2. Molecular Biological Studies of the Pathogenesis in Inflammatory Bowel Disease

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General features of inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic relapsing and remitting inflammatory condition of the gastrointestinal tract which comprises Crohn's disease (CD) and ulcerative colitis (UC). The pathogenesis of IBD remains unknown, although in recent years more data have become available. Both CD and UC result from interrelated genetic and environmental factors that are channeled through an abnormality in mucosal immune function, possibly due to dysregulated or excessive T helper cell responses. Strengthened by promising experimental data, research in this field is rapidly expanding. These revealed that the pathophysiology of CD and UC is quite different. Namely, functional abnormalities of monocyte/macrophage and Th1-type immune response exist in CD, while functional abnormalities of colonic epithelial cells and dysregulation of T/B cells are suggested in UC (1, 2).

Pathogenesis of Crohn's disease

Crohn's disease is characterized by transmural inflammation that could affect any part of the gastrointestinal tract, and the disease relapses and remits throughout its course. Genetic contribution is believed to play an important role in the pathogenesis of CD, compared with UC. Monozygotic twin concordance, familial predisposition and segregation analyses have demonstrated that genetic factors confer the susceptibility to the disease. Although several loci for CD have now been identified, most notably, the pericentromeric region of chromosome 16, designated as *IBD1* (3), no specific gene had been identified. Recently, it has been demonstrated that three variants in the coding region of the *NOD2* gene, located on chromosome 16q12, are associated with CD in Western countries (4–6). Notably, Ogura et al demonstrated that *NOD2* is an intracellular receptor for lipopolysaccharide and is exclusively expressed in monocyte/macrophage lineage cells (7). However, we have demonstrated that none possessed these common *NOD2* variants among the subjects in three independent study groups, including CD pa-

tients, UC patients and healthy controls (8). These results indicate that common variants in *NOD2* found in Caucasian patients with CD are not associated with CD in the Japanese population.

It is well established that the activation of macrophages and their cytokine production is important in Th1 immune responses in CD (Fig. 1) (9, 10). We demonstrated that IL-12 and IL-18 was produced from activated macrophages and they synergistically affect T helper cells to produce IFN- γ , and lead to chronic intestinal inflammation in CD (11). Next, we assessed the role of macrophages and of IL-18 in 2,4,6-trinitrobenzen sulfonic acid (TNBS) colitis, the murine model of intestinal inflammation that mimics the immunologic characteristics of human CD. TNBS was unable to induce significant colitis in the IL-18 $^{-/-}$ mice and the administration of the neutralizing anti-IL-18 antibody resulted in an attenuation of TNBS colitis. Moreover, the administration of an antibody against macrophages conjugated with the saporin toxin (anti-Mac-1-saporin) resulted in a dramatic attenuation of mucosal inflammation with a marked decrease of macrophages (12). These results underscore the pivotal role of macrophages and macrophage-derived IL-18 in the pathogenesis of CD.

Based upon these central roles of activated macrophages in the pathogenesis of CD, novel therapies directed against activated macrophages or cytokines produced by activated macrophages in patients with CD have been developed. Among these, only one therapy that is already approved for clinical use is the chimeric monoclonal anti-TNF- α antibody infliximab (13). TNF- α mainly produced by activated macrophages induces upregulation of proinflammatory mediators, metalloproteinases and adhesion molecules. A series of clinical trials have demonstrated convincing evidence for a pivotal role of TNF- α in CD. Infliximab neutralizes soluble TNF- α and binds to membranous TNF- α , causes lysis of TNF- α -expressing cells by antibody-dependent and complement-dependent cellular cytotoxicity, and induces apoptosis of activated macrophages. Studies in animal colitis models have demonstrated that antibodies against IL-12 can prevent or treat intestinal inflammation. A phase II dose-finding trial with a human anti-IL-12 antibody in patients with active CD is currently underway in western countries.

Figure 1. Pathogenesis of Crohn's disease.

Pathogenesis of ulcerative colitis

Ulcerative colitis is chronic idiopathic inflammation of the rectal and colonic mucosa. There is a possibility that UC might be a sort of syndrome. Some might be viral/bacterial infections and some might be autoimmune. However, according to the evidence, the central core of UC is thought to be an organ-specific autoimmune disease accompanied by many extra-colonic manifestations. The low rates of concordance in monozygotic twin pairs with UC compared with twins with CD suggest that environmental and immunologic factors are more important than genetic factors in the pathogenesis of UC. The presence of autoantibodies, such as anticolon antibody, antimucin antibody, or antitropomyosin antibody, is suggestive of a Th2 pathogenesis in contrast with a role for Th1 in CD. There are several conflicting immunological findings, however, we are assured that some immunological abnormalities may induce colonic mucosal inflammation. The effectiveness of thymectomy (14) and the recent success of the treatment with cyclosporine (15) indicate that T cells with certain abnormalities play an important role in the pathogenesis of UC (Fig. 2).

Two signals provided through the T cell receptor and costimulatory molecule are required for T cell activation. We demonstrated that colonic epithelial cells from inflamed mucosa of UC expressed CD86, but not CD80. In contrast, colonic epithelial cells from control subjects, UC patients at remission stages, or patients with CD did not express CD86 (16). Together with the upregulation of MHC class II antigen on epithelial cells from inflamed mucosa, colonic epithelial

cells may act as antigen presenting cells, and contribute to the activation of T cells through costimulatory molecule CD86 expression in inflamed mucosa of UC.

Among the autoantibodies described, autoantibodies against colonic epithelial cells (anticolon antibody) are frequently found in the serum of UC patients, especially those of chronic continuous type (17). These antibodies have been shown to contribute to the destruction of colonic mucosa through antibody-dependent cell-mediated cytotoxicity mechanisms against colonic epithelial cells. To determine the specific antigen(s) on colonic epithelial cells, we established B cell clones that produced anticolon antibody from inflamed mucosa of UC patients and analyzed the variable region of immunoglobulin heavy chain (VH) genes. All clones from UC used a restricted VH3 gene family, whereas diverse VH gene families were used by clones from normal controls. The analysis of nucleotide sequences indicated that these clones were derived from various germline gene segments (18). These results suggest that a particular antigenic stimulus contributes to the pathogenesis of UC, and that the colonic antigens or epitopes recognized by this autoantibody in UC is heterogeneous.

How are T cells regulated and activated in normal and inflamed colonic mucosa? We have demonstrated that intestinal epithelial cells produce interleukin-7 (IL-7) and that IL-7 serves as a regulatory factor for the proliferation of mucosal lymphocytes (19). IL-7 expression in the inflamed colonic mucosa of UC at chronic active stage was notably decreased

Figure 2. Pathogenesis of ulcerative colitis.

compared with normal colonic mucosa or colonic mucosa of UC at remission stage (20). Decreased production of IL-7 probably due to the depletion of IL-7-producing goblet cells results in a decrease in apoptotic cell death of activated mucosal lymphocytes expressing IL-7 receptor (IL-7R). These activated mucosal lymphocytes eventually cause and maintain inflammation in the intestinal mucosa. We have developed a novel therapy targeting these infiltrated activated mucosal lymphocytes expressing IL-7R, and obtained promising results in chronic colitis in T cell receptor α knockout mice, a murine model of human UC.

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