LECTURE

Pathogenesis of Gastritis in Ileitis-prone SAMP1/Yit Mice

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Inflammatory bowel disease is a chronic inflammatory disease of the gut which manifests as ulcerative colitis or Crohn's disease. One of the most studied animal models of spontaneous Crohn's disease is the senescence-accelerated mouse (SAMP1/Yit strain) model.[1] In SAMP1/Yit mice, although many immunological responses are perturbed, some evidence suggests that the primary defect lies in the epithelial cell barrier. In the process of studying epithelial permeability, we observed that the stomach in SAMP1/Yit mice also had increased permeability. Upon further examination, these mice were shown to have marked, chronic gastritis with focal to diffuse aggregates of mononuclear cells of mixed lineages. These aggregates were located predominantly in the oxyntic mucosa, with occasional lesions in the forestomach but with relatively fewer cellular infiltrates in the antral mucosa. Real-time reverse transcription (RT) PCR showed an increase in several helper T cell (Th cell)-derived pro-inflammatory cytokines in the gastric mucosa of SAMP1/Yit mice. However, many of the cells in the aggregates of SAMP1/Yit mice were B cells. SAMP1/Yit B cells exacerbate ileitis when co-transferred into immunodeficient recipients. The gastritis also reflects a contribution by B cells. As SAMP1/Yit mice were derived from AKR mice, we examined AKR mice and determined that they too have an increased occurrence of gastritis, although they do not develop ileitis. B cells contributed to the gastric inflammation in these mice also. Thus, SAMP1/Yit mice display gastritis as well as ileitis, and B cells appear to play a role in the pathogenesis of inflammation at both sites. This lecture will discuss some of the mechanisms that may account for these different manifestations of gastrointestinal disease. (Keio J Med 60 (2) : 65–68, June 2011)

Keywords: gastritis, ileitis, Crohn's disease, B cell

Introduction

The gastrointestinal tract faces the unique immunological challenge of coping with a dynamic and vast array of dietary and microbial antigens. Chronic inflammatory disease of the intestine as well as the stomach of adults and children[2–4] arises in genetically susceptible individuals that fail to properly regulate host responses to these luminal antigens. The importance of gastrointestinal infections in Japan is illustrated by the frequency of *Helicobacter pylori* infection[5] and its key role in the pathogenesis of gastric cancer.[6,7] Another example of chronic inflammatory disease in the gut is inflammatory bowel disease, which manifests as ulcerative colitis or Crohn's disease. Crohn's disease tends to affect the terminal ileum, but in humans, the disease can arise virtually anywhere within the gastrointestinal tract.

Animal Models of Inflammatory Bowel Disease

For many years, it was believed that intestinal immune responses were carefully regulated to prevent excessive amounts of inflammation caused by luminal antigens. This notion was confirmed when genetic engineering was applied broadly to manipulate gene expression in mice. It quickly became apparent that disruption of several different genes leads to the development of colitis.[8–10] It is now appreciated that disrupting the expression of several genes encoding proteins that function in a related signaling pathway often leads to disease. For example,

deficiencies in *Il-10*,[10] its receptor[11] or downstream signaling molecules[12] all result in colitis. *Il-10* receptors are broadly expressed in cells in the gut[13,14] and contribute to anti-inflammatory responses. It is intellectually satisfying that an entire pathway related to a receptor, its ligand and signaling molecules all contribute to the control of host responses. However, there are many gene knockout models that target unrelated pathways that still result in the development of intestinal inflammation. For example, mutations affecting the epithelial cell barrier,[15] nerves,[16] and innate and adaptive responses[17,18] can all lead to colitis. These observations speak to the diverse aspects of the host response that contribute to the optimal control of the host response to luminal antigens.

While the appearance of intestinal inflammation in various mouse models differs, most of this inflammation is focused in the colon and, in general, provide a better model of ulcerative colitis than Crohn's disease.[19] In some mouse models, such as the *Il-10* knockout mouse, inflammation of the small intestine has been observed[10] in addition to the more common colitis. Two other models, the SAMP1/Yit[1,20] mouse and the TNF$^{\Delta ARE}$[21, 22] mouse, develop inflammation in the terminal ileum rather that the colon and thus are often preferred as a model with which to study Crohn's disease.

The Role of the Microbiome in Gastrointestinal Inflammation

While luminal antigens, particularly microbes, have long been believed to be factors that trigger or ameliorate disease, direct evidence supporting this notion has emerged with the use of gnotobiotic mice. When colitis-prone mice are housed in germ-free conditions, almost all of the strains remain healthy.[2] For example, germ-free *Il-10*-deficient mice do not develop disease.[23] Using germ-free mice, it is possible to identify a single species, or a limited numbers of species, as triggers of gastroenteric disease. While the intestinal microbe *Helicobacter hepaticus* can exacerbate disease in *Il-10*-deficient mice,[24] it was insufficient to trigger colitis in germ-free mice.[23] In fact, in wildtype mice, *H. hepaticus* induces a regulatory helper T cell that prevents inflammation by this organism.[25] While some bacteria may promote disease[26] and others attenuate it,[27] in general, colitis that occurs in various knockout mice results from an inappropriate intestinal immune and inflammatory response to the normal gastrointestinal microbiome that is usually not pathogenic in wildtype mice. This is consistent with the fact that so many genetically engineered mice develop colitis spontaneously without any intentional manipulation of their microbiome.

Characterization of the SAMP1/Yit Mouse as a Model of Crohn's Disease

Senescence-accelerated mouse (SAM) strains were originally derived from AKR mice.[28] One of these strains, the SAMP1/Yit mouse, was subsequently shown to have many manifestations resembling human Crohn's disease, including discontinuous and transmural chronic inflammation of the terminal ileum and, in some cases, perianal fistulae.[1,20,29] Other studies have shown that ileitis is associated with pro-inflammatory cytokine production – Th1 responses in younger mice[20] but also Th2 cytokines,[30] particularly as the mice mature.[31]

Extraintestinal Manifestations in the SAMP1/Yit Mouse

Although many immunological responses are perturbed in the SAMP1/Yit mouse, some evidence suggests that the primary defect lies in the epithelial cell barrier. In the process of studying intestinal epithelial permeability,[32] we showed that the stomach in SAMP1/Yit mice also has a disrupted epithelial cell barrier function.[33] Upon further examination, it became obvious that the permeability defect in the SAMP1/Yit stomach was associated with marked gastritis (Ernst et al, manuscript in preparation). The histological changes include focal to diffuse aggregates of mononuclear cells of different lineages, including CD3$^+$ T cells and B220$^+$ B cells. These aggregates are located predominantly in the oxyntic mucosa with occasional lesions in the forestomach, but with fewer cellular infiltrates in the antral mucosa. Inflammation in the oxyntic mucosa mimics that in the gerbil model of *H. pylori*-induced gastritis and parietal cell atrophy.[6] Infiltration with polymorphonuclear cells (including neutrophils) was remarkably modest. Realtime RT PCR shows an increase in many of the pro-inflammatory Th cell-derived cytokines in the gastric mucosa of SAMP1/Yit mice (Ernst et al, manuscript in preparation).

The majority of cells in the gastric aggregates of SAMP1/Yit mice are B cells. As SAMP1/Yit mice are derived from AKR mice, we examined AKR mice and determined that they too have significant gastritis. B cells also contribute to the gastric inflammation in AKR mice.

B cells appear to have pathogenic roles in multiple tissues. The recruitment of B cells exacerbates inflammation in the ileum[34]; this recruitment is dependent on beta 7 integrin.[35] The role of these B cells in the pathogenesis of ileitis is not entirely clear. It has been suggested that B cells from SAMP1/Yit mice express glucocorticoid-induced TNFR-related protein (GITR) ligand which, when engaged into its receptor GITR, decreases the function of regulatory helper T cells.[34] Interestingly, B cells in SAMP1/Yit mice produce less *Il-10* and TGF-β,[36] which may affect their function and favor a role in the pathogenesis of inflammation. Thus, it is possible that the B

cell defect(s) in SAMP1/Yit mice affects their regulatory function; these defective B cells could contribute directly to inflammation, perhaps through the production of antibodies leading to immune complex-mediated damage.

In the original description of the disease in SAMP1/Yit mice, the authors reported "no significant extraintestinal manifestations" detected in the stomach, liver, spleen, thymus and various lymph nodes.[20] Subsequently, it became clear that these mice also have some skin lesions as well as mononuclear cell aggregates in their livers,[1] and, based on our studies, gastritis. There are several possible explanations for this discrepancy. First, there could be genetic drift between strains housed at different institutions. While possible, this seems unlikely since SNP analysis of the founding and current strains at our institution appear genetically identical (Drs. Jesus Rivera-Nieves and M. McDuffie, personal communication). Moreover, SAMP1/Yit mice were originally derived from AKR mice,[28] which also develop gastritis. Thus, the tendency to develop gastritis may lie in the AKR background that is embedded in SAMP1/Yit mice. Another interesting avenue to explore is the role of epigenetic control of the disease.

The inability to detect gastritis previously could have been due to differences in the microbiome in the mice now compared to the initial studies. This is indeed possible because bacteria can at least exacerbate and/or hasten the onset of lesions in SAMP1/Yit mice. In other cases, the presence of certain bacterial species can attenuate the ileitis.[37–39] (It should be noted that, in view of the presence of gastritis, the effects of inflammation on gastric pH may favor the ability of probiotics to be protective, and, in turn, these bacteria may not necessarily colonize human Crohn's disease patients that have normal gastric acid.) Importantly, histological review of stomachs from germ-free mice shows occasional evidence of the aggregates that are typical of this form of gastritis (unpublished observation 2005), and inflammatory changes are also found in the ileum.[40] Thus, inflammation in the gastrointestinal tract of SAMP1/Yit mice is not entirely dependent on a full microbiome, although it is exacerbated by the presence of microbial communities. It is possible that dietary antigens or microbial products within mouse chow were sufficient to trigger these lesions; however, further study is required. A very likely factor in the failure to recognize gastritis in SAMP1/Yit mice previously is that many scientists reviewing histology in murine models may not have a complete awareness of some of the subtle aspects of comparative pathology. Thus, they may have simply failed to recognize gastritis in these animals, especially if they were being compared with AKR, which also have gastritis.

Summary

In summary, the SAMP1/Yit mouse continues to provide an interesting model for studying the pathogenesis of inflammatory diseases. However, disease in these mice is not limited to the ileum but is also manifest broadly in the skin, liver and, in particular, the stomach. How these lesions relate to each other and whether they reflect a common mechanism related to the pathogenesis of Crohn's disease remains unclear. It is more likely that they differ significantly in their tissue-specific pathogenesis, but distinct pro-inflammatory mechanisms may emerge due a general defect in SAMP1/Yit mice.

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