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IL-12, But Not IFN-γ, Plays a Major Role in Sustaining the Chronic Phase of Colitis in IL-10-Deficient Mice[1]

Natalie J. Davidson,[2]* Susan A. Hudak,* Robin E. Lesley,* Satish Menon,* Michael W. Leach,† and Donna M. Rennick*

IL-10-deficient (IL-10$^{-/-}$) mice develop chronic enterocolitis mediated by CD4$^+$ Th1 cells producing IFN-γ. Because IL-12 can promote Th1 development and IFN-γ production, the ability of neutralizing anti-IL-12 mAb to modulate colitis in IL-10$^{-/-}$ mice was investigated. Anti-IL-12 mAb treatment completely prevented disease development in young IL-10$^{-/-}$ mice. Treatment of adult mice resulted in significant amelioration of established disease accompanied by reduced numbers of mesenteric lymph node and colonic CD4$^+$ T cells and of mesenteric lymph node T cells spontaneously producing IFN-γ. In contrast, anti-IFN-γ mAb had minimal effect on disease reversal, despite a significant preventative effect in young mice. These findings suggested that IL-12 sustains colitis by supporting the expansion of differentiated Th1 cells that mediate disease independently of their IFN-γ production. This conclusion was supported by the finding that anti-IL-12 mAb greatly diminished the ability of a limited number of CD4$^+$ T cells expressing high levels of CD45RB from diseased IL-10$^{-/-}$ mice to expand and cause colitis in recombination-activating gene-2$^{-/-}$ recipients, while anti-IFN-γ mAb had no effect. Furthermore, IL-12 could support pathogenic IL-10$^{-/-}$ T cells stimulated in vitro in the absence of IL-2. While these studies show that IL-12 plays an important role in sustaining activated Th1 cells during the chronic phase of disease, the inability of anti-IL-12 mAb to abolish established colitis or completely prevent disease transfer by Th1 cells suggests that additional factors contribute to disease maintenance. ***The Journal of Immunology,*** **1998, 161: 3143–3149.**

Inflammatory bowel diseases (IBD)[3] in humans, categorized as Crohn's disease or ulcerative colitis, are complex disorders of unknown etiology (1). Numerous mouse models of experimentally induced or spontaneously occurring colitis are now available with which to examine the pathogenesis of IBD (2–5). A pathogenic role for CD4$^+$ T cells has been demonstrated in many of these models, including IL-10$^{-/-}$ (6, 7), IL-2$^{-/-}$ (8), and TCRα$^{-/-}$ (9) mice; a trinitrobenzene sulfonic acid (TNBS)-induced colitis model (10); the SCID mouse CD4$^+$ (CD45RB^{high}) T cell transfer model (11, 12); and mice expressing a human CD3ε transgene (13). Furthermore, IFN-γ production appears to be a hallmark of CD4$^+$ T cells involved in intestinal inflammation (6, 14–17). IFN-γ may function in numerous ways to affect inflammation, such as augmenting the production of proinflammatory mediators by activated Mϕ, influencing cell trafficking by regulating adhesion molecule expression, and altering the permeability of the intestinal epithelial barrier (18). The potential importance of this cytokine in contributing to pathogenesis is further suggested by the finding that neutralization of IFN-γ using mAb will significantly attenuate colitis development in both young IL-10$^{-/-}$ mice (3, 7) and SCID mice reconstituted with naive CD4$^+$CD45RB^{high} T cells (19).

IL-12 has been shown to drive Th1 differentiation and IFN-γ production (20, 21). In Crohn's disease patients, the expression and release of IL-12 by lamina propria mononuclear cells combined with an increased production of IFN-γ by gut lymphocytes imply that this form of IBD involves a Th1 response (1, 22, 23). A direct role for IL-12 in promoting T cell-mediated colonic inflammation has been investigated in two different models of colitis. Neutralizing anti-IL-12 mAb abrogated TNBS-induced colitis (14, 24) and prevented immunization-induced colitis in IL-2$^{-/-}$ mice (25). Moreover, anti-IL-12 mAb treatment significantly reduced IFN-γ production by colonic lamina propria T cells. Taken together, such results suggest that both IL-12 and IFN-γ may play pivotal roles in the pathogenesis of TNBS- and immunization-induced inflammation.

In the present study we have assessed the role that IL-12 may play in the chronic, nonresolving colitis that develops spontaneously in IL-10$^{-/-}$ mice. In contrast to TNBS-induced colitis (14), the intestinal lesions in IL-10$^{-/-}$ mice are discontinuous and affect primarily the mucosa and submucosa, although transmural lesions are occasionally present (2, 3). While our studies have formally demonstrated that IFN-γ-producing CD4$^+$ T cells mediate colitis in IL-10$^{-/-}$ mice, these studies have also demonstrated that IFN-γ itself contributes to the induction phase of the disease in neonates but is not necessarily required for sustaining the chronic phase of the disease in adult mice (3, 6, 7). As one of the major functions of IL-12 is to optimize IFN-γ production by Th1 cells, we have examined the ability of neutralizing anti-IL-12 mAb to modulate colitis in IL-10$^{-/-}$ mice, with special emphasis on established disease that appears to be IFN-γ independent.

Materials and Methods

Mice

IL-10$^{-/-}$ mice generated on a 129-Ola background and backcrossed onto a 129/SvEv background were exclusively used in this study. These mice

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[3] Abbreviations used in this paper: IBD, inflammatory bowel disease; IL-10$^{-/-}$, IL-10-deficient; TNBS, trinitrobenzene sulfonic acid; Mϕ, macrophage; wt, wild type; RAG-2$^{-/-}$, recombinase-activating gene-2-deficient; ELISPOT, enzyme-linked immunospot assay; MLN, mesenteric lymph node.

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were derived by cesarean section under specific pathogen-free conditions at Simonsen Laboratories (Gilroy, CA) and were maintained in microisolator cages in the Animal Care Facility of the DNAX Research Institute (Palo Alto, CA). Wild-type (wt) 129/SvEv and immunodeficient 129/SvEv RAG-2$^{-/-}$ mice (2–4 mo old) were purchased from Taconic Farms (Germantown, NY).

Induction of colitis in immunodeficient RAG-2$^{-/-}$ mice by adoptive transfer of IL-10$^{-/-}$ CD4$^+$CD45RB^{high} T cells

IL-10$^{-/-}$ CD4$^+$ splenic T cells were enriched by red cell lysis and magnetic bead depletion using lineage-specific mAb supernatants (10%, v/v; B220 (B cells), 8C5 (neutrophils), Mac-1 (Mϕ), and Ter119 (erythrocytes)) and purified anti-CD8 (10 μg/ml; PharMingen, San Diego, CA). mAb-stained cells were removed in a magnetic field using goat anti-rat IgG (Fc)- and anti-rat IgG (H+L)-coated magnetic beads (PerSeptive Diagnostics, Cambridge, MA). The remaining cells were stained with CD4-phycoerythrin (5 μg/ml; Caltag Laboratories, South San Francisco, CA) and CD45RB-FITC (5 μg/ml; PharMingen) for cell sorting. Two-color cell sorting was performed using a FACStar Plus (Becton Dickinson, Mountain View, CA); the sorted populations were >98% pure upon reanalysis. For colitis induction, 5×10^4 sorted CD4$^+$CD45RB^{high} splenic T cells were injected i.p. into RAG-2$^{-/-}$ recipients. Mice were sacrificed and analyzed for bowel inflammation 7 to 8 wk after cell transfer.

Sorted CD4$^+$CD45RB^{high} splenic T cells were also cultured before injection into RAG-2$^{-/-}$ recipients. Cells were cultured on anti-CD3-coated (10 μg/ml, 4 h) 24-well plates in RPMI (JRH, Lenexa, KS) supplemented with 10% FBS (HyClone, Logan, UT), 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM HEPES buffer, and 5×10^{-5} M 2-ME. Cultures were supplemented with cytokines or mAb as follows: 10 ng/ml IL-12 (R&D Systems, Minneapolis, MN), 500 U/ml IL-2 (S. Zurawski, DNAX), 50 μg/ml anti-IL-2 mAb (clone 1A12, DNAX), and 100 μg/ml anti-IL-12 (clone C17.8.20; see below). Three different culture conditions were employed: IL-2 plus IL-12, IL-2 plus anti-IL-12, and anti-IL-2 plus IL-12. After 16 days of culture, the progeny of 5×10^4 cells cultured/well (the number of cells injected into RAG-2$^{-/-}$ mice immediately after sorting) were retrieved, counted, and injected i.p. into a single RAG-2$^{-/-}$ mouse (five mice per group for each culture condition). Hence, RAG-2$^{-/-}$ recipients of T cells cultured in IL-2 plus IL-12 received 22×10^6 cells, recipients of cells cultured in IL-2 plus anti-IL-12 mAb received 19×10^5 cells, and mice injected with cells cultured in anti-IL-2 mAb plus IL-12 received 26×10^5 cells. The experiment was performed in this manner because the primary objective was to determine whether any pathogenic T cells remained after culture. Furthermore, our previous studies have suggested that a 100-fold increase in cell number over that which will cause colitis in RAG-2$^{-/-}$ recipients does not result in disease of significantly greater severity (6) (see also Table V).

In vivo treatment with mAb and IL-10

All mAb and cytokines were delivered by i.p. injection. The anti-IL-12 hydridoma, C17.8.20 (26), was provided by Dr. G. Trinchieri (The Wistar Institute, Philadelphia, PA), and the mAb was purified at Harlan Bioproducts for Science (Madison, WI). Purified rat anti-mouse IFN-γ (XMG1.2) was provided by Dr. J. Abrams (DNAX). Adult IL-10$^{-/-}$ mice (≥3 mo old) were treated for 8 wk with 2 mg of mAb/wk. Ten-day-old IL-10$^{-/-}$ mice were initially injected with 0.5 mg of mAb, 1 mg the following week, and then 2 mg/wk for 6 wk. RAG-2$^{-/-}$ recipients of IL-10$^{-/-}$ T cells were treated with 2 mg mAb/wk for 6 wk beginning at the time of cell transfer. An isotype-matched, control mAb was administered in an identical manner. Recombinant mouse IL-10 (DNAX) (3) was administered on a daily basis at 40 μg/mouse in 100 μl of HBSS for 6 wk. HBSS (100 μl) alone served as a control.

Histology and immunohistochemistry

Histologic examination of mouse large intestine was performed in a blinded fashion by the same pathologist (M. W. Leach) using formalin-fixed tissue sections stained with hematoxylin and eosin as previously described (3, 6). As the lesions in IL-10$^{-/-}$ mice can be multifocal, longitudinal sections of the entire length of the colon were evaluated, taking into account both the number of lesions and their severity (3). Each region of the colon (cecum, ascending, transverse and descending colon, and rectum) was graded semiquantitatively as 0 (no change) to 5 (most severe change). The grading represents an increasing degree of inflammation and epithelial cell hyperplasia. Moderate to severe cases exhibited an increasing incidence of transmural inflammation, goblet cell loss, crypt abscesses and ulceration, and severe colitis-incorporated lamina propria fibrosis. The summation of the score for each segment of the colon provides a total disease score per mouse (from 0–25), where 0 indicates no change, >0 to 5 indicates mild disease, >5 to 10 indicates mild to moderate disease, >10 to 20 indicates moderate to severe disease, and >20 to 25 indicates severe colitis (data are presented as the mean ± SD).

The frequency of colonic CD4$^+$ T cells (mean number of cells per high power field, ×400) and MHC class II expression (as determined by the mAb MTS6) by colon epithelial cells were assessed by indirect immunofluorescence of frozen tissue sections in conjunction with an anticytokeratin reagent to detect epithelial cells as previously described (3).

Detection of IFN-γ by ELISPOT or ELISA

The ELISPOT, to detect spontaneous IFN-γ production by freshly isolated cells, was based on the method of Rönnelid and Klareskog (27). Briefly, MLN cells were plated at 5×10^5 cells/well on Immulon II plastic plates (Dynatech, Chantilly, VA) coated overnight with 10 μg/ml anti-IFN-γ mAb (clone R46A2, DNAX). IFN-γ production was detected using 1 μg/ml biotinylated anti-IFN-γ (clone XMG1.2, PharMingen), followed by streptavidin alkaline phosphatase and a 5-bromo-4-chloro-3-indolyl-phosphate substrate (Promega, Madison, WI). All the IFN-γ spots in each well were counted using a microscope.

IFN-γ production by CD3-stimulated T cells was assessed as follows. MLN cells were plated at 5×10^6 in CD3-coated (10 μg/ml, 4 h) 24-well plates in 1 ml of RPMI (JRH) supplemented with 10% FBS (HyClone) and containing 5 μg/ml anti-CD28 (PharMingen). The cells were incubated in a humidified chamber (37°C, 5% CO_2) for 48 h, at which time culture supernatants were collected, and IFN-γ production was determined by specific ELISA as described previously (28).

T cell proliferation assay

Sorted CD4$^+$CD45RB^{high} spleen T cells were plated in CD3-coated (10 μg/ml, 4 h) round-bottom 96-well plates at 2×10^4/well in a total of 100 μl RPMI (JRH) supplemented with 10% FBS (HyClone). Additions included 10 ng/ml IL-12, 500 U/ml IL-2, 10 ng/ml IFN-γ (DNAX), 50 μg/ml anti-IL-2 mAb, 100 μg/ml anti-IL-12 mAb, and 100 μg/ml anti-IFN-γ mAb. Cells were incubated for 3, 6, 9, or 12 days in a humidified chamber (37°C, 5% CO_2). [^{3}H]TdR (Amersham, Arlington Heights, IL) was added at a final concentration of 1 μCi/well for the last 12 h of incubation, and the cells were harvested onto glass filters. The level of [^{3}H]TdR incorporation was measured using a Wallac 1205 Betaplate scintillation counter (Pharmacia, Piscataway, NJ).

Results

Anti-IL-12 mAb treatment of neonatal and diseased, adult IL-10$^{-/-}$ mice

As one of the functions of IL-12 is to drive Th1 differentiation (20, 21), we examined the effects of blocking IL-12 in the IL-10$^{-/-}$ mouse model of colitis. Ten-day-old IL-10$^{-/-}$ mice, which are disease free (3), were treated with anti-IL-12 mAb for 8 wk. Colitis was completely prevented in all but one mouse (Table I). Mucosal inflammation and epithelial cell hyperplasia were absent in anti-IL-12 mAb-treated mice (Fig. 1*A*); the tissue was indistinguishable from that of normal mice (data not shown). In contrast, mice treated with control mAb showed mild to moderate disease (Table I) involving multifocal to coalescing inflammation with some epithelial hyperplasia and crypt elongation with depletion of mucous in goblet cells (Fig. 1*B*).

When adult IL-10$^{-/-}$ mice with established colitis (>3 mo old) were treated with anti-IL-12 mAb, the disease was significantly ameliorated, but was not reversed (Table I). The inflammation and epithelial cell hyperplasia present in the anti-IL-12 mAb-treated mice (Fig. 1*C*) were qualitatively similar to those in the control mAb-treated group, but were less severe. The moderate to severe disease in adult mice receiving control mAb resulted in prominent epithelial cell hyperplasia, depletion of mucous in goblet cells, crypt loss, ulceration, and transmural inflammation (Fig. 1*D*). Anti-IL-12 mAb also reduced blood granulocyte numbers by 65% in adult mice, an indication of reduced systemic inflammation. In contrast, treatment of adult IL-10$^{-/-}$ mice with neutralizing mAb to IFN-γ resulted in a small reduction in colitis severity but not

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Table I. *Effects of anti-IL-12 or anti-IFNγ mAb treatment on the incidence and severity of colitis in IL-10$^{-/-}$ mice*

mAb Treatment of IL-10$^{-/-}$ Mice[a]	Number of Mice Affected	Disease Score Distribution (0–25)[b]					Mean Disease Score (0–25)[b]
		0	>0–5	>5–10	>10–20	>20–25	
Ten-day-old mice							
Anti-IL-12	1/16 (6%)	15	1	0	0	0	1.0
Control mAb	19/19 (100%)	0	1	16	2	0	8.6 ± 1.1
Three-month-old adult mice							
Anti-IL-12	23/25 (92%)	2	20	2	1	0	3.3 ± 2.5*
Anti-IFN-γ	16/16 (100%)	0	0	9	7	0	9.3 ± 2.7*
Control mAb	22/22 (100%)	0	0	6	16	0	11.7 ± 2.1

[a] Mice treated with 2 mg mAb i.p. per week for 8 wk.
[b] Mean severity of colonic lesions in affected mice; refer to *Materials and Methods* for a description of histologic analysis. Data incorporates two to three independent experiments for each age group.
* Denotes a significant difference to corresponding control mAb score (assessed by using an unpaired Student's *t* test).

incidence (Table I), suggesting that anti-IL-12 mAb treatment does not ameliorate colitis by reducing IFN-γ production.

Effects of combined IL-10 and anti-IL-12 mAb treatment on established disease in IL-10$^{-/-}$ mice

To determine whether the absence of endogenous IL-10 production hampered the ability of anti-IL-12 mAb to completely reverse established colitis in adult IL-10$^{-/-}$ mice we combined daily administration of 40 μg/mouse recombinant mouse IL-10 with weekly doses of anti-IL-12 mAb. Consistent with our previous findings, treatment of adult IL-10$^{-/-}$ mice with IL-10 resulted in an amelioration of colitis; however, the lowered mean disease score was not statistically significant (Table II). Again, anti-IL-12 mAb treatment significantly ameliorated colitis, but this result was not improved by coadministration of IL-10. In fact, the mean disease score of these mice was higher than that of anti-IL-12 mAb-treated mice. One explanation is that this randomized group of mice may have contained a greater proportion of animals with more advanced disease. Although the cause is unknown, we nevertheless concluded that the inability of neutralizing IL-12 to completely reverse colitis was not simply due to the absence of endogenously produced IL-10.

Effects of anti-IL-12 mAb treatment on T cells in adult IL-10$^{-/-}$ mice

To investigate the mechanism by which neutralizing IL-12 ameliorated colitis in adult IL-10$^{-/-}$ mice, we assessed T cell numbers in the spleen, MLN, and colon as well as IFN-γ production by

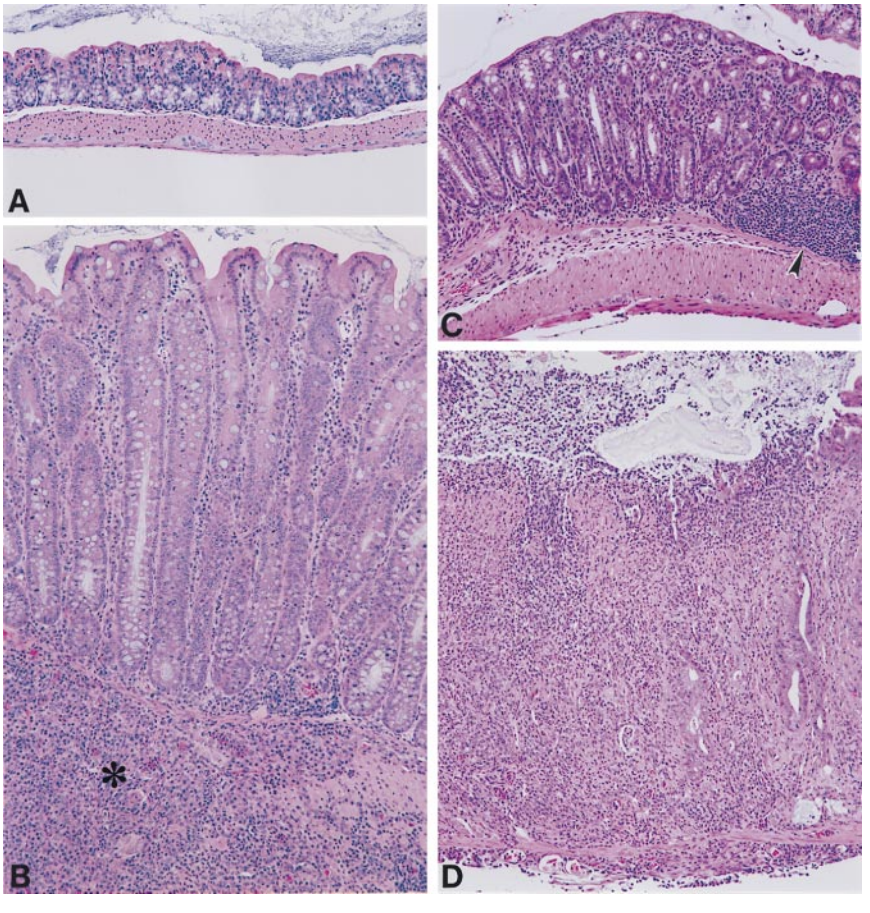

FIGURE 1. Photomicrographs of the proximal colon of IL-10$^{-/-}$ mice treated with anti-IL-12 or control mAb. The colon of a 10-day-old IL-10$^{-/-}$ mouse treated for 8 wk with anti-IL-12 (*A*) or control mAb (*B*) is shown. The former shows no inflammation or epithelial cell hyperplasia, in contrast to the latter (* designates prominent submucosal inflammation). Treatment of adult IL-10$^{-/-}$ mice with anti-IL-12 (*C*) reduces colitis to mild inflammation and epithelial hyperplasia (arrowhead indicates normal gut-associated lymphoid tissue), whereas control mAb-treated mice (*D*) show severe colitis with transmural inflammation, ulceration, and loss of intestinal glands. This change was among the most severe observed in this group. All photomicrographs are at the same magnification (×100).

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Table II. *Effects of IL-10 plus anti-IL-12 mAb treatment on colitis in adult IL-10$^{-/-}$ mice*

Treatment[a]	Mean Disease Score (0–25)[b]
Control mAb	8.6 ± 2.2
IL-10	5.0 ± 4.0
Anti-IL-12	2.3 ± 2.9*
IL-10 plus anti-IL-12	5.0 ± 3.7

[a] Mice (six to eight per group) were treated with 40 μg/day IL-10 and/or 2 mg mAb/wk i.p. for 6 wk.
[b] Mean severity of colonic lesions; refer to *Materials and Methods* for a description of histologic analysis.
* Denotes a significant difference to control mAb score (assessed by using an unpaired Student's *t* test).

MLN T cells. While anti-IL-12 mAb treatment had little effect on the number of CD4$^+$ T cells in the spleen in adult IL-10$^{-/-}$ mice, the number of CD4$^+$ T cells in both the MLN and colon was reduced to half that in control mAb-treated mice (Table III). Treatment with anti-IFN-γ mAb similarly reduced MLN, but not colonic or splenic, CD4$^+$ T cell numbers. Although both mAb treatments significantly reduced MLN T cell numbers, this number was still much greater than that in wt mice (Table III), consistent with residual disease. Interestingly, the failure of anti-IFN-γ mAb treatment to reduce the frequency of colonic CD4$^+$ T cells (Table III) correlated with its inability to significantly reverse established disease (Table I). Combined, these results imply that intestinal disease is driven by the presence of pathogenic T cells in the colon, which, in turn, is apparently IL-12 dependent.

Using ELISPOT, we found that 100-fold fewer MLN cells were spontaneously producing IFN-γ following anti-IL-12 treatment (Table III). Aberrant MHC class II expression by colonic epithelial cells is known to be induced by IFN-γ (1–3). The lack of epithelial MHC II expression within the colon of anti-IFN-γ-treated IL-10$^{-/-}$ mice confirmed that most, if not all, IFN-γ was being neutralized (Table III) even though these mice had colitis (Table I). While it cannot be absolutely stated that our mAb treatments completely neutralized their target cytokines, the lack of epithelial MHC class II expression in the colon of mice treated with anti-IL-12 mAb combined with the markedly reduced frequency of MLN cells spontaneously producing IFN-γ provide a good indication that IL-12 was effectively neutralized. However, the in vitro stimulation of T cells from anti-IL-12 or control mAb-treated mice resulted in comparable levels of IFN-γ, demonstrating that anti-IL-12 treatment had not caused a selective loss of Th1 cells with the potential to produce IFN-γ.

Effect of neutralizing anti-IL-12 mAb on the establishment of colitis in RAG-2$^{-/-}$ mice transplanted with T cells from diseased IL-10$^{-/-}$ mice

We have previously demonstrated that IL-10$^{-/-}$ T cells will transfer colitis into immunodeficient RAG-2$^{-/-}$ mice, resulting in an inflammation that is more diffuse but qualitatively similar to that in IL-10$^{-/-}$ mice (6, 7). In this model, CD4$^+$CD45RB^{high} T cells (5×10^4) from diseased adult IL-10$^{-/-}$ mice can be transferred into a lymphocyte-deficient host, facilitating assessment of the ability of anti-IL-12 to prevent the establishment of colitis by a fixed number of pathogenic T cells. Within 6 wk all RAG-2$^{-/-}$ recipients of IL-10$^{-/-}$ T cells developed a moderate to severe colitis (Table IV). While anti-IL-12 mAb treatment of such mice significantly controlled the establishment of colitis, 50% of treated mice still showed mild to moderate disease. When spleen and MLN T cell numbers were examined in transplanted RAG-2$^{-/-}$ mice, it was clear that blocking IL-12 significantly inhibited the ability of T cells to expand within the host; the numbers were 8-fold fewer in the MLN and 2.5-fold fewer in the spleen (Table IV). These data imply that IL-12 plays an important role in the expansion of pathogenic T cells, and that the degree of this expansion is directly related to the incidence and severity of disease.

IFN-γ-induced MHC class II expression by colon epithelial cells was also assessed in RAG-2$^{-/-}$ recipients of IL-10$^{-/-}$ CD4$^+$CD45RB^{high} T cells. Even though anti-IL-12 mAb-treated recipients had residual disease, the aberrant expression of MHC II by colonic epithelial cells was completely inhibited (Table IV), further indicating that colitis can be sustained by fully differentiated Th1 cells independently of IFN-γ. This hypothesis was supported by the finding that neutralizing anti-IFN-γ mAb had no significant effect on the disease score or the incidence of colitis induced in RAG-2$^{-/-}$ mice by IL-10$^{-/-}$ CD4$^+$CD45RB^{high} T cells compared with those in control mAb-treated mice. Hence, we conclude that the primary role of IL-12 in the IL-10$^{-/-}$ mouse model of colitis is not to induce IFN-γ production by pathogenic Th1 cells, but, rather, to promote their proliferation.

IL-12 alone sustains pathogenic T cells in vitro

Given the apparent ability of IL-12 to effect the expansion of pathogenic T cells in vivo, a direct effect of IL-12 on the expansion of T cells was investigated in a proliferation assay using plate-bound CD3 stimulation of purified IL-10$^{-/-}$ CD4$^+$CD45RB^{high} T cells. We assessed T cell proliferation at 3, 6, 9, and 12 days and found that the response to a specific set of cytokines and/or mAb showed the same trend at all time points tested. Representative data for day 6 are presented in Figure 2. IL-2 supported modest

Table III. *Effects of anti-IL-12 and anti-IFN-γ mAb treatment on T cells in adult IL-10$^{-/-}$ mice*

mAb Treatment[a]	Number of CD4$^+$ T Cells: Spleen (×10^{-6})	Number of CD4$^+$ T Cells: MLN (×10^{-6})	Frequency of Colonic CD4$^+$ T Cells[b]	Number of Cells Spontaneously Producing IFN-γ per 5 × 10^5 MLN Cells[c]	IFN-γ Production per 2 × 10^5 CD4$^+$ MLN T Cells (ng/ml)[d]	MHC II Expression on Colonic Epithelial Cells[e]
Anti-IL-12	8.6 ± 3.9	5.1 ± 4.8*	35 ± 7*	0.4 ± 1.4*	1.1 ± 0.4	−
Anti-IFN-γ	5.6 ± 2.9	5.6 ± 2.8*	61 ± 15	ND	ND	−
Control mAb	7.5 ± 3.1	10.5 ± 5.9	61 ± 16	42 ± 22	1.0 ± 0.3	+
WT[f]	3.9 ± 0.6	0.27 ± 0.03	22 ± 6	ND	ND	−

[a] Mice treated with 2 mg mAb per week for 8 wk.
[b] Mean number of colonic CD4$^+$ T cells per 10 to 15 high power-fields (×400) assessed by immunohistochemistry.
[c] Spontaneous IFN-γ production was assayed by ELISPOT.
[d] IFN-γ production was assayed by ELISA following *in vitro* stimulation and correlated to the absolute number of CD4$^+$ T cells in each culture.
[e] MHC class II expression by colonic epithelial cells was assessed by immunohistochemistry.
[f] Values for unmanipulated 129/SvEv WT mice ($n = 6$).
* Denotes a significant difference to corresponding control mAb score (assessed by using an unpaired Student's *t* test).

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Table IV. *Effect of anti-IL-12 or anti-IFNγ mAb treatment on the incidence of colitis in RAG-2$^{-/-}$ recipients of adult IL-10$^{-/-}$ spleen CD4$^+$CD45RB^{high} T cells*

mAb Treatment of Rag-2$^{-/-}$ Recipients of IL-10$^{-/-}$ T Cells[a]	Number of Mice Affected	Disease Score Distribution (0–25)[b]					Mean Disease Score (0–25)[b]	Number of CD4$^+$ T cells		MHC II Expression on Colonic Epithelial Cells[c]
		0	> 0–5	> 5–10	> 10–20	> 20–25		spleen ($\times 10^{-6}$)	MLN ($\times 10^{-6}$)	
Anti-IL-12	9/18 (50%)	9	4	4	1	0	6.1 ± 3.8*	0.6 ± 0.3*	0.05 ± 0.03*	−
Anti-IFN-γ	8/8 (100%)	0	1	4	3	0	9.6 ± 3.0	ND	ND	−
Control mAb	15/15 (100%)	0	1	4	11	0	11.9 ± 3.1	1.6 ± 0.7	0.40 ± 0.21	+

[a] A total of 5×10^4 IL-10$^{-/-}$ CD4$^+$CD45RB^{high} T cells were transferred per RAG-2$^{-/-}$ recipient mouse, 2 mg mAb administered i.p. on day 0 (coincident with cell transfer) and weekly for 6 wk.

[b] Mean severity of colonic lesions in affected mice assessed at 7 wk post-cell transfer; refer to *Materials and Methods* for a description of histologic analysis. Data incorporates two to three independent experiments.

[c] MHC class II expression by colonic epithelial cells assessed by immunohistochemistry.

* Denotes a significant difference to corresponding control mAb score (assessed by using an unpaired Student's *t* test).

proliferation of IL-10$^{-/-}$ T cells, which was dramatically increased by the addition of IL-12, but was completely unaffected by IFN-γ. Interestingly, IL-12 supported T cell proliferation even in the presence of neutralizing anti-IL-2 mAb. Hence, IL-12 directly influenced the proliferation of CD4$^+$CD45RB^{high} T cells. As it has been previously reported that naive murine T cells do not respond in vitro to IL-12 alone (29), and that CD45RB^{high} is not a reliable marker of naive T cells (30), we concluded that IL-10$^{-/-}$ CD4$^+$CD45RB^{high} T cells from diseased mice incorporated a significant proportion of previously activated cells.

In addition to assessing the proliferative effects of IL-12, we examined its ability to maintain the pathogenicity of IL-10$^{-/-}$ CD4$^+$CD45RB^{high} T cells in vitro. Table V indicates that the ability of IL-10$^{-/-}$ CD4$^+$CD45RB^{high} T cells to induce colitis in RAG-2$^{-/-}$ recipient mice was dependent upon the presence of IL-12 during the culture period. Pathogenic T cells could only be recovered from cultures containing IL-12, and most strikingly, these cells could be supported by IL-12 alone. Interestingly, greater numbers of cultured T cells than of freshly sorted cells were required to induce colitis in RAG-2$^{-/-}$ mice. The transfer of 5×10^4 cultured CD4$^+$CD45RB^{high} T cells was not able to induce colitis (data not shown), suggesting that the T cells were either less pathogenic after culture or that a subset of nonpathogenic T cells also expanded under these culture conditions. Nevertheless, our combined data indicate that the maintenance of pathogenic CD4$^+$CD45RB^{high} T cells is highly dependent upon IL-12.

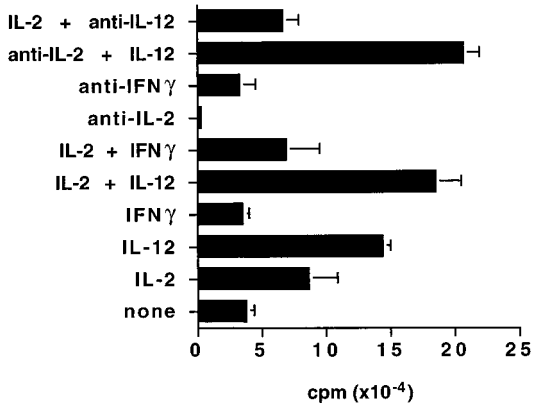

FIGURE 2. Effect of IL-12 on T cell proliferation. Sorted IL-10$^{-/-}$ CD4$^+$CD45RB^{high} T cells were cultured at 2×10^4/well on CD3-coated plates in the presence of cytokines and/or mAb as indicated. Proliferation was assessed after 6 days of culture by incorporation of [^{3}H]TdR (counts per minute). Data are the mean ± SEM of three independent experiments.

Discussion

Our previous studies have demonstrated that enterocolitis in IL-10$^{-/-}$ mice is mediated by IFN-γ-producing Th1 cells (3, 6). It is well established that IL-12 promotes both Th1 differentiation and IFN-γ production (20, 21) and that IL-12 production by activated APC is suppressed by IL-10 (31). Therefore, IL-12 was a prime candidate for inducing colitis in IL-10$^{-/-}$ mice. Herein we have shown that treatment of neonatal IL-10$^{-/-}$ mice with anti-IL-12 mAb completely prevented colitis development. The absence of any pathologic changes in the colons of these mice suggested that neutralization of IL-12 suppressed the generation of pathogenic Th1 cells from a naive population. Neutralization of IL-12 using mAb has been shown to be effective in preventing disease in two other Th1-dependent colitis models, TNBS-induced colitis (14, 24) and immunization-induced colitis in IL-2$^{-/-}$ mice (25). In both cases the beneficial effects of this treatment were ascribed to a reduction in IFN-γ production. We have previously reported that colitis in neonatal IL-10$^{-/-}$ mice can also be prevented by administration of IL-10 (3). This result was probably achieved primarily through the inhibitory activities of IL-10 on the production of IL-12 by accessory cells (31). The ability of either anti-IL-12 mAb or IL-10 to prevent the establishment of Th1-mediated inflammation was also shown in a model of TNBS-induced colitis (24). Furthermore, anti-IL-12 mAb treatment has been reported to prevent other Th1-mediated inflammatory diseases, including experimental autoimmune encephalomyelitis (32) and experimental autoimmune uveoretinitis (33). Thus, IL-12 appears to play a central role in the induction of Th1-mediated inflammation regardless of the initiating event or target organ.

Table V. *Pathogenic potential of IL-10$^{-/-}$ CD4$^+$ CD45RB^{high} T cells cultured with or without IL-12*

Culture Conditions[a]	Mean Disease Score in RAG-2$^{-/-}$ Recipients of Cultured Cells (0–25)[b]
IL-2 + IL-12	11.8 ± 1.9
IL-2 + anti-IL-12	0.5 ± 1.0
Anti-IL-2 + IL-12	7.6 ± 3.3
Freshly isolated[c]	9.0 ± 2.7

[a] Sorted T cells were cultured on CD3-coated plates for 16 days with cytokines and mAb as indicated (refer to *Materials and Methods* section for details of culture conditions).

[b] Cultured cells were injected i.p. into RAG-2$^{-/-}$ mice ($n = 5$) and the mean severity of colonic lesions assessed 8 wk post-cell transfer (refer to *Materials and Methods* section for a description of histologic analysis).

[c] Cells (5×10^4) were immediately injected i.p. into RAG-2$^{-/-}$ recipient mice ($n = 5$) following sorting.

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The primary goal of this study, however, was to examine the role of IL-12 in chronic colitis. We found that anti-IL-12 mAb treatment was able to significantly ameliorate ongoing disease in adult IL-10$^{-/-}$ mice as well as the establishment of colitis in RAG-2$^{-/-}$ recipients of pathogenic IL-10$^{-/-}$ CD4^{+}CD45RB^{high} T cells. Interestingly, this beneficial effect was not due to the ability of anti-IL-12 mAb treatment to inhibit IFN-γ production by primed Th1 cells. Our previous studies have shown that while IFN-γ played a significant role in disease induction, it was apparently not required for maintaining the chronic phase of disease in IL-10$^{-/-}$ mice (3, 7). We have extended this observation by demonstrating that the neutralization of IFN-γ had no effect on disease establishment in RAG-2$^{-/-}$ recipients of pathogenic IL-10$^{-/-}$ T cells. Our results appear to contrast with those reported by Powrie et al., who showed that anti-IFN-γ mAb treatment of SCID recipients of CD4^{+}CD45RB^{high} T cells could prevent the establishment of colitis (19). However, naive WT T cells were transferred in this model, whereas the donor CD4^{+}CD45RB^{high} T cells from diseased IL-10$^{-/-}$ mice are not exclusively naive, as suggested by their ability to proliferate in vitro to IL-12 in the absence of IL-2 (Fig. 2). Taken together, it appears that IL-12 has a more complex role in maintaining colitis than that of simply promoting IFN-γ production.

The ability of anti-IL-12 mAb treatment to ameliorate colitis in adult IL-10$^{-/-}$ mice correlated with reduced CD4^{+} T cell numbers in the MLN and colon. That this mAb treatment was directly affecting the expansion of pathogenic Th1 cells was further validated using the T cell transfer model, in which anti-IL-12 mAb treatment markedly inhibited the expansion of a limited number of pathogenic T cells. Furthermore, IL-12 supported the anti-CD3-induced proliferation of IL-10$^{-/-}$ T cells in vitro. Studies in both human and murine systems have demonstrated that IL-12 will enhance the proliferation and cytokine production of activated T cells, although the response of murine Th1 clones is reported to be dependent upon CD28-B7 costimulation and the presence of IL-2 (20, 29, 34). However, we have found that IL-12 is able to support the proliferation of pathogenic IL-10$^{-/-}$ CD4^{+}CD45RB^{high} T cells in vitro independently of IL-2 or CD28-B7 costimulation. Hence, our studies suggest that IL-12 plays a major role in the perpetuation of colitis as a growth stimulus for differentiated Th1 cells.

The ability of anti-IL-12 mAb to reverse established Th1-mediated disease has also been examined in the TNBS-induced colitis model (14). Neurath et al. (14) have reported that administration of anti-IL-12 mAb abrogated established TNBS-induced colitis. While anti-IL-12 mAb treatment greatly ameliorated colitis in IL-10$^{-/-}$ mice, disease was not completely reversed. The discrepancy between these studies most likely reflects inherent differences between the two colitis models. Although mediated by CD4^{+} T cells, TNBS-induced colitis is ultimately resolved with time (14, 35). In contrast, colitis in IL-10$^{-/-}$ mice occurs spontaneously and progresses into a chronic, nonresolving disease. It could be argued that the absence of endogenously produced IL-10 in our colitis model hampered the effectiveness of the anti-IL-12 treatment of adult mice because of the persistent inflammatory activities of Mϕ. However, the failure to reverse colitis with a combined treatment of anti-IL-12 mAb and daily administration of IL-10 does not support this hypothesis. As we have previously reported that IL-10 treatment of adult IL-10$^{-/-}$ mice will ameliorate, but not reverse, established disease (3), it would appear that neither IL-10 nor anti-IL-12 mAb treatment is able to completely regulate the activity of pathogenic Th1 cells in IL-10$^{-/-}$ mice. Studies involving TNBS-induced colitis have suggested that disease abrogation by anti-IL-12 mAb may be the result of increased production of the immunosuppressive cytokine, TGF-β, and augmented apoptosis of T cells in peripheral lymphoid tissues (10, 24, 36). While TGF-β may have some role in the effect of anti-IL-12 treatment on colitis in IL-10$^{-/-}$ mice, our in vivo and in vitro results suggest that IL-12 is a significant growth stimulus for pathogenic T cells.

Although our data suggest that IL-12 is crucial for the optimal expansion of differentiated pathogenic T cells, modest numbers of pathogenic T cells were maintained in the absence of IL-12. Similarly, mild experimental autoimmune encephalomyelitis developed in 40% of mice treated with anti-IL-12 (32), and the severity and incidence of collagen-induced arthritis were reduced in IL-12-deficient mice (37). A pathogenic Th1 response may also be maintained, in the absence of IL-12, by cytokines such as IFN-γ-inducing factor (IL-18) (38, 39) or by inflammatory cytokines such as TNF-α (20, 40). However, it is unlikely that TNF-α contributes to the chronic phase of disease in IL-10$^{-/-}$ mice. This conclusion is based on our studies showing that anti-TNF-α mAb treatment was unable to diminish colitis in adult IL-10$^{-/-}$ mice (7), and that combined anti-IL-12 and anti-TNF-α mAb treatment was no more effective than anti-IL-12 alone (N. J. Davidson, unpublished observations). Identifying factors that may compensate for the critical activities of IL-12 will aid in advancing the development of effective therapeutic strategies for chronic T cell-mediated inflammatory disorders.

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