Published in final edited form as:
Gastroenterology. 2011 January ; 140(1): 254–264.e2. doi:10.1053/j.gastro.2010.09.047.

Colitis and intestinal inflammation in *IL10 −/−* mice results from IL-13Rα2-mediated attenuation of IL-13 activity

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Abstract

Background & Aims—The immunoregulatory cytokine interleukin (IL)-10 is required to maintain immune homeostaisis 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 mice (dKO). 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 *Trichuris muris*, production of IL-13Rα2 increased, resulting in decreased IL-13 bioactivity and increased intestinal 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* infecion. In both models, resistance to IFN-γ and IL-17-mediated intestinal

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Wilson – study design, acquisition and analysis of data, co-wrote the paper, Ramalingam – acquisition of data, Rivollier - study design, acquisition and analysis of data, Shenderov - acquisition of data, M. Mentink – acquisition of data, Madala - acquisition of data, Cheever - study design, acquisition and analysis of data, Artis - study design, Kelsall - study design and co-wrote the paper, Wynn - study design, analysis and interpretation of data, and drafting of the manuscript.

Disclosures: i. None of the authors have any financial arrangement to disclose.
ii. This works was supported by the intramural program of the NIH/NIAID.
iii. The manuscript was written exclusively by the authors listed above.

inflammation was associated with increased IL-13 activity. Colitis susceptibility was restored when the dKO mice were injected with monoclonal antibodies against IL-13, confirming its protective role.

Conclusion—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 pro-inflammatory 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

helminth; IBD

Introduction

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 non-hematopoietic cells, including epithelial, 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 (CD) and ulcerative colitis (UC) are believed to be induced by distinct immunological mechanisms[4], with mixed Th1/Th17 responses mediating CD[5] and persistent Th2-type responses triggering UC[6]. In the case of Crohn's disease, a variety of mechanisms have been shown to limit the production of IFN-γ/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 two 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 *T. muris* infection or following exposure to the non-steroidal 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[21], resulting in decreased IL-13 bioactivity and markedly increased IFNγ/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 chain is primarily expressed on epithelial cells, smooth muscle, and fibroblasts, they also illustrate a novel mechanism for cells of non-hematopoietic 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[18], which 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 – 8 week old mice were obtained from Taconic. Animals were housed under specific pathogen-free conditions at the NIH in an American Association for the Accreditation of Laboratory Animal Care–approved facility. The NIAID 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 euthanized at day 14 for analysis.

Trichuris muris infection

Mice were infected orally with 200 embryonated *T. muris* eggs, as described[19, 20].

Histopathology

For histopathological analyses, tissues were fixed in 4% phosphate buffered formalin and embedded in paraffin for sectioning. Wright's Giemsa, hematoxylin and eosin (H&E) or alcian blue periodic acid Schiff (AB-PAS) stains were used. Sub-mucosal inflammation, intramuscular inflammation, mucus and ulcer frequency and severity were scored by a blinded observer on a 1–4+ basis. Eosinophil score was based on % eosinophilia. The same individual scored all histological 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 10μg/ml of *T.muris* antigen[19, 20]. For in-vitro Th1 and Th17 differentiation, FACS-purified naïve $CD4^+CD62L^{hi}CD44^{lo}$ T cells were stimulated under Th17 (rIL-6 (R&D, 20ng/ml), rhTGFβ (R&D, 5ng/ml), anti-IL-4 (11D11, 10 μg/ml) and anti-IFNγ (XMG1.1, 10μg/ml)) or Th1 (IL-12 (R&D, 10ng/ml) and anti-IL-4 (11D11, 10μg/ml)) conditions with or without rIL-13 at indicated concentrations.

Polymorphonucelar cell (PMN) Analysis

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

RT-PCR

RNA was isolated from tissue or cells in 1 ml TRIZOL reagent (Invitrogen) and processed as previously described [21, 22]. Real-time RT-PCR was performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). mRNA levels for each sample were normalized to hypoxanthine guanine phosphoribosyl transferase (HPRT). Primers were either designed using Primer Express software (version 2.0; Applied Biosystems) or adopted from previously published primer sequences[21, 22].

ELISA

Cytokines were measured by ELISA using Immulon 2HB plates (Thermo) and manufacturers guidelines. Paired capture and detection antibodies from R&D for IL-17A, IFNγ, IL-4, IL-10 and IL-13 were used. Plates were washed with 0.05% Tween 20 in PBS (PBST) and blocked with 5% milk in PBST. Recombinant cytokine standards (R&D) were

used to assess quantity using a standard curve, with OD acquired at 405 nm in an ELISA reader.

Flow cytometry

Following a 3 hour incubation with phorbol 12-myristate 13-acetate (PMA, 10ng/ml), Ionomycin (1μg/ml) and brefeldin A (BFA, 10μg/ml), cells were stained with antibodies diluted in PBS with 0.5% BSA (Sigma-Aldrich) and 0.05% sodium azide (Sigma-Aldrich) for 20 minutes at 4°C. Surface molecule staining (CD4 (BD), CD25 (eBioscience), CD69 (BioLegend), CD44 (BD), CD62L (BD) followed by fixation and permeabilization (BD Cytofix/Cytoperm™) and intracellular staining (IL-17A (BD), IFNγ (BD), Foxp3 (eBioscience)) were carried out on freshly isolated cells. The expression of surface molecules and intracellular cytokines were analyzed on a BD LSR II flow cytometer using FlowJo v.8 software (Tree Star).

Statistical analysis

Data sets were compared using a Mann Whitney test or Kruskal-Wallis test where appropriate, using Prism software v5. Differences were considered significant (*) at $P < 0.05$.

Results

IL-17A and IFN-γ-associated colitis is reduced in the absence of IL-13Rα2

IL-10 is a critical immunoregulatory cytokine, which maintains intestinal homeostasis and symbiosis with enteric microflora. Germ-line deletion of IL-10 in mice results in spontaneous colitis after 3–4 months, driven by dysregulated immune responses to colonic flora [16]. The onset of colitis can be accelerated and synchronized by feeding mice piroxicam, a non-steroidal anti-inflammatory drug (NSAID)[23]. It has been demonstrated that piroxicam-induced colitis in *il10*$^{-/-}$ mice is attenuated following intestinal helminth infection[15], with infection-driven Th2 responses correlating with protection. Because the bioactivity of IL-13 is attenuated by the IL-13 decoy receptor, IL-13Rα2 [22, 24], we tested the role of IL-13Rα2 in piroxicam-induced colitis by deleting IL-13Rα2 on the colitis-prone *il10*$^{-/-}$ background (dKO, *il10*$^{-/-}$*il13Rα2*$^{-/-}$). In contrast to *il10*$^{-/-}$ mice, *il10*$^{-/-}$*il13Rα2*$^{-/-}$dKO mice were protected from both sub-mucosal and intramuscular inflammation, with less severe colonic ulcers (Fig. 1A). Intestinal pathology in *il10*$^{-/-}$ mice correlated with weight loss 14 days post treatment and increased polymorphonuclear cells (PMN) (Fig. 1B). dKO mice however did not lose weight and had significantly reduced circulating PMN's compared to *il10*$^{-/-}$ mice, correlating with reduced disease severity. These findings suggest that either IL-13Rα2 directly induces disease[25, 26], or that IL-13Rα2 functions as a decoy receptor and suppresses a critical anti-inflammatory function of IL-13.

IL-17A and IFNγ are elevated in IBD patients [27, 28] and both are thought to mediate colitis following piroxicam treatment [29], similar to other models of colitis [5, 30]. As expected, significant numbers of IL-17A and IFNγ-producing T cells were observed in the MLN (Fig. 1C) and to a greater extent in the lamina propria of the colon (Fig. 1D) of *il10*$^{-/-}$ mice following piroxicam treatment. Strikingly however, the frequency and total number of Th1−/−Th17 cells were reduced in dKO mice compared to *il10*$^{-/-}$ mice, while the number of CD25^{+}Foxp3^{+} Treg cells was not significantly different. Consistent with this, several Th1-Th17 associated cytokines and chemokines, measured in colon-derived mRNA, were also significantly reduced in the absence of IL-13Rα2 (Sup. Fig. 1), providing an explanation for the reduced circulating PMN's. Expression of two IL-13/STAT-6-regulated genes, *Chi3l3* (Ym1) and *Arg1*, were markedly increased in the dKO mice (Fig. 1E) compared to *il10*$^{-/-}$ mice, indicating that IL-13Rα2 was attenuating IL-13 activity in the

disease prone *il10*$^{-/-}$ mice. Furthermore, IL-22, a cytokine which can facilitate mucosal wound healing and protect mice from IBD[31], was also significantly elevated in the colon of dKO mice (Fig. 1E). Together these data demonstrate that enhanced IL-13 bioactivity, uncovered in the absence of IL-13Rα2, significantly suppresses Th1/Th17-mediated inflammation and immunopathology. Consequently, these data suggest that the IL-13 decoy receptor (IL-13Rα2) like IL-10, controls susceptibility to Th1/Th17-driven inflammation in the gastrointestinal tract.

Lethal gastrointestinal helminth infection is reversed in the absence of IL-13Rα2

Following infection of genetically resistant mice with the gastrointestinal helminth parasite *T. muris*, immunity is mediated by a CD4$^+$ Th2 response[32], with IL-13 playing a dominant role in resistance[33]. We showed that IL-10 is critically involved in generating a polarized and protective IL-13 response during *T. muris* infection[20]. In the absence of IL-10, parasites are not expelled and the mice develop a lethal intestinal inflammatory response (Fig 2A). The intestinal pathology in *il10*$^{-/-}$ mice is characterized by reductions in goblet cells, Gob5 mRNA expression, eosinophils, and mucus secretion (Fig. 2B and 2C) and correspondingly increased production of IFN-γ and IL-17A relative to WT mice (Fig 2D). Prior studies conducted with *il10*$^{-/-}$*il12p40*$^{-/-}$ double KO mice suggested that the IFN-γ/IL-17A axis plays a critical pathogenic role in this model [20]. Following the observation that IL-13 decoy receptor critically controls susceptibility to piroxicam-induced colitis, we performed a similar series of experiments with *T. muris* infected *il10*$^{-/-}$ mice and examined whether the development of lethal Th1/Th17-driven intestinal inflammation during infection is controlled by IL-13 and/or IL-13Rα2.

Strikingly, although 100% of the *il10*$^{-/-}$ mice succumbed within 40 days of infection, 80% of the dKO mice survived greater than 100 days (Fig. 2A), despite the fact that they still harbored parasites (Fig. 2A, **photomicrographs**). Although the quantity of IL-13 in the caecum was similar for all genotypes (Sup. Fig 2), the dKO mice displayed enhanced IL-13 effector function compared to *il10*$^{-/-}$ mice, confirmed by increased *gob5* mRNA and AB-PAS staining (Fig. 2B), increased *ccl11* (eotaxin) and tissue eosinophilia (Fig. 2B, C) and increased *retlna* (FIZZ-1) mRNA responses in the caecum (Sup. Fig 2). As with piroxicam, *T. muris*-infected *il10*$^{-/-}$ mice developed significant IL-17A and IFN-γ responses. In contrast, dKO mice displayed minimal IFN-γ/IL-17 responses (Fig. 2D), which resulted in reduced intestinal inflammation compared to *il10*$^{-/-}$ mice (Fig. 2C). IFNγ-mediated *cxcl10* (IP-10) production was also decreased in the dKO (Sup. Fig 2). Thus, although the dKO mice remained infected (>100 days), the enhanced IL-13 effector response revealed in the absence of IL-13Rα2, led to the suppression of IFN-γ/IL-17 responses (Fig 2D), resulting in markedly decreased morbidity and prevention of mortality.

IL-13 negatively regulates Th17 cell differentiation and cytokine secretion *in vitro*

Deletion of IL-13Rα2 on the *il10*$^{-/-}$ background resulted in increased IL-13 bioactivity and reduced IFN-γ/IL-17A responses in both models. This is consistent with a recent study that reported exaggerated IL-17A responses in the absence of IL-4 and IL-13[34]. Although IL-13 has potent anti-inflammatory activity in vivo[35] the mechanisms by which IL-13Rα2 controls pro-inflammatory Th17 responses remains unclear. Given the dramatic effect IL-13Rα2 deficiency had on the development of the pathogenic Th1/Th17 responses, we investigated if IL-13 signaling was responsible for the altered response. FACS-purified naïve CD4$^+$ T cells were polarized under Th1, Th2 or Th17 conditions and examined for cytokine production and cytokine receptor expression. As expected, IL-4R mRNA was up-regulated during Th2 polarizing conditions, with only marginal expression detected on Th17 cells (Fig. 3A). Unexpectedly however, IL-13Rα1 was significantly up-regulated on both Th1 and Th17 cells (Fig 3A), confirming a previous observation[18]. To investigate the functional

significance of type II IL-4 receptor expression (IL-4Rα and IL-13Rα1), we polarized naïve $CD4^+$ T cells under Th1 and Th17 conditions in the presence of recombinant IL-13. Although IL-13 had no direct impact on Th1 polarized cells (Sup. Fig 3), the frequency of Th17 cells and secretion of IL-17A was significantly reduced in the presence of IL-13 (Fig 3B), suggesting that IL-13 signaling was negatively regulating the development of Th17 cells, confirming recent observations by Newcomb and colleagues[18]. Thus, IL-13 likely antagonizes $CD4^+$ Th17 responses by both direct and indirect mechanisms[36].

IL-13 suppresses Th1 and Th17 cells *in vivo* and protects mice from lethal intestinal inflammation

To determine whether IL-13 was responsible for the reduced Th1/Th17 responses in dKO mice, *T. muris*-infected dKO mice were treated with a neutralizing anti-IL-13 mAb. Efficient neutralization of IL-13[37] was confirmed by the marked decrease in gob 5 mRNA (Fig. 4D) and reduced mucus staining observed in the caecum of anti-IL-13-treated WT mice (Fig. 4E). As expected, infected *il10*$^{-/-}$ mice displayed significant weight loss (Fig. 4A), marked mortality (Fig. 4B), reduced gob5 mRNA expression (Fig. 4D) and decreased mucus producing cells (Fig. 4E). These observations correlated with significant increases in parasite-specific IFN-γ and IL-17A production in the mesenteric lymph nodes (Fig. 4C), increased sub-mucosal inflammation (Fig 4E), and heightened IL-13Rα2 expression in the caecum (Fig. 4D). In contrast, *il10*$^{-/-}$*il13Rα2*$^{-/-}$ dKO mice displayed a completely opposite phenotype, suggesting that, as observed with piroxicam (Fig 2), the absence of the IL-13 decoy receptor was sufficient to restore the protective effects of IL-13. To confirm this hypothesis, additional dKO mice were treated with anti-IL-13 mAb. Similar to *il10*$^{-/-}$ control group, anti-IL-13 treated dKO animals lost weight, displayed reduced mucus responses (Fig. 4D, 4E), and rapidly succumbed to the infection. Production of the pro-inflammatory cytokines IFN-γ and IL-1A7 was also restored when IL-13 was neutralized (Fig. 4C and 4D), confirming that IL-13Rα2 was functioning as a decoy receptor for IL-13 in the gastrointestinal tract and blocking the protective potent protective effects of IL-13.

Discussion

Following piroxicam treatment or infection with *T. muris*, IFN-γ and IL-17A-driven intestinal inflammation correlated with elevated IL-13Rα2. Deletion of IL-13Rα2 abrogated IFN-γ and IL-17A and significantly attenuated the degree of inflammation in both models. These data suggest that IL-13Rα2 is directly responsible for the development of inflammation, as suggested in a different model of colitis[25]. Alternatively, these observations suggest that IL-13Rα2 was blocking IL-13[22, 24], and that IL-13 functions as a negative regulator of IFN-γ and IL-17A. Following piroxicam treatment or *T. muris* infection of *il10*$^{-/-}$*il13Rα2*$^{-/-}$ dKO mice, we observed evidence of increased IL-13 activity (increased ym-1 and arg-1 or elevated goblet cell and mucus responses, respectively) and decreased IFN-γ and IL-17A, supporting the latter model. Thus, increased IL-13 activity in the absence of IL-13Rα2 led us to hypothesize that IL-13Rα2 blocks the protective effects IL-13. We tested this hypothesis by neutralizing IL-13 in *il10*$^{-/-}$*il13Rα2*$^{-/-}$ dKO mice, which restored IFN-γ and IL-17A responses, inflammation and mortality following *T. muris* infection. These observations clearly indicate that the balance between IL-13 and the IFN-γ/IL-17A axis tightly regulates the degree of intestinal inflammation. Furthermore these data also demonstrate that IL-13Rα2 is intricately involved in the regulation of this balance and consequently, the development of IBD.

Because CD and UC have distinct inflammatory etiologies, with Th1-Th17-associated Crohn's disease developing in the small and large bowel[38] and IL-13-mediated ulcerative colitis occurring primarily in the colon[39], the suppression of IL-13 activity by the decoy receptor may be a critical event in the genesis of Th1-Th17 driven inflammation in the

gastrointestinal tract. In our studies, the enhanced IL-13 activity observed in the colon of *il10*$^{-/-}$*il13Rα2*$^{-/-}$ mice suppressed the development of Th1-Th17–associated colitis. These data present a new mechanism of IL-13-mediated control of Th1/17 responses. They may also explain why ulcerative colitis and Crohn's disease are rarely if ever identified in the same individual[40], as the key inducer of UC (IL-13) appears to be a negative regulator of Th1-Th17-associated CD.

Interestingly, a recent paper by Shea-Donohue and colleagues[41] showed that IL-13 and IL-13Rα2 are expressed much more in the colon than small intestine. Thus, changes in IL-13 and IL-13Rα2 expression might be expected to have a much larger impact on the development of Th1-Th17-mediated inflammation in the colon than in other areas of the gastrointestinal tract. However, the marked absence of IL-13Rα2 in the small bowel might also lead to increased IL-13 bioactivity in this region and thus provide critical protection from Th1-Th17-dependent colitis, while increasing the risk of IL-13-driven UC. Clinical findings support the notion that IL-13 may be protective in Crohn's disease, as Crohn's disease patients produce less IL-4 and IL-13 and their PBMC's are hypo-responsive to IL-13[42].

The hypothesis that IL-13 can cross-regulate the development of Th17 cells is supported by the inverse relationship we observed between IL-17A and IL-13. Although IFNγ and IL-17A production was more marked in the absence of IL-10, they also increased when WT mice were treated with anti-IL-13, further supporting a role for IL-13 in the suppression of IFN-γ/IL-17A production in the gut. Together, these observations suggest that in addition to inducing goblet cell hyperplasia[43], epithelial cell turnover[44], and production of Relm-β (Fizz-2/*Retnlb*)[19], IL-13 also plays a key protective role in the gut by suppressing CD4^{+} Th1 and Th17 cell development[33]. When viewed together, these studies reveal a novel protective pathway for IL-13 and its decoy in the control of Th1/Th17-mediated inflammation in the gastrointestinal tract.

In contrast to our findings, two related studies investigating the role of IL-13Rα2 in a model of chronic trinitrobenzene sulfonic acid (TNBS)-induced colitis concluded that IL-13Rα2 functions as a signaling receptor for IL-13 [25, 26]. Using either a soluble IL-13Rα2-Fc protein or IL-13Rα2-specific small interfering RNA's to block IL-13Rα2, they concluded that IL-13 signals through IL-13Rα2 and induces IL-13-dependent fibrosis. Although the TNBS model was not employed in our studies, we found no evidence that IL-13Rα2 was functioning as a signaling receptor for IL-13. In fact, our data suggested that IL-13Rα2 primarily blocks IL-13 effector functions, supporting its role as a decoy receptor. We concluded that by blocking IL-13 activity, IL-13Rα2 plays an indispensable role in the genesis of Th1/Th17-associated intestinal pathology in *il10*$^{-/-}$ mice.

The explanation for the different conclusions is not clear, although the sIL-13Rα2-Fc blocker used in their studies binds IL-13, not IL-13Rα2; therefore it is not capable of distinguishing between IL-13Rα1 and IL-13Rα2-mediated effects. The use of small interfering RNAs in vivo may also have important off-target effects[45]. Our studies with *il13Rα2*$^{-/-}$ and *il10*$^{-/-}$*il13Rα2*$^{-/-}$ dKO mice all point to IL-13Rα2 functioning as a decoy receptor for IL-13 in the gastrointestinal tract.

In contrast to the well-known pathogenic properties of IL-13 [46, 47], to our knowledge, this is the first study to provide evidence of a tissue-protective role for IL-13 in the GI tract. A similar protective role for IL-13 has been described in the liver following ischemia/reperfusion injury[48] and in the CNS following MOG_{33-55} immunization[49]. In both cases, pro-inflammatory cytokine production was suppressed, either following rIL-13 treatment or following the induction of IL-13 by IL-25, with hepatic Kupfer cells[48] and APC's[49]

identified as the key targets of IL-13. It has previously been reported that IL-13 can abrogate Th1 development indirectly [50], by modulating APC function (reviewed in [35, 51]); however, this is one of the first studies implicating IL-13 as a negative regulator of Th17 development in vivo.

Previously, using models of pulmonary and hepatic inflammation, we demonstrated that IL-13Rα2 is upregulated by IL-4 and IL-13 and suppressed by IFNγ in vivo[52]. The soluble form of the IL-13Rα2 was also found in abundance in un-manipulated mice[22] and consistent with our findings, was expressed at quite high levels in the colon[41]. In the lung and liver the decoy IL-13Rα2 restricts the pro-fibrotic and pathogenic activities of IL-13 during allergic inflammation and chronic helminth infection[22]. Therefore, in some circumstances, disrupting IL-13Rα2 might have unintended consequences such as exacerbating IL-13-driven diseases like allergic asthma or infection induced hepatic fibrosis [21, 46]. This report identifies a similar role for IL-13Rα2 in the intestine, however instead of restricting IL-13 and preventing IL-13 related pathology, our data suggest that IL-13Rα2 is key to generating IFNγ/IL-17A-driven inflammation and immunopathology in the bowel. The heightened susceptibility of IL-10-deficient mice to colitis is clearly controlled by this mechanism, as *il10*$^{-/-}$ mice developed increased IL-13Rα2 responses and reduced IL-13 activity during *T. muris* infection or following treatment with piroxicam. In both cases this led to the development of IFNγ/IL-17-associated colitis and intestinal inflammation. Consequently, reagents that specifically target and inhibit the IL-13 decoy receptor and enhance IL-13 effector function might offer a new strategy to prevent or reverse inflammatory bowel disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge the meticulous care of all animals by Nicole Mahoney, Joy McFarlane, Jose Encarnacion, Lauren Donato and SoBran staff. We thank Mary Collins and Marion Kasaian at Wyeth LLC for providing IL-13Rα2-deficient mice. We thank Sandra D. White, Robert W. Thompson and NIH Clinical Center staff for additional animal care, technical assistance and CBC processing. We also thank Dr. Luke Barron and Dr. Kevin Vanella for helpful and constructive discussion. This research was supported by the Intramural Research Program of the NIH, NIAID.

Abbreviations

dKO double knockout

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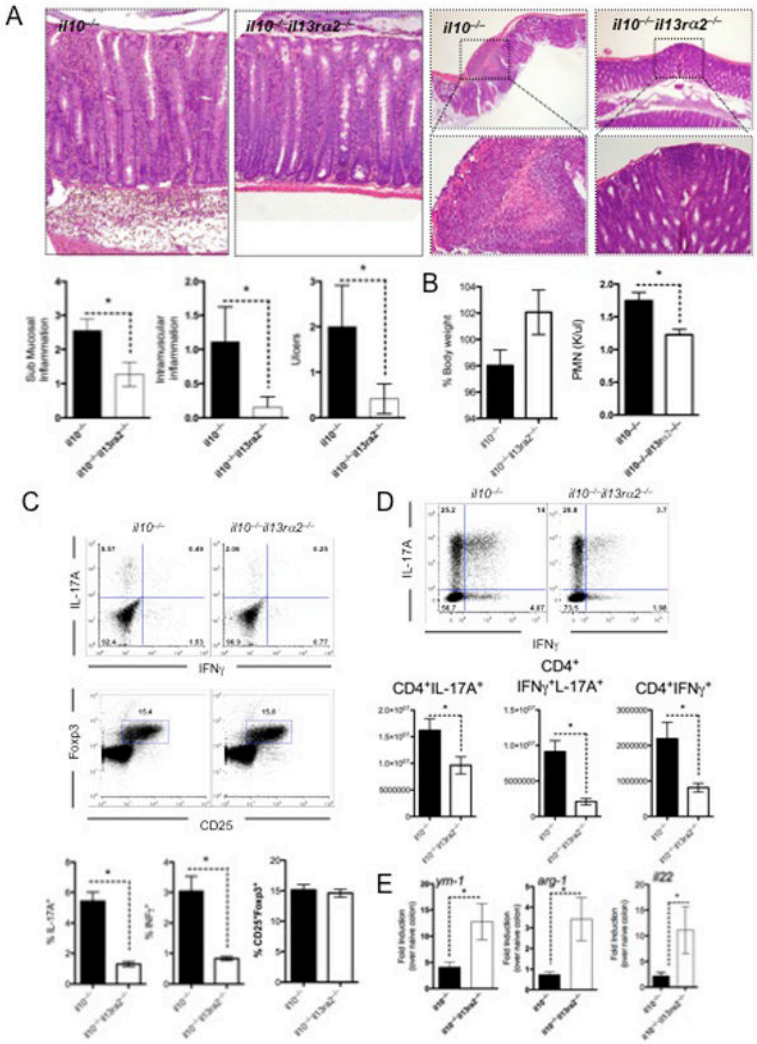

Figure 1. Deletion of IL-13Rα2 suppresses IL-17A and IFNγ production and protects *il10*$^{-/-}$ mice from piroxicam-induced colitis

Mice were fed piroxicam infused food for 14 days, with colitis assessed on day 14. Five animals per group were used with 1 of 2 experiments shown. Mean±SEM are shown with * $p<0.05$ considered statistically significant.

A- 5μm sections of paraffin-embedded colon were stained with H&E and assessed for pathological abnormalities.

B- Animal weights and circulating PMN's measured in whole blood after 14 days of piroxicam-infused food.

C- Mesenteric lymph node (MLN) cells stimulated with PMA and ionomycin in the presence of BFA and stained with anti-mouse CD4, CD25, IL-17A and IFNγ and Foxp3.

D- Lamina propria lymphocytes isolated and quantified at day 14 were stimulated with PMA and ionomycin in the presence of BFA and stained with anti-mouse CD4, IL-17A and IFNγ.

E- RNA was extracted from the colon of mice, 14 days post piroxicam exposure with ym-1, arg1 and *il22* gene transcripts quantified and expressed relative to HPRT.

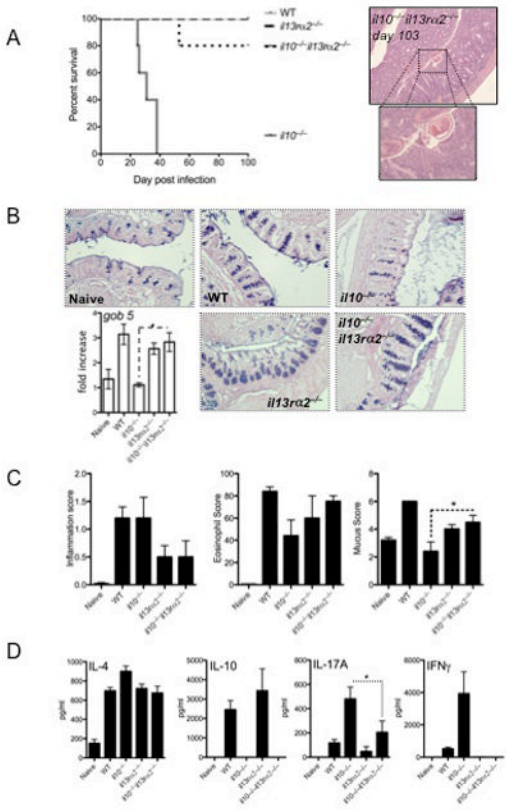

Figure 2. Lethal intestinal helminth infection in *il10*$^{-/-}$ mice is reversed by deleting IL-13R*α*2

Mice were infected with 200 *T. muris* eggs and monitored for survival or euthanized and assessed on day-15. Five animals per group were used with 1 of 3 experiments shown. Mean ±SEM are shown with $p<0.05$ considered statistically significant.

A- Survival of mice with evidence of infection at day 103.

B- 5μm sections of paraffin-embedded caecum were stained with AB-PAS and assessed for goblet cell frequency and gob5 gene expression.

C- Inflammation, Eosinophilia and mucus-producing cells was scored from 5μm sections of paraffin-embedded caecum.

D- Mesenteric lymph node cells re-stimulated with 10μg of *T. muris* antigen were cultured for 4 days. IL-4, IL-10, IL-17A and IFNγ production were measured by ELISA.

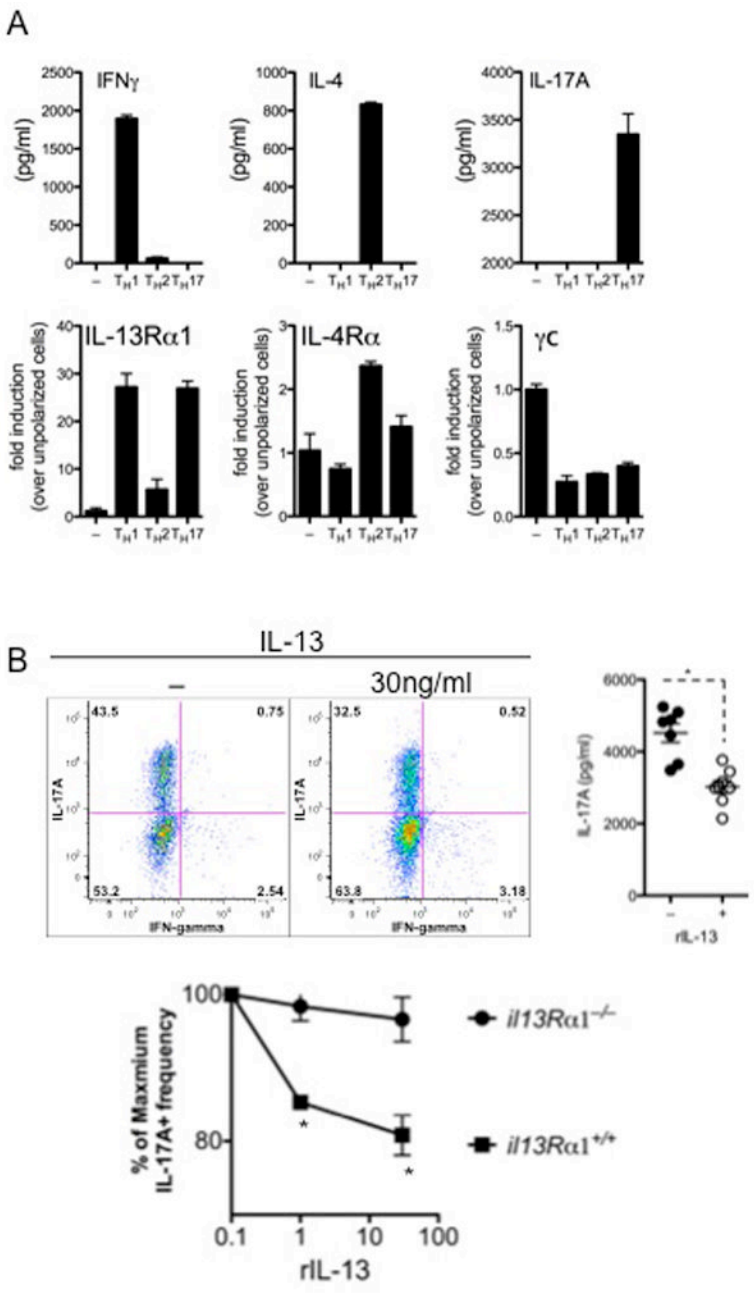

Figure 3. In vitro Th17 cells possess a functional IL-13 receptor that regulates IL-17A production

FACS-purified naïve $CD4^{+}CD62L^{hi}CD44^{lo}$ T cells were stimulated under Th1, Th2 or Th17 conditions. One of 4 experiments is shown. Mean±SEM are shown with $p<0.05$ considered statistically significant.

A- Canonical Th1 (IFNγ), Th2 (IL-4) and Th17 (IL-17A) cytokines were measured in culture supernatants. mRNA transcripts for *il13Rα1*, *il4rα* and *γC* were measured from polarized cells.

B- Th17-polarized cells cultured with rIL-13. Th17 frequency and IL-17A secretion were measured by flow cytometry and ELISA, respectively.

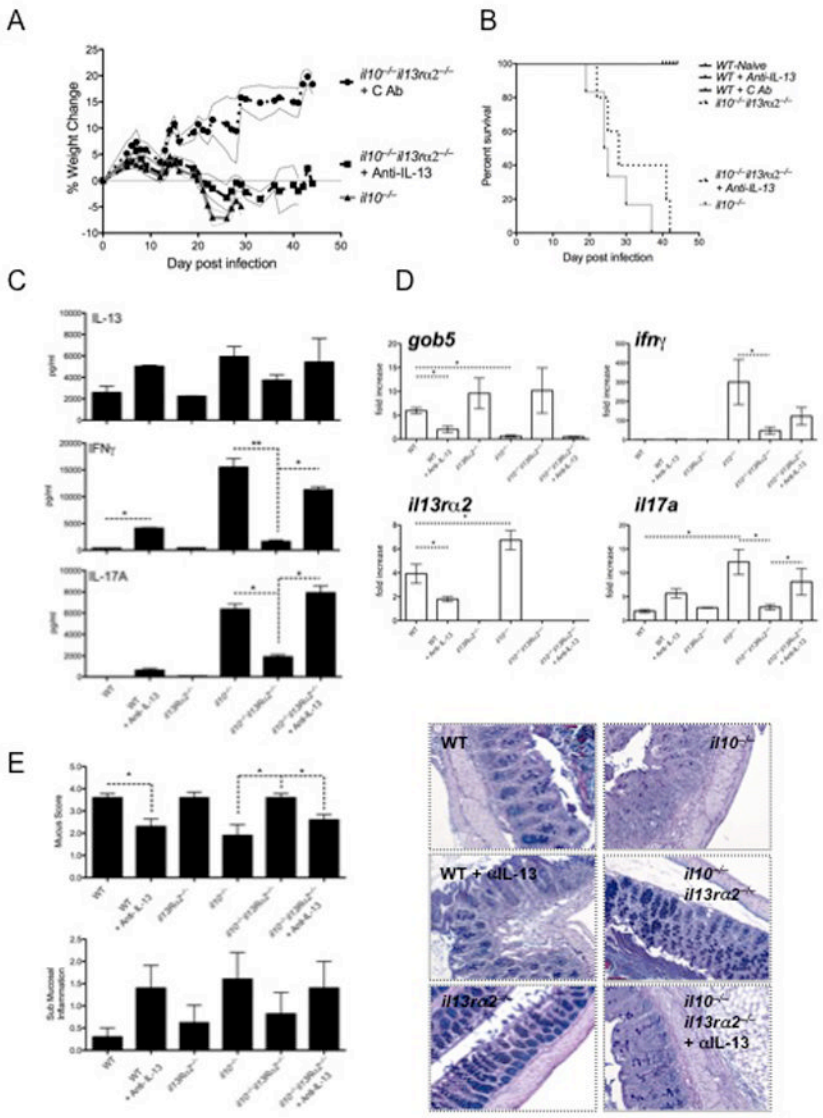

Figure 4. IL-13 regulates Th17 and Th1 responses and protects mice from a lethal infection

Mice were infected with 200 *T. muris* eggs and monitored for weight loss and survival or euthanized and assessed at day-21. Animals were treated with 500μg of anti-IL-13 mAb per week starting on day 1. Five animals per group were used with 1 of 2 experiments shown. Mean±SEM are shown with $p<0.05$ considered statistically significant.

A- Weight monitored daily.

B- Survival of mice.

C- Mesenteric lymph node cells re-stimulated with 10μg of *T. muris* antigen were cultured for 4 days. IL-13, IFNγ and IL-17A production was measured by ELISA.

D- RNA isolated from the caecum was reverse transcribed and assessed for *gob5*, *ifnγ*, *il13rα2* and *il17a* transcripts.

E- 5μm sections of paraffin-embedded caecum were stained with Giemsa and AB-PAS (shown) and assessed for submucosal inflammation and mucus-producing cell frequency.