional features indicate a prominent role of dendritic cells in IBD pathogenesis. Epithelial cells are also involved in innate immunity. Interestingly, ileal Paneth cells also express the NOD2 protein, and their production of mucosal α-defensins is decreased in CD patients with NOD2 mutations, perhaps leading to an impaired resistance against enteric microorganisms and eventually contributing to bacteria-induced inflammation[48].

Another crucial component of innate immunity is the TLRs, cell surface molecules that detect microbial infection and trigger antimicrobial host defense responses[49]. TLRs are abundantly expressed on the surface of monocytes, macrophages, and dendritic and epithelial cells and, in addition to recognizing pathogenic microorganisms, are essential to identify the commensal microflora and maintain intestinal homeostasis[50]. Alterations of TL3 and

TLR4 expression by intestinal epithelial cells have been described in IBD, suggesting the possibility that abnormal bacterial sensing contributes to disease pathogenesis[51]. Because both NOD2 and TLRs are involved in innate immunity and recognition of and response to bacteria, much attention has been recently devoted to their biological interrelationship and the possibility of functional abnormalities in IBD, and CD in particular. Monocyte-derived macrophages of CD patients carrying homozygous mutations of NOD2 show clear-cut defects of IL-1β and IL-8 production upon activation by MDP or TNF-α[52]. Moreover, the synergism between MDP and TLR ligands that causes a substantial upregulation of TNF-α and IL-1β production in normal peripheral blood mononuclear cells is lost using cells from CD patients with double mutant genotypes[53]. Thus, these preliminary reports point to the existence of generalized major defects of innate immune responses mediated *via* pattern recognition receptors in CD.

Nonimmune cells

Other cell types participate in the chronic inflammatory response of IBD, including epithelial, mesenchymal and endothelial cells, and platelets, which actually exert many of the functions traditionally attributed to classical immune cells, such as cytokine production or expression of MHC class II antigens.

Initial evidence that intestinal epithelial cell (IEC) function may be altered in IBD was acquired when immunohistochemical studies showed that IEC inappropriately expressed the class Ⅱ antigens HLA-DR in actively inflamed mucosa of UC and CD patients[54]. Later on, after the demonstration that normal IECs have antigen-presenting capacity and preferentially stimulate CD8+ suppressor T-cells, a report showed that IEC from IBD mucosa fail to induce such cells and instead activate CD4+ T-cells, and thus potentially amplify intestinal inflammation[55]. More recently, IBD IECs were reported to inappropriately express members of the B7 family of co-stimulatory molecules[56], a finding suggesting possible alterations in B7-ICOS costimulatory pathways in IBD. These reports, together with the above-mentioned altered expression of TLRs in IBD[51], provide support for the notion that IECs have a role in IBD pathogenesis, but to fully understand their functional relevance will require additional investigation.

The involvement of fibroblasts in IBD has been traditionally viewed as one restricted to production in the extracellular matrix and the pathogenesis of a common and serious complication; e.g., intestinal fibrosis[57,58]. However, fibroblasts are also involved in gut injury because they represent a major source of matrix metalloproteinases (MMPs), a family of proteolytic enzymes directly responsible for tissue destruction during inflammation[59,60]. Of special importance is the observation that interaction with activated T-cells is a major pathway of fibroblast activation and MMP production, a phenomenon that links together fibroblast function, adaptive immunity, and gut tissue injury[61]. In reality, the functional interaction of mucosal fibroblasts with the surrounding microenvironment is physically more complex and functionally more important than previously recognized. A recent report showed that activation of fibroblasts through the CD40 pathway induces the upregulation of cell adhesion molecules and production of chemokines which, in turn, induce the migration of T-cells through local microvascular cells[62]. Therefore, mucosal fibroblasts must also be considered as active rather than passive participants in IBD pathogenesis.

Endothelial cells play an essential role in inflammation due to their central "gatekeeper" function, which controls the quality and quantity of leukocytes that transmigrate from the vascular into the interstitial space. This process is complex and is mediated by a number of molecules, including cytokines, chemokines and adhesion molecules. A key observation that opens the whole field of functional vascular biology in IBD is that human intestinal microvascular endothelial cells (HIMEC) isolated from CD and UC mucosa exhibit a significantly higher cytokine-mediated leukocyte binding capacity compared to HIMEC from normal mucosa[63], a phenomenon secondary to their chronic exposure to the inflammatory milieu of the IBD mucosa[64]. Increased leukocyte adhesion by IBD HIMEC is apparently due to their deficient production of inducible nitric oxide (NO) synthase[65]. This also causes a microvascular endothelial dysfunction in IBD due to a loss of NO-dependent dilation that may lead to reduced perfusion, poor wound healing, and maintenance of inflammation[66].

The role of platelets in IBD has been known for quite some time, but primarily because of their involvement in thrombotic events which are relatively common in CD and UC patients[67]. However, platelets have increasingly acquired a strong immunological connotation through the demonstration of their initiator or amplificatory role in immunity and inflammation, which is mostly mediated through the CD40/CD40 ligand pathway[68]. Platelets exist in an activated state in the peripheral circulation of IBD patients, and the elevated levels of soluble CD40 ligand present in their systemic circulation are mostly of platelet origin, apparently due to platelet activation in the inflamed intestinal microvascular bed[69]. More importantly, recent studies have shown that platelets trigger a CD40-dependent inflammatory response in the microvasculature of IBD patients[70], thus closely linking this unique cell type to the process of IBD pathogenesis.

CONCLUSIONS

Since the recognition of IBD as a perplexing and challenging clinical entity, the investigation of its pathogenic mechanisms has gone through repeated cycles of new hopes, new knowledge, and new realities. Infectious, allergic, dietary, psychosocial, environmental, microbial, vascular, metabolic, immune and other based theories have been put forward, most of them to be rebuked, if not ridiculed[71]. At the moment, we appear to have settled down on a unifying but still wide-ranging hypothesis that IBD results from complex interactions between evolving environmental changes induced by society's progress, a still undefined number of predisposing genetic mutations, an incredibly complex gut microbiota that may be constantly varying, and the intricacies of

individual immune systems[72]. The ability to integrate all these various components into a single cohesive and logical pathway of disease that explains all aspects of IBD appears still a bit distant at the moment. On the other hand, if we look back at where we stood only two or three decades ago, the progress achieved in our understanding of IBD pathogenesis and the way it has changed our approach to therapy is just short of spectacular.

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