Lessons From Genetically Engineered Animal Models
XII. IL-10-deficient (IL-10<sup>−/−</sup>) mice and intestinal inflammation

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**Rennick, Donna M., and Madeline M. Fort.** Lessons From Genetically Engineered Animal Models. XII. IL-10-deficient (IL-10<sup>−/−</sup>) mice and intestinal inflammation. Am J Physiol Gastrointest Liver Physiol 278: G829–G833, 2000.— Interleukin (IL)-10<sup>−/−</sup> mice spontaneously develop intestinal inflammation characterized by discontinuous transmural lesions affecting the small and large intestine and by dysregulated production of proinflammatory cytokines. The uncontrolled generation of IFN-γ-producing CD4<sup>+</sup> T cells (Th1 type) has been shown to play a causal role in the development of enterocolitis affecting these mutants. This article discusses studies of IL-10<sup>−/−</sup> mice that have investigated the role of enteric organisms in triggering intestinal disease, the mediators responsible for initiating and maintaining intestinal disease, the role of IL-10 in the generation and/or function of regulatory cells, and the results of IL-10 therapy in experimental animal models of inflammatory bowel disease (IBD) and human patients with IBD.

interleukin; counterregulation by IL-10; proinflammatory cytokines; Th1-mediated intestinal disease; IL-10 therapy

BEFORE THE GENERATION of interleukin (IL)-10<sup>−/−</sup> mice, it had already been established that IL-10 was an anti-inflammatory molecule capable of suppressing the in vitro production of numerous cytokines by macrophages, dendritic cells, T cells, and natural killer (NK) cells (21). IL-10 was firmly established as an essential regulator of mucosal responses when it was recognized that enterocolitis was manifested by IL-10<sup>−/−</sup> mice and by mice with a disruption in the gene encoding an IL-10 receptor component (CRF2-4<sup>−/−</sup>) (16, 30).

In addition to IL-10<sup>−/−</sup> mice, other rodents with gene-targeted mutations develop intestinal disease due to immune imbalances created by either deleting or overexpressing genes encoding cytokines or other immune components. For example, intestinal inflammation was present in rodents rendered deficient in IL-2, IL-2Ra, T cell receptor (TCR)-α, TCR-β, G<sub>01,2</sub>, or transforming growth factor (TGF)-β, as well as in transgenic rodents overexpressing IL-7, tumor necrosis factor (TNF), and human leukocyte antigen (HLA)-B27 (4). Although these genetically engineered mutants share many abnormalities consistent with tissue inflammation, they also exhibit distinct pathological changes and cytokine profiles that have been associated with either Crohn’s disease (CD) or ulcerative colitis (UC) in humans. Currently, IL-10<sup>−/−</sup> mice and other genetic mutants are being studied in an effort to answer fundamental questions about the pathogenic mechanism(s) specific or unique to each model and to determine how information obtained from these studies may delineate the multifactorial processes involved in human CD and UC.

**GENES, GERMS, AND INTESTINAL DISEASE**

Human inflammatory bowel disease (IBD) is generally described as a condition that occurs in genetically susceptible individuals because of an aberrant immune response to enteric antigens. Although the particular antigens responsible for causing human IBD have remained elusive, enteric flora have proven sufficient to trigger mucosal inflammation in IL-10<sup>−/−</sup> mice, IL-2<sup>−/−</sup> mice, TCR-α<sup>−/−</sup> mice, and HLA-B27 transgenic rats, since none of these mutants develop intestinal disease under germ-free conditions. In the case of IL-10<sup>−/−</sup> mice, their microbial milieu plays a major role in determining the characteristics of their disease. IL-10<sup>−/−</sup> mutants derived under conventional conditions developed discontinuous transmural lesions affecting both the upper and lower gastrointestinal tract. Other pathological changes included epithelial hyperplasia, mucin depletion, crypt abscesses, ulcers, and thickening of the bowel wall (2, 16). In contrast, IL-10<sup>−/−</sup> mice derived under specific pathogen-free (SPF) conditions developed a mild-to-moderate inflammation restricted almost entirely to the colon. Furthermore, unlike IL-10<sup>−/−</sup> mice raised under conventional conditions, the SPF mutants developed fewer systemic complications (i.e., anemia, wasting, splenomegaly, and hepatitis) until late in the progression of their colitis. Although
IL-10−/− mice are prone to developing transmural disease throughout the intestinal tract similar to Crohn’s patients, the distribution and severity of their lesions were dependent on the enteric stimuli encountered by the host.

Germ-free and SPF IL-10−/− mice have been used for microbial reconstitution experiments in an effort to define the bacterial species capable of triggering enterocolitis. Germ-free IL-10−/− mice repopulated with six defined bacterial strains, including Bacteroides vulgatus, developed a very mild form of colitis compared with transgenic HLA-B27 rats colonized by the same bacteria (28). Although these studies illustrate that ordinary resident enteric bacteria are sufficient to initiate disease in genetically susceptible animals, the particular bacterial species involved may vary considerably between different hosts. Helicobacter species are among the commensal organisms incriminated in the development of intestinal inflammation in humans and rodents. Helicobacter hepaticus, in particular, is known to induce chronic mucosal inflammation in immune-deficient mice and was identified as part of the complex intestinal flora present in IL-10−/− mice generated under conventional conditions. However, H. hepaticus was not essential for mucosal disease in IL-10−/− mice, since this organism was eliminated from SPF mutants that eventually develop colitis. Nevertheless, infecting SPF mutants with H. hepaticus significantly exacerbated their colitis (17). Interestingly, these infected mice still did not show evidence of inflammation in the upper gastrointestinal tract. So far, the microbial species capable of triggering pathogenic changes in the small intestine and stomach of IL-10−/− mice remain undefined.

Other investigations have suggested that the presence of certain types of bacteria, such as Lactobacillus species, may actually diminish intestinal inflammation. When the enteric flora of neonatal IL-10−/− mice were analyzed, there were decreased levels of Lactobacillus species combined with increased levels of aerobic adherent and translocated bacteria (18). Normal colonization by Lactobacillus species was established in young IL-10−/− mice through the rectal delivery of Lactobacillus reuteri or oral lactulose therapy. Restoring the balance between Lactobacillus species and other bacterial strains prevented the development of colitis in mutants housed in SPF conditions.

The large number of genetically engineered mutations that lead to IBD in rodents, in part, explains why no consistent pattern of inheritance has been associated with human susceptibility to CD or UC. Nonparametric linkage studies of affected siblings have suggested a linkage between genes of the major histocompatibility complex (MHC) class II and UC. However, it appears that other genetic factors must contribute to disease expression because disease does not occur in all siblings bearing the same MHC class II allele. To investigate the impact of inheritable factors on disease expression in IL-10−/− mice, mutants were generated on three genetic backgrounds with different MHC alleles. It was determined that enterocolitis developed earlier and was more severe in IL-10−/− 129/SvEv (H-2b) and BALB/c (H-2k) strains compared with IL-10−/− C57BL/6 (H-2b) strain (2). Not surprisingly, TCR-α−/− mice backcrossed onto several genetic backgrounds also showed differences in the severity of their colitis (20). By comparison, the C57BL/6 background conferred increased resistance to both IL-10−/− and TCR-α−/− mice. Strikingly, the BALB/c background resulted in increased disease severity in IL-10−/− mice but not in TCR-α−/− mice. These preliminary observations demonstrate that inheritable genetic factors can profoundly modify disease expression initiated by a single gene mutation, be it defective IL-10 or TCR-α synthesis. Furthermore, the modifier genes involved in disease expression will vary depending on the particular genetic defect predisposing the host to IBD. It is anticipated that genetically engineered and spontaneous models (C3H/HeJ Bir mice) of IBD with relatively defined backgrounds may be useful in identifying the combination of human genes affecting susceptibility to CD and UC.

COLITIS AND ADENOCARCINOMAS

During our analysis of IL-10−/− mice, we noted epithelial extension/invasion into the underlying submucosa, tunica muscularis, and serosa. The highest incidence occurred in severely diseased IL-10−/− mice on BALB/c and 129 SvEv backgrounds (2). Epithelial extension/invasion was most frequently found in the ascending colon and often involved well-differentiated and organized cells. However, in some cases the glandular structures were irregular, with back-to-back growth and slight loss of epithelial cell nuclear polarity. These irregular glandular structures were described as adenocarcinomas and were similar to those found in Gaα2−/− mice and in mice expressing a dominant-negative N-cadherin (2, 14, 26).

Because IL-10−/− mice failed to show signs of metastatic disease, we questioned whether the presence of microinvasive epithelium represented neoplasia or hyperplasia. Recently, IL-10−/− mice were crossed to p53−/− mice, since p53 tumor suppressor gene mutations are common events in colon cancer. At 4 mo of age, p53−/− mice developed thymic lymphomas, which was the expected phenotype. Age-matched IL-10−/− mice had developed colitis and microinvasive colonic epithelium, whereas the IL-10−/− p53−/− double mutants had high-grade adenocarcinomas of the colon (3). In one case, there were metastases to lymph nodes and the serosal surface of the stomach. Colon carcinomas occur in CD and UC patients with a similar frequency (3–5%). Although risk factors have not been defined, histological dysplasia in association with chronic intestinal inflammation has been documented in cases of CD. Comparative studies of IL-10−/− p53−/− mice generated on different genetic backgrounds may prove invaluable in identifying the complexity of genetic factors determining the incidence of colitis-associated colon cancer.
IL-10-DEFICIENT MICE AND INTESTINAL INFLAMMATION

Th1-TYPE CD4⁺ T CELLS INDUCE ENTEROCOLITIS IN IL-10⁻/⁻ MICE

Identifying the immune abnormalities, which play a causal role in the development of enterocolitis in IL-10⁻/⁻ mice, has been the major focus of many studies. Analysis of the inflammatory cells present in the mucosa and submucosa revealed large numbers of macrophages, B cells, plasma cells, and CD4⁺ αβ TCR⁺ T cells (2). Additionally, numerous cytokines (i.e., IL-1, IL-6, IL-12/p40, and TNF-α) were abnormally upregulated in these colonic tissues. Circulating colon-reactive IgG was also detected, suggesting that autoantibodies may contribute to tissue damage. Despite the plethora of immune cell types and mediators found to be abnormally upregulated in these mutants, it was concluded that enterocolitis in IL-10⁻/⁻ mice is mediated by the unregulated actions of CD4⁺ T cells. This conclusion was based on studies showing that T cell-deficient IL-10⁻/⁻ mice failed to develop disease and CD4⁺ T cells isolated from the colons of IL-10⁻/⁻ readily transferred colitis to immunodeficient recombination activating gene (Rag)2⁻/⁻ recipients. Moreover, the CD4⁺ T cells present in the colons and draining mesenteric lymph nodes of IL-10⁻/⁻ mice produced predominately IFN-γ, implicating Th1-type cells in the pathogenic process. Infusing neonatal IL-10⁻/⁻ mice with anti-IFN-γ monoclonal antibodies (MAbs) significantly diminished the onset and severity of intestinal disease, thus confirming a causal role for this cytokine. Importantly, anti-IL-12 MAb treatment completely prevented disease in young mutants (6, 7). Presumably, in the absence of IL-10 suppression, dysregulated IL-12 production is sufficient to generate large numbers of Th1 cells producing IFN-γ.

CD4⁺ T cells are major effectors, causing intestinal inflammation in many animal models of IBD. Like IL-10⁻/⁻ mice, many were characterized by dominant Th1 cytokine profiles (IFN-γ and TNF) and developed transmural lesions characteristic of CD. These include IL-2⁻/⁻ mice, trinitrobenzene sulfonic acid (TNBS)-induced colitis, immunization-induced colitis in IL-2⁻/⁻ mice, and the scid and Tgε26-transfer models (4). Recently, another class of CD4⁺ T cells producing predominately Th2 cytokines (IL-4) has been shown to cause intestinal inflammation in TCR-α⁻/⁻ mice and oxazolone-induced colitis (5, 19). This latter group developed superficial ulcersations of the epithelium characteristic of UC.

The distinction between Th1-mediated, CD-like models and Th2-mediated, UC-like models is an important step in identifying common pathogenic pathways that may be operational in human IBD. Importantly, most of the CD-like models, including IL-10⁻/⁻ mice, share a dependency on dysregulated IL-12 production as an essential step in generating pathogenic Th1-mediated responses (4, 7, 11, 22, 29). Nevertheless, differences have been observed among these CD-like models with respect to the specific Th1-type mediators that function as initiators of intestinal disease. For example, IFN-γ was identified as a major mediator initiating colitis in IL-10⁻/⁻ neonates, whereas TNF was not involved in this pathogenic process even though abnormally high levels of TNF were detected (2, 6). TNBS-induced colitis and the Tgε26-transfer model of colitis appear to have little or no dependence on IFN-γ; instead, TNF/TNF-related molecules have been implicated as mediators of disease (4, 15, 23). The scid transfer model of colitis is partially dependent on both IFN-γ and TNF (25). The particular cytokines that are the dominant mediators of CD in humans are also likely to vary, and these differences may be responsible for the heterogeneous forms of CD manifested by these patients.

Because IL-12 plays a central role in the majority of Th1-mediated models of IBD, it has been identified as a target for therapeutic intervention. Another potential target is nuclear factor (NF)–κB, a transcription factor that regulates the expression of various genes encoding proinflammatory cytokines. Increased expression of NF–κB was found in lamina propria macrophages from IL-10⁻/⁻ mice and mice with TNBS-induced colitis (24). The inhibition of NF–κB activity by the administration of a p65 antisense oligonucleotide significantly reduced weight loss and histopathology in IL-10⁻/⁻ mice. This treatment also abrogated established TNBS-induced colitis and was associated with decreased production of IL-1, IL-6, and TNF-α by lamina propria macrophages. These studies have shown convincingly that blocking NF–κB signaling is an effective treatment for two models of colitis (24).

WHICH CYTOKINES SUSTAIN THE CHRONIC STAGE OF ENTEROCOLITIS?

Although IL-12 and IFN-γ are key mediators responsible for inducing enterocolitis in young IL-10⁻/⁻ mice, their role in sustaining/perpetuating the chronic phase of disease was uncertain. We found that treating diseased IL-10⁻/⁻ adults with anti-IFN-γ MAb for 8 wk had no effect on their disease progression, indicating that IFN-γ was not required once disease was established (5, 6, 7). In contrast, anti-IL-12 MAb treatment ameliorated ongoing disease since both the number and severity of lesions in adult IL-10⁻/⁻ mice were reduced (7). The beneficial effect of anti-IL-12 treatment was accompanied by a reduction in the number of CD4⁺ T cells present in the intestines and draining lymph nodes, suggesting that IL-12 is necessary for sustaining the proliferation and/or viability of chronically activated Th1 cells. It has also been shown that, although early treatment with IL-10 prevented enterocolitis from developing in neonatal IL-10⁻/⁻ mice, IL-10 treatment of diseased adults diminished but did not cure their ongoing disease. It is possible that IL-10 may ameliorate ongoing disease by suppressing IL-12 production because decreased IL-12/p40 mRNA expression was observed in IL-10 treated mice. This possibility was further supported by the failure of combined anti-IL-12 MAb and IL-10 treatment to be more effective than using either reagent alone.

The residual inflammation detected in adult IL-10⁻/⁻ mice following anti-IL-12 MAb treatment implied that additional factors were contributing to disease mainte-
nance (7). We have investigated whether TNF, IL-1, or IL-6 is involved in the chronic phase of disease even though they were not required for the inductive phase. When neutralizing antibodies specific for these cytokines were given, separately or jointly, disease progression was not affected. In contrast to our findings, TNF plays a significant role in perpetuating the chronic phase of TNBS-induced colitis and dextran sulfate sodium (DSS)-induced colitis. Administering anti-TNF MAb or anti-IL-12 MAb was very effective and often completely ablated established intestinal inflammation in these induced models of colitis (10, 15). Our current studies are focused on identifying late-acting mediators that work in conjunction with IL-12 to perpetuate disease in IL-10−/− mice. Candidate molecules include IL-7, which stimulates the proliferation of intestinal mucosal lymphocytes and is present in the sera of IBD patients (31).

**IL-10 AND REGULATORY T CELLS**

There is considerable evidence that regulatory CD4+ T cells are required to establish normal intestinal immune responses. Regulatory CD4+ T cells, expressing low levels of CD45RB molecules, were first functionally characterized in the scid transfer model of colitis. In cotransfer experiments, CD4+ CD45RB<sub>low</sub> T cells were able to prevent the induction of colitis caused in scid mice implanted with CD4+ CD45RB<sub>high</sub> naive T cells. Initially it was shown that the ability of the CD45RB<sub>low</sub> T subset to suppress colitis was ablated by anti-TGF-β mAb treatment (25). It was reported recently that anti-IL-10 receptor mAb treatment also ablated the protection provided by CD45RB<sub>low</sub> T cells (1). Thus both TGF-β and IL-10 appear necessary for proper T cell regulation of mucosal responses in this model. A critical role for IL-10 is also evident from studies of transgenic mice. When normally pathogenic CD4+ CD45RB<sub>high</sub> T cells were isolated from mice expressing an IL-10 transgene under the control of the IL-2 promoter, they not only failed to cause colitis on transfer into scid mice, they inhibited the pathogenic activity of cotransferred CD4+ CD45RB<sub>high</sub> T cells. Importantly, IL-10 has recently been identified as a growth factor required for the in vitro generation of a murine regulatory T cell line (Tr1) (12). Tr1 cells derived from IL-10-supplemented cultures share many properties in common with naturally occurring CD4+ CD45RB<sub>low</sub> regulatory T cells. Both populations produce IL-10 and TGF-β and are capable of preventing colitis by CD45RB<sub>high</sub> T cells on cotransfer into scid mice (12, 25).

In the scid transfer model mentioned above, the anti-IL-10 receptor MAb treatment, which ablated the protective effects of the CD45RB<sub>low</sub> T cells, was started at the time of T cell reconstitution (CD45RB<sub>high</sub> plus CD45RB<sub>low</sub> T cells) (1). Therefore, it was unclear whether IL-10 production by regulatory cells was required for their own expansion to prevent disease in the transplanted host, required as the mediator of their suppressive actions, or both. To address this question, a series of experiments were conducted with IL-10−/− mice. It was found that inhibition of colitis development in young IL-10−/− mice was dependent on the continuous administration of IL-10 because intestinal inflammation began to develop once treatment ceased. Moreover, CD4+ CD45RB<sub>low</sub> T cells, isolated from disease-free mutants after continuous treatment with IL-10, showed no signs of regulatory activities because they still failed to prevent colitis in Rag2−/− mice when cotransferred with CD4+ CD45RB<sub>high</sub> T cells. In fact, the CD4+ CD45RB<sub>low</sub> T cells from IL-10-treated mice caused colitis in Rag2−/− when transferred in the absence of CD4+ CD45RB<sub>high</sub> T cells (6). Although these studies do not preclude a role for IL-10 in the generation of regulatory T cells, it is apparent that CD4+ CD45RB<sub>low</sub> T cells from IL-10-treated IL-10−/− mice are still functionally impaired due to their inability to directly produce IL-10.

**PROSPECTS FOR IL-10 THERAPY OF IBD**

The overproduction of immune-inflammatory cells and their mediators has consistently been implicated in the pathogenesis of human IBD. Moreover, patients often experience intermittent remission and reactivation of their disease, suggesting a fluctuating balance between pro- and anti-inflammatory cell types and mediators. Expectations that exogenously administered IL-10 may be an effective therapeutic treatment for IBD patients are based largely on the outcome of murine studies. Theoretically, IL-10 treatment could dampen the activities of proinflammatory cells and at the same time augment the development/activities of regulatory T cells to counterbalance the large number of chronically activated effectors present in established mucosal lesions. In the case of experimental models of IBD, it has already been shown that continuous IL-10 administration prevented intestinal disease in neonatal IL-10−/− mice, in scid and Rag2−/− transfer models of colitis, in granulomatous enterocolitis induced in rats by bacterial cell wall polymers, and in DSS-induced and TNBS-induced colitis (2, 6, 10, 13, 15). IL-10 was also administered after the establishment of disease in some of these models and was shown to have beneficial effects.

Will IL-10 infusions prove effective in suppressing human IBD? In short-term studies, IL-10 administered rectally to a small number of UC patients resulted in improved histological scores and diminished cytokine production by lamina propria and circulating mononuclear cells (9, 27). As encouraging as these results appear, the benefits of long-term treatment with IL-10 are not yet known. Multiple cytokines are dysregulated in IBD patients, and those involved in perpetuating the chronic phase of disease may differ between individuals, as in the case of experimental animal models. Therefore, the most successful therapeutic strategies will probably include combinations of drug, cytokine-based (i.e., IL-10) and antibody-based (i.e., anti-TNF) treatments.
REFERENCES


