

Review

Experimental Models of Inflammatory Bowel Disease

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Abstract. The etiology and pathogenesis of inflammatory bowel disease (IBD) remains unsolved, but improved experimental models of enterocolitis have led to progress. Intestinal inflammation and experimental IBD can be induced by chemical or dietary factors or by microbial products. Many animal models of IBD can be used to evaluate new anti-inflammatory drugs. These models, however, usually demonstrate acute, self-limiting colitis. The spontaneous colitis models developed in the cotton-top tamarin monkey and the C3H/HeJBir mouse mimic more features of human IBD. Inflammation is chronic and is under genetic control. The differential genetic susceptibility of inbred rat strains to chronic inflammation have been exploited. Lewis rats injected with bacterial products, peptidoglycan polysaccharide or indomethacin develop chronic relapsing enterocolitis, whereas closely related Buffalo or Fisher rat strains develop only transient inflammation. These models are also useful to test the specific inhibition of inflammatory mediators and target molecules. Over-expression (transgenic) or deletion (knockout) of specific genes have led to the development of rodent models of spontaneous colitis. Inflammation arises from a number of mutations of immunomodulatory molecules, supporting the concept of genetic heterogeneity for IBD. The results obtained from experimental models have generated new hypotheses, expanded human studies, and suggested novel forms of therapy for IBD patients.

Key words: experimental enterocolitis; kininogen; plasma kallikrein; intestinal inflammation; animal models.

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are complex disorders characterized by chronic, local, and systemic inflammation and a spontaneously relapsing course. These idiopathic disorders show both genetic and environmental components, immunologic alterations, and activation of proteolytic cascades²³. The pathogenesis of IBD is not completely understood, but considerable progress has been made owing to the development of novel animal models⁵. The question arises as to which model of IBD is the most useful? An appropriate response is that the value of each model depends on the particular application. Animal models

have been used to study each phase of inflammation. The multiple factors contributing to IBD may be divided into major components: environmental, genetic, immunologic, inflammatory, and repair mechanisms. The preexisting environmental, genetic, and immunologic backgrounds are required for the initiation of early events in the inflammatory process^{6, 27}.

Most inflammatory cascades represent a final common pathway to tissue damage. Environmental factors include non-physiological bacterial flora, drugs, smoking and stress factors. Genetic components consist of human major histocompatibility complex molecules or epithelial proteins such as the HLA component. Studies

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at the genomic level have identified a linkage to specific chromosomes which increase susceptibility to both human CD and UC on chromosomes 3, 7, and 12²⁶ and a locus on chromosome 16 exclusively in CD.

Cell-related immunity may be due to altered function of specific T cells (regulatory or effector), T cell-specific cytokines, B cell-related immunoglobulins or innate effector cells (mainly neutrophils and macrophages). T helper (Th)-lymphocyte (CD4⁺) cytokine secretion phenotype has an important influence on the chronicity of intestinal inflammation in IBD. The Th1 lymphocyte appears to be selectively activated in CD, as demonstrated by increased interleukin 2 (IL-2) and interferon γ (IFN- γ), whereas in UC, activation of the Th2 lymphocyte with a cytokine profile of IL-4 and IL-10 predominates. In addition, complement and kallikrein-kinin system (KKS) activation may play a role, as well as such inflammatory mediators as eicosanoids, lysosomal enzymes, and coagulation/fibrinolytic components. Interactions between environmental, genetic and immunologic factors appear to be crucial to the development of IBD. It is apparent from animal models that the initiating and perpetuating mechanisms are frequently distinct⁶. Episodic infection and environmental toxins may induce transient injury. The normal response is suppression of inflammation¹⁶; however, in genetically susceptible hosts the response that leads to chronic inflammation may be amplified. According to the inducing factors used in experimental IBD, animal models may be divided into those induced by exogenous factors (chemicals or bacterial components), genetic models that may be due to altered genetically determined host susceptibility or spontaneous mutation, genetically engineered models, and immunologic models^{8, 19}. Many of the models induced by exogenous toxins, acetic acid, trinitrobenzene sulfonic acid in ethanol, formalin, and 5% dextran sulfate sodium (DDS) have been used to evaluate new anti-inflammatory drugs. These models, however, usually demonstrate acute, self-limiting colitis. New insights into the pathogenesis of IBD have been made by considering the differential genetic susceptibility of different inbred rat strains.

We have employed two experimental models of enterocolitis: an enterocolitis model initiated by peptidoglycan-polysaccharide from group A streptococci (PG-APS) and an indomethacin-induced model. Lewis rats injected with PG-APS or indomethacin develop chronic relapsing enterocolitis with extraintestinal manifestations, whereas inbred Buffalo or Fisher rats develop only transient acute inflammation and no systemic manifestations. Female Lewis rats, the highest responders, develop acute intestinal inflammation that peaks 1–2 days



Fig 1. Gross features of inflammation in Lewis rats 3 weeks after intramural PG-APS injection. A dense adhesion covers the cecum. The liver is enlarged with multiple nodules. (Reproduced with permission from reference³⁰)

after PG-APS injection, gradually decreases over the next 10 days and spontaneously reactivates beginning on day 14, accompanied by peripheral erosive arthritis, granulomatous hepatitis, normochronic anemia, and leukocytosis with histological findings of intestinal fibrosis and granulomas resembling CD (Fig. 1). Inflammation induced by PG-APS, similar to human IBD, is mediated by a large number of cellular and humoral inflammatory cascades which liberate soluble mediators, including cytokines, prostanoids and bradykinin^{22, 32}. Bradykinin is a product of kallikrein digestion of high molecular weight kininogen. Plasma kallikrein is a chemotactic enzyme for neutrophils and induces these cells to release elastase³⁰. We have applied a specific kallikrein inhibitor (P8720) to evaluate a direct relationship between the KKS activation and inflammatory changes.

We found that a specific kallikrein inhibitor decreased both acute²⁹ and chronic³³ granulomatous intestinal inflammation as measured by blinded gross and histologic scores (Fig. 2), indicating that spontaneous reactivation of enterocolitis and extraintestinal inflammation is in part due to the selective KKS activation in susceptible Lewis rats. Inhibition of this pathway may have a potential for human IBD therapy.

The PG-APS-induced model of colitis has unique features that mimic changes in human IBD. Inflammation is induced by an environmentally relevant antigen and has a spontaneous relapsing course. The chronicity of intestinal and extraintestinal inflammation in this model depends on the host genetic background, since closely related rat strains only have acute transient colitis. Inflammation is granulomatous and transmural. Cyclosporin prevents and treats the chronic phase of

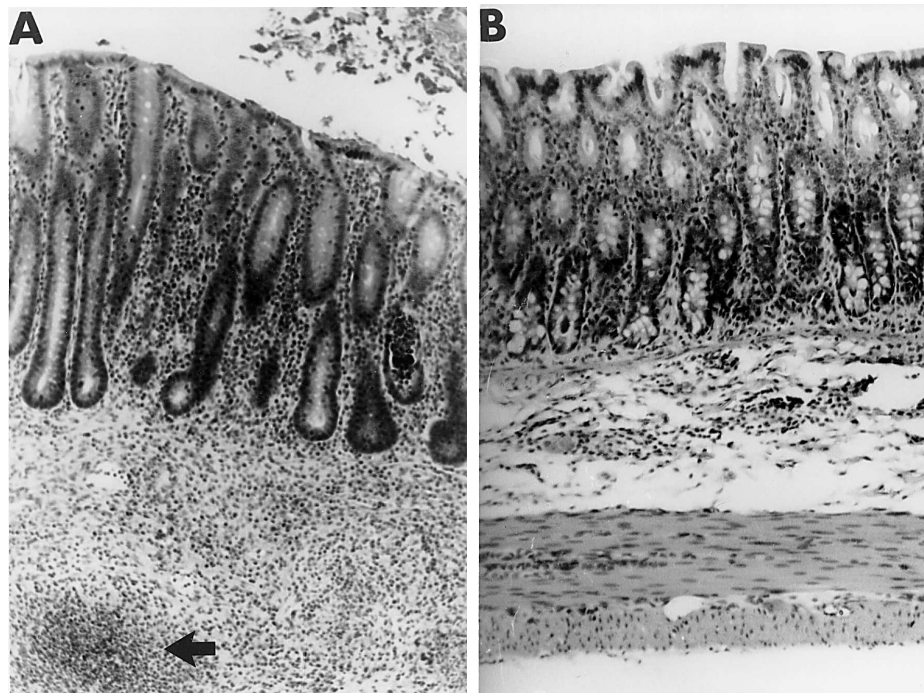


Fig. 2. Histological changes in Lewis rat intestine 3 weeks after PG-APS injection. **A** – granulomatous, transmural inflammation in a representative rat cecum with fibrosis, **B** – rat cecum after PG-APS injection and treatment with specific kallikrein inhibitor, with only minimal inflammatory changes. Original magnification $\times 25$. (Reproduced with permission from reference³³)

PG-APS-induced colitis²⁴ and chronic lesions do not develop in athymic nude Lewis rats, indicating that T cells play a critical role in the chronic phase of this model. Treatment with human recombinant IL-1 receptor antagonist¹⁵ as well as specific plasma kallikrein inhibits and diminishes the acute and chronic phase of enterocolitis, showing that both pathways have a pathogenic role. However, the use of this model is limited by an artificial non-physiological injection of bacterial products, and requirement of surgical procedures. Genetic mutations in the Lewis rat have been hypothesized but still not identified.

In the second model, we modified the indomethacin-induced enterocolitis to induce chronic intestinal inflammation in genetically susceptible rats²⁵. Lewis rats injected with indomethacin (7.5 mg/kg per day for 2 days, a sublethal dose) develop acute intestinal inflammation manifested grossly by a thickening of the bowel wall and mesenteric hemorrhage, and characterized by mesentery adhesion and multiple mucosal ulcers of the small intestine. Histologically, at day 2 the ulcers showed necrosis of the entire mucosa with several submucosal inflammations, with neutrophils and macrophages as the predominant infiltrating cells. At day 14 the rats exhibited chronic intestinal inflammation with longitudinal ulcers. Histologic ulcers, crypt abscesses in the mucosa, and transmural inflammation with fibrosis and massive thickening of the submucosa were present (Fig. 3). In contrast, the control rats, which were treated with buffer, had only minimal in-

flammatory changes. In the chronic phase, intestinal inflammation is associated with a marked hepatosplenomegaly, anemia, and a decrease of total body weight³⁷. KKS activation occurred in association with both the acute and chronic phases of intestinal injury, indicating that KKS activation is not limited to intestinal inflammation induced by exogenous bacterial products, e.g. PG-APS. Thus, we concluded that the activation of KKS is integrally involved in intestinal inflammation in genetically susceptible hosts. This conclusion is supported by our recent observation that genetically kininogen-deficient Lewis rats exhibited a decrease in chronic intestinal and systemic inflammation after being challenged with PG-APS⁹.

Our modified indomethacin-induced enterocolitis model has the advantages of induction by an easily administered, clinically relevant compound as well as the distinct acute and chronic phases and extraintestinal manifestations. Ulcerations with inflammation persist for approximately 3 months in inbred Lewis rats, but only for 2 weeks in inbred Fisher rats, indicating differences in genetic susceptibility among inbred rat strains. In contrast to intestinal inflammation triggered by PG-APS, the indomethacin model is characterized by large numbers of ulcers in the mid-small intestine. This model has also been useful in identifying the role of luminal factors, especially normal bacterial flora, in intestinal inflammation as well as in drug screening. However, the inflammation has no relapsing course. Moreover, the intestinal pathologic changes represent

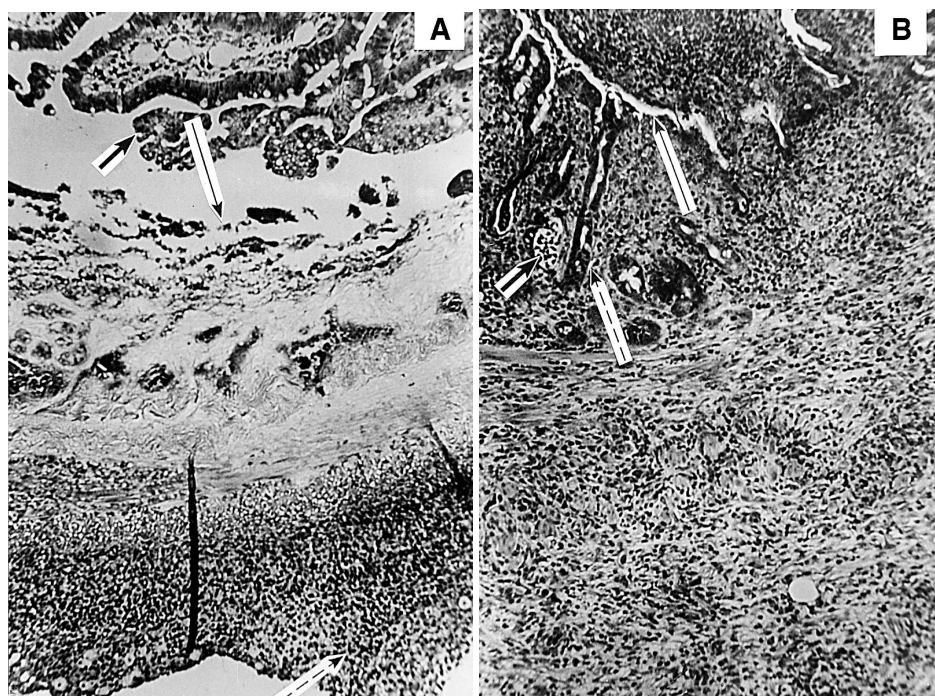


Fig. 3. Histological features of intestinal inflammation induced by indomethacin in Lewis rats. **A** – at day 2 after indomethacin treatment, a large necrotic ulcer of the entire mucosa with neutrophilic and mononuclear cell infiltration, **B** – at day 14, an ulcer, a crypt abscess, and glandular dysplasia are visible. Transmural inflammation with thickening of the submucosa is evident. Original magnification $\times 60$

intestinal injury rather than chronic inflammation. The role of immunologic factors in indomethacin-induced models is still not defined, but, for the first time, our results document a significant increase in intestinal IL-1 β in both the acute and chronic phases of inflammation.

Spontaneous colitis develops in the cotton-top tamarin, a monkey native to Columbia, South America¹⁴. Cotton-top tamarin colitis mimics many features of human IBD. Inflammation is chronic, relapsing, and is related to yet unidentified genetic mutations. Likely possibilities include class I of MHC or genes involved in mucous production. Genetic susceptibility is implicated by the lack of colitis in other types of tamarins. The disease is spontaneous and has relapses and remissions. The sequence of chronic colitis leads to dysplasia and then to colon cancer, typically after 5–8 years.

A murine model is useful to test novel therapeutic agents and to study neurogenic or stress factors. Selective breeding of C3H/HeJ mice, a strain shown to have a propensity to develop colitis, resulted in a new substrain, C3H/HeJBir, that spontaneously develops colitis³⁴. C3H/HeJBir CD4⁺ cells are strongly reactive to the antigens of enteric bacterial flora³ and provide a direct demonstration that cells reactive to conventional antigens of enteric bacterial flora can mediate chronic IBD.

Recent advances in molecular biologic techniques have led to the development of new genetically engineered rodent models of spontaneous colitis. Deletion

(knockout) of specific cytokine genes, including IL-2 or IL-10, transforming growth factor β (TGF- β) gene, T cell receptor (TCR) genes, and G protein genes (important in signal transduction) result in spontaneous colitis in mice. Over-expression (transgenic) of HLA-B 27/ β 2 microglobulin genes in rats results in a model of spontaneous colitis.

The role of tumor necrosis factor (TNF) in the pathogenesis of human IBD has been demonstrated by successful treatment using anti-TNF antibodies³⁶. A recent experimental study documented the deletion of TNF AU-rich elements from the mouse genome, which affect the mechanism responsible for TNF mRNA stabilization¹⁰. TNF AU-rich elements are a target for a signal regulating cell cytotoxicity and apoptosis, leading to human-like intestinal and joint inflammation²⁴. IL-2 is a cytokine produced by activated Th1 that promotes growth and expansion of T cells, differentiation of B cells, and activation of macrophages and NK cells. IL-2 gene knockout mice develop pancolitis and anemia²¹. Some immune abnormalities (a high number of activated T cells expressing CD44 and CD69) suggest that a shift of T cell responses to the Th2 pattern is critical in this model.

IL-10 is also a regulatory cytokine produced mainly by T cells and macrophages. IL-10 is a potent inhibitor of macrophages and the Th1 cell subset, and normally down-regulates T cell reactivity to enteric bacterial flora. IL-10 gene knockout mice develop anemia, growth retardation, and chronic IBD, indicating that

deficient production of IL-1 could lead to chronic IBD¹¹. The gene encoding either receptor α or receptor β of T cells has been deleted by a targeted mutation and the affected mice develop chronic diarrhea with rectal prolapse. The number of γ/δ cells in the intestine increases does that of the B cells, producing immunoglobulin (Ig) A, IgG and IgM. The deregulated B cell response may be due to an autoantibody. The spontaneous colitis in this model is the result of a lack of appropriate α/β T cell-mediated suppression of B cells¹⁷.

Severe combined immunodeficient (SCID) mice have a spontaneous mutation that, with a deficiency of both T cells and B cells, may be reconstituted completely by the transfer of normal B cells and T cells. In contrast, the transfer of a subset of CD45 cells in SCID mice expressing the surface molecule CD45RB has been found to result in a disease of chronic diarrhea and wasting¹⁸. CD45-low cells secrete Th2 lymphokine profile, whereas spleen-derived memory type CD4⁺ (CD45RB) T cells secrete predominantly Th1 lymphokines, suggesting that a disturbed balance between Th1 and Th2 lymphokine production may lead to chronic colitis.

The recent therapeutic approach of gene therapy strategies using adenoviral vectors encoding the immunoregulatory cytokines may prove to be a potent strategy for the treatment of chronic IBD. A daily injection of IL-10 in IL-10 knockout mice does not induce remission; however, rectal administration of an adenoviral vector encoding murine IL-10 induces colonic IL-10 release, is therapeutic in this model, and can induce IL-10 expression in colonic epithelial cells *in vitro*¹³. In addition, IL-10 gene transfer prevents experimental colitis in rats¹.

In other models of colitis, immunostimulatory DNA sequences (ISS), common in certain bacteria and viral genomes, are found to prevent and attenuate the severity of intestinal inflammation³⁰. Amelioration of colonic inflammation in these models is explained by ISS-induced anti-apoptotic effects on colonic epithelium and inhibition of colonic proinflammatory cytokine generation. ISS may have a role in normal intestinal homeostasis, in amelioration of colitis, and, therefore, possibly in IBD treatment. Intestinal inflammation develops in response to gene-specific deletion and can provide insight into the mechanisms of inflammation.

One lesson from these models is that intestinal inflammation develops as a result of defective immunosuppression. The models of TCR deletion and CD15RB-lymphocytes in SCID mice demonstrates the effect of ineffective T lymphocyte suppression. The other models, e.g. IL-2 knockout mice and TCR knockout mice, indicate mechanisms of colitis resulting from uncon-

trolled B lymphocyte activation in the absence of regulatory T cells.

The human class I HLA-B27 molecule is associated with susceptibility to ankylosing spondylitis. Using recombinant DNA technology, transgenic rats have been created which express a copy of HLA-B27/ β 2 microglobulin gene and develop colitis with associated features resembling the human spondyloarthropathies associated with IBD²⁰. Clinically, the disease can be transferred by bone marrow cells from B27-positive donors to irradiated B27-negative recipients (over-expression), indicating that the HLA-B27 syndrome is mediated by immunocytes. In addition, successful bone marrow engraftment from non-transgenic rats could suggest bone marrow transplantation in IBD therapy.

Our models illuminate the role of the bacterial flora in intestinal inflammation. The critical pathologic role of ubiquitous normal bacteria is illustrated by the lack of colitis in IL-2 knockout mice and transgenic HLA-B27/B2 microglobulin mice living in germ-free conditions. Luminal bacterial and bacterial products also contribute to the inflammatory response in the indomethacin-induced colitis model. Finally, the propensity to develop colon cancer can be evaluated in DDS-induced colitis model, the HLA-B27/ β 2 microglobulin transgenic rat³⁵ model, as well as in the cotton-top tamarin.

The injured gut has a remarkable and rapid healing activity. TGF- β , a macrophage product, can stimulate collagen synthesis by fibroblasts or intestinal smooth muscle cells and suppress immunologic activity by inhibition of lymphocyte proliferation. The strain of TGF- β knockout mouse develops colitis probably mediated, in part, by a defective suppression of lymphocyte^{12, 33}. The short-chain fatty acid, mainly sodium butyrate (normally produced in the colon from fiber polysaccharides that escape from digestion), has been found to improve experimental colitis as well as non-specific proctitis in humans²⁸. Sodium butyrate is able to increase intestinal transglutaminase activity² (an enzyme which, in hemostasis, is equivalent to factor XIIIa)³¹. In fact, supplementation of both transglutaminase and factor XIII has been shown to be efficacious in both experimental colitis and human IBD, in all probability by improving the intestinal ulcer healing process^{4, 31}. The observation from animal models indicates that similar lesions can arise from different immunoregulatory defects, suggesting that human IBD could arise from a number of mutations and a wide variety of molecules, and supporting the concept of the genetic heterogeneity of IBD. The results obtained from experimental models can generate new hypo-

theses and expand human studies but do not replace them. The models have already suggested novel forms of therapy for IBD patients.

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