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IL-4 Exacerbates Disease in a Th1 Cell Transfer Model of Colitis

Madeline M. Fort,* Robin Lesley,* Natalie J. Davidson,* Satish Menon,* Frank Brombacher,† Michael W. Leach,§ and Donna M. Rennick*

IL-4 is associated with Th2-type immune responses and can either inhibit or, in some cases, promote Th1-type responses. We tested the effect of IL-4 treatment on the development of inflammation in the CD4+CD45RBhigh T cell transfer model of colitis, which has been characterized as a Th1-dependent disease. IL-4 treatment significantly accelerated the development of colitis in immunodeficient recipients (recombinase-activating gene-2 (Rag2)−/−) of CD4+CD45RBhigh T cells. Quantitative analysis of mRNA expression in the colons of IL-4-treated mice showed an up-regulation of both Th1- and Th2-associated molecules, including IFN-γ, IP-10, MIG, CXCR3, chemokine receptor-8, and IL-4. However, cotreatment with either IL-10 or anti-IL-12 mAb effectively blocked the development of colitis in the presence of exogenous IL-4. These data indicate that IL-4 treatment exacerbates a Th1-mediated disease rather than induces Th2-mediated inflammation. As other cell types besides T cells express the receptor for IL-4, the proinflammatory effects of IL-4 on host cells in Rag2−/− recipients were assessed. IL-4 treatment was able to moderately exacerbate colitis in Rag2−/− mice that were reconstituted with IL-4Rα-deficient (IL-4Rα−/−) CD4+CD45RBhigh T cells, suggesting that the IL-4 has proinflammatory effects on both non-T and T cells in this model. IL-4 did not cause colitis in Rag2−/− mice in the absence of T cells, but did induce an increase in MHC class II expression in the lamina propria of the colon, which was blocked by cotreatment with IL-10. Together these results indicate that IL-4 can indirectly promote Th1-type inflammation in the CD4+CD45RBhigh T cell transfer model of colitis. The Journal of Immunology, 2001, 166: 2793–2800.
reconstituted with wild-type (WT) bone marrow cells, immunodeficient recipients of CD4+ CD45RB<sup>high</sup> T cells, and trinitrobenzenesulfonic acid (TNBS)-treated SJL/J mice all develop Th1-type colitis (28–32). In many of these models disease is either prevented, or at least ameliorated, by early treatment with IL-10 or neutralizing Ab to IL-12 (26, 28, 30). The role of IL-4 in Th1-type intestinal disease is less clear. In one study IL-4 treatment did not suppress the development of colitis in acid mice transplanted with CD4<sup>+</sup> CD45RB<sup>high</sup> T cells (33). In contrast, infection of mice with adenovirus containing the IL-4 gene reduced inflammation in rats with TNBS-induced colitis (34).

We have evaluated the effect of IL-4 treatment in a modified version of the original T cell transfer model of colitis (29, 35). In this modified model the transfer of CD4<sup>+</sup> CD45RB<sup>high</sup> T cells from either WT mice or IL-10<sup>-/-</sup> mice with established Th1-type disease into 129 SveV Rag<sup>2/-</sup> mice results in colitis characterized by diffuse inflammatory cellular infiltrates, epithelial hyperplasia, and, when severe, ulcers and transmural inflammation. The resulting colitis can be significantly ameliorated by treatment with anti-IL-12 mAb or rIL-10 in vivo (36, 37). We transferred CD4<sup>+</sup> CD45RB<sup>high</sup> T cells from either WT or IL-10<sup>-/-</sup> mice into Rag<sup>2/-</sup> immunodeficient mice and treated them daily with IL-4. Our data show that IL-4 treatment has the ability to exacerbate Th1-type colonic inflammation in this model.

Materials and Methods

Mice

Recombinase-activating gene 2-deficient (Rag2<sup>-/-</sup>) mice on either 129 SveV or BALB/c background and WT 129 SveV mice were obtained from Taconic Farms (Germantown, NY) or from a colony maintained at the DNAx Animal Care Facility. IL-10<sup>-/-</sup> 129 SveV mice and IL-4R<sup>-/-</sup> o-chain-deficient (IL-4R<sup>α/-</sup>) BALB/c mice were from colonies maintained under specific pathogen-free conditions at the DNAx Animal Care Facility (38, 39).

In vivo treatments

Purified recombinant murine IL-4 and IL-10 were made as previously described and contained <0.1 U/ml mouse protein of endotoxin (37, 40). Mice were given 10–20 μg of IL-4 i.p. daily for 28 days. For IL-10 treatment, mice were given 20 μg i.p. daily. Saline (1× HBSS; BioWhittaker, Walkersville, MD) was given daily as a negative control. Purified anti-murine IL-12 mAb (C17.8.20) or isotype control mAb (MP4–25D2, anti-human IL-4; no cross-reactivity with murine IL-4) was given to mice i.p. weekly at 2 mg/dose for the duration of the experiment. All cytokine and Ab treatments were started on the day of T cell transfer (day 0).

Cell isolations and transfers

CD4<sup>+</sup> CD45RB<sup>high</sup> splenic T cells were obtained by cell sorting. Briefly, splenocytes were first enriched for CD4<sup>+</sup> T cells by red cell lysis and magnetic bead depletion using lineage-specific rat mAbs supernatants (10%, v/v); B220 (B cells) and Ter119 (erythrocytes). mAb-stained cells were removed in a magnetic field using goat-anti rat IgG (Fc) and goat anti-anti IgG (H+L)-coated magnetic beads (PerSeptive Diagnostics, Cambridge, MA). The remaining cells were then stained with anti-CD4-FFITC and anti-CD45RB-PE (both from PharMingen, San Diego, CA). Two-color cell sorting was performed using a FACStar Plus (Becton Dickinson, Mountain View, CA). The sorted CD4<sup>+</sup> CD45RB<sup>high</sup> T cells were >98% pure upon reanalysis. The purified CD4<sup>+</sup> CD45RB<sup>high</sup> cells (2 × 10<sup>5</sup>) were injected i.p. into 129 SveV or BALB/c Rag2<sup>-/-</sup> recipient mice, depending on the donor cells used. Four weeks after T cell transfer, mice were sacrificed and analyzed for the presence of enterocolitis.

Histologic analysis of colitis

Microscopic examination of mouse colons was performed in a blinded fashion by the same pathologist (M.W.L.) on formalin-fixed tissue sections stained with hematoxylin and eosin as previously described (36). Longitudinal sections of the entire length of the colon were evaluated, taking into account both the number of lesions and their severity. Five regions of the colon (c Cecum, ascending, transverse and descending colon, and rectum) were graded semiquantitatively as 0 (no change) or 1 (most severe change).

The grading represents the incidence and severity of inflammatory lesions based on infiltrates, goblet cell loss, crypt abscesses, ulcerations, and fibrosis. The summation of the score for each of five segments of the colon provides a total disease score per mouse (from 0–25), where 0–1 indicates no change, 2–5 indicates mild disease, 6–10 indicates moderate disease, and 11–20 indicates severe disease. No mice in these studies have a score >20, because such severe disease results in death.

Quantitative mRNA (TaqMan) analysis in colon

Total RNA was isolated from whole colon samples using Qiagen RNeasy columns (Qiagen, Valencia, CA), according to the manufacturer’s instructions. Total RNA (5 μg) was reverse transcribed into cDNA using random hexamers (Promega, Madison, WI). The expression of IFN-γ, IL-4, MIG, IP-10, eotaxin, TARC, CCR3, CCR8, CXCR3, and TNF-α was determined by a method for real-time quantitative PCR using the ABI 7700 sequence detector system (Perkin-Elmer Applied Biosystems, Foster City, CA). Briefly, 50 ng of total cDNA was in a reaction volume of 25 μl that contained final concentrations of 1× PCR buffer; 200 μM dATP, dCTP, and dGTP; 400 μM dUTP; 4 mM MgCl<sub>2</sub>; 1.25 U of AmpliTaq DNA polymerase; 0.5 μM of Amp-erase uracil-N-glycosylase; 900 nM of each primer; and 250 nM probe. The thermal cycling conditions included 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of amplification at 95°C for 15 s and 55°C for 1 min for denaturing and annealing-extension, respectively. Sense and antisense primers as well as probes used for the detection of the genes of interest were predeveloped TaqMan assay reagents (Perkin-Elmer Applied Biosystems). Primers and probes were designed to ensure that no cross-reactivity with other genes would occur. Each primer-probe pair was tested on a panel of cDNA plasmids containing a variety of cytokines, chemokines, and chemokine receptors. The probes for each message were labeled at the 5′ end with a reporter fluorescent dye, FAM, and at the 3′ end with a quencher fluorescent dye, TAMRA. Fluorescence detection of FAM was performed at the end of each cycle. The quantity of cDNA of the gene of interest was directly related to the amount of FAM detected after 40 cycles. cDNA plasmids containing the gene of interest were used as a standard curve, ranging from 100 to 0.01 pg. From this standard curve, the amount of cDNA of the gene of interest was calculated in femtograms per 50 ng of total cDNA. As an internal control, 18S ribosomal RNA (rRNA) expression was measured in each sample in a multiplex assay; the probe for rRNA was labeled at the 5′ end with the fluorophore fluorescent dye VIC. The amount of 18S RNA was correlated to the cycle at which VIC fluorescence was first detected (cycle threshold value). To correct for any variation in the amount of RNA between individual samples, the mean cycle threshold value for 18S RNA was calculated for all samples and subtracted from each individual cycle threshold value, then this difference was raised to the second power and multiplied by the FAM value (in femtograms per 50 ng of total cDNA) for each sample. Thus, the amount of cDNA of the gene of interest in each sample could be directly compared with the amounts detected in all other samples.

Immunohistochemical analysis

Briefly, colon pieces from saline- and IL-4-treated Rag2<sup>-/-</sup> mice were cleaned of feces and frozen in tissue-freezing medium (Triangle Biomedical Sciences, Durham, NC). Frozen blocks were cut on a cryostat, and 8-μm-thick sections were cut on gelatinized glass slides, dried, and fixed for 10 min with acetone. Sections were preincubated with 1× PBS (GenMate, Kaysville, UT) and 10% normal mouse serum (NMS; Jackson ImmunoResearch Laboratories, West Grove, PA) for 15 min. Rat anti-mouse I-Ab (PharMingen) was diluted in PBS/10% NMS and incubated on sections for 1 h at room temperature. Rat IgG<sub>2b</sub> (PharMingen) was used as an isotype control. Sections were rinsed in 1× PBS and stained with HRP-conjugated sheep anti-IgG (The Binding Site, Birmingham, U.K.) diluted in PBS/10% NMS for 30 min at room temperature. AEC (Vector, Burlingame, CA) was used as a substrate for HRP. Sections were counterstained with hematoxalin (Vector), and then sequentially rinsed in H<sub>2</sub>O, 1% ammonium hydroxide, and PBS. Sections were mounted with coverslips and observed by light microscopy.

Statistics

All data were analyzed using a statistical program (InStatP, GraphPad, San Diego, CA). Student’s t test or nonparametric Mann-Whitney test was used to determine statistical significance between groups, with p ≤ 0.05 considered significant.
Normal appearing colon from nonreconstituted Rag2−/− mice. Purified 2 × 10^6 CD4+CD45RB_b high T cells from either WT (A) or IL-10−/− (B) mice were transferred into Rag2−/− mice. Mice were treated daily i.p. with saline or 20 μg of IL-4 for 28 days. At the end of 28 days mice were sacrificed, and the colons of individual mice were scored for colitis by histological analysis (see Materials and Methods). Each symbol represents an individual animal. Bar indicates mean colitis score for each group. A and B, IL-4-treated mice had significantly higher colitis scores compared with saline-treated mice (p = 0.002 and p = 0.016, respectively, by Mann-Whitney U test).

Results

IL-4 exacerbates disease in the CD4+ CD45RB_b high T cell transfer model of colitis

To test the effect of IL-4 on a Th1-type murine model of colitis, WT CD4+CD45RB_b high T cells were transferred into immunodeficient Rag2−/− 129 SvEv mice, and the recipient mice were treated daily with either saline (vehicle control) or 20 μg IL-4. In this model untreated recipient mice develop moderate to severe colitis 8–12 wk after T cell transfer (29, 36, 37). However, IL-4 treatment exacerbated the disease such that the majority of mice were moribund 4 wk after T cell transfer. Therefore, the experiment was terminated, and the mice were analyzed histologically for signs of colitis. Five of eight mice had moderate to severe colitis by 4 wk, while saline-treated mice had few histological signs of disease at that time point (Fig. 1A). As WT CD4+CD45RB_b high T cells are uncommitted to either a Th1 or a Th2 phenotype, we considered the possibility that IL-4 treatment was pushing naive T cells to an inflammatory Th2 phenotype, rather than having a proinflammatory effect on Th1-type T cells. Therefore, we repeated the experiment using CD4+CD45RB_b high T cells from 4- to 6-mo-old IL-10−/− 129 SvEv mice that already had signs of chronic Th1-type colitis (29, 40). The IL-10−/− CD4+CD45RB_b high T cells caused mild colitis in recipient Rag2−/− mice within 4 wk (Fig. 1B; mean colitis score, 3.3; Fig. 2A). However, IL-4 treatment was able to clearly exacerbate the colitis seen in Rag2−/− recipients of IL-10−/− CD4+CD45RB_b high T cells (Figs. 1B and 2B; mean colitis score, 10.9). Because, in our hands, IL-10−/− CD4+CD45RB_b high T cells cause more consistent and aggressive disease in Rag2−/− recipients than T cells from WT mice, we performed all subsequent transfer experiments with CD4+CD45RB_b high T cells from IL-10−/− mice. (M. Fort and D. Remnick, unpublished observations).

IL-4 treatment does not switch the colitis to a Th2-type inflammation

The ability of exogenous IL-4 to exacerbate colitis in this T cell transfer model of colitis lead us to investigate whether IL-4 induced a switch from a Th1-dependent to a Th2-dependent disease. Therefore, we looked for differential gene expression in the colons of Rag2−/− mice 28 days after reconstitution with IL-10−/− CD4+CD45RB_b high T cells and treatment with either saline or IL-4. Using a very sensitive, quantitative method of PCR analysis, colonic total RNA was tested for the presence of mRNA of various Th1- and Th2-type-associated cytokines, chemokines, and chemokine receptors. IL-4 treatment induced a significant increase in the

FIGURE 1. In vivo treatment with IL-4 accelerates the colitis caused by CD4+CD45RB_b high T cells. Purified 2 × 10^6 CD4+CD45RB_b high T cells from either WT (A) or IL-10−/− (B) mice were transferred into Rag2−/− mice. Mice were treated daily i.p. with saline or 20 μg of IL-4 for 28 days. At the end of 28 days mice were sacrificed, and the colons of individual mice were scored for colitis by histological analysis (see Materials and Methods). Each symbol represents an individual animal. Bar indicates mean colitis score for each group. A and B, IL-4-treated mice had significantly higher colitis scores compared with saline-treated mice (p = 0.002 and p = 0.016, respectively, by Mann-Whitney U test).

FIGURE 2. IL-4 exacerbates colitis in T cell-reconstituted Rag2−/− mice. Representative photomicrographs of the descending colon of Rag2−/− mice. When present, colitis was usually diffuse. A, Example of moderate colitis in a mouse receiving IL-10−/− CD4+CD45RB_b high T cells and treated with saline. This was the most severe disease seen in this group at 4 wk post-transfer. B, Example of severe disease typically seen in mice receiving IL-10−/− CD4+CD45RB_b high T cells and treated with IL-4. There is greater epithelial hyperplasia and more inflammation that separates intestinal glands and extends into the submucosa than shown in A. A multinucleated giant cells is also present (left side at the base of the glands). C and D, Normal appearing colon from mice receiving IL-10−/− CD4+CD45RB_b high T cells and treated with IL-4 and IL-10 (C) or with IL-4 and anti-IL-12 mAb (D). E and F, Normal-appearing colon from nonreconstituted Rag2−/− mice treated with saline (E) or IL-4 (F). Hematoxylin and eosin, ×100.
expression of TNF-α and IFN-γ mRNA, as well as in that of the IFN-γ-induced chemokines MIG and IP-10 (Fig. 3 and data not shown). In addition, IL-4 treatment up-regulated the expression of mRNA for CXCR3, which is the receptor for MIG and IP-10 and is expressed on Th1-type CD4+ T cells (41). In all samples tested the mRNA expressions of IL-12p35 and IL-12p40 were below the detection limits of the system (data not shown). IL-4 treatment also induced a significant increase in IL-4 mRNA expression in the colon, but not in the mRNA expression of other Th2-associated molecules, such as IL-5, IL-13, eotaxin, and TARC (Fig. 3 and data not shown). CCR4 and CCR3, which are preferentially expressed on Th2-type T cells and are the receptors for TARC and eotaxin, respectively, were not up-regulated with IL-4 treatment (Fig. 3 and data not shown) (32). Expression of CCR8, which is expressed on Th2-type T cells and monocytes, was significantly increased (Fig. 3) (41–43). Thus, IL-4 treatment in vivo can up-regulate the colonic mRNA expression of Th1-associated cytokines and chemokines as well as some Th2-associated molecules.

To elucidate the relative importance of the up-regulated Th1-associated molecules seen in the colons of IL-4-treated animals, we tested the ability of inhibitors of Th1-type responses to counteract disease. First, we examined the effect of blocking IL-12 with neutralizing Ab. CD4+ CD45RB<sup>high</sup> T cells from aged IL-10<sup>−/−</sup> mice were transferred into Rag2<sup>−/−</sup> recipient mice, and the recipients were treated with either saline or IL-4 (daily) and with anti-IL-12 mAb or an isotype-matched control mAb (weekly) for 28 days. As shown in Figs. 2D and 4, Rag2<sup>−/−</sup> recipients of IL-10<sup>−/−</sup> CD4<sup>+</sup>CD45RB<sup>high</sup> T cells cotreated with IL-4 and anti-IL-12 mAb developed significantly less disease than recipients cotreated with IL-4 and isotype control mAb (mean colitis score, 2.4 and 10.9, respectively; p < 0.001). Quantitative PCR analysis of gene expression in the colons of mice cotreated with anti-IL-12 mAb and saline or IL-4 showed decreased mRNA expression of Th1-associated genes compared with isotype control-cotreated animals (data not shown). The gene expressions of MIG, IL-10, and CXCR3 were decreased with anti-IL-12 mAb cotreatment by 35- to 125-fold, 8- to 32-fold, and 3-fold, respectively. The expression of IFN-γ mRNA was completely suppressed in the colons of recipients cotreated with saline and anti-IL-12 mAb, but was only partially suppressed (3-fold decrease) in colons of recipients cotreated with IL-4 and anti-IL-12 mAb. This could explain the mild disease seen in IL-4- and anti-IL-12 mAb-cotreated mice compared with the almost complete absence of disease seen in saline- and anti-IL-12 mAb-cotreated mice (Fig. 4). There was no change in the gene expression of IL-4, CCR4, or CCR8 in the colons of anti-IL-12 mAb-treated vs isotype control-treated mice (data not shown).

![FIGURE 3](http://www.jimmunol.org/)  
**FIGURE 3.** IL-4 treatment in vivo results in higher mRNA expression of both Th1- and Th2-associated molecules. IL-10<sup>−/−</sup> CD4<sup>+</sup>CD45RB<sup>high</sup> T cells were transferred into Rag2<sup>−/−</sup> recipient mice, which were treated daily with IL-4 or saline. After 4 wk total RNA was isolated from the whole colon of each mouse, reverse transcribed to cDNA, and tested by quantitative PCR analyses for the presence of IFN-γ, MIG, IP-10, CXCR3, IL-4, IL-5, CCR4, and CCR8 transcripts. □, saline-treated animals; ■, IL-4-treated animals. Data are representative of two separate experiments, with each bar representing the average of four or five mice. * Significantly different from saline-treated mice (p < 0.05).

![FIGURE 4](http://www.jimmunol.org/)  
**FIGURE 4.** The proinflammatory effects of IL-4 treatment are inhibited by cotreatment with anti-IL-12 mAb or IL-10. Rag2<sup>−/−</sup> mice were reconstituted with 2×10<sup>5</sup> IL-10<sup>−/−</sup> CD4<sup>+</sup>CD45RB<sup>high</sup> T cells and treated daily with saline or IL-4. Mice were also cotreated on a weekly basis with 2 mg i.p. of either anti-IL-12 mAb or an isotype control mAb. A separate group of mice was cotreated with IL-4 and IL-10 daily. After 28 days mice were sacrificed, and the severity of colitis was determined; each symbol represents an individual mouse. Bar indicates mean colitis score for each group. In the groups treated with isotype control mAb, IL-4-treated animals had significantly higher colitis scores than saline-treated mice (p < 0.0001). Among mice treated with IL-4, those cotreated with anti-IL-12 mAb had significantly lower colitis scores (p < 0.001). Mice cotreated with IL-4 and IL-10 had significantly lower colitis scores than IL-4/isotype control mAb-treated controls (p < 0.0001). Data are pooled from three separate experiments.
To confirm that IL-4 was acting by exacerbating a Th1 response, the ability of IL-10 to ameliorate the effects of IL-4 was also tested. Daily IL-10 cotreatment completely suppressed the development of colitis in the presence of IL-4 (Figs 2C and 4). These data suggest that the colitis seen in IL-4-treated recipients was dependent on the production of IL-12 and was inhibited by the presence of exogenous IL-10, and thus fits the paradigm of a Th1-type inflammatory disease.

IL-4 need not act directly on T cells to augment a Th1-type inflammatory response

The receptor for IL-4 consists of the IL-4R α-chain and IL-2R γ-chain and is expressed on many cell types, including T cells, B cells, monocytes, and nonhemopoietic cells, including intestinal epithelial cells (44–47). Therefore, IL-4 treatment in vivo may accelerate colitis by acting on T cells, microenvironmental cells, or both. To differentiate the effects of exogenous IL-4 on the Rag2−/− host cells vs donor T cells, we made use of T cells from mice that are deficient in the IL-4R α-chain (IL-4Rα−/−). CD4+CD45RBhigh T cells from IL-4Rα−/− BALB/c mice were transferred into Rag2−/− BALB/c mice, and the mice were treated for 28 days with either IL-4 or saline. As shown in Fig. 5, saline-treated recipients of IL-4Rα−/− T cells developed mild-moderate colitis (mean score, 4.8) within 4 wk. Of the IL-4-treated recipients, 9 of 13 mice had moderate to severe disease, even though the transferred T cells could not respond to IL-4 (Fig. 5). However, because of the large variability in disease score within each group, the results were not statistically significant. Nevertheless, the trend toward exacerbated disease following IL-4 treatment suggests a proinflammatory effect on the microenvironmental cells that express functional IL-4R.

To test whether IL-4 treatment could induce an inflammatory response in the absence of T cells, nonreconstituted Rag2−/− mice were treated daily with IL-4 or saline control for 21–28 days. As shown in Table I, 9 of 10 nonreconstituted Rag2−/− mice treated with IL-4 remained disease free, and only one mouse had very mild colitis. Histological analysis failed to show obvious increases in mononuclear or polymuclear infiltrates in the colons of IL-4-treated Rag2−/− mice (Fig. 2, E and F). To determine whether there were changes in macrophage or dendritic cell populations, immunohistochemical analyses of frozen sections from colon tissue were performed. No difference in the expression of F4/80, CD11b, CD11c, or CD4 was found in colons from IL-4-treated nonreconstituted Rag2−/− mice compared with saline-treated controls (data not shown). However, there was a consistent increase in MHC class II expression in the lamina propria (LP) of IL-4-treated nonreconstituted Rag2−/− mice (Fig. 6). Furthermore, cotreatment of Rag2−/− with IL-10 suppressed this increase in MHC class II expression in the LP by IL-4 (Fig. 6). Thus, IL-4 treatment may promote colitis in the presence of T cells by increasing Ag presentation by resident macrophages and/or dendritic cells. Treatment of nonreconstituted Rag2−/− mice with IL-4 did cause a 3-fold increase in spleen size (Table I) due to extramural inflammatory disease. The inability of IL-4 to inhibit a T cell transfer model of colitis has been previously described by others (48). The splenomegaly was not significantly altered by cotreatment with IL-10. As IL-10 effectively blocked the exacerbation of colitis and the increased MHC class II expression in the LP, the development of extramural inflammatory disease and splenomegaly appeared to be a distinct effect of IL-4 treatment. These data show that IL-4 can have effects on hemopoietic cells in the absence of T or B cells, but the development of colitis is strictly T cell dependent in this model.

Discussion

Our results clearly show that IL-4, which is typically associated with Th2-type responses, exacerbated an intestinal Th1-type inflammatory disease. The inability of IL-4 to inhibit a T cell transfer model of colitis has been previously described, but our data extend these results to show that IL-4 treatment significantly accelerates the onset and the severity of the colitis seen in Rag2−/− recipients of either WT or IL-10−/− CD4+CD45RBhigh T cells (37). Cotreatment with either IL-10 or anti-IL-12 mAb greatly diminished the effects of IL-4 on the development of colitis. Both anti-IL-12 mAb and IL-10 treatments have been shown to prevent colitis in several models of Th1-type colitis, including IL-10−/− mice and the transfer of WT or IL-10−/− CD4+CD45RBhigh T cells to immunodeficient mice (29, 36, 37, 49). Our observation that IL-4 can accelerate an IL-12-dependent model of colitis is distinct from Th2-type models of colitis, which are strictly dependent on the presence of IL-4. For example, BALB/c mice treated intrarectally with TNBS and SJL/J mice treated intrarectally with oxazolone will develop intestinal inflammation that is ameliorated by treatment with anti-IL-4 mAb, but not by anti-IL-12 mAb (50, 51).

The conclusion that IL-4 treatment promoted a Th1-type inflammatory response is further supported by the greatly enhanced gene expression of IFN-γ, MIG, IP-10, and CXCR3 in the colons of IL-4-treated mice. Furthermore, anti-IL-12 mAb cotreatment reduced the expression of Th1-associated genes as well as the severity of disease. MIG and IP-10 are both produced by monocytes and lymphocytes in response to IFN-γ. MIG has chemotactic activity for activated T cells, while IP-10 is a chemoattractant for activated CD4+ T cells, monocytes, and NK cells (52). CXCR3 is the receptor for both MIG and IP-10 and has been shown to be

![Image](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/byguestonNovember13,2017)
expressed on Th1-type CD4$^{+}$ T cells, but not Th2-type cells (41). In addition, of the strictly Th2-associated molecules examined, only the expression of IL-4 was increased. The increase in expression of CCR8 could be due to an influx of Th2-type T cells or monocytes into the colon (42, 43). Because the expressions of CCR4 and CCR3, which are specific to Th2 cells, were not also up-regulated, we favor the possibility that the increased expression of CCR8 was by monocytes. Together, these data indicate that the exacerbation of colitis by IL-4 treatment is due to an influx of Th1-type T cells and, potentially, monocytes.

The ability of IL-4 to exacerbate a T cell transfer model of colitis is contrary to the observations by others that IL-4 gene transfer significantly ameliorated inflammation in a rat model of TNBS-induced colitis (34). Possible explanations for the disparity in the results with IL-4 treatment include differences in the animals used (rats vs mice), induction of disease (chemical vs T cell), and method of administration of IL-4 (adenoviral vector vs daily injections of protein). Another critical difference may be the dose of IL-4 received. For example, we observed that saline-treated recipients of IL-4R$^{a-/-}$ T cells developed more severe colitis than saline-treated recipients of either WT or IL-10$^{-/-}$ T cells (compare Figs. 1 and 5). This observation could be explained by the hypothesis that low amounts of endogenous IL-4 are inhibitory to the development of Th1-type inflammatory responses, but that higher amounts of IL-4 augment those same responses. An alternative explanation is the strain differences between the IL-4R$^{a-/-}$ donor mice (BALB/c) and the WT and IL-10$^{-/-}$ donor mice (129 SvEv). Therefore, IL-4 may have either suppressive or proinflammatory effects, depending on the dose, genetic background, and cell types present at the site of inflammation.

The proinflammatory effects of IL-4 on Th1 responses in vivo appear to contradict considerable evidence that IL-4 inhibits such responses in vitro. IL-4 is known to enhance T cell proliferation, to drive the differentiation of naive CD4$^{+}$ T cells toward a Th2 phenotype, and to suppress the production of IFN-γ by CD4$^{+}$ T cells (46). IL-4 also has been shown to inhibit IFN-γ-induced responses by macrophages and their production of proinflammatory molecules, such as IL-1α, IL-5, IL-12, GM-CSF, and TNF-α (45, 46). However, under certain in vitro conditions, IL-4 has been shown to possess proinflammatory activities on APC. Monocytes that have been pretreated with IL-4 for an extended period actually have enhanced, rather than suppressed, IL-12 production in response to LPS or Staphylococcus aureus (53). IL-4 also has been shown to act synergistically with IL-12 in vitro to stimulate IFN-γ secretion by splenic dendritic cells (54). Furthermore, IL-4 inhibits IL-10 production by monocytes and up-regulates MHC class II, B7.1, and B7.2 expression on APC (45, 46).

The results of our studies in vivo support observations that IL-4 can have proinflammatory effects on accessory cells. For example, IL-4 treatment was able to moderately exacerbate colitis when the T cells could not respond directly to IL-4. In parallel studies, we observed an increase in MHC class II expression on cells in the colonic LP of IL-4-treated nonreconstituted Rag2$^{-/-}$ mice, which is in agreement with the ability of IL-4 to up-regulate MHC class II expression on monocytes in vitro (46). In addition, IL-10 was able to block the IL-4-induced increased MHC class II expression in the LP and, more importantly, abrogated the ability of IL-4 to exacerbate colitis upon T cell transfer. It is also possible that IL-10 may be able to block enhanced IL-12 production by APC (45). Nevertheless, our studies with IL-4R$^{a-/-}$ T cells show that IL-4

**FIGURE 6.** IL-4 treatment increased MHC class II expression in the LP of Rag2$^{-/-}$ mice. Immunohistochemical analysis of frozen sections (8 μm) from nonreconstituted Rag2$^{-/-}$ mice treated for 21 days with saline (A), IL-4 (B), or IL-4 plus IL-10 (C). All images are ×200. Sections were stained with anti-I-A$^{b}$ mAb (red) and counterstained with hematoxylin (blue). D, Colon section from saline-treated mouse stained with isotype control mAb.
may have some direct proinflammatory effect on Th1 cells. It is also likely that IL-4 has proinflammatory effects on nonhemopoietic cells. Colonic epithelial cells express IL-4R and can increase ICAM-1 expression in response to IL-4, and thus may contribute to the exacerbation of disease in this model (47, 55).

While there is much evidence to support the role of IL-4 in allergic Th2-type responses, the role of IL-4 in the development of an IL-12-dependent, IFN-γ-producing immune response in vivo needs to be more fully elucidated. In particular, which microenvironmental conditions cause IL-4 to favor a Th2 vs Th1 inflammatory response remains to be determined. By developing a better understanding of the interaction of Th1-associated and Th2-associated cytokines, more effective therapeutics for chronic inflammatory diseases can be designed.

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