Colitis and Intestinal Inflammation in *IL10*$^{-/-}$ Mice Results From IL-13Rα2–Mediated Attenuation of IL-13 Activity

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BACKGROUND & AIMS: The cytokine interleukin (IL)-10 is required to maintain immune homeostasis in the gastrointestinal tract. IL-10 null mice spontaneously develop colitis or are more susceptible to induction of colitis by infections, drugs, and autoimmune reactions. IL-13 regulates inflammatory conditions; its activity might be compromised by the IL-13 decoy receptor (IL-13Rα2). **METHODS:** We examined the roles of IL-13 and IL-13Rα2 in intestinal inflammation in mice. To study the function of IL-13Rα2, *il10*$^{-/-}$ mice were crossed with *il13rα2*$^{-/-}$ to generate *il10*$^{-/-}$ *il13rα2*$^{-/-}$ double knockout (dKO) mice. Colitis was induced with the gastrointestinal toxin piroxicam or *Trichuris muris* infection. **RESULTS:** Induction of colitis by interferon (IFN)-γ or IL-17 in IL-10 null mice requires IL-13Rα2. Following exposure of *il10*$^{-/-}$ mice to piroxicam or infection with *T muris*, production of IL-13Rα2 increased, resulting in decreased IL-13 bioactivity and increased inflammation in response to IFN-γ or IL-17A. In contrast to *il10*$^{-/-}$ mice, dKO mice were resistant to piroxicam-induced colitis; they also developed less severe colitis during chronic infection with *T muris* infection. In both models, resistance to IFN-γ and IL-17–mediated intestinal inflammation was associated with increased IL-13 activity. Susceptibility to colitis was restored when the dKO mice were injected with monoclonal antibodies against IL-13, confirming its protective role. **CONCLUSIONS: Colitis and intestinal inflammation in *IL10*$^{-/-}$ mice results from IL-13Rα2–mediated attenuation of IL-13 activity. In the absence of IL-13Rα2, IL-13 suppresses proinflammatory Th1 and Th17 responses. Reagents that block the IL-13 decoy receptor IL-13Rα2 might be developed for inflammatory bowel disease associated with increased levels of IFN-γ and IL-17.**

Keywords: Colitis; Helminth; IBD; Th17.

BASIC–ALIMENTARY TRACT

Studies have suggested that immune homeostasis in the gastrointestinal tract is maintained by a variety of immunoregulatory mechanisms.[1] Although regulatory T cells play a critical role,[2] recent studies have suggested that resident nonhematopoietic cells, including epithelial cells, smooth muscle, and fibroblasts, are critically involved in maintaining homeostasis in the gut.[3] However, the mechanisms by which these cells regulate immune homeostasis in the intestine remain incompletely defined.

Crohn's disease and ulcerative colitis are believed to be induced by distinct immunologic mechanisms,[4] with mixed Th1/Th17 responses mediating Crohn's disease[5] and persistent Th2-type responses triggering ulcerative colitis.[6] In the case of Crohn's disease, a variety of mechanisms have been shown to limit the production of interferon (IFN)-γ/interleukin (IL)-17A and development of severe disease, including regulatory cell populations,[7] immunoregulatory cytokines,[8] and anti-inflammatory proteins.[9] However, the cytokine IL-10 has emerged as a key suppressive mediator. Indeed, animal studies[10] and genetic linkage association studies have revealed an important protective role for IL-10 in colitis.[11–13] Although Th2 cytokines, in particular IL-4 and IL-13, can also antagonize Th1/Th17 responses, the mechanisms by which Th2 responses regulate the development of colitis remain much less clear.[14,15]

Here, using 2 independent models of chemical- and infection-induced intestinal inflammation, we show that the development of Th1/Th17-dependent disease in *il10*$^{-/-}$ mice[16] is tightly regulated by the IL-13 decoy receptor (IL-13Rα2). During *Trichuris muris* infection or following exposure to the nonsteroidal anti-inflammatory drug piroxicam (a gastrointestinal toxin),[17] production of IL-13Rα2 increased in the absence of IL-10, consistent with our previous studies in the lung and liver,[18] resulting in decreased IL-13 bioactivity and markedly increased IFN-γ/IL-17A–driven intestinal inflammation. As such, these studies reveal a previously unrecognized role for IL-13 and its decoy receptor in the regulation of Th1/Th17 responses in the gut. Because the IL-13Rα2

Abbreviations used in this paper: AB-PAS, Alcian blue periodic acid–Schiff; dKO, double knockout; IFN, interferon; IL, interleukin; IL-13Rα2, interleukin-13 decoy receptor; *Trichuris muris*, *T muris*.

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chain is primarily expressed on epithelial cells, smooth muscle, and fibroblasts, they also illustrate a novel mechanism for cells of nonhematopoietic origin to control IFN-γ/IL-17–mediated intestinal inflammation. Finally, using in vitro polarized $CD4^+$ T cells, we confirm that Th17 cells express a functional IL-13 receptor[19] that, when activated with IL-13, can directly reduce the frequency of Th17 cells and secretion of IL-17A, thus providing an additional mechanism for IL-13 to limit Th17-dependent pathology in the gastrointestinal tract.

Materials and Methods

Animals

Female C57BL/6, BALB/c, BALB/c *il13rα2*$^{-/-}$, *il13rα1*$^{-/-}$, *il10*$^{-/-}$, and *il10*$^{-/-}$*il13rα2*$^{-/-}$ 6- to 8-week-old mice were obtained from Taconic (Hudson, NY). Animals were housed under specific pathogen-free conditions at the National Institutes of Health in an American Association for the Accreditation of Laboratory Animal Care–approved facility. The National Institute of Allergy and Infectious Diseases Animal Care and Use Committee approved all experimental procedures. A minimum of 5 mice per group was used in each experiment unless indicated.

Piroxicam-Induced Colitis

Animals were fed normal animal chow mixed with piroxicam (200 ppm) for 14 consecutive days. Animals were weighed daily and killed at day 14 for analysis.

T muris *Infection*

Mice were infected orally with 200 embryonated *T muris* eggs, as described.[20,21]

Histopathology

For histopathologic analyses, tissues were fixed in 4% phosphate-buffered formalin and embedded in paraffin for sectioning. Wright's Giemsa, H&E, or Alcian blue periodic acid–Schiff (AB-PAS) stains were used. Submucosal inflammation, intramuscular inflammation, and mucus and ulcer frequency and severity were scored by a blinded observer on a scale of 1 to 4+. Eosinophil score was based on percent eosinophilia. The same individual scored all histologic features and had no knowledge of the experimental groups.

In Vitro Cell Culture

Lymph node cells were isolated, washed, and plated at 5×10^5 cells per well of a 96-well plate and stimulated with 10 μg/mL of *T muris* antigen.[20,21] For in vitro Th1 and Th17 differentiation, fluorescence-activated cell sorter–purified naive $CD4^+CD62L^{hi}CD44^{lo}$ T cells were stimulated under Th17 (recombinant IL-6 [R&D Systems, 20 ng/mL], recombinant human transforming growth factor β [R&D Systems, 5 ng/mL], anti–IL-4 [11D11, 10 μg/mL], and anti–IFN-γ [XMG1.1, 10 μg/mL]) or Th1 (IL-12 [R&D Systems, 10 ng/mL] and anti–IL-4 [11D11, 10 μg/mL]) conditions with or without recombinant IL-13 at indicated concentrations.

Polymorphonuclear Cell Analysis

EDTA-treated blood was processed for automated counting using Vista Analyzer (Siemens, Deerfield, IL).

Reverse-Transcription Polymerase Chain Reaction

RNA was isolated from tissue or cells in 1 mL TRIzol reagent (Invitrogen, Carlsbad, CA) and processed as previously described.[18,22] Real-time reverse-transcription polymerase chain reaction was performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Carlsbad, CA). Messenger RNA (mRNA) levels for each sample were normalized to hypoxanthine guanine phosphoribosyl transferase. Primers were either designed using Primer Express software (version 2.0; Applied Biosystems) or adopted from previously reported primer sequences.[18,22]

Enzyme-Linked Immunosorbent Assay

Cytokines were measured by enzyme-linked immunosorbent assay using Immulon 2HB plates (Thermo Scientific, Rockford, IL) and the manufacturer's guidelines. Paired capture and detection antibodies from R&D Systems for IL-17A, IFN-γ, IL-4, IL-10, and IL-13 were used. Plates were washed with 0.05% Tween 20 in phosphate-buffered saline and blocked with 5% milk in Tween 20 in phosphate-buffered saline. Recombinant cytokine standards (R&D Systems) were used to assess quantity using a standard curve, with OD acquired at 405 nm in an enzyme-linked immunosorbent assay reader.

Flow Cytometry

Following a 3-hour incubation with phorbol myristate acetate (10 ng/mL), ionomycin (1 μg/mL), and brefeldin A (10 μg/mL), cells were stained with antibodies diluted in phosphate-buffered saline with 0.5% bovine serum albumin (Sigma–Aldrich, St. Louis, MO) and 0.05% sodium azide (Sigma–Aldrich) for 20 minutes at 4°C. Surface molecule staining (CD4 [BD Biosciences, San Diego, CA], CD25 [eBioscience, San Diego, CA], CD69 [BioLegend, San Diego, CA], CD44 [BD Biosciences], CD62L [BD Biosciences]) followed by fixation and permeabilization (BD Cytofix/Cytoperm; BD Biosciences) and intracellular staining (IL-17A [BD Biosciences], IFN-γ [BD Biosciences], Foxp3 [eBioscience]) was performed on freshly isolated cells. The expression of surface molecules and intracellular cytokines were analyzed on a BD LSR II flow cytometer (BD Biosciences) using FlowJo version 8 software (Tree Star, Ashland, OR).

Statistical Analysis

Data sets were compared with a Mann–Whitney test or Kruskal–Wallis test where appropriate, using