T Cell–mediated Pathology in Two Models of Experimental Colitis Depends Predominantly on the Interleukin 12/Signal Transducer and Activator of Transcription (Stat)-4 Pathway, but Is Not Conditional on Interferon γ Expression by T Cells

By Stephen J. Simpson,* Samir Shah,* Martina Comiskey,* Ype P. de Jong,* Baoping Wang,* Emiko Mizoguchi,‡ Atul K. Bhan,‡ and Cox Terhorst*

*From the *Division of Immunology, Beth Israel Deaconess Medical Center and the ‡Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02115*

Summary

The requirements for interleukin (IL)-12/signal transducer and activator of transcription (Stat)-4 signaling and induction of T cell–specific interferon (IFN)-γ expression in the development of T helper cell (Th)1–type pathology were examined in two different models of experimental colitis. In each model, abnormal reconstitution of the T cell compartment in immunodeficient mice by adoptive cell transfer leads to a wasting syndrome and inflammation of the colon, induced by IFN-γ and tumor necrosis factor (TNF)-α–producing T cells. We show here that treatment with anti–IL-12 antibodies in one of the models, or reconstitution with T cells from Stat-4–deficient (Stat-4^{null}) mice in both models resulted in a milder disease in the majority of recipient animals, compared with those that were left untreated or that had been reconstituted with *wt* cells. Protected mice in each group also harbored lower frequencies of IFN-γ–producing T cells than did diseased mice, suggesting that effects on wasting and colitis resulted from the attenuation of IFN-γ expression by T cells. To test whether the development of pathogenic T cells in the two colitis models was directly dependent on T cell–specific IFN-γ expression, IFN-γ^{null} donors were used for T cell reconstitution in each system. Surprisingly, large numbers of IFN-γ^{null}–reconstituted mice developed wasting and colitis, which in many cases was of comparable severity to that seen in animals reconstituted with *wt* cells. Furthermore, T cells from these animals expressed TNF-α, demonstrating that they had retained the ability to produce another proinflammatory cytokine. Taken together, these results demonstrate that in some forms of chronic experimental colitis the development of pathogenic T cells is influenced predominantly, though not exclusively, by IL-12 via the actions of Stat-4 proteins. Furthermore, our data suggest that in the models of colitis studied here the effects of IL-12/Stat-4 or other Th1 promoting pathways are not limited to the induction of IFN-γ gene expression in T lymphocytes.

Several rodent models of chronic intestinal inflammation share features of immunopathology with human inflammatory bowel disease (IBD),[1] which exists in the two distinct forms of Crohn's disease and ulcerative colitis (1–6). In particular, the finding that human and many animal forms of IBD show evidence of aberrant Th1 responses has come under close scrutiny (5, 7–12). Most recently, it has been proposed that mucosal inflammation, such as that found in IBD, emerges from an alteration in the normal balance between the effects of proinflammatory cytokines such as IFN-γ and regulatory cytokines such as transforming growth factor β (TGF-β; reference 13). The observation that TGF-β–deficient mice develop inflammation of various tissues, including the intestine, provides strong evidence in support for this (14). IL-12, a cytokine produced by activated macrophages and dendritic cells, plays a central role in the generation of Th1-type responses, characterized predominantly by the induction of IFN-γ production in T cells (15–20). In light of this, elucidation of the mechanisms

Simpson and Shah contributed equally to this work.

[1]*Abbreviations used in this paper:* BM→Tgε26, (C57BL/6 × CBA/J)F1; BMC; bone marrow cells; IBD; inflammatory bowel disease; Stat, signal transducer and activator of transcription; TGF, transforming growth factor.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/98/04/1225/10 $2.00
Volume 187, Number 8, April 20, 1998 1225–1234
http://www.jem.org

by which IL-12 might promote mucosal inflammation is of clear importance for understanding the pathogenesis of IBD.

Evidence that IL-12 is important in intestinal inflammation has recently been provided in an experimental model of acute granulomatous colitis, induced by administration of trinitrobenzene sulphonic acid (TNBS), which could be prevented by treatment with an anti–IL-12 antibody (21). The absence of IFN-γ expression in protected animals in these experiments was consistent with other data that has shown that IL-12 activity correlates strongly with IFN-γ expression by T cells (19, 22, 23). Recent studies have extended these observations by demonstrating that induction of IFN-γ expression in Th1 cells depends largely upon the activity of intracellular signal transducer and activator of transcription 4 (Stat-4) proteins, which mediate signals via the IL-12 receptor (24–28). Although IL-12 activates other members of the Stat protein family (including Stat-3; reference 27), the effects of this cytokine on Th1 cell development have been shown to depend specifically on expression of Stat-4. Consistent with this, T cells from Stat-4^{null} mice are almost completely unable to produce IFN-γ in response to IL-12 and show reduced Th1 responses equivalent to those in IL-12^{null} mice (28–30).

Although the IL-12/Stat-4 pathway clearly predominates in the development of Th1 type T cells, Stat-4–deficient T cells are nevertheless capable of limited IFN-γ production in response to IL-12–independent stimuli, such as CD3–IL-2 costimulation (28, 29). Additionally, IFN-γ has been shown to efficiently augment IL-12–mediated differentiation of Th1 cells (31). These data leave open the possibility that pathways other than that provided by IL-12 and Stat-4 contribute to the expression of IFN-γ and development of Th1-mediated inflammation. Furthermore, such experiments beg the question as to whether induction of IFN-γ gene expression in T cells does in fact represent the critical step in the development and action of pathogenic Th1 type cells. For example, it is possible that T cells could be induced into a Th1-like state in the absence of autocrine IFN-γ expression, and induce pathology through expression of other proinflammatory mediators. In this study we sought to examine these questions by testing the extent to which Stat-4 signaling and the resulting IFN-γ production by T cells contribute to pathology in two distinct Th1 models of colitis.

In each colitis model, wasting and intestinal pathology has been shown to be generated by the abnormal reconstitution of the T cell compartment in nonallogeneic, immunodeficient mice. In the first system, we have previously shown that disease develops after bone marrow cell (BMC) reconstitution of T cell and NK cell–deficient Tgε26 (C57BL/6 × CBA/J) mice, using (C57BL/6 × CBA/J)F1 donor animals (BM→Tgε26) (32–34). In the second system, IBD was originally shown to be induced upon reconstitution of CB.17 *Scid* mice with CD45RB^{hi} CD4^{+} T cells from *wt* Balb/c or CB.17 animals (35–39). In both models, strong evidence exists to suggest that pathology develops through a lack of normal T cell regulation. In the case of the CD45RB^{hi} transfer model, this occurs due to the absence of CD45RB^{lo} CD4^{+} T cells, which exert their regulatory effects through the expression of IL-10 and TGF-β (38). Similarly, we have provided evidence in the BM→Tgε26 model that abnormal T cell regulation, resulting from aberrant development of thymus-derived T cells, is causal in the development of pathology in these mice (34). Central to our hypothesis is the observation that the thymi of Tgε26 mice lack a normal stromal architecture and thus are unable to support normal ontogeny of *wt* donor–derived thymocytes supplied by bone marrow inoculation. Consequently, we have suggested that this defect in thymocyte development prevents the establishment of a regulated T cell repertoire in BM→Tgε26 mice.

In both the BM→Tgε26 and CD45RB^{hi} transfer models, the notion that T cell dysregulation leads to aberrant Th1 responses is underscored by the presence of very large numbers of activated IFN-γ– and TNF-α–secreting T cells in the peripheral lymphoid tissue and the colon (38, 40). However, despite the similarity of the T cell phenotype in each model, the resulting pathology in each case is clearly distinct. Thus, in the BM→Tgε26 mice, inflammation is limited to the mucosa of the colon and shares some features of UC, including crypt abscess formation, crypt cell proliferation, and extensive mononuclear cell infiltration. Although these are also characteristic of the CD45RB^{hi} transfer model, colitis in these mice displays additional features resembling some of those observed in Crohn's disease, including transmural inflammation, formation of granulomas, and occasional involvement of the distal small intestine. These distinctions provide a useful means by which to compare mechanisms important in alternative forms of chronic intestinal inflammation. Furthermore, since each system uses adoptive transfer of cells into T–cell deficient host animals, both are amenable to a novel experimental design whereby alternative chimeras can be generated using donors genetically deficient in specific proteins of interest. Using this as one of our approaches, we investigated the requirements for IL-12/Stat-4 signaling and induction of T cell–specific IFN-γ gene expression in the development of pathology in each form of colitis.

Materials and Methods

Mice. Donor (C57BL/6 × CBA/J)F1, C57BL/6, C57BL/6/*Scid*, and C57BL/6/IFN-γ^{null} mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The Stat-4^{null} mice were a gift from Dr. J.N. Ihle (St. Jude's Children's Hospital, Memphis, TN). 129/SvEv RAG-2^{null} and *wt* mice were purchased from Taconic Farms (Germantown, NY). The Tgε26 recipient mice were generated as previously described by transgenic overexpression of a full-length human CD3ε gene (32) and bred on the original C57BL/6 × CBAJ background in the Beth Israel Deaconess Medical Center animal facility. All mice were kept under standard conditions in microisolator cages with autoclaved food, water, and bedding. In RAG^{null} and *Scid* reconstitution experiments, recipients were between 4 and 6 wk of age. Tgε26 recipient mice were between 5 and 10 wk of age.

Cell and Bone Marrow Purification and Cell Transfer. The CD45RB^{hi} model was generated as described by Powrie et al. with minor modifications (37). CD4$^+$ T cells obtained from the spleens of donor animals and were enriched by magnetic sorting. For cell purification, the following biotinylated anti–mouse antibodies were used to label non-CD4$^+$ T cells isolated from the spleens of donor mice: B220 (RA3-6B2), Mac-1 (M1/70), Gr-1(RB6-8C5), and CD8α (53-6.7). Magnetically labeled streptavidin beads (Miltenyi Biotec Inc., Sunnyvale, CA) were used to bind the biotinylated antibodies. All antibodies were obtained from PharMingen (San Diego, CA). Negative selection was accomplished using a MACS magnetic cell sorter (Miltenyi Biotec Inc.). The enriched CD4$^+$ cells were then labeled for cell sorting with FITC-conjugated CD45RB (16A) and PE-conjugated CD4 (Becton Dickinson, Mountain View, CA). Subsequently, cells were sorted under sterile conditions by flow cytometry for CD4$^+$ CD45RB^{hi} cells, using criteria similar to that described in previous studies (37). This resulted in a >98% pure population of T cells. Harvested cells were resuspended at 10^6/400 ml PBS (no FCS) and injected into the tail veins of recipient mice.

For the BM→Tgε26 model, bone marrow was prepared as previously described (34). In brief, BMCs were depleted of T cells using two rounds of treatment with anti-Thy1.2 antibody (30-H12) followed by rabbit complement lysis (Cedarlane Labs, Westbury, NY). This procedure resulted in <0.1% CD4$^+$ and CD8α$^+$ cells within the inoculum, as determined by flow cytometry. Bone marrow recipients were total body irradiated with a sublethal dose (400 rads) 6–8 h before transplant or were treated with 5-Fluorouracil (75 mg/kg) 48 h before transplantation. 5×10^6–10^7 cells were resuspended in 400 ml PBS (no FCS) and transferred by tail vein injection.

To reduce the possibility of graft versus host disease (GVHD) in the CD45RB^{hi} model, cell transfers were made, where possible, between mice of the same backgrounds. Stat-4^{null} mice were of the mixed background C57BL/6 × 129/SvEv, making responses against segregated minor antigens from the 129/SvEv donor background a possibility. However, no evidence of GVHD, such as skin inflammation, was observed in any of the animals studied. In the Tgε26 bone marrow transfer model, significant differences were present between donor and recipient mice in some cases. However, preconditioning of bone marrow was effective in depleting the BMCs of T cells (see above). Inflammation of the skin and small bowel were not apparent in the mice included in this study.

Antibody Treatment. The anti–IL-12 antibody (clone C 17.8; reference 16) was a gift from Dr. T. Veldman (Genetics Institute, Cambridge, MA). 260 μg of purified anti–IL-12 antibody (or vehicle control [PBS]) was injected intraperitoneally into mice on a weekly basis starting at the time of BMC transfer.

Disease Monitoring and Scoring. Mice were weighed twice a week and monitored for appearance and signs of loose stool and diarrhea. In the BM→Tgε26 model, the level of wasting was determined by percentage of loss of weight from the starting weight, measured at the time of transplant. Weights were subsequently ranked 1 point for every 10% of body weight (or part thereof) lost: 0 = no weight loss; 1 = 1–10% weight loss; 2 = 11–20% weight loss; and 3 = ⩾21% weight loss. At necropsy animals were assessed for the level of colitis, which they displayed using two parameters: colon thickening and stool consistency, which were subsequently ranked as follows: for colon enlargement, 0 = no colon thickening; 1 = mild thickening; 2 = moderate thickening; 3 = extensive thickening; and for stool consistency, 0 = normal beaded stool; 1 = soft stool; 2 = diarrhea; and an additional point was added if gross blood was noted. The total score given for disease was the combined scores for colon thickening, stool consistency, and weight loss, divided by three.

Histological Analysis. Colon tissue samples were fixed in 10% buffered formalin and embedded in paraffin. Sections (4 μm) were cut and placed on gelatin-coated microscope slides for staining with hematoxylin and eosin using standard techniques as previously described (34). The severity of colitis was determined based on histological examination of the distal colon, whereby the extent of colonic inflammation was graded on a scale of 0–3 in each of three criteria: cell infiltration, thickening of the bowel wall, and the number of crypt abscesses. Histological grades were assigned in a blinded fashion by the same pathologist (A.K. Bhan).

Cell Preparations and Cytokine Analysis. Mesenteric lymphocytes were harvested and single cell suspensions prepared in PBS with 2% FCS (Intergen, Purchase, NY). Viable cells were counted and determined by trypan blue exclusion. Surface and cytoplasmic staining and FACS® (Becton Dickinson, Indianapolis, IN) analysis were performed as previously described (40). All antibodies were purchased from PharMingen and were conjugated to PE, FITC, or biotin. In the case of biotin-conjugated antibodies, labeling was accomplished by secondary staining with streptavidin-RED670 (GIBCO BRL). Cytoplasmic staining for IFN-γ (clone XMG1.2) and TNF-α (clone MP6-XT22) was done as previously described (40). In brief, freshly isolated cells were stimulated overnight with plate-bound anti-CD3 (clone 145-2C11). Brefeldin A was added for the final 2 h of incubation at a final concentration of 10 mg/ml. Cells were stained with anti-CD4 (clone RM4-5) and anti–CD3-biotin (145-2C11), washed, and fixed with 2% paraformaldehyde (Sigma Chemical Co., St. Louis, MO). Cells were permeabilized with Saponin (0.2% in PBS) to facilitate cytoplasmic cytokine staining. Cells were analyzed by flow cytometry using a FACscan® flow cytometer in conjunction with FACScan® software (Becton Dickinson) and CellQuest Software (Becton Dickinson).

Results

The IL-12/Stat-4 Pathway Predominates in Development of Wasting and Intestinal Pathology in Two Models of Colitis. To examine the role of IL-12 in the BM→Tgε26 colitis model, Tgε26 mice were transplanted with BMCs from (C57BL/6 × CBA)F1 donors as previously described (34) and given a weekly dose of anti–IL-12 antibody, or vehicle control (PBS). The requirement for Stat-4 expression in T cells was tested by comparing disease in Tgε26 mice transplanted with BMCs from Stat-4^{null} mice or *wt* control animals of the same genetic background (129/SvEv). Using a similar approach in the CD45RB^{hi} model, we used 129/SvEv RAG^{null} mice, rather than CB.17/*SCID* mice, as recipients, allowing us to maintain an equivalent genetic background to the Stat-4^{null} donor animals (see Materials and Methods). RAG^{null} mice were thus reconstituted with FACS®-sorted splenic CD4$^+$ CD45RB^{hi} T cells from Stat-4^{null} mice (Stat-4^{null}→RAG^{null}). Control RAG^{null} mice in these experiments were transplanted with *wt* 129/SvEv CD4$^+$ CD45RB^{hi} T cells (129/*wt*→RAG^{null}).

Treatment of BM→Tgε26 mice with anti–IL-12 antibody resulted in a significant reduction in disease compared with control (untreated) mice. A gross clinical disease activ-

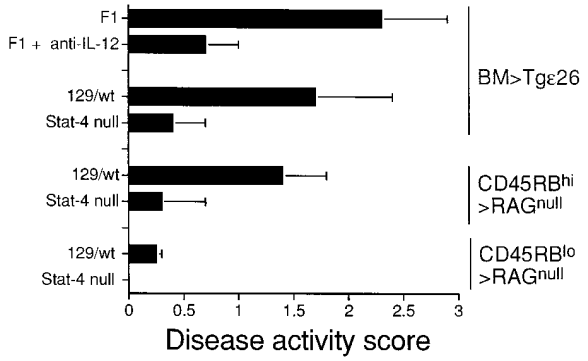

Figure 1. Disease is reduced in the absence of the IL-12/Stat-4 pathway. Disease activity scores of Tgε26 mice that were transplanted with (C57BL/6 × CBA/J)F1 BMCs (F1→Tgε26) and received a weekly dose of anti–IL-12 antibody are compared with PBS-treated F1→Tgε26 mice (untreated). Tgε26 and RAG^{null} mice were reconstituted with Stat-4^{null} BMCs and $CD45RB^{hi}$ $CD4^+$ T cells, respectively, and compared with animals that received 129/SvEv/*wt* cells. For comparison, results are also presented from RAG^{null} mice that received $CD45RB^{lo}$ $CD4^+$ T cells from *wt* and Stat-4^{null} donors. Clinical disease activity scores were determined according to parameters of weight loss, colon thickening, and stool consistency (described in Materials and Methods). The means of collective individual scores, obtained from three separate experiments, are plotted ± SD (BM→Tgε26 and $CD45RB^{hi}$ transfer groups, n = 10–15/group; $CD45RB^{lo}$, n = 3–4/group).

ity score, which measures stool consistency, gross colon enlargement, and weight loss, is shown in Fig. 1. The difference in the extent of disease was most apparent between 4 and 5 wk after BMC transfer, where control animals showed signs of severe cachexia, and in many cases diarrhea which was occasionally accompanied by visible blood. By comparison, anti–IL-12–treated animals showed reduced wasting and generally did not develop diarrhea. At necropsy, the colons of treated animals were also visibly less thickened than those of control mice. Similarly, the majority of Tgε26 reconstituted with Stat-4^{null} BMCs showed milder disease compared with control animals that had received *wt* BMC (Fig. 1). 129/*wt*→RAG^{null} mice developed wasting and colitis 7–10 wk after cell transfer. In contrast, $Stat^{null}$→RAG^{null} retained a healthy appearance over the same period of time and showed mild colitis at necroscopy, similar to that seen in RAG^{null} mice that had received sorted $CD4^+$ $CD45RB^{lo}$ T cells (36, 37).

The reduced severity of disease found in each experimental group of mice was reflected both in the extent of loss of body mass (Fig. 2 *A*) and for the most part in the level of mucosal inflammation in the large bowel, determined by examination of histological tissue sections (Fig. 2 *B*). Thus, the extent of mucosal inflammation in all anti–IL-12–treated F1→Tgε26 mice, and the majority of Stat-4^{null} reconstituted mice, was lower than in control animals. Nevertheless, significant histological colitis could be detected in a proportion of Stat-4^{null} recipient animals, even in cases where severe gross colitis (extensive colon thickening and diarrhea) was not apparent. In Stat-4^{null}→RAG^{null} experiments, a large majority of animals showed considerably lower grades of colitis than did control mice. Representative histological sections from colons of $CD45RB^{hi}$→RAG^{null} and BM→Tgε26 mice from different groups are shown in Fig. 3, illustrating the principal differences between mild and severe forms of colitis in each model. The limited extent of mucosal inflammation in most of the Stat-4^{null}-recipients and in those subjected to anti–IL-12 antibody treatment was evident from the nominal level of cellular infiltration, crypt cell hyperplasia, and the paucity of crypt cell abscesses formation. However, it was noted, in the colons of some Stat-4^{null}→RAG^{null} mice, that even in the absence of continuous mucosal inflammation there was a moderate frequency of focal inflammatory involvement. In some cases this was accompanied by the appearance of granulomas, a characteristic feature of the $CD45RB^{hi}$ transfer model (Fig. 3 *D*). Collectively, the use of anti–IL-12 antibodies and Stat-4^{null} mutant mice as donors revealed a

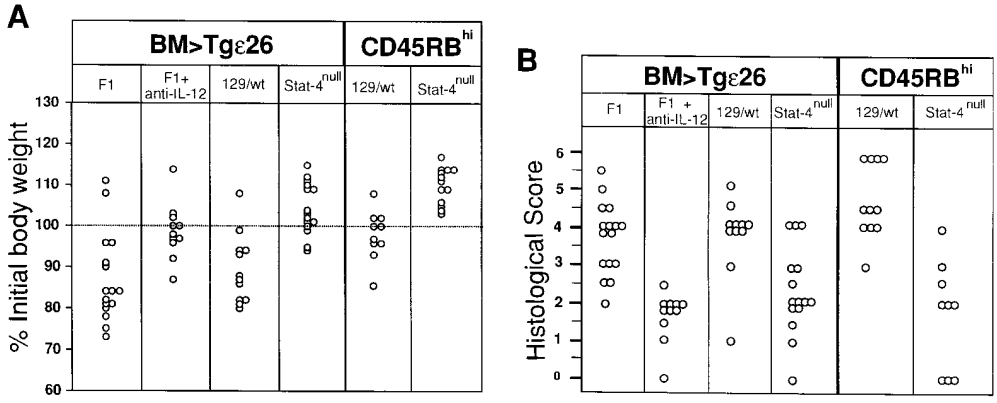

Figure 2. Wasting and colitis are both affected by the absence of IL-12/Stat-4 signaling. The divisible components of disease, weight loss and histological colitis, are represented. (*A*) Weight loss. The body weights of animals measured at the end of each experiment was divided by the starting body weight (on the day of cell transfer), to calculate the percentage of starting body weight. Each point represents data from an individual animal. (*B*) The extent of mucosal inflammation was examined histologically and graded (see Materials and Methods). Represented are the combined scores for cell infiltration (0–3) and colon thickening (0–3) on a scale of 1–6. Each point represents the score assigned to an individual animal.

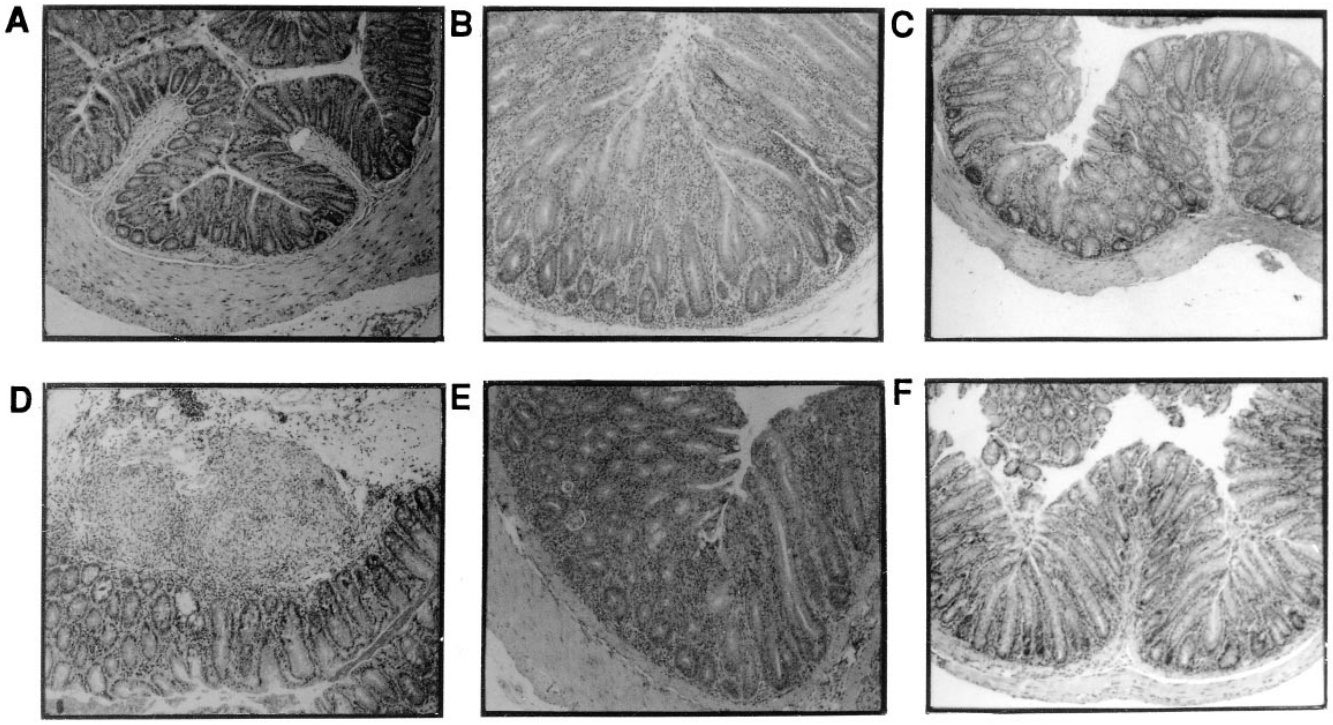

Figure 3. Histology of colitis. Sections taken from the distal region of colon are compared after hematoxylin and eosin staining. Objectives are all ×10 and scoring is as described in Materials and Methods and Fig. 2. (*A*) Normal colon taken from an untransplanted *Scid* mouse. (*B*) Severe disease (6+) in a RAG^{null} mouse transplanted with $CD45RB^{hi}$ T cells from a 129/SvEv/*wt* donor showing extensive cellular infiltration and crypt destruction. (*C*) Mild disease (2+) in a RAG^{null} mouse transplanted with $CD45RB^{hi}$ T cells from a Stat-4^{null} donor. The basic structure of the mucosa is intact, but reveals some infiltration and crypt distortion. (*D*) Mild disease (2+) and presence of a granuloma in a RAG^{null} mouse transplanted with $CD45RB^{hi}$ T cells from a Stat-4^{null} donor. (*E*) Severe disease (5+) in a Tgε26 mouse transplanted with *wt* (129/SvEv) bone marrow. Evident is extensive crypt elongation, and a pronounced cellular infiltration. (*F*) Mild disease (2+) in a BM→Tgε26 mouse treated with a weekly dose of anti–IL-12 antibody. This section reveals a low level of cellular infiltrate, moderate crypt elongation, and reduced thickening of the bowel wall.

predominant role for IL-12 and Stat-4 proteins in the development of both the wasting syndrome and colon inflammation in two distinct models of IBD.

IL-12 and Stat-4 Cooperate in the Expression of IFN-γ by Th1 Cells. To examine whether disease correlated directly with the level of Th1 involvement in the two colitis models, we examined the development of Th1-type T cells in each system. Since previous studies have shown a direct effect of IL-12 and Stat-4 on the production of IFN-γ by T cells, we used cytoplasmic staining of IFN-γ and FACS® analysis to determine the frequencies of T cells capable of IFN-γ expression. As shown in Fig. 4, the frequency of IFN-γ–producing $CD4^+$ T cells in the mesenteric lymph nodes (MLNs) in anti–IL-12–treated F1→Tgε26 mice and in Stat-4^{null}→Tgε26 and Stat-4^{null}→RAG^{null} mice was 60–80% lower than in control animals. Additionally, numbers of colon lamina propria $CD4^+$ T cells capable of IFN-γ production were also markedly reduced in protected animals (data not shown). Previous reports have suggested that in the absence of Stat-4 signaling T cells develop a Th2-like phenotype (29). Examination of IL-4 and IL-10 expression by MLN T cells in Stat-4^{null} recipients revealed no detectable expression of either cytokine (data not shown).

IFN-γ–deficient T Cells Mediate Disease and Produce TNF-α. Together, the above data implicate a causal relationship between the numbers of T cells capable of IFN-γ production and the extent of wasting and colitis. We reasoned that if induction of IFN-γ gene expression did in fact represent a critical step in the development of pathogenic Th1 cells, then the ablation of this, specifically in T cells, should be sufficient to prevent disease. To test this, we reconstituted the T cell compartment of Tgε26 and *Scid* or RAG^{null} mice using IFN-γ–deficient donors (IFN-γ^{null} mice; reference 41).

In the BM→Tgε26 model, animals injected with BMCs from IFN-γ^{null} donors backcrossed to the C57BL/6 background were compared with controls injected with C57BL/6/*wt* BMCs. In the $CD45RB^{hi}$ transfer model, $CD45RB^{hi}$ $CD4^+$ T cells from IFN-γ^{null} mice were transferred into C57BL/6/*Scid* or RAG^{null} mice. *Scid* and RAG^{null} mice transplanted with C57BL/6/*wt* $CD4^+$ $CD45RB^{hi}$ T cells served as controls in these experiments. Fig. 5 shows the cumulative results from these experiments, which compare the mean disease activity scores from each group. In both BM→Tgε26 and $CD45RB^{hi}$→*Scid* RAG^{null} animals little difference was apparent between the overall gross disease in each group of mice (Fig. 5). However, in some cases, weight loss and/or histological colitis were milder in IFN-γ^{null}–recipient mice (Fig. 6, *A* and *B*). A large number of IFN-γ^{null}→Tgε26 mice showed a range of histological scores and, in some cases, despite showing clinical evidence of significant colitis,

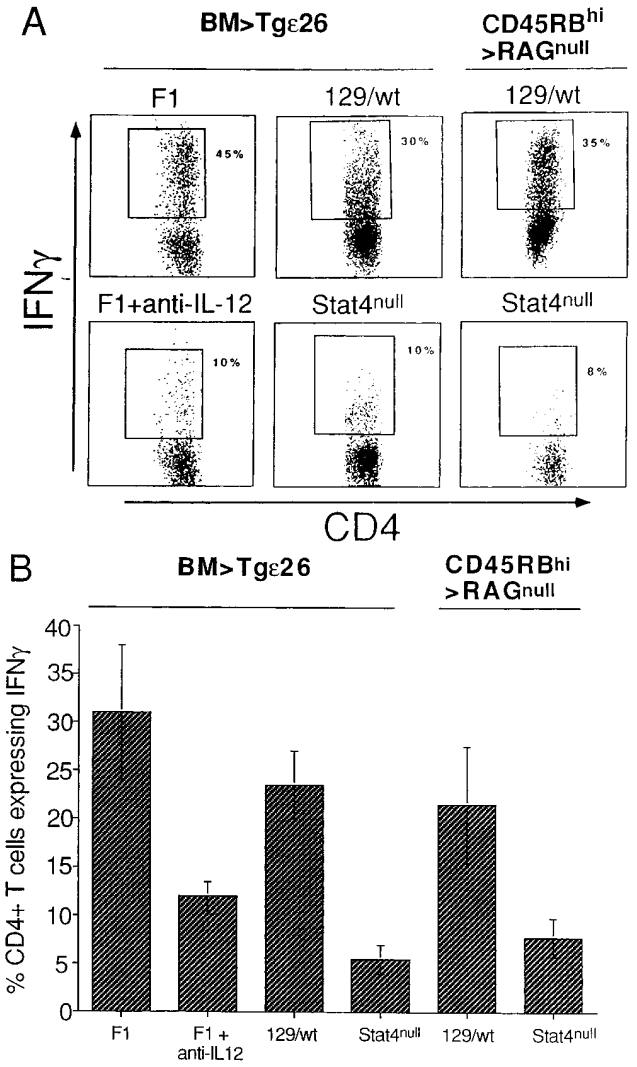

Figure 4. The effect of the IL-12/Stat-4 pathway on IFN-γ production by T cells. T cells from the MLNs from each group of BM→Tgε26 and CD45RB^{hi}→RAG^{null} mice were compared for production of IFN-γ. T cells were stimulated overnight using anti-CD3 antibody and stained for cytoplasmic expression of IFN-γ, and the frequency of cytokine positive cells was assessed by flow cytometry. (*A*) Dot plots comparing the frequency IFN-γ production by CD4$^+$ MLN T cells from BM→Tgε26 mice treated with anti–IL-12 antibody or PBS, Stat-4^{null}→Tgε26 and *wt*→Tgε26 controls, and CD45RB^{hi}→RAG^{null} mice that had received Stat-4^{null} and 129/SvEv/*wt* (129/*wt*) cells. Plots show staining of cytoplasmic IFN-γ after analytical gating on CD4$^+$ T cells. Gates are drawn after determination of background staining after preblocking with anti–IFN-γ antibodies and staining with isotype control antibodies of irrelevant specificity. The percentages of positive cells falling in the designated gates are shown. Frequencies of T cells from healthy *wt* mice expressing IFN-γ after identical CD3 stimulation or T cells from each of the represented groups without CD3 staining fall below 1% (data not shown) (*B*) The percentages of IFN-γ–positive T cells from the MLNs of different groups of mice were determined. Shown are the mean values ± SD calculated for each group (n = 5–9/group).

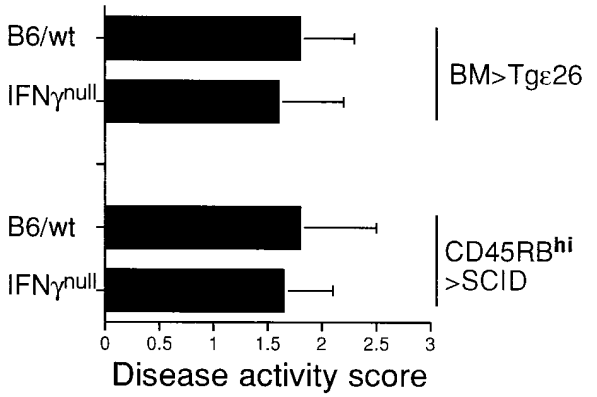

Figure 5. T cells deficient in IFN-γ production can cause disease in each of the colitis models. Tgε26 mice and C57BL/6/*Scid* or RAG^{null} mice received CD45RB^{hi} CD4$^+$ T cells and BMCs, respectively, from C57BL/6/IFN-γ^{null} donors, and were monitored for disease. These animals were compared with mice that received cells from C57BL/6/*wt* (B6/*wt*) donors. Plotted are the mean clinical disease activity scores ± 1 SD for each group of animals (n = 4–12 mice/group).

revealed comparatively mild inflammation of the mucosa by histological examination (Fig. 6 *B*). In CD45RB^{hi}→*Scid*/RAG^{null} experiments, most animals showed visible signs of wasting. However, due to their young age at the time of cell transfer, many of these mice increased in body mass due to compensatory growth. The extent of colon involvement observed in the large majority of CD45RB^{hi}→*Scid*/RAG^{null} mice was equivalent whether or not animals had received IFN-γ^{null} or C57BL/6/*wt* T cells. Representative histological sections from mice that developed colitis in the presence of IFN-γ^{null} T cells are shown in Fig. 7. In each case the salient features of pathology were similar to those in animals reconstituted with *wt* T cells.

Finally, we observed that large numbers of T cells from IFN-γ–reconstituted animals expressed a pattern of cell surface markers consistent with an activation or memory status, including low CD45RB and L-selectin expression and elevated CD69 expression (data not shown). To examine whether, in the absence of IFN-γ expression, these T cells retained other characteristics consistent with a pathogenic phenotype, their ability to produce TNF-α was tested. As shown in Fig. 8, equivalent frequencies of CD4$^+$ T cells from IFN-γ^{null} and *wt* recipients in each model expressed TNF-α. Taken together, these data demonstrate that without producing IFN-γ themselves, T cells in each of the IBD models were nevertheless capable of mediating significant levels of wasting and colitis that correlated with the retained capacity of these cells to produce a proinflammatory cytokine.

Discussion

In our study we used a novel experimental approach to examine the mechanisms leading to development and action of pathogenic Th1-type T cells in colitis. The significant effect of Stat-4 deficiency in limiting progression of disease in both the BM→Tgε26 and the CD45RB^{hi} transfer systems establishes a principal role for this pathway in the development of pathogenic Th1-type T cells in colitis. Furthermore, the similar attenuation of both disease and T cell–specific IFN-γ expression seen in anti–IL-12–treated

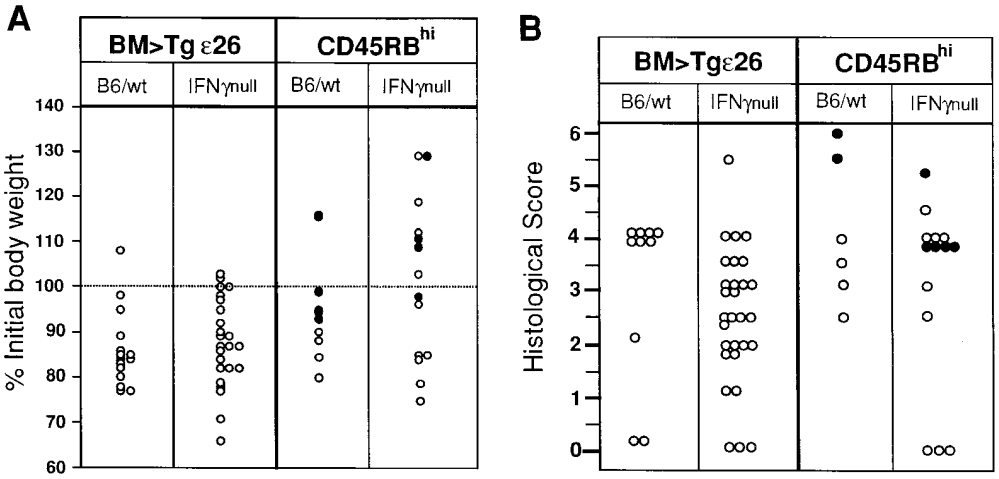

Figure 6. Wasting and colitis in the absence of IFN-γ expression in T cells. (*A*) Change in body weights and (*B*) histological colitis are represented as described in Fig. 2 in animals reconstituted with IFN-γ^{null} T cells. In the CD45RB^{hi} transfer model, closed symbols represent RAG^{null}, rather than *Scid* recipients mice.

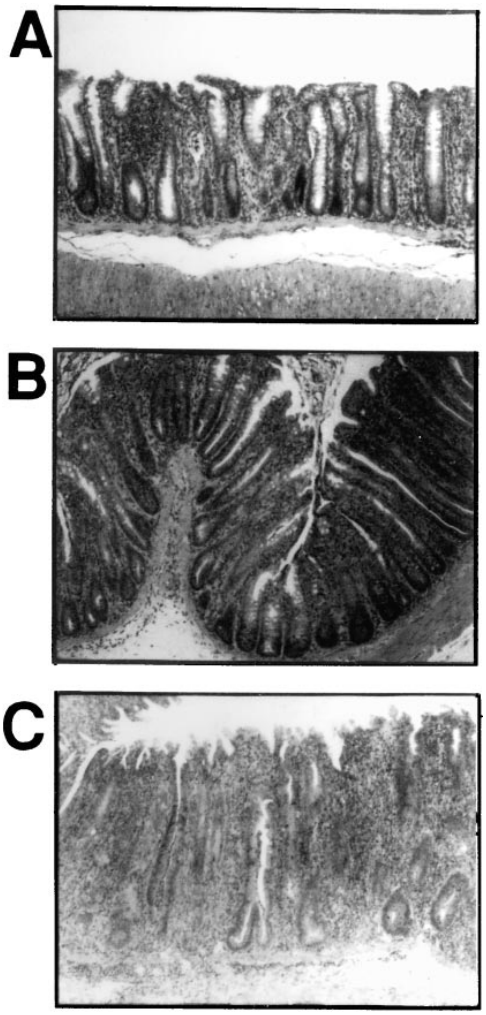

Figure 7. Histological colitis in IFN-γ^{null} T cell–reconstituted mice. Sections taken from distal colon are shown from (*A*) an IFN-γ^{null}→Tgε26 mouse with mild colitis (2+ histological score), (*B*) IFN-γ^{null}→Tgε26 mouse severe colitis (4+ histological score), and (*C*) IFN-γ^{null}→*Scid* mouse with severe colitis (5+ histological score).

BM→Tgε26 mice and Stat-4^{null}→Tgε26 mice underscores the link between the actions of IL-12 and Stat-4. In the two colitis models studied here, inhibition of IL-12 and/or Stat-4 signaling affected two divisible aspects of disease, colitis and wasting, implicating involvement of Th1 cells in both components of pathology.

To an extent, our experimental results appear consistent with those obtained in the more acute TNBS-induced model of colitis (21). However, we observed that although disease was for the most part attenuated in the absence of IL-12/Stat-4 signaling, many animals nevertheless revealed evidence of pathology. Furthermore, this correlated with the continued (although strongly reduced) presence of T cells capable of IFN-γ expression. These data differ from those in the TNBS colitis model, where an almost com-

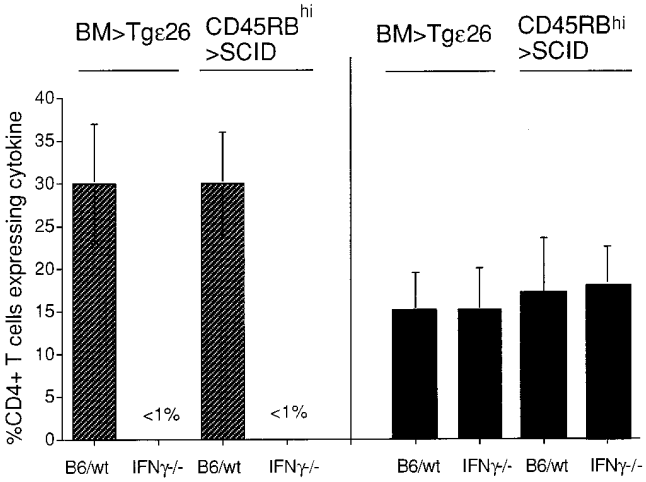

Figure 8. T cells from IFN-γ^{null}–recipient mice produce TNF-α. MLN T cells were examined for expression of IFN-γ (*hatched bars*) and TNF-α (*closed bars*) using flow cytometry after overnight in vitro stimulation with an anti-CD3 antibody. The mean percentages of cytokine-positive cells in each group are shown ± SD (n = 5–10/group).

plete inhibition of both disease and IFN-γ production were observed after anti–IL-12 treatment. These differences make apparent that the mechanisms of pathology in colitis models studied here and that induced by TNBS are not equivalent, and they suggest that factors other than IL-12 contribute to the development of more chronic forms of intestinal inflammation. In this capacity, the recently described IFN-γ–inducing factor (IL-18) is a potential candidate, since this cytokine has been shown to have a significant effect on the long-term ability of T cells to produce IFN-γ (42–44). By inference it is likely that this could significantly affect the development of pathogenic Th1-like T cells.

Notwithstanding the above arguments, the results from our study suggest a degree of independence between the actions of IL-12 and/or other Th1-promoting cytokines and the specific expression of IFN-γ by T cells. Thus, although the absence of IFN-γ expression by T cells altered the severity of colitis in many cases, the overall effects on disease were less pronounced than might have been anticipated. On first assessment this is surprising, principally for three reasons. First, the dependence of IFN-γ expression on IL-12 signaling suggests that the effects of IL-12 (via Stat-4) result from its ability to induce IFN-γ transcription in T cells (22). Second, IFN-γ has been shown to be required for efficient IL-12 priming of Th1 cells, presumably through autocrine expression (45–47). Principally, this appears to be achieved through the upregulation of the IL-12 receptor (48–49). Third, a general consensus exists that the well-documented proinflammatory effects of IFN-γ are central to pathology in IBD (4, 7, 13). The validity of this last assumption in experimental colitis has been strengthened by a previous study by Powrie et al., who demonstrated that anti–IFN-γ treatment was effective in reducing disease in the $CD4^+$ $CD45RB^{hi}$ transfer model (38). It is possible that in this latter case anti–IFN-γ antibody treatment might block all available IFN-γ. By contrast, in our experiments non-T cells, resident in the host animals, might remain a sufficient source of IFN-γ to promote effects on both T cells and macrophages. However, the development of colitis in the absence of IFN-γ expression is not unprecedented, since $IL\text{-}2^{null}$ mice, which succumb to a Th1-type colitis similar to that seen in BM→Tgε26 mice, also develop disease after import of the $IFN\text{-}\gamma^{null}$ mutation (Zand, M., C. Stevens, and T. Strom, personal communication). In addition, a recent study by Berg et al. revealed that antibody neutralization of IFN-γ in $IL\text{-}10^{null}$ mice was sufficient to prevent disease only when administered to animals at 3 wk of age and not in animals aged 3 mo or older (50). Thus, although IFN-γ was required to establish colitis in $IL\text{-}10^{null}$ mice, other inflammatory mediators were clearly sufficient to mediate disease once a pathogenic T cell phenotype had been established.

In two autoimmune models classically associated with Th1-type T cell responses, it has been observed that Th2 cells are also capable of mediating disease (51, 52). These studies support the contention that, in some cases, immunopathology normally associated with Th1-type responses can also be attributable to cells that have undergone immune deviation, leading to the expression of Th2-associated cytokines. However, in our studies we were unable to detect any IL-4 or IL-10 expression in the absence of either $Stat\text{-}4^{null}$ or IFN-γ expression by T cells. Although these findings do not unequivocally demonstrate lack of immune deviation, they do argue that this is not predominant in the colitis models studied here.

Collectively, the results offered here lend credence to the argument that Th1-type cells develop pathogenicity via a complex range of mechanisms, not all of which fall under the influence of IL-12 or their ability to produce IFN-γ. Ultimately, this is most likely achieved by the concerted expression of a range of cytokines and cytotoxic molecules which could act directly or indirectly to promote inflammation. TNF-α, which is produced by T cells as well as macrophages, is a cytokine that encompasses these characteristics and is known to induce potent inflammatory effects. Consistent with this, we have shown that in colitic mice T cells expressed TNF-α at a similar frequency whether IFN-γ was expressed by the same cells or not. Furthermore, we have found that anti–TNF-α antibody treatment is highly effective in reducing disease in the BM→Tgε26 model.[2] Similarly, Powrie et al. demonstrated an effect of anti–TNF-α treatment in the $CD45RB^{hi}$ → *Scid* model (38). These data are consolidated by the finding that anti–TNF-α antibody therapy has been shown to be efficacious in treating Crohn's disease (53).

In summary, our study offers insights into the requirements for both the development and pathogenic activity of Th1-type cells in two distinct models of inflammatory colitis. The observations that IL-12 is upregulated in human IBD (7) and that interruption of the TNF-α pathway inhibits Crohn's disease correlate well with our observations in the two models studied here (38).[2] Further dissection of the pathways which lead to aberrant Th1 pathology in animal models of IBD will undoubtedly provide a useful source of information for future therapy in IBD.

[2]Mackay, F., J. Browning, P. Lawton, S. Shah, M. Comiskey, A.K. Bhan, E. Mizoguchi, C. Terhorst, and S. Simpson, manuscript submitted for publication.

We thank Dr. James Ihle for providing us with the $Stat\text{-}4^{null}$ mice and Dr. T. Veldman for providing anti–IL-12 antibody.

This work was supported by grants from the Crohn's and Colitis Foundation of America (C. Terhorst) and from the National Institutes of Health (P30 DK-43551 and RO1 DK-47677 to A.K. Bhan). Samir A. Shah

is a Howard Hughes Medical Institute Physician Postdoctoral Fellow. Stephen J. Simpson was supported by a Research Fellowship Award from the Crohn's and Colitis Foundation of America.

Address correspondence to Cox Terhorst, Division of Immunology, Beth Israel Hospital, 330 Brookline Ave., Harvard Medical School, Boston, MA 02115. Phone: 617-667-7147; Fax: 617-667-7140.

Stephen J. Simpson's present address is Education and Research Centre, St. Vincent's Hospital, Dublin 4, Ireland.

Received for publication 22 September 1997 and in revised form 22 December 1997.

References

1. Podolsky, D.K. 1991. Inflammatory bowel disease. *N. Engl. J. Med.* 325:928–937.
2. MacDonald, T.T. 1994. Gastrointestinal inflammation. Inflammatory bowel disease in knockout mice. *Curr. Biol.* 3: 261–263.
3. Sartor, R.B. 1995. Current concepts of the etiology and pathogenesis of ulcerative colitis and Crohn's disease. *Gastroenterol. Clin. North Am.* 24:475–507.
4. Sartor, R.B. 1994. Cytokines in intestinal inflammation: pathophysiological and clinical considerations. *Gastroenterology.* 106:533–539.
5. Simpson, S.J., G.A. Hollander, E. Mizoguchi, A.K. Bhan, B. Wang, and C. Terhorst. 1996. Defects in T-cell regulation: lessons for inflammatory bowel disease. *In* Essentials of Mucosal Immunology. M.F. Kagnoff and H. Kiyono, editors. Academic Press Inc., San Diego, CA. 291–304.
6. Stenson, W.F. 1994. Animal models of inflammatory bowel disease. *In* Inflammatory Bowel Disease: From Bench to Bedside. S.R. Targan, editor. Williams and Wilkins, Baltimore, MD. 180–192.
7. Parronchi, P., P. Romagnani, F. Annunziato, S. Sampognaro, A. Becchio, L. Giannarini, E. Maggi, C. Pupilli, F. Tonelli, and S. Romagnani. 1997. Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. *Am. J. Pathol.* 150:823–832.
8. Fiocchi, C., D.G. Binion, and J.A. Katz. 1994. Cytokine production in the human gastrointestinal tract during inflammation. *Current Opinion in Gastroenterology.* 2:639–644.
9. Murata, Y., Y. Ishiguro, J. Itoh, A. Munakata, and Y. Yoshida. 1995. The role of proinflammatory and immunoregulatory cytokines in the pathogenesis of ulcerative colitis. *J. Gastroenterol.* 30(Suppl.):56–60.
10. Niessner, M., and B.A. Volk. 1995. Altered Th1/Th2 cytokine profiles in the intestinal mucosa of patients with inflammatory bowel disease as assessed by quantitative reversed transcribed polymerase chain reaction (RT-PCR). *Clin. Exp. Immunol.* 101:428–435.
11. Radford-Smith, G., and D.P. Jewell. 1996. Cytokines and inflammatory bowel disease. *Bailliere's Clin. Gastroenterol.* 10: 151–164.
12. Powrie, F. 1995b. T cells in inflammatory bowel disease: protective and pathogenic roles. *Immunity.* 3:171–174.
13. Strober, W., B. Kelsall, T. Marth, B. Ludviksson, R. Erhardt, and M. Neurath. 1997. Reciprocal IFN-γ and TGF-β responses regulate the occurrence of mucosa inflammation. *Immunol. Today.* 18:61–64.
14. Shull, M.M., I. Ormsby, A.B. Kier, S. Pawlowski, R.J. Diebold, M. Yin, R. Allen, C. Sidman, G. Proetzel, D. Calvin, et al. 1992. Targeted disruption of the mouse transforming growth factor–beta 1 gene results in multifocal inflammatory disease. *Nature.* 359:693–699.
15. Trinchieri, G. 1995. Interleukin-12: a pro-inflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol.* 13:251–276.
16. Ozmen, L., M. Pericin, J. Hakimi, R.A. Chizzonite, M. Wysocka, G. Trinchieri, M. Gately, and G. Garotta. 1994. Interleukin 12, interferon γ, and tumor necrosis factor α are the key cytokines of the generalized Shwartzman reaction. *J. Exp. Med.* 180:907–915.
17. Gerosa, F., C. Paganin, D. Peritt, F. Paiola, M.T. Scupoli, M. Aste-Amezaga, I. Frank, and G. Trinchieri. 1996. Interleukin-12 primes human CD4 and CD8 T cell clones for high production of both interferon γ and interleukin 10. *J. Exp. Med.* 183:2559–2569.
18. Heufler, C., F. Koch, U. Stanzl, G. Topar, M. Wysocka, G. Trinchieri, A. Enk, R.M. Steinman, N. Romani, and G. Schuler. 1996. Interleukin-12 is produced by dendritic cells and mediates T helper 1 development as well as interferon-γ production by T helper 1 cells. *Eur. J. Immunol.* 26:659–668.
19. Wysocka, M., M. Kubin, L.Q. Vieira, L. Ozmen, G. Garotta, P. Scott, and G. Trinchieri. 1995. Interleukin-12 is required for interferon-gamma production and lethality in lipopolysaccharide-induced shock in mice. *Eur. J. Immunol.* 25:672–676.
20. Gracie, J.A., and J.A. Bradley. 1996. Interleukin-12 induces interferon-γ–dependent switching of IgG alloantibody subclass. *Eur. J. Immunol.* 26:1217–1221.
21. Neurath, M.F., I. Fuss, B.L. Kelsall, E. Stuber, and W. Strober. 1995. Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J. Exp. Med.* 182:1281–1290.
22. Ryffel, B. 1997. Interleukin-12: role of interferon-γ in IL-12 adverse effects. *Clin. Immunol. Immunopathol.* 83:18–20.
23. Schmitt, E., P. Hoehn, C. Huels, S. Goedert, N. Palm, E. Rude, and T. Germann. 1994. T helper type 1 development of naive CD4+ T cells requires the coordinate action of interleukin-12 and interferon-γ and is inhibited by transforming growth factor–β. *Eur. J. Immunol.* 24:793–798.
24. Cho, S.S., C.M. Bacon, C. Sudarshan, R.C. Rees, D. Finbloom, R. Pine, and J.J. O'Shea. 1996. Activation of STAT4 by IL-12 and IFN-α: evidence for the involvement of ligand-induced tyrosine and serine phosphorylation. *J. Immunol.* 157: 4781–4789.
25. Bacon, C.M., E.F.R. Petricoin, J.R. Ortaldo, R.C. Rees, A.C. Larner, J.A. Johnston, and J.J. O'Shea. 1995. Interleukin 12 induces tyrosine phosphorylation and activation of STAT4 in human lymphocytes. *Proc. Natl. Acad. Sci. USA.* 92:7307–7311.
26. Ihle, J.N. 1996. STATs: signal transducers and activators of transcription. *Cell.* 84:331–334.

27. Jacobson, N.G., S.J. Szabo, R.M. Weber-Nordt, Z. Zhong, R.D. Schreiber, J.E. Darnell, Jr., and K.M. Murphy. 1995. Interleukin 12 signaling in T helper type 1 (Th1) cells involves tyrosine phosphorylation of signal transducer and activator of transcription (Stat)3 and Stat4. *J. Exp. Med.* 181: 1755–1762.
28. Thierfelder, W.E., J.M. van Deursen, K. Yamamoto, R.A. Tripp, S.R. Sarawar, R.T. Carson, M.Y. Sangster, D.A. Vignali, P.C. Doherty, G.C. Grosveld, and J.N. Ihle. 1996. Requirement for Stat-4 in interleukin-12–mediated responses of natural killer and T cells. *Nature*. 382:171–174.
29. Kaplan, M.H., Y.L. Sun, T. Hoey, and M.J. Grusby. 1996. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. *Nature*. 382:174–177.
30. Magram, J., S.E. Connaughton, R.R. Warrier, D.M. Carvajal, C.Y. Wu, J. Ferrante, C. Stewart, U. Sarmiento, D.A. Faherty, and M.K. Gately. 1996. IL-12–deficient mice are defective in IFNγ production and type 1 cytokine responses. *Immunity*. 4:471–481.
31. Wenner, C.A., M.L. Guler, S.E. Macatonia, A. O'Garra, and K.M. Murphy. 1996. Roles of IFN-γ and IFN-α in IL-12–induced T helper cell–1 development. *J. Immunol*. 156:1442–1447.
32. Wang, B., C. Biron, J. She, K. Higgins, M.J. Sunshine, E. Lacy, N. Lonberg, and C. Terhorst. 1994. A block in both early T lymphocyte and natural killer cell development in transgenic mice with high-copy numbers of the human CD3ϵ gene. *Proc. Natl. Acad. Sci. USA*. 91:9402–9406.
33. Hollander, G.A., B. Wang, A. Nichogiannopoulou, P.P. Platenberg, W. Van Ewijk, S.J. Burakoff, J.C. Gutierrez-Ramos, and C. Terhorst. 1995. Developmental control point in induction of thymic cortex regulated by a subpopulation of prothymocytes. *Nature*. 373:350–353.
34. Hollander, G.A., S.J. Simpson, E. Mizoguchi, A. Nichogiannopoulou, J. She, J.C. Gutierrez-Ramos, A.K. Bhan, S.J. Burakoff, B. Wang, and C. Terhorst. 1995. Severe colitis in mice with aberrant thymic selection. *Immunity*. 3:27–38.
35. Powrie, F., M.W. Leach, S. Mauze, L.B. Caddle, and R.L. Coffman. 1993. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in CB-17 *Scid* mice. *Int. Immunol*. 5:1461–1471.
36. Morrissey, P.J., K. Charrier, S. Braddy, D. Liggitt, and J.D. Watson. 1993. $CD4^+$ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified $CD4^+$ T cells. *J. Exp. Med*. 178:237–244.
37. Powrie, F., M.W. Leach, and S. Mauze. 1994. Inhibition of TH1 responses prevents IBD in *SCID* mice reconstituted with $CD45RB^{high}$ CD4+ T cells. *Immunity*. 1:553–562.
38. Powrie, F., J. Carlino, M.W. Leach, S. Mauze, and R.L. Coffman. 1996. A critical role for transforming growth factor-β but not interleukin 4 in the suppression of T helper type 1–mediated colitis by $CD45RB^{low}$ $CD4^+$ T cells. *J. Exp. Med*. 183:2669–2674.
39. Powrie, F., R. Correa-Oliveira, S. Mauze, and R.L. Coffman. 1994. Regulatory interactions between $CD45RB^{high}$ and $CD45RB^{low}$ $CD4^+$ T cells are important for the balance between protective and pathogenic cell–mediated immunity. *J. Exp. Med*. 179:589–600.
40. Simpson, S.J., G.A. Hollander, E. Mizoguchi, D. Allen, B.P. Wang, and C. Terhorst. 1997. Expression of pro-inflammatory cytokines by $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ T cells in an experimental model of colitis. *Eur. J. Immunol*. 27:17–25.
41. Dalton, D.K., S. Pitts-Meek, S. Keshav, I.S. Figari, A. Bradley, and T.A. Stewart. 1993. Multiple defects of immune cell function in mice with disrupted interferon-γ genes. *Science*. 259:1739–1742.
42. Kohno, K., J. Kataoka, T. Ohtsuki, Y. Suemoto, I. Okamoto, M. Usui, M. Ikeda, and M. Kurimoto. 1997. IFN-γ–inducing factor (IGIF) is a costimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. *J. Immunol*. 158:1541–1550.
43. Micallef, M.J., T. Ohtsuki, K. Kohno, F. Tanabe, S. Ushio, M. Namba, T. Tanimoto, K. Torigoe, M. Fujii, M. Ikeda, et al. 1996. Interferon-γ–inducing factor enhances T helper 1 cytokine production by stimulated human T cells: synergism with interleukin-12 for interferon-γ production. *Eur. J. Immunol*. 26:1647–1651.
44. Okamura, H., H. Tsutsi, T. Komatsu, M. Yutsudo, A. Hakura, T. Tanimoto, K. Torigoe, T. Okura, Y. Nukada, K. Hattori, et al. 1995. Cloning of a new cytokine that induces IFN-γ production by T cells. *Nature*. 378:88–91.
45. Gollob, J.A., H. Kawasaki, and J. Ritz. 1997. Interferon-γ and interleukin-4 regulate T cell interleukin-12 responsiveness through the differential modulation of high-affinity interleukin-12 receptor expression. *Eur. J. Immunol*. 27:647–652.
46. Bradley, L.M., D.K. Dalton, and M. Croft. 1996. A direct role for IFN-γ in regulation of Th1 cell development. *J. Immunol*. 157:1350–1358.
47. Nakamura, T., R.K. Lee, S.Y. Nam, E.R. Podack, K. Bottomly, and R.A. Flavell. 1997. Roles of IL-4 and IFN-γ in stabilizing the T helper cell type 1 and 2 phenotype. *J. Immunol*. 158:2648–2653.
48. Szabo, S.J., A.S. Dighe, U. Gubler, and K.M. Murphy. 1997. Regulation of the interleukin (IL)-12R β2 subunit expression in developing T helper 1 (Th1) and Th2 cells. *J. Exp. Med*. 185:817–824.
49. Rogge, L., L. Barberis-Maino, M. Biffi, N. Passini, D.H. Presky, U. Gubler, and F. Sinigaglia. 1997. Selective expression of an interleukin 12 receptor component by human T helper 1 cells. *J. Exp. Med*. 185:825–831.
50. Berg, D.J, N. Davidson, R. Kuhn, W. Muller, S. Menon, G. Holland, L. Thompson-Snipes, M.W. Leach, and D. Rennick. 1996. Enterocolitis and colon cancer in interleukin-10–deficient mice are associated with aberrant cytokine production and $CD4^+$ Th-1–like responses. *J. Clin. Invest*. 98:1010–1020.
51. Lafaille, J.J., F. van de Keere, A.L. Hsu, J.L. Baron, W. Haas, C.S. Raine, and S. Tonegawa. 1997. Myelin basic protein–specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from disease. *J. Exp. Med*. 186:307–312.
52. Pakela, S., M.O. Kurrer, and J.D. Katz. 1997. T helper cells (Th2) T cells induce acute pancreatitis and diabetes in immune-compromised nonobese diabetic (NOD) mice. *J. Exp. Med*. 186:299–306.
53. van Dullemen, H.M., S.J. van Deventer, D.W. Hommes, H.A. Bijl, J. Jansen, G.N. Tytgat, and J. Woody. 1995. Treatment of Crohn's disease with anti–tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology*. 109: 129–135.