Pathogenesis of Inflammatory Bowel Diseases

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Our understanding of IBD pathogenesis has been increasing rapidly. The genetically determined interplay between the commensal microbiota, intestinal epithelial cells, and the immune system has been appreciated deeply. The interplay is also considered to be modified by specific environmental factors. This review examines the recent findings from the animal and human studies on IBD pathogenesis and the implications for future effective therapies. (Intest Res 2010;8:9-17)

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INTRODUCTION

Inflammatory bowel diseases (IBD), notably ulcerative colitis (UC) and Crohn’s disease (CD) are clinically distinct entities that have different characteristics and affect the colon, and any part of the gastrointestinal tract, respectively. The discordance of IBD among monozygotic twins along with increased incidence of IBD in immigrants to high-prevalence countries, shows that environmental factors should play an important role in their development. From an Asian perspective, several epidemiological studies suggest the increased incidence and prevalence of IBD, which also imply the importance of environmental factors in IBD pathogenesis. Enormous success of tumor necrotic factor (TNF-α) blockade in IBD treatment in 1990s opened a new era of investigation for IBD pathogenesis. Genetically determined interactions between the human intestinal microbiota and mucosal immune system and how environmental factors modify the relationships are particularly relevant in IBD development. The aim of this review is to integrate recent findings in the genetics, microbiology, and immunology of IBD and emphasize the dynamic interplay between these components to understand the entire IBD pathogenesis.

GENETICS

Genetic studies, including candidate gene approaches, linkage mapping studies, and particularly genome-wide association studies (GWASs), have significantly advanced our understanding on the importance of genetic susceptibility in IBD. NOD2 gene is the most studied and well-known gene mutation with an increased susceptibility to the development of Crohn’s disease. NOD2 gene is located on chromosome 16q12 and its intracellular protein activates NF-κB and mitogen-activated protein (MAP) kinase pathways in response to stimulation by peptidoglycan fragment muramyldipeptide in the cell walls of both Gram-positive and Gram-negative bacteria. Faulty in NOD2 function is considered to lead to an altered innate immune response to bacteria and resultant uncontrolled inflammation. Three variants (Arg702Trp, Gly908Arg, Leu1007fsinsC) are associated with increased risk of ileal and ileocolonic, but not colonic CD. Interestingly enough, these mutations are associated with unique disease phenotypes, such as younger age of onset and fibro-stenotic CD. IL23R is involved in the IL12/23 pathways of inflammation. The glutamine allele of Arg381Gln is much less common than the arginine allele, and appears to protect...
against development of CD in both non-Jewish [odds ratio (OR)=0.26, 95% confidence interval (CI) (0.15 to 0.43)] and Jewish [OR=0.45, 95% CI (0.27 to 0.73)] case-control cohorts. IL23R gene on chromosome 1p31 encodes a subunit of the receptor for the pro-inflammatory cytokine interleukin-23. Consequently, blockade of the IL-23 signaling pathway would be a rational therapeutic target for IBD.

ATG16L mutation is associated with a process called autophagy, where cellular ‘debris’ are degraded by lysosomes, and can be used as an innate immune response to clear intracellular pathogens. Cadwell et al. reported that compared to ileal samples from CD patients that had no risk alleles, ileal specimens from CD patients homozygous for the ATG16L1 risk allele demonstrated Paneth cell abnormalities. A SNP (Ala281Thr) was found to be highly associated with CD, with the less common threonine allele being protective. Failed stimulation of autophagy is likely to be associated with impaired response of the innate immunity to intestinal microbial flora.

Interestingly enough, IBD risk loci are significantly different among different ethnic populations. No association was found between NOD2 variants and CD among Asian populations. GWA study, which was conducted in Japanese IBD patients and controls, identified significant association between SNPs and haplotypes within TNFSF15, which is an enhancer of interferon (IFN)-γ production in T-cells and natural killer cells. Less significant association was also found in the study of Caucasian IBD families. Recently, three new susceptibility loci: the immunoglobulin receptor gene FCGR2A (rs1801274, P=1.56×10(-12)), a locus on chromosome 13q12 (rs17085007, P=6.64×10(-8)) and the glycoprotein gene SLC26A3 (rs2108225, P=9.50×10(-8)) were identified in a two-stage GWA study, followed by a replication study including 1384 Japanese UC patients and 3,057 controls. Particularly, FCGR2A was reported to be associated with other autoimmune diseases, suggesting the relevance of immune complex pathway in UC pathogenesis.

**DYSBIOSIS**

It has become quite clear from genetic studies that genetics alone is insufficient to explain the development of IBD. Monozygotic twin studies show the concordance rate for disease being 40-60%. It is well accepted that the environment, particularly gut flora, must play a key role in the development of disease.

A broken balance in the proportions of “protective” and “harmful” bacteria has been termed “dysbiosis”, which is now considered to be vital in the pathogenesis of IBD. Higher concentrations of mucosal bacteria were found in IBD patients than in normal controls and the concentrations increased progressively with the severity of disease, both in inflamed and non-inflamed colon. It also has long been recognized that bacteria are essential to the development of IBD. Patients with IBD are empirically known to respond favorably to antibiotic treatment and faecal diversion and have higher antibody titers against indigenous bacteria than unaffected individuals. Furthermore, inflammation is particularly pronounced in the intestine where higher number of bacteria is present such as in terminal ileum and colon. A recent meta-genomic study compared the microbiota of patients with IBD with that of non-IBD controls by a culture-independent rRNA sequence analysis and revealed a statistically significant difference in their composition. Specifically, the microbiota of IBD patients showed abnormal microbial composition that was characterized by depletion of two phyla of bacteria, the Firmicutes and Bacteriodetes, which are both prominently represented in non-IBD controls. However, it is unknown whether these altered microbiota in IBD patients is the primary pathogenesis of IBD or just the secondary outcome of the inflammation.

The term probiotics is used to describe dietary microorganisms that are beneficial to the health of the host. Recent evidence also suggests that the induction of Treg cells by these microorganisms is essential in their ability to suppress inflammation and disease. Treatment of colitis mice with the probiotic cocktail VSL#3 increased the production of IL-10 and the proportion of TGF β-expressing T cells. Furthermore, transfer of lamina propria mononuclear cells from VSL#3-treated mice prevented colitis in recipient mice. The suggested probiotic mechanisms include not only immunomodulation, which was described above, but also competitive exclusion of microbial pathogens,
antimicrobial activity, and enhanced barrier activity, and T-cell apoptosis.\textsuperscript{31}

Intestinal mucosal immune system consists of orchestra of various innate and acquired immune mechanisms, which will be discussed below and summarized in Fig. 1.

**INTESTINAL EPITHELIUM**

Intestinal epithelium is a physical barrier that prevents excessive bacteria and other antigens from entering into the circulation. Normal barrier function is dependent

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**Fig. 1.** Intestinal mucosal immune system. Intestinal mucosal immune system consists of orchestra of various innate and acquired immune mechanisms. Innate immune mechanism includes barrier function of intestinal epithelial cells, secretion of antimicrobial peptide such as α-defensins from paneth cells, innate microbial sensing by epithelial cells and dendritic cells through TLRs or NOD, and activation of macrophage and dendritic cells, which leads to phagocytosis or proinflammatory cytokine production. Acquired immune mechanism includes activation of Th1, Th2 or Th17 effector T cells, which leads to production of effector cytokines, cellular immunity of killer T cells, and secretion of IGA by plasma cells. Regulatory T cells suppress effector T cells in the lymphoid organ and lamina propria, which contribute to the homeostasis of intestinal immune system.
upon the intercellular junctions. But in IBD patients, the paracellular space has increased permeability and the regulation of tight junction is disruptive. Another barrier mechanism consists of specialized cells such as goblet cells and Paneth cells. Goblet cells play an important role for regulating the production of mucus, as well as for repairing epithelium and regulating inflammation. Paneth cells secrete antimicrobial peptides such as α-defensins into the small intestinal lumen. In ileal Crohn’s disease patients, α-defensins in Paneth cells are deficient, which result in increased exposure to intestinal microbiota and subsequent inflammation. Other possible disorders of epithelial functions were derived from the results of genome-wide association maps. For example, polymorphisms close to the gene encoding EP4 (PTGER4) were implicated in Crohn’s disease. Moreover, a variant in a genomic region that includes the gene encoding MUC19, one of gel-forming mucins, has been associated with Crohn’s disease.

Endoplasmic reticulum (ER)-mediated stress responses in intestinal epithelial cells are enhanced in IL-10 deficient mice as well as in IBD patients, thus may contribute to the chronic intestinal inflammation. The transcription factor XBP1 is a key component of the endoplasmic reticulum (ER) stress response, which is a cellular response triggered by various environmental changes. An association of XBP1 variants and both UC and Crohn’s disease was identified. IL-22 is known to contribute to increased epithelial resistance to injury by microbial infection in gut. Recently, a population of NKp46+ cells that expressed RORγt, which is a transcription factor previously associated with lymphoid tissue-inducer cell, were found. They also produced interleukin-22, a member of IL-10 family of cytokines, suggesting their role as detectors of epithelial cell damage.

Notch signaling regulates differentiation and proliferation of intestinal epithelial cells. In ulcerative colitis patients, Notch signaling is activated in increased number of epithelial cells, resulting in goblet cell depletion and ectopic expression of PLA2G2A. Also, inhibitor of Notch signaling significantly exacerbated a mice model of colitis, by suppressing the regenerative response of the epithelium. Thus, Notch signaling may function as a key molecular pathway in epithelial regeneration of IBD patients.

**INNATE IMMUNITY**

Innate immunity is the ancient form of immunity that is not specific for pathogens. It is responsible for the initial and rapid immune responses, which localize and eradicate invading pathogens for the host’s survival. In the intestine, innate immunity includes the epithelial barrier and phagocytic cells within the lamina propria (macrophages, dendritic cells [DCs], and neutrophils). They express various types of innate immune receptors such as toll-like receptor (TLR) and Nod-like receptor (NLR), which recognize general microbial patterns, in contrast to antigen specific receptors such as T cell receptors or immunoglobulins of the adaptive immune system. These receptors not only mediate defense against luminal microbiota but also regulate tolerance of epithelial and antigen presenting cells. The down-regulation of the expression and response of these receptors limit activation of intestinal epithelial cells by luminal microbes, which contribute to homeostasis of intestine. Sampling of intestinal microbiota is also important for the regulation of intestinal immune system. Microbial sampling occurs by translocation of microbes across epithelial cells or M cells in Peyer’s patches, by immunoglobulin or dendritic cells. Activated antigen presenting cells then present peptide antigen to T cells in secondary lymphoid organs, such as mesenteric lymph nodes and Peyer’s patches. This encounter initiates the subsequent adaptive immune response.

NOD2 is an intracellular sensor of peptidoglycan, a component of bacterial cell walls. As mentioned above, it is reported that three polymorphism of NOD2 gene increase frequency of CD in European or American, but not Asian populations. The activation of the NOD2 protein by bacterial peptidoglycan activates NF-κB and MAP kinase signaling pathway, which leads to the production of proinflammatory cytokines such as TNF or IL-1β. These NOD2 carriers show decreased secretion of proinflammatory cytokines and decreased activation of NF-κB on stimulation of NOD2 with bacterial peptidoglycan. How NOD2 impairment increases susceptibility to CD is unknown, but it
suggests deep association of pathogenesis of IBD with innate immune system.

Alteration of TLR3 and TLR4 expression of intestinal epithelial cells of IBD patients has been reported. In addition, some groups reported that polymorphism of TLR4 gene is associated with the development of UC and CD.

**ADAPTIVE IMMUNITY**

Despite the increased knowledge about the role of innate immunity in the intestinal inflammation, the adaptive immunity, specifically helper T cells (Th), has been most closely correlated with IBD pathogenesis. Adaptive immunity is characterized by specificity and memory and is mostly mediated by lymphocytes that express antigen receptors on their surface. When naïve T cells are presented specific antigen by antigen-presenting cells in secondary lymphoid organ, they differentiate to effector T cells. Following the elimination of antigen, most of effector cells undergo apoptosis, and then a small number of memory T cells survive. At the second attack of antigen, memory T cells rapidly differentiate to effector cells and exert effector function, which is the system of immunological memory. CD4+ effector T cells can be divided into three different subgroups, Th1, Th2 or Th17, which secrete characteristic types of cytokine. Th1 cells produce IFN-γ and TNF-α, and play an important role for the protection against intracellular microbes. Th2 cells produce IL-4, IL-5 and IL-13 and they direct the immune response against extracellular pathogens including parasites. Th1 differentiation is mainly driven by IL-12 under the control of transcription factor T-bet. On the other hand, Th2 differentiation is induced by IL-4 under the control of GATA-3. In addition to these subgroups, Th17 cytokines were elevated several animal models of IBD that had been thought as Th1 or Th2 type disease. IL-23p19 transgenic mice develop systemic autoimmune disease including chronic colitis. Some models aggravate colitis when they are administered with rIL-23. In addition, neutralization or genetic defect of IL-23 ameliorates colitis in some models. As mentioned above, in human, several GWASs revealed highly significant associations between Th17/IL-23 pathway and IBD. Elevated expression of IL-17, IL-21, IL-22, IL-23, RORγt and IL-23R in inflamed mucosa of CD and UC patient is reported. Furthermore, specific subset of macrophages, which secreted IL-23, TNF and IL-6, are increased in intestinal mucosa of CD patient. Recently, anti-p40 monoclonal antibodies have been reported to effective for CD, which suggest the importance of Th17/IL23 pathway in IBD.

Regulatory T cells (Treg) are special subset of T cells, which suppress the effector function of other T cells, and play important roles in the homeostasis of intestinal mucosal immune system. Regulatory T cells consist of some different groups. Although how regulatory T cells suppress effector function is not fully understood, inhibitory cytokines such as IL-10 or TGF-β is thought to play important roles. CD4+CD25+ Treg that develops in thymus is referred to as ‘natural’ Treg (nTreg). These cells express the transcription factor Foxp3, which is master regulator of nTreg. Recently, it has been reported that CD4+CD25−Foxp3− T cells can be induced by TGF-β in the absence of IL-6 in vitro. This Treg should be referred to as ‘induced’ Treg (iTreg). Scurfy mice, which defect Foxp3 gene, develop
severe autoimmune disease including chronic colitis. Furthermore, in several animal models, dysfunction or depletion of Treg cause excess activation of effector T cells against intestinal microbiota, which lead to chronic colitis. In human, patients of CD and UC have decreased number of CD4^{+}CD25^{+} Treg cells during active disease. Although several studies showed that intestinal inflamed mucosa of CD and UC patient contain increased number of Treg compared to healthy control, colonic biopsies from IBD patient have a reduced proportion of Treg compared with those from non-IBD patient such as diverticulitis. Regulatory function of Treg form IBD patient was not impaired. These findings suggest that collapse of balance between Treg and effector T cells may occur in IBD, rather than their defect or dysfunction.

IL-10 is important inhibitory cytokine. IL-10^{−/−} mice develop chronic colitis. There is a genetic association between IL10 and UC. In addition, it was reported that uncommon recessive loss-of-function mutation in either IL10RA or IL10RB resulted in CD. IL-2 is essential maintenance factor for CD4^{+}CD25^{+}Foxp3^{+} Treg. Indeed, IL-2^{−/−} mice develop chronic colitis as a result of reduced number of Treg. It is reported that T cells in the intestinal mucosa of UC and CD patient produce lower amount of IL-2 than those of healthy control, which may be one of mechanisms of relative decreased proportion of Treg in colonic mucosa of IBD patient.

IL-7 is homeostatic cytokine that is secreted by stromal cells in the bone marrow and thymus and other epithelial cells including intestinal goblet cells. IL-7 is a critical key cytokine controlling survival of peripheral resting CD4^{+} T cells, including naïve and memory cells but not effector cells. We have assumed that colitogenic memory T cells, which survive for life long even in remission, play important roles in the perpetuation of IBD, and noticed IL-7 as the important survival factor for these cells. We have previously demonstrated that IL-7 transgenic mice developed chronic colitis that mimicked histopathological characteristics of human IBD. In addition, the selective elimination of CD4^{+}IL-7R_{α}^{high} T cells by anti-IL-7R_{α} mAb or genetic depletion of IL-7 ameliorated colitis in animal model. These findings suggest that therapeutic approaches targeting systemic IL-7 may be epoch-making therapy for the treatment of IBD, which can “reset” the pathogenic memory.

**LEUKOCYTE MIGRATION AND MICROVASCULATURE**

The entry of T cells into intestinal tissues is regulated by adhesion molecules such as selectins and integrins, and chemokines. Activated T cells in the secondary lymphoid organs become gut-tropic cells by expressing the integrin α_{4}β_{7} and the chemokine receptor CCR9. In the priming of gut-homing specificity, the retinol metabolite, retinoic acid, was reported to enhance the expression of the adhesion molecules. Medications that stop the entry of T cells into the gut would be expected to have a beneficial effect on intestinal inflammation. Currently, α_{4}β_{7}-based anti-integrin therapy seems to be beneficial for a subgroup of, but not all IBD patients, suggesting its simultaneous effect on the suppressor cell group.

In IBD, microvasculature contributes to chronic inflammation by altered leukocyte recruitment, impaired perfusion, and angiogenesis leading to tissue remodeling. Consequently, microvasculature is considered to be another therapeutic target for IBD.

**LIPID METABOLISM**

PPAR (peroxisome proliferator-activated receptor)-γ is a lipid-activated transcription factor and expressed in liver, adipose tissue, IECs, and hematopoietic cells involved in the regulation of lipid metabolism, inflammation, and cancer. PPAR-γ is prominently expressed in the intestinal epithelium, which is dependent on the intestinal microbiota, and regulated by microbial metabolites. UC patients show decreased expression of PPAR-γ in IECs but not in hematopoietic cells, suggesting the impaired PPAR-γ signaling in UC pathogenesis. In animal models, conditional PPAR-γ depletion in the intestinal epithelium leads to spontaneous colitis, increased susceptibility to DSS colitis, and prevention of the beneficial effects by PPAR-γ agonists. Recently, the association between PPAR-γ signaling and ER stress is suggested. Pioglitazone was
reported to reduce ER stress and prevent diabetes by pancreatic islet cell protection.\textsuperscript{99}

### CONCLUSION

Advancing understanding in the complex interactions between genetics, environmental factors such as commensal intestinal bacteria, and immunological factors in IBD is expected to allow us to identify several key points where intestinal inflammation can be modulated, and expand our treatment options for IBD.

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