The Journal of Immunology

IL-7 Deficiency Prevents Development of a Non-T Cell Non-B Cell-Mediated Colitis

Ursula von Freeden-Jeffry, Natalie Davidson, Rhonda Wiler, Madeline Fort, Stefan Burdach and Richard Murray

J Immunol 1998; 161:5673-5680; ;
http://www.jimmunol.org/content/161/10/5673

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

IL-7 Deficiency Prevents Development of a Non-T Cell Non-B Cell-Mediated Colitis[1]

Ursula von Freeden-Jeffry,[2]* Natalie Davidson,* Rhonda Wiler,* Madeline Fort,* Stefan Burdach,[3†] and Richard Murray[2,4]*

IL-7 is a stromal cell-derived cytokine with a well-established physiologic role in lymphocyte biology. This report describes an unexpected role for IL-7 in the development of colitis in a T and B cell-deficient environment. Recombination-activating gene-2 (RAG-2)-deficient mice (RAG-2$^{-/-}$) were exposed to and subsequently maintained a horizontally transmitted microbial flora that included *Helicobacter hepaticus*. These animals mounted a strong myeloid cell response and developed both systemic and local signs of a severe colitis. A striking infiltration of F4/80 and MHC class II-positive cells was seen in the colon and cecum of animals undergoing the disease. Mice mutant for both IL-7 and RAG-2 (IL-7/RAG-2$^{-/-}$) that were colonized by the same flora showed no signs of myeloid responses or colitis, indicating that IL-7 plays a critical role in exacerbating a non-T cell/non-B cell-mediated chronic inflammatory response. Recombinant IL-10 protein therapy was able to prevent the occurrence of colitis in susceptible mice, suggesting a pivotal role for macrophages. The implications of a role for IL-7 in this disease model with respect to human inflammatory bowel disease are discussed. ***The Journal of Immunology*, 1998, 161: 5673–5680.**

Genetic analysis in mice has shown that among the cytokines that interact with the common γ-chain receptor, IL-7 is unique in its effect on the T- and B-cell lineages (1). The mechanism of IL-7 function has now been linked to the *bcl-2* anti-apoptosis pathway, indicating a role in the maintenance of lymphocyte viability (2, 3), particularly at the developmental point of Ag receptor rearrangement (4).

Aside from the role for IL-7 on T and B cells, little is known about the in vivo actions of IL-7 on other cell types. It has been established that IL-7 stimulates myeloid colony formation in vitro (5), regulates Janus kinase-3 phosphorylation in human monocytes (6), induces cytokine secretion and tumoricidal activity by human monocytes (7), stimulates NK cells (8), and has a supportive effect on eosinophil progenitors (9). As a pharmacologic agent in vivo, IL-7 administration mobilizes myeloid progenitor cells from the mouse bone marrow to peripheral sites (10, 11). Despite these findings, no obvious abnormality in myelopoietic cells or cells of the NK lineage was apparent in IL-7-deficient (IL-7$^{-/-}$)[5] mice under normal conditions.

The intestinal epithelium as well as intestinal human goblet cells have been identified as prominent sites for the expression of IL-7 (12). A relatively common pathologic condition at this anatomical site is inflammatory bowel disease (IBD), a heterogeneous disease occurring in 0.2% of humans (13). It is generally believed that this disease involves an altered or aberrant intestinal immune response, perhaps in the context of certain micro-organisms (14). This concept is supported by findings that a wide variety of mutant mice that harbor mutations affecting the inflammatory or immune response have a complex spectrum of pathologic abnormalities in the digestive tract (15). Typically these disorders do not occur in germfree animals (16, 17), establishing the importance of a microbial trigger that may subvert the altered immune response toward chronic pathology (18). In particular, *Helicobacter hepaticus* has been shown to cause colitis in immunodeficient mice, such as SCID, but causes no intestinal pathology in immunocompetent animals (19). Moreover, *H. hepaticus* has been detected in immunocompetent mice from major mouse vendors (20).

One approach to understanding this etiologically diverse disease in humans is to determine which molecules, pathways, and mechanisms are operating in genetically modified disease models. Herein we describe a T cell- and B cell-independent inflammation in the colon and cecum of RAG-2-deficient mice colonized with *H. hepaticus* (Hh flora^{+}), akin to the pathology described in SCID mice (19), and show that the initiation and/or maintenance of this disease are dependent on IL-7. We characterize the cell populations responding in the disease state by in situ immunofluorescence and FACS analysis, and show that rIL-10 protein therapy is also an effective method of preventing the occurrence of colitis.

Materials and Methods

RAG-2$^{-/-}$ and IL-7/RAG-2$^{-/-}$ mice

All animals were derived from one common breeder pair on a 129 SvEv/129 Ola hybrid background. Genomic DNA was isolated from tails and was digested with either *Eco*RI and *Xba*I for screening of RAG-2 deficiency or with *Bam*HI and *Xba*I for screening of IL-7 deficiency as described in the original publications of the single gene-targeted mice (1, 21). All mice were housed in a specific pathogen-free barrier facility in enhanced microisolator cages and screened quarterly for viral, bacterial, and

*Department of Immunobiology, DNAX Research Institute, Palo Alto, CA 94304; †Department of Pediatrics and Biomedical Research Center, Heinrich Heine University, Düsseldorf, Germany

Received for publication May 5, 1998. Accepted for publication July 13, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] DNAX Research Institute of Molecular and Cellular Biology is supported by the Schering-Plough Corp. S.B. is supported by the German BMBF/BEU BioRegio 311661 and Elterninitiative Kinderkrebsklinik Düsseldorf.

[2] Current address: Eos Biotechnology, Inc., 225A Gateway Blvd., South San Francisco, CA 94080.

[3] Current address: Children's Hospital Medical Center and BioCenter, University of Halle-Wittenberg, 06097 Halle, Germany.

[4] Address correspondence and reprint requests to Dr. Rich Murray, Eos Biotechnology, Inc., 225A Gateway Blvd., South San Francisco, CA 94080. E-mail address: rmurray@eosbiotech.com

[5] Abbreviations used in this paper: IL-7$^{-/-}$, IL-7 deficiency; IBD, inflammatory bowel disease; Hh flora, *Helicobacter hepaticus*-containing flora; RAG-2$^{-/-}$, recombination-activating gene-2 deficiency; IL-7/RAG-2$^{-/-}$, IL-7 and recombination-activating gene-2 deficiency; SAA, serum amyloid A; WBC, white blood cells.

0022-1767/98/$02.00

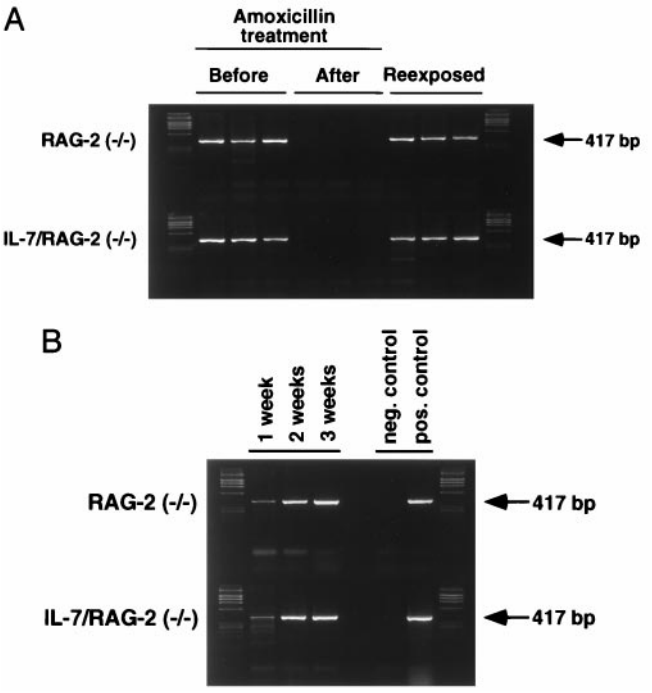

FIGURE 1. Detection of *H. hepaticus* in RAG-2$^{-/-}$ and IL-7/RAG-2$^{-/-}$ mice by PCR. *A*, *H. hepaticus*-specific PCR analysis of DNA prepared from feces of RAG-2$^{-/-}$ or IL-7/RAG-2$^{-/-}$ mice harboring *H. hepaticus*, after treatment for 14 days with amoxicillin and after re-exposure to Hh flora. *B*, PCR analysis of DNA prepared from pooled feces of 1-, 2-, and 3-wk-old mice exposed to Hh flora from either RAG-2$^{-/-}$ or IL-7/RAG-2$^{-/-}$ strains. DNA amounts were equalized by comparing genomic DNA intensities in ethidium bromide-stained gels. A 417-bp PCR product indicated the presence of *H. hepaticus*.

parasitic agents at the Research Animal Diagnostic and Investigative Laboratory in Columbia, MO. The DNAX animal facility is an American Association for the Accreditation of Laboratory Animal Care-accredited institution. Treatment and care of the animals were in accordance with the institutional guidelines.

H. hepaticus *screening by PCR*

DNA from fecal samples were prepared by first homogenizing the sample with a QIAshredder (Qiagen, Hilden, Germany) and then processing it through a QIAamp column (QIAamp Tissue Kit, Qiagen). DNA amounts were equalized by comparing genomic DNA intensities in ethidium bromide-stained gels. PCR was performed following the protocol of Shames et al. (20). The primer sequences chosen were specific for *H. hepaticus* (5′, 5′-GCA TTT GAA ACT GTT ACT CTG-3′; 3′, 5′-CTG TTT TCA AGC TCC CCG AAG-3′). A 417-bp PCR product indicated the presence of *H. hepaticus*.

Hh flora exposure

Exposure to Hh flora of PCR-negative mice was accomplished by using the natural means of transmission. Either newborn mice from Hh flora$^-$ mothers were marked, mixed, and then raised by known infected, Hh flora$^+$ foster parents, or Hh flora$^-$ adult mice from both strains were cohoused and exposed weekly to bedding from cages of Hh flora$^+$ mice. The acquisition of *H. hepaticus* was confirmed in each case by PCR.

Antibiotic treatment

Animals were treated with 50 mg/kg/day amoxicillin via addition to the drinking water for 14 days following the suggested treatment of Russel et al. (22). Treatment was started after weaning at age 21 days. This treatment ablated the presence of *H. hepaticus* in RAG-2$^{-/-}$ and IL-7/RAG-2$^{-/-}$ mice shown by *H. hepaticus*-specific PCR and of the inflammatory pathology in the RAG-2$^{-/-}$ group.

Peripheral blood analysis

Blood samples were obtained via the tail vein. Blood cell concentrations were assessed using an automated cell counter (model 901, Serono-Baker Diagnostics, Allentown, PA). For differential counts, blood smears were stained with Wright-Giemsa stain and counted under a light microscope. Serum amyloid A (SAA) levels were measured by ELISA using a kit supplied by BioSource International (Camarillo, CA) according to the manufacturer's direction.

Histologic analysis

Cecums were removed, trimmed of fat and connective tissue, fixed in 10% neutral buffered formalin, routinely processed, sectioned at 5 μm, and stained with either hematoxylin and eosin or Steiner's silver stain (23) for light microscopic examination.

Immunofluorescence labeling of tissue sections

Colon tissue was immersed in Tissue-Tek embedding compound (Miles Scientific, Elkhart, IN) and snap-frozen on liquid nitrogen. Sections (4 μm) were cut using a Reichert-Jung Frigocut cryostat (Cambridge Instruments, Buffalo, NY) and immediately air-dried onto glass microscope slides. Tissue sections were simultaneously stained with specific mAb and anti-cytokeratin using a two-step protocol as previously described (24). The mAb used were rat anti-mouse F4/80 tissue culture supernatant (clone A3-1; 1/50 dilution: BMA, Westerbury, NY), rat anti-mouse MTS6 (anti-I-A, I-E; 1/10 dilution: provided by Dr. R. L. Boyd, Monash University, Melbourne, Australia), and rabbit anti-bovine cytokeratin (broad spectrum antiserum; 1/100 dilution; Dakopatts, Santa Barbara, CA). Second-step Ab were goat anti-rat IgG (γ+L)-FITC (8 μg/ml; Caltag, Burlingame, CA) and rhodamine isothiocyanate-conjugated goat anti-rabbit IgM and IgG (H+L) (25 μg/ml; Southern Biotechnology Associates, Birmingham, AL).

Flow cytometric analysis

Peritoneal lavage cells were collected by washing the peritoneal cavity with 10 ml of cold PBS supplemented with 10% FCS. After washing, cells

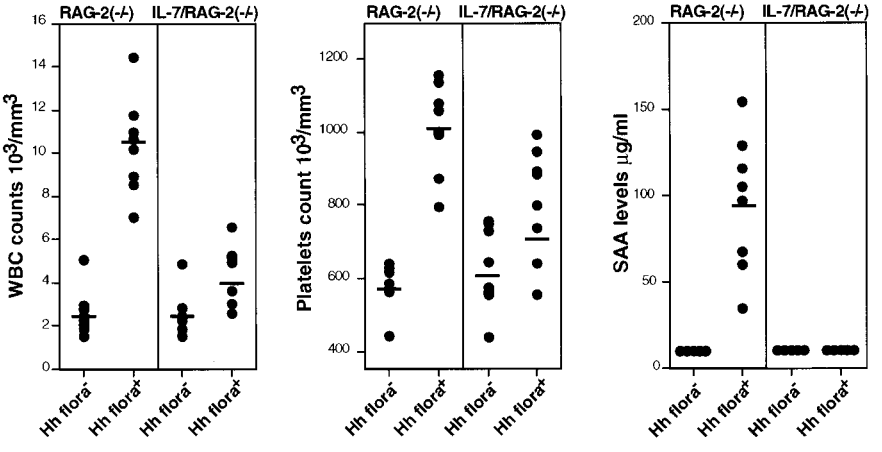

FIGURE 2. Development of systemic inflammatory parameters in RAG-2$^{-/-}$ and IL-7/RAG-2$^{-/-}$ mice after exposure to Hh flora. Newborn mice of either the RAG-2$^{-/-}$ or the IL-7/RAG-2$^{-/-}$ strain were mixed and raised by Hh flora$^+$ nursing mothers. WBC counts, platelets counts, and SAA levels were measured at the age of 8 wk (eight mice per group).

Table I. *Peripheral blood counts in Hh flora$^-$ and Hh flora$^+$ RAG-2$^{-/-}$ and IL-7/RAG-2$^{-/-}$ mice*[a]

	Hh flora$^-$		Hh flora$^+$	
	RAG-2$^{-/-}$	IL-7/RAG-2$^{-/-}$	RAG-2$^{-/-}$	IL-7/RAG-2$^{-/-}$
WBC (10^3/ml)	3.23 ± 0.48	2.95 ± 1.04	8.9 ± 2.55	4.16 ± 1.47
Monocytes (%)	39.0 ± 7.8	39.2 ± 8.0	25.8 ± 7.1	34.7 ± 9.7
Neutrophils (%)	52.0 ± 8.4	50.6 ± 7.7	66.0 ± 10.8	58.3 ± 10.1
Eosinophils (%)	6.0 ± 3.3	5.7 ± 4.9	6.5 ± 3.3	4.2 ± 2.3
Other cells[b](%)	2.4 ± 2.5	4.0 ± 2.5	1.7 ± 1.9	2.8 ± 2.3
Platelets (10^3/ml)	662 ± 36	691 ± 48	1036 ± 121	801 ± 153

[a] Blood samples were obtained via the tail vein of adult mice. Cell counts were determined using an automated cell counter. For differential counts, blood smears were stained with Wright-Giemsa stain, and cells were counted using a light microscope. Results are expressed as the mean ± SD ($n > 6$).

[b] Lymphoid-like morphology, presumably NK cells.

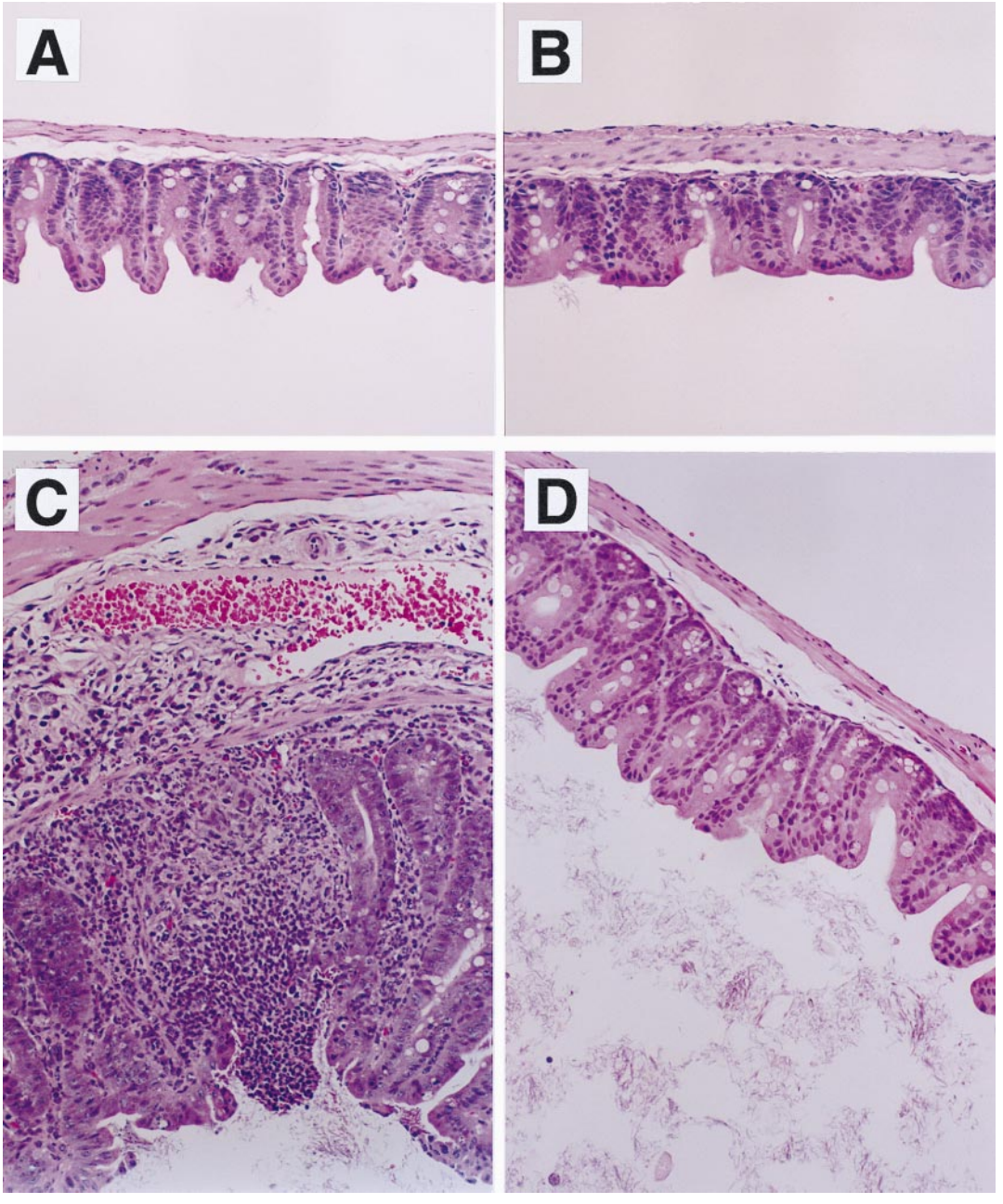

FIGURE 3. Representative histologic analysis of cecum tissue from *H. hepaticus*-unexposed and -exposed RAG-2$^{-/-}$ and IL-7/RAG-2$^{-/-}$ mice. Cecum tissues from a RAG-2$^{-/-}$ Hh flora$^-$ mouse (*A*), an IL-7/RAG-2$^{-/-}$ Hh flora$^-$ mouse (*B*), a RAG-2$^{-/-}$ Hh flora$^+$ mouse (*C*), and an IL-7/RAG-2$^{-/-}$ Hh flora$^+$ mouse (*D*) are shown. Magnification, ×65.

were incubated with CD16/CD32 mAb (1/50; PharMingen, San Diego, CA) for 10 min at 4°C to block nonspecific binding. Three-color staining was then performed using biotinylated F4/80 mAb (1/100; Caltag), phycoerythrin-conjugated DX-5 mAb (1/100; PharMingen), and FITC-conjugated anti-CCR-3 mAb (1/200; DNAX, Palo Alto, CA) followed by staining with Tri-Color-conjugated streptavidin (1/100; Caltag). Cell washes and Ab dilutions were performed in PBS containing 2% FCS. Each incubation was performed at 4°C for 20 min. Cells were analyzed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) equipped with CellQuest software (Becton Dickinson). Cell sorting was performed using a FACStar Plus (Becton Dickinson); sorted populations were >99% pure upon reanalysis.

Treatment with rIL-10

Hh flora$^+$ RAG-2$^{-/-}$ mice received daily i.p. injections of 20 μg of rIL-10 beginning at 3 wk of age and were cohoused with untreated litter mates. Mice were killed after 6 wk of treatment for pathologic evaluation. Production and purification of rIL-10 were previously described (25).

Results

Development of colitis in RAG-2$^{-/-}$ and IL-7/RAG-2$^{-/-}$ mice

Routine breeding of RAG-2$^{-/-}$ and IL-7/Rag-2$^{-/-}$ revealed rectal prolapse formation in a large percentage of RAG-2$^{-/-}$ mice, but not in IL-7/RAG-2$^{-/-}$ mice. Comprehensive screening of animals from both colonies for a large panel of viruses, parasites, and bacteria showed *H. hepaticus* colonization, but no other unusual findings (data not shown).

This observation prompted us to design studies to examine a potential pathologic role for IL-7 in the development of this inflammatory disorder. A PCR assay (20) was employed to survey for the status of *H. hepaticus* colonization. Two methods were then used to control the status of *H. hepaticus* colonization in experimental groups of animals. First, mice of both genotypes that were *H. hepaticus* PCR positive, but before the onset of pathology, were treated with amoxicillin according to established protocols (22). The amoxicillin treatment resulted in a conversion of the PCR signal from positive to negative. Subsequent re-exposure to Hh flora was accomplished by cohousing PCR-negative animals with PCR-positive mice (Fig. 1*A*). Second, newborn litters of each genotype were marked, then mixed to nurse on mothers known to harbor Hh flora. PCR analysis was performed each week for 2 wk and showed a similar acquisition of *H. hepaticus* in both groups (Fig. 1*B*). By these two designs, experimental groups of animals could acquire the flora that contained *H. hepaticus* by a natural mode of transmission. Animals are hereafter referred to as Hh flora$^+$ or Hh flora$^-$.

Newborn RAG-2$^{-/-}$ and IL-7/RAG-2$^{-/-}$ mice were cohoused and raised with either Hh flora$^+$ or Hh flora$^-$ nursing mothers. After 8 wk animals were examined for white blood cell (WBC) counts, platelet counts, and SAA, all bloodstream diagnostic markers of generalized inflammation. Fig. 2 shows a significant increase in all these parameters in the RAG-2$^{-/-}$ Hh flora$^+$ group of mice compared with normal values in RAG-2$^{-/-}$ Hh flora$^-$ mice. In contrast (and relative to the data from RAG-2$^{-/-}$ mice), IL-7/RAG-2$^{-/-}$ Hh flora$^+$ mice showed only a minimal increase in WBC counts, a moderate increase in platelet counts, and no increase in SAA compared with IL-7/RAG-2$^{-/-}$ Hh flora$^-$ mice. In a separate experiment with animals treated in the same way, the differential subpopulations of WBC were investigated (Table I). While WBC and platelet counts were elevated, the percent WBC composition was not significantly different, indicating a generalized hemopoietic response in the blood. Identical results were obtained when PCR$^-$ adult animals became Hh flora$^+$ (data not shown).

Five animals in each of the following four groups were sacrificed for examination of their digestive tracts: 1) RAG-2$^{-/-}$ Hh flora$^-$, 2) RAG-2$^{-/-}$ Hh flora$^+$, 3) IL-7/RAG-2$^{-/-}$ Hh flora$^-$, and 4) IL-7/RAG-2$^{-/-}$ Hh flora$^+$. Microscopic analysis of RAG-2$^{-/-}$ Hh flora$^-$ mice revealed normal physiology in both the colon and, as shown, the cecum (Fig. 3*A*). In contrast, all RAG-2$^{-/-}$ Hh flora$^+$ mice showed severe pathologic changes, consisting of markedly hyperplastic epithelia, a depletion of goblet cells, and focal epithelial necrosis and ulceration. Areas underlying and adjacent to the ulcerations were expanded by edema and were infiltrated with inflammatory cells consisting of macrophages, neutrophils, and eosinophils (Fig. 3*C*). The pathology occurred in discontinuous patches along the colon (data not shown). In contrast, pathologic changes were not observed in the IL-7/RAG-2$^{-/-}$ mice regardless of whether the animals were Hh flora$^-$ (Fig. 3*B*) or Hh flora$^+$ (Fig. 3*D*). Cecum tissue sections stained with Steiner's silver stain (23) from RAG-2$^{-/-}$ Hh flora$^+$ (Fig. 4*A*) or IL-7/RAG-2$^{-/-}$ Hh flora$^+$ (Fig. 4*B*) showed characteristic helical organisms that were indicative of *H. hepaticus*. Both groups of mice had similar patterns of staining for these organisms, providing an additional method to compare and monitor acquisition of the Hh

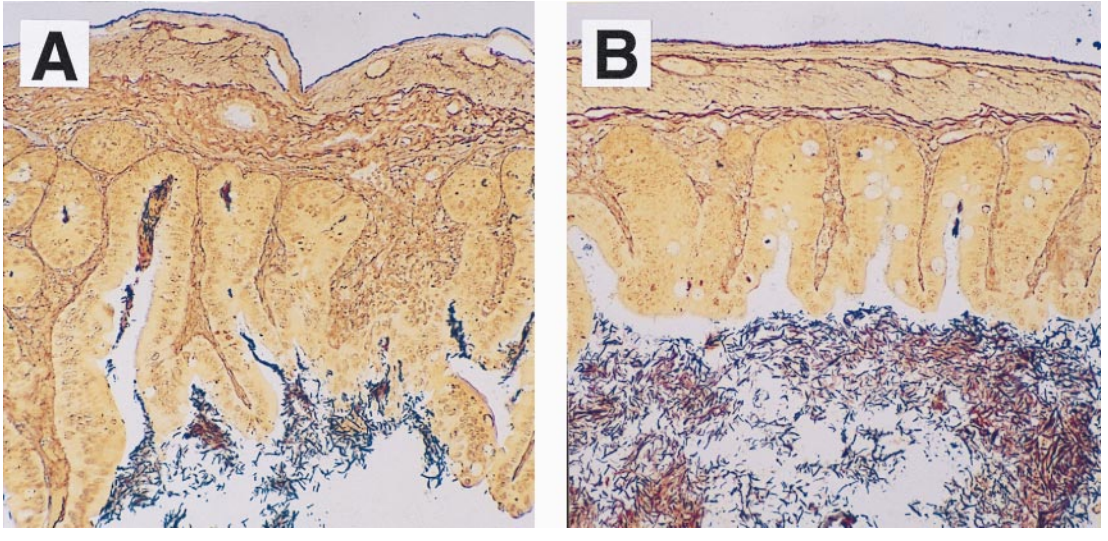

FIGURE 4. Histologic detection of helical organisms with Steiner's silver stain in the cecum. *A*, Cecum of a 4-wk-old *H. hepaticus*-exposed RAG-2$^{-/-}$ mouse; *B*, cecum of a 4-wk-old *H. hepaticus*-exposed IL-7/RAG-2$^{-/-}$ mice. Magnification, ×65.

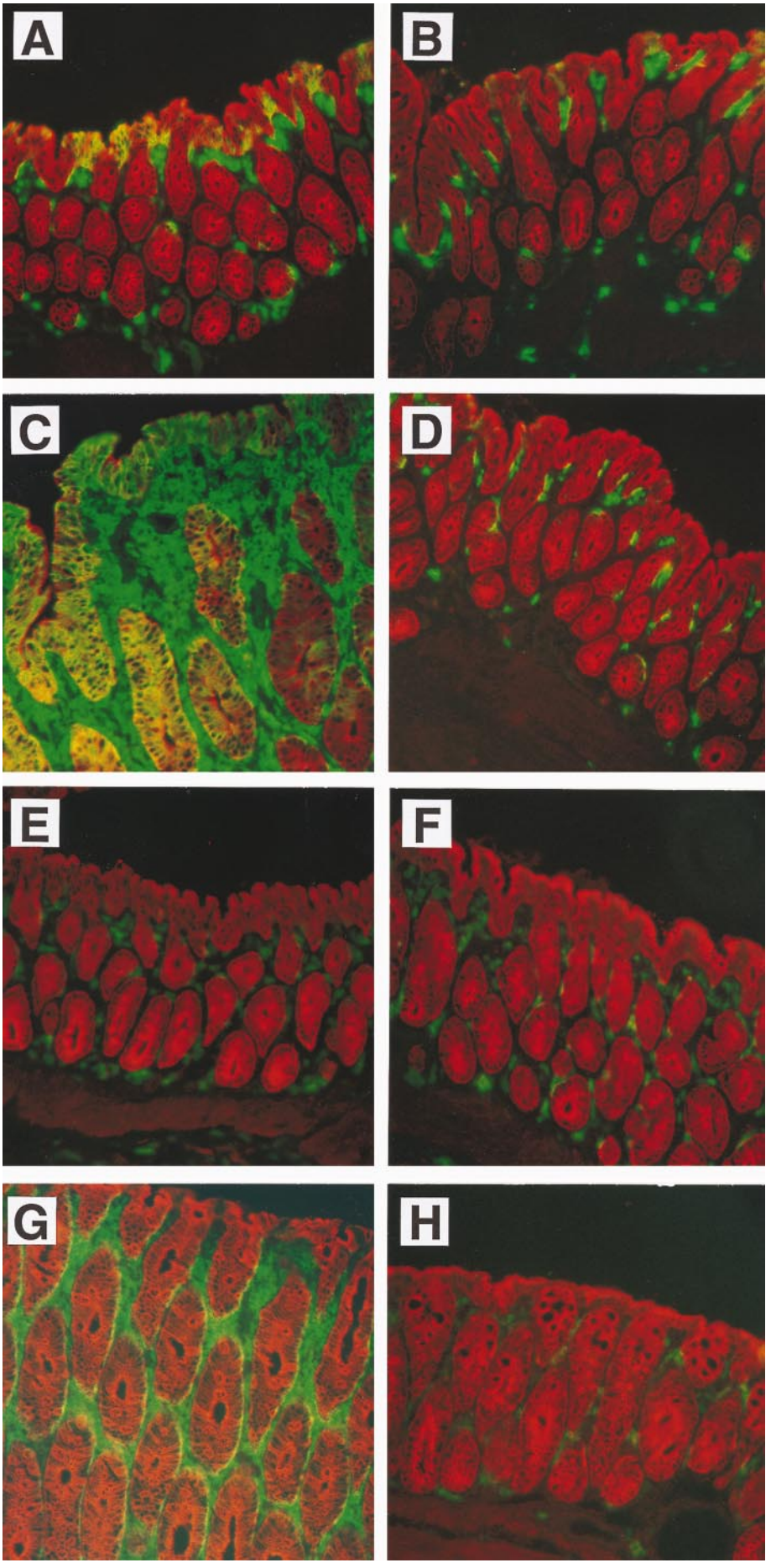

FIGURE 5. Phenotypic analysis of colonic cells from *H. hepaticus*-unexposed and -exposed RAG-2$^{-/-}$ and IL-7/RAG-2$^{-/-}$ mice. Rhodamine (red) labeling of cytokeratin defines epithelial cells. FITC (green) labeling defines staining with the cell markers MTS6 (MHC class II) or F4/80 (macrophages). Yellow staining indicates coexpression of cytokeratin and the specific cell marker. MHC class II staining of colon tissue from 3-mo-old RAG-2$^{-/-}$ Hh flora^{-} mice (*A*), IL-7/RAG-2$^{-/-}$Hh flora^{-} mice (*B*), RAG-2$^{-/-}$ Hh flora^{+} mice (*C*), and IL-7/RAG-2$^{-/-}$ Hh flora^{+} mice (*D*) is shown. F4/80^{+} macrophages in 3-mo-old RAG-2$^{-/-}$ Hh flora^{-} mice (*E*), IL-7/RAG-2$^{-/-}$ Hh flora^{-} mice (*F*), RAG-2$^{-/-}$ Hh flora^{+} mice (*G*), and IL-7/RAG-2$^{-/-}$ Hh flora^{+} mice (*H*) are shown. Magnification, ×65.

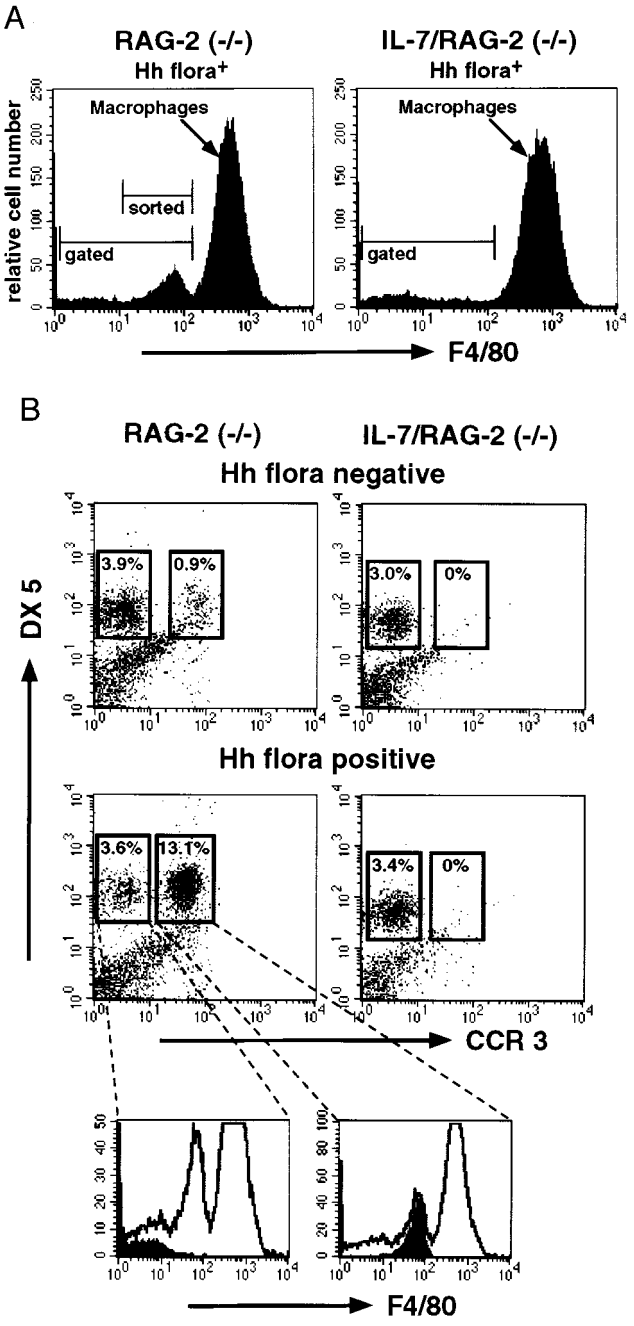

FIGURE 6. Detection of eosinophils and NK cells in the peritoneal cavity. *A*, F4/80 surface expression on peritoneal lavage cells from RAG-$2^{-/-}$ Hh flora$^+$ and IL-7/RAG-$2^{-/-}$ Hh flora$^+$ mice. The sorted population (see text) as well as the gated population shown in *B* are indicated. *B*, Surface expression of CCR-3 and DX-5 on F4/80^{low} and F4/80$^-$ peritoneal lavage cells from *H. hepaticus*-unexposed and -exposed RAG-$2^{-/-}$ and IL-7/RAG-$2^{-/-}$ mice. DX-5 single-positive cells represent NK cells that are F4/80$^-$. DX-5/CCR-3 double-positive cells represent eosinophils that are F4/80^{low}, as shown in the *two lower panels*.

flora. Interestingly, the inflamed nature of the tissue in RAG-$2^{-/-}$ mice showed closer contact and a deeper penetration of the bacteria into the crypt structures compared with tissues from IL-7/RAG-$2^{-/-}$ Hh flora$^+$ mice.

Myeloid and epithelial cell responses in the colitis

A hallmark of colitis is the up-regulation of MHC class II, particularly expression by colonic epithelial cells (26). Using in situ immunofluorescent techniques, RAG-$2^{-/-}$ Hh flora$^-$ mice, which have normal intestinal physiology, showed MHC class II staining in patches, primarily at the luminal epithelial surface (Fig. 5*A*). In contrast, intense MHC class II staining was observed throughout the entire tissue section in RAG-$2^{-/-}$ Hh flora$^+$ mice (Fig. 5*C*). Large numbers of infiltrating cells were MHC class II positive, as were most epithelial cells. Neither group of IL-7/RAG-$2^{-/-}$ mice, whether Hh flora$^-$ or Hh flora$^+$, showed an up-regulation of MHC class II expression (Fig. 5, *B* and *D*, respectively). The occurrence of infiltrating macrophages was also assessed using the F4/80 mAb. Only diseased mice, RAG-$2^{-/-}$ Hh flora$^+$ animals, showed a markedly enhanced frequency of F4/80-positive cells infiltrating into the tissue (Fig. 5, *E–H*). Further characterization of cell populations from the peritoneal cavity showed an unusual F4/80^{low} subset, present only in RAG$^{-/-}$ Hh flora$^+$ animals (Fig. 6*A*). To investigate the nature of these cells we sorted the F4/80^{low} population by flow cytometry, followed by cytospinning and staining with Wright-Giemsa stain. All cells were eosinophils (data not shown). To further investigate these cells we performed a three-color FACS analysis using the mAb F4/80, a mAb to the eosinophil chemokine receptor CCR-3,[6] and the mAb DX-5, which recognizes NK cells. F4/80 bright cells (macrophages) were excluded from analysis by gating, and the staining pattern for the remaining cells is shown in Fig. 6*B*. The DX-5$^+$/CCR-3$^+$/F4/80^{low} phenotype defined the eosinophil population. Only RAG-$2^{-/-}$ Hh flora$^+$ mice with colitis showed robust eosinophilia, in contrast to the IL-7/RAG-$2^{-/-}$ mice, regardless of the Hh flora status. To our knowledge, DX-5 staining on eosinophils has not previously been described. It is unclear whether this is an unusual feature of IL-7/RAG-$2^{-/-}$ mice or a more generalized feature of mouse eosinophils. The number of DX-5$^+$/CCR-3$^-$/F4/80$^-$ NK cells (Fig. 6*B*) in the peritoneal lavage was similar for both genotypes of Hh flora$^-$ and Hh flora$^+$ animals. Attempts to detect DX-5-positive cells with an NK cell morphology in inflamed colon tissue sections were unsuccessful.

Protection from colitis afforded by rIL-10 therapy

The anti-inflammatory properties of IL-10 are well documented (27), and IL-10-deficient mice develop colitis (17, 28). In light of these data and the nature of the inflammation in the RAG-$2^{-/-}$ Hh flora$^+$ mice, we investigated the potential of rIL-10 protein therapy to protect against IL-7-dependent colitis in the model described herein. Weanling RAG-$2^{-/-}$ Hh flora$^+$ untreated control mice and RAG-$2^{-/-}$ Hh flora$^+$ mice treated with rIL-10 for 6 wk were investigated for a blood response and intestinal pathology. WBC and platelet counts showed that rIL-10 therapy reduced the disease-associated WBC count to normal and reduced, at least to a moderate degree, the platelet count (data not shown). Histology revealed that rIL-10 therapy completely protected all the treated animals, whereas all the control animals developed disease, as shown in the representative Fig. 7, *A* and *B*, respectively. Given the well-established down-regulatory effects of rIL-10 on macrophages, these data suggest a critical role for macrophages in this disease.

Discussion

The physiologic role of IL-7 in the function and development of T and B cell populations is well established (1). The data in this manuscript identified a novel in vivo role for IL-7 as a pathogenic

[6] J. C. Grimaldi, N. Yu, G. Grunig, B. W. P. Seymour, F. Cottrez, D. S. Robinson, N. Hosken, W. G. Ferlin, X. Wu, H. Soto, A. O'Garra, K. S. Soo, M. C. Howard, and R. L. Coffman. Depletion of eosinophils in mice using antibodies specific for C-C chemokine receptor 3 (CCR3). *Submitted for publication.*

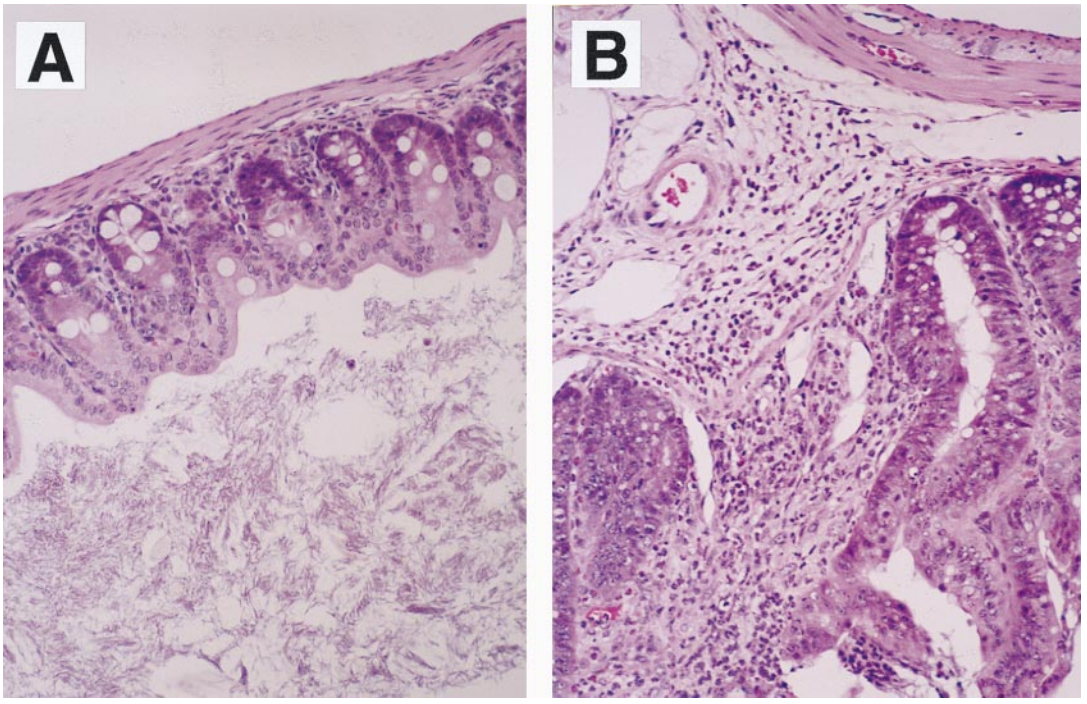

FIGURE 7. Representative histologic analysis of cecum tissue from *H. hepaticus*-exposed RAG-2$^{-/-}$ mice treated with rIL-10. *A*, Cecum sections from a 9-wk-old RAG-2$^{-/-}$ Hh flora^{+} mouse treated with rIL-10 i.p. daily starting at 3 wk of age; *B*, cecum tissue from a 9-wk-old RAG-2$^{-/-}$ Hh flora^{+} mouse that was a cohoused untreated littermate. Magnification, ×65.

factor in colitis independent of its role in T and B cell biology. IL-7 mediated a critical role, either directly or indirectly, in initiating or maintaining the disease state.

Both genetic and environmental factors are implicated in human IBDs (29). Aberrant immune or inflammatory responses triggered by microbial organisms may be important in the sequence of events leading to pathology. However, it is also clear that defects in epithelial cell factors can predispose mice toward colitis (30). Given the complexity of potential interactions of the various cell populations that are likely to be involved in human IBD, one approach to understanding disease mechanisms is to take advantage of the controlled genetics and environmental conditions afforded by mouse models.

A variety of mouse models of colitis have recently been characterized (31), many involving gene knockout studies. While all these models are unlikely to represent genetic replicas of the human disease, each may point to a mechanistic pathway worthy of pursuit in understanding the nature of the disease. Indeed, most of the gene knockout models that develop colitis, such as IL-10-deficient (17), IL-2-deficient (16), and TCRα-deficient mice (32) unexpectedly developed intestinal pathology, emphasizing the complex and poorly understood immune and inflammatory regulation along the digestive tract.

While most mouse models established the importance of T cells in the disease, the occurrence of colitis in SCID mice offered a different perspective (19). It is clear that the intestinal pathophysiology, similar to that in the T cell-driven models, can occur independently of T cells. Furthermore, in relation to human IBD, numerous studies have implied that the presence of particular microbial flora correlates with disease incidence (14). This conclusion was supported by the finding that antibiotic treatment of postsurgery human patients with metronidazole had shown efficacy (33). Within the context of T cell-independent chronic inflammation and microbial organisms, we describe herein a myeloid response in RAG-2$^{-/-}$ Hh flora^{+} animals that is either directly or indirectly dependent on IL-7.

The acquisition and colonization of RAG-2$^{-/-}$ mice by Hh flora resulted in a peripheral increase in WBC and platelets. In the cecum and colon, a substantial infiltration of macrophages and granulocytes accompanied the chronic disease. In contrast, the colonization of IL-7/RAG-2$^{-/-}$ mice with Hh flora did not result in any signs of disease or inflammation. The development of IL-7-dependent colitis invoked a number of potentially new and unexplored mechanisms. For example, IL-7 may be involved in initiating migratory events to the bowel, although IL-7 alone did not appear to be directly chemotactic for these cell populations (our unpublished observations). However, other cytokines have been characterized as an initiating signal for chemokine gene expression (34). Alternatively, IL-7 might be involved in maintaining an inflammatory state in the colon, perhaps by acting as a cell maintenance factor for myeloid cells, in a manner comparable to the IL-7 *bcl-2*-dependent maintenance of lymphocytes (4). Indeed, IL-7 has been shown to effectively stimulate the monocyte and macrophage lineage (5). The proximity of IL-7 production by mucosal epithelia (12) and its potential interaction with infiltrating F4/80 positive cells further the hypothesis that IL-7 may regulate inflammation via macrophages.

IL-10 is well appreciated for its anti-inflammatory activities and its inhibitory effect on macrophages (27). These data combined with the observation of colitis in IL-10-deficient mice have formed the basis of human clinical trials using rIL-10 as a therapeutic protein for Crohn's disease patients (35). In the colitis model described herein, rIL-10 administration is able to prevent the occurrence of disease, further supporting the idea of a primary role for macrophages in this disease model.

It was recently reported (36) that mice expressing an IL-7 transgene in the colonic mucosa develop colitis, consistent with the data in this paper indicating a pathogenic role for IL-7. The model

presented here indicates a previously unsuspected and dominant role for IL-7 in driving inflammation promoted by gut flora, but only in the absence of lymphocytes. While this function may be surprising for IL-7, these data suggest that complex interactions are occurring between distinct cell types that may depend on similar molecules for intestinal homeostasis. For example, the γδ T cell population in a normal mouse intestine is strictly dependent on the presence of IL-7 (37), implying that normal T cell populations may be required to keep innate inflammatory mechanisms in check. In this sense, it is reasonable to hypothesize that variations in the adaptive immune response may lead to the lack of appropriate control of innate inflammatory mechanisms in addition to the more traditional concept of an aggressive and pathogenic T cell. This type of adaptive cell loss of function may include pathways that mediate anti-inflammatory properties. Ultimately, the lessons learned from the approach of mouse genetics and disease modeling may allow for a broader understanding of the mechanistic complexities of this ill-defined disease in humans.

Acknowledgments

We thank L. Lucian and T. McNeil for expert technical assistance. We also thank C. Grimaldi for production and labeling of the CCR-3 mAb, and Dr. S. Menon for providing the rIL10.

References

1. von Freeden-Jeffry, U., P. Vieira, L. A. Lucian, T. McNeil, S. E. Burdach, and R. Murray. 1995. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. *J. Exp. Med. 181:1519.*
2. Akashi, K., M. Kondo, U. von Freeden-Jeffry, R. Murray, and I. L. Weissman. 1997. Bcl-2 rescues T lymphopoiesis in interleukin-7 receptor-deficient mice. *Cell 89:1033.*
3. Maraskovsky, E., L. A. O'Reilly, M. Teepe, L. M. Corcoran, J. J. Peschon, and A. Strasser. 1997. Bcl-2 can rescue T lymphocyte development in interleukin-7 receptor-deficient mice but not in mutant rag-$1^{-/-}$ mice. *Cell 89:1011.*
4. von Freeden-Jeffry, U., N. Solvason, M. Howard, and R. Murray. 1997. The earliest T lineage-committed cells depend on IL-7 for Bcl-2 expression and normal cell cycle progression. *Immunity 7:147.*
5. Jacobsen, F. W., O. P. Veiby, C. Skjonsberg, and S. E. Jacobsen. 1993. Novel role of interleukin 7 in myelopoiesis: stimulation of primitive murine hematopoietic progenitor cells. *J. Exp. Med. 178:1777.*
6. Musso, T., J. A. Johnston, D. Linnekin, L. Varesio, T. K. Rowe, J. J. O'Shea, and D. W. McVicar. 1995. Regulation of JAK3 expression in human monocytes: phosphorylation in response to interleukins 2, 4, and 7. *J. Exp. Med. 181:1425.*
7. Alderson, M. R., T. W. Tough, S. F. Ziegler, and K. H. Grabstein. 1991. Interleukin 7 induces cytokine secretion and tumoricidal activity by human peripheral blood monocytes. *J. Exp. Med. 173:923.*
8. Lynch, D. H., and R. E. Miller. 1990. Induction of murine lymphokine-activated killer cells by recombinant IL-7. *J. Immunol. 145:1983.*
9. Vellenga, E., M. T. Esselink, J. Straaten, B. K. Stulp, J. T. De Wolf, R. Brons, J. Giannotti, J. W. Smit, and M. R. Halie. 1992. The supportive effects of IL-7 on eosinophil progenitors from human bone marrow cells can be blocked by anti-IL-5. *J. Immunol. 149:2992.*
10. Faltynek, C. R., S. Wang, D. Miller, E. Young, L. Tiberio, K. Kross, M. Kelley, and E. Kloszewski. 1992. Administration of human recombinant IL-7 to normal and irradiated mice increases the numbers of lymphocytes and some immature cells of the myeloid lineage. *J. Immunol. 149:1276.*
11. Grzegorzewski, K., K. L. Komschlies, M. Mori, K. Kaneda, N. Usui, C. R. Faltynek, J. R. Keller, F. W. Ruscetti, and R. H. Wiltrout. 1994. Administration of recombinant human interleukin-7 to mice induces the exportation of myeloid progenitor cells from the bone marrow to peripheral sites. *Blood 83:377.*
12. Watanabe, M., Y. Ueno, T. Yajima, Y. Iwao, M. Tsuchiya, H. Ishikawa, S. Aiso, T. Hibi, and H. Ishii. 1995. Interleukin 7 is produced by human intestinal epithelial cells and regulates the proliferation of intestinal mucosal lymphocytes. *J. Clin. Invest. 95:2945.*
13. Barkin, R., and J. H. Lewis. 1992. Overview of inflammatory bowel disease in humans. *Semin. Vet. Med. Surg. (Small Anim) 7:117.*
14. MacDonald, T. T. 1995. Breakdown of tolerance to the intestinal bacterial flora in inflammatory bowel disease (IBD). *Clin. Exp. Immunol. 102:445.*
15. Powrie, F., and M. W. Leach. 1995. Genetic and spontaneous models of inflammatory bowel disease in rodents: evidence for abnormalities in mucosal immune regulation. *Ther. Immunol. 2:115.*
16. Sadlack, B., H. Merz, H. Schorle, A. Schimpl, A. C. Feller, and I. Horak. 1993. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell 75:253.*
17. Kuhn, R., J. Lohler, D. Rennick, K. Rajewsky, and W. Muller. 1993. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell 75:263.*
18. Sartor, R. B. 1995. Microbial factors in the pathogenesis of Crohn's disease, ulcerative colitis and experimental intestinal inflammation. In *Inflammatory Bowel Disease,* 4th Ed. J. B. Kirsner and R. J. Shorter, eds. Williams & Wilkins, Baltimore, pp. 96–124.
19. Ward, J. M., M. R. Anver, D. C. Haines, J. M. Melhorn, P. Gorelick, L. Yan, and J. G. Fox. 1996. Inflammatory large bowel disease in immunodeficient mice naturally infected with *Helicobacter hepaticus*. *Lab. Anim. Sci. 46:15.*
20. Shames, B., J. G. Fox, F. Dewhirst, L. Yan, Z. Shen, and N. S. Taylor. 1995. Identification of widespread *Helicobacter hepaticus* infection in feces in commercial mouse colonies by culture and PCR assay. *J. Clin. Microbiol. 33:2968.*
21. Shinkai, Y., G. Rathbun, K. P. Lam, E. M. Oltz, V. Stewart, M. Mendelsohn, J. Charron, M. Datta, F. Young, A. M. Stall, et al. 1992. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell 68:855.*
22. Russell, R. J., D. C. Haines, M. R. Anver, J. K. Battles, P. L. Gorelick, L. L. Blumenauer, M. A. Gonda, and J. M. Ward. 1995. Use of antibiotics to prevent hepatitis and typhlitis in male scid mice spontaneously infected with *Helicobacter hepaticus*. *Lab. Anim. Sci. 45:373.*
23. Garvin, W., A. Fathi, and F. Bigelow. 1985. Modified Steiner for the demonstration of spirochetes. *J. Histotechnol. 8:1344.*
24. Davidson, N. J., M. W. Leach, M. M. Fort, L. Thompson-Snipes, R. Kuhn, W. Muller, D. J. Berg, and D. M. Rennick. 1996. T helper cell 1-type $CD4^+$ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. *J. Exp. Med. 184:241.*
25. Berg, D. J., R. Kühn, K. Rajewsky, W. Müller, S. Menon, N. Davidson, G. Grünig, and D. Rennick. 1995. Interleukin-10 is a central regulator of the response to LPS in murine models of endotoxin shock and the Shwartzman reaction but not endotoxin tolerance. *J. Clin. Invest. 96:2339.*
26. Brandtzaeg, P., T. Halstensen, and K. Kett. 1992. Immunopathology of inflammatory bowel disease. In *Inflammatory Bowel Disease,* Ch. 5. R. P. MacDermott and W. F. Stenson, eds. Elsevier Science Publishers, New York, pp. 95–136.
27. Fiorentino, D. F., A. Zlotnik, T. R. Mosmann, M. Howard, and A. O'Garra. 1991. IL-10 inhibits cytokine production by activated macrophages. *J. Immunol. 147:3815.*
28. 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(+) TH1-like responses. *J. Clin. Invest. 98:1010.*
29. Sartor, R. B. 1995. Current concepts of the etiology and pathogenesis of ulcerative colitis and Crohn's disease. *Gastroenterol. Clin. North Am. 24:475.*
30. Mashimo, H., D. C. Wu, D. K. Podolsky, and M. C. Fishman. 1996. Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. *Science 274:262.*
31. Elson, C. O., R. B. Sartor, G. S. Tennyson, and R. H. Riddell. 1995. Experimental models of inflammatory bowel disease. *Gastroenterology 109:1344.*
32. Mombaerts, P., E. Mizoguchi, M. J. Grusby, L. H. Glimcher, A. K. Bhan, and S. Tonegawa. 1993. Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. *Cell 75:274.*
33. Rutgeerts, P., M. Hiele, K. Geboes, M. Peeters, F. Penninckx, R. Aerts, and R. Kerremans. 1995. Controlled trial of metronidazole treatment for prevention of Crohn's recurrence after ileal resection. *Gastroenterology 108:1617.*
34. Romano, M., M. Sironi, C. Toniatti, N. Polentarutti, P. Fruscella, P. Ghezzi, R. Faggioni, W. Luini, V. van Hinsbergh, S. Sozzani, et al. 1997. Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity 6:315.*
35. van Deventer, S. J., C. O. Elson, and R. N. Fedorak. 1997. Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn's disease. *Crohn's Dis. Study Group Gastroenterol.* 113:383.
36. Watanabe, M., Y. Ueno, T. Yajima, S. Okamoto, T. Hayashi, M. Yamazaki, Y. Iwao, H. Ishii, S. Habu, M. Uehira, et al. 1998. Interleukin 7 transgenic mice develop chronic colitis with decreased interleukin 7 protein accumulation in the colonic mucosa. *J. Exp. Med. 187:389.*
37. Moore, T. A., U. von Freeden-Jeffry, R. Murray, and A. Zlotnik. 1996. Inhibition of gamma delta T cell development and early thymocyte maturation in IL-$7^{-/-}$ mice. *J. Immunol. 157:2366.*