T Helper Cell 1–type CD4+ T Cells, but Not B Cells, Mediate Colitis in Interleukin 10–deficient Mice

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Summary

Mice rendered deficient in the production of interleukin 10 (IL-$10^{-/-}$) develop a chronic inflammatory bowel disease (IBD) that predominates in the colon and shares histopathological features with human IBD. Our aim was to identify which cell type(s) can mediate colitis in IL-$10^{-/-}$ mice. We detected an influx of immunoglobulin-positive cells into the colon and the presence of colon-reactive antibodies in the serum of IL-$10^{-/-}$ mice. To assess a pathogenic role for B cells, we generated a B cell–deficient ($B^{-/-}$) strain of IL-$10^{-/-}$ mice. $B^{-/-}$IL-$10^{-/-}$ mice acquired a severe colitis analogous to that of IL-$10^{-/-}$ mice, implying that B cells were not the primary mediator of IBD in this model. A series of cell transfer experiments was performed to assess a pathogenic role for T cells. When IL-$10^{-/-}$ T cell–enriched lamina propria lymphocytes (LPL) or intraepithelial lymphocytes (IEL) were transferred into immunodeficient recombinase-activating gene (RAG)-$2^{-/-}$ recipients, a mild to severe colitis developed, depending on the cell number transferred. Lymphocytes recovered from the colon of transplanted RAG-$2^{-/-}$ mice with colitis were predominantly $\alpha\beta$TCR$^+$CD4$^+$, including a large proportion of CD4$^+$CD8α^+ cells. These cells were also CD45RB$^{-/low}$ and CD44$^+$, indicative of an activated/memory population. Individual populations of CD4$^+$CD8α^-, CD4$^+$CD8α^+ and CD4$^-$CD8α^+ T cells were then isolated from the lamina propria compartment of IL-$10^{-/-}$ mice and transferred into RAG-$2^{-/-}$ recipients. Only IL-$10^{-/-}$ CD4-expressing LPL, including both the CD4$^+$CD8α^- and CD4$^+$CD8α^+ populations, induced colitis in recipient mice. Interferon-γ, but little to no IL-4, was produced by CD4$^+$CD8α^- and CD4$^+$CD8α^+ LPL recovered from the inflamed colons of RAG-$2^{-/-}$ recipients implicating a T helper cell 1 (TH1)–mediated response. We thus conclude that colitis in IL-$10^{-/-}$ mice is predominantly mediated by TH1-type $\alpha\beta$TCR$^+$ T cells expressing CD4 alone, or in combination with the CD8α molecule.

Human inflammatory bowel disease (IBD)[1] is divided into two major categories, ulcerative colitis and Crohn's disease (1–3). Both diseases exhibit a characteristic profile of chronic inflammation, the former involving the large bowel, particularly the distal colon and rectum, and the latter including small bowel inflammation. Whereas little is known regarding the etiology of these disorders, IBD is thought to be amplified or perpetuated as a result of an uncontrolled immune response to enteric antigens, presumably bacterial in origin (4, 5).

Both the humoral and cell-mediated components of the immune system have been implicated in the pathogenesis of human IBD and, more recently, murine models of IBD. Abs reactive with enteric flora such as *Campylobacter jejuni*, which also react with gut tissue and peripheral blood components, have been identified in human patients, particularly those with ulcerative colitis (1–3, 6, 7). An IgG1 Ab reactive with colonic epithelial cells has been described that can be found colocalized with activated complement components in tissue sections of human colon implicating autoAb in tissue damage (8, 9). A pathogenic role for B cells and Abs has also been suggested by murine models of IBD. Anticolon Abs have been detected in IL-$2^{-/-}$ mice (10). IBD in TCR and MHC mutant mice, which are almost devoid of mature CD4$^+$ T cells, is postulated to result from a lack of T cell–mediated suppression of B cells and

[1] *Abbreviations used in this paper:* AMCA, aminomethylcoumarin acetate; $B^{-/-}$, B cell–deficient mice; H & E, hematoxylin and eosin; IBD, inflammatory bowel disease; IEL, intraepithelial lymphocyte(s); IL-$10^{-/-}$, IL-10–deficient mice; LPL, lamina propria lymphocyte(s); RAG-$2^{-/-}$, recombinase-activating gene 2–deficient mice; SAA, serum amyloid A; WT, wild type (IL-$10^{+/+}$).

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thus the loss of oral tolerance to enteric antigens (11). A pathogenic role for T cells has been suggested by the increased frequency of highly activated T cells present in both gut tissues and peripheral blood of IBD patients (1–3). Furthermore, increased serum levels of IL-2 receptor and enhanced production of IFN-γ and TNF-α by mononuclear cells isolated from the gut correlate well with disease activity (1–3). IBD can also be induced in immunodeficient mice by reconstitution with peripheral T cells transgenic for the human CD3ε molecule (12) or normal CD45-$RB^{hi}CD4^+$ splenic T cells (13, 14). Additionally, indirect evidence that T cells can mediate colitis in IL-$2^{-/-}$ mice has been recently reported (15). Together, these data strongly support the hypothesis that B and/or T cells, and their products, are key mediators in certain types of human and murine IBD.

IBD, resembling Crohn's disease in humans, is a predominant feature of mice with a targeted disruption of the IL-10 gene (IL-$10^{-/-}$; 16, 17, 18). IL-10 is normally produced by a variety of cell types, including lymphocytes and macrophages, and whereas it displays a broad range of activities, one of its major roles is that of suppressing cell-mediated immune and inflammatory responses (19, 20). Therefore, it has been proposed that IBD develops in specific pathogen-free IL-$10^{-/-}$ mice as a result of an uncontrolled immune response to normal enteric flora, and thus IL-10 is essential in regulating mucosal immunity (16–18). Whether an aberrant response to enteric flora is primarily mediated by T and/or B lymphocytes has not yet been elucidated. An extensive mononuclear cell infiltrate is present in the mucosa and submucosa of the colon and cecum of IL-$10^{-/-}$ mice with colitis (16–18). Many of these cells are plasma cells and elevated levels of IgA or IgG1 can be detected in the serum of IL-$10^{-/-}$ mice (16). The cells comprising the inflammatory infiltrate also include a high incidence of phenotypically activated/memory $CD4^+CD8\alpha^-$ and $CD4^+CD8\alpha^+$ $\alpha\beta TCR^+$ T cells (17, 18). Furthermore, $CD4^+$ T lymphocytes isolated from these mice produce high levels of IFN-γ, but not IL-4, implicating a TH1 response in the pathogenesis of IBD in IL-$10^{-/-}$ mice. Hence, we employed the IL-$10^{-/-}$ mice model of colitis to ascertain the respective roles of B and T cells in mediating IBD.

Materials and Methods

Mice. IL-$10^{-/-}$ mice generated by targeted gene disruption on a C57BL/6/129-Ola background and wild-type (WT) littermate controls were provided by W. Müller. IL-$10^{-/-}$ mice and WT littermates bred onto a 129/SvEv background were kindly provided by Dr. R. Coffman (DNAX). B cell–deficient mice ($B^{-/-}$) generated on a C57BL/6/129/Sv background by targeted disruption of the membrane exon of the Ig μ chain gene (21) were kindly provided by Dr. A. O'Garra (DNAX). $B^{-/-}$ IL-$10^{-/-}$ mice were generated at DNAX. All mouse strains were derived by cesarean section under specific pathogen-free conditions at Simonsen Laboratories (Gilroy, CA) and maintained in microisolator cages in the Animal Care Facility of the DNAX Research Institute. Immunodeficient 129/SvEv recombinase-activating gene 2–deficient (RAG-$2^{-/-}$) mice were obtained from Taconic Farms, Inc. (Germantown, NY) and maintained at DNAX as described above.

Abs. All mAbs were diluted in PBS containing 1% (vol/vol) heat-inactivated FCS (PBS/FCS). For cell sorting, anti–mouse CD4 conjugated to FITC (clone RM4-5, 10 μg/ml) and PE-conjugated anti-CD8α (clone 53.67, 4 μg/ml; both from PharMingen, San Diego, CA) were used. The following mAbs were used for flow cytometric analysis: Tri-Color™–conjugated anti–mouse CD4 (clone YTS 191.1, 2 μg/ml; Caltag Laboratories, South San Francisco, CA); anti-CD8α-PE; anti-CD3-FITC (clone 145-2C11, 5 μg/ml); anti-αβTCR-PE (clone H57-597, 1 μg/ml); anti-γδTCR-FITC (clone GL-3, 5 μg/ml); anti-CD45RB-FITC (clone 16A, 5 μg/ml); and anti-CD44-PE (clone IM7, 1 μg/ml; all from PharMingen). Isotype controls included rat IgG2a-FITC (Caltag), rat IgG2a-PE (PharMingen), and rat IgG2b-Tri-Color™ (PharMingen). Immunohistochemistry employed the following mAbs: purified, unconjugated anti-CD4 (clone YTS 191.1, 4 μg/ml; Caltag); purified, unconjugated anti-CD8α (clone 53.67, 4 μg/ml; PharMingen); donkey anti–mouse IgG (H+L) conjugated to aminomethylcoumarin acetate (AMCA; 30 μg/ml; Jackson ImmunoResearch Laboratories Inc., West Grove, PA); rabbit anti–bovine cytokeratin (broad spectrum anti–sera, 1:100 dilution; Dakopatts, Santa Barbara, CA); goat anti–rat IgG (γ+L)-FITC (8 μg/ml; Caltag); rhodamine isothiocyanate–conjugated goat anti–rabbit IgM and IgG (H+L, 25 μg/ml); and goat anti–mouse IgG (γ-specific; 10 μg/ml; both from Southern Biotechnology Associates Inc., Birmingham, AL).

Peripheral Blood Analysis. Blood samples were obtained from RAG-$2^{-/-}$ recipients of IL-$10^{-/-}$ or WT cells at regular intervals via the tail vein. Blood cell concentrations were assessed using an automated counter (model 901; Serono Diagnostics, Allentown, PA). Serum amyloid A (SAA) levels were determined by ELISA using a kit supplied by Biosource (Camarillo, CA) as per the manufacturer's direction. The statistical significance between experimental groups was evaluated using a Student's unpaired *t* test.

Histology and Immunohistochemistry. A histological examination of the following tissues was performed: stomach, duodenum, ileum, cecum, colon, rectum, heart, liver, lung, spleen, and kidney. Tissues were isolated, trimmed of fat and connective tissue, either fixed in 10% formaldehyde or immersed in Tissue-Tek embedding compound (Miles Scientific, Elkhart, IN), and snap frozen in liquid nitrogen. Paraffin-embedded sections (6 μm) were stained with hematoxylin and eosin (H & E). Sections were graded in a blinded fashion by the same pathologist (M.W. Leach). Mouse frozen colon sections (4 μm) were cut using a Reichert-Jung Frigocut cryostat (Cambridge Instruments Inc., Buffalo, NY) and immediately air-dried onto glass microscope slides. Monkey (*M. nemestrina*) tissue sections (MeDiCa, Carlsbad, CA), including colon, kidney, salivary gland, and esophagus, were used to ascertain the presence of tissue-reactive serum Ab.

Mouse tissue sections were simultaneously stained with specific mAb and anticytokeratin using a two-step protocol. Sections were first rinsed in 1% (vol/vol) FCS in PBS and then incubated with 30 μl of primary mAb (as described above) and rabbit anti-cytokeratin in a humidified staining box for 20 min at room temperature. Sections were washed three times for 5 min each by immersion in PBS. Sections were then incubated in a mixture of FITC-conjugated goat anti–rat IgG (γ+L) and rhodamine-conjugated goat anti–rabbit IgM and IgG (H+L) for a further 20 min and washed again as above. Monkey colon sections were stained, using the above protocol, with serum obtained from IL-$10^{-/-}$ or WT mice which was diluted 1:10 and 1:100 in PBS. Goat anti–mouse IgG-FITC was used to reveal mouse serum autoAbs. All

tissue sections were mounted in Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA) under glass coverslips. Sections were examined, and photomicrographs taken, using a Zeiss Axioskop fluorescence microscope (Carl Zeiss Inc., San Leandro, CA) with 1600 ASA professional print film (Eastman Kodak, Rochester, NY).

Lymphocyte Isolation and Flow Cytometry. Gut lymphocytes were isolated from WT and IL-$10^{-/-}$ mice with rectal prolapses at 4–8 mo of age according to a modified version of Lefrançois (22). Either the entire intestine or the large bowel only was isolated, trimmed of fat and connective tissue, and lymphoid aggregates, including Peyer's patches, were removed. Intestinal tissue, after extensive washing in PBS to remove fecal matter, was chopped and incubated in 2 mM EDTA in PBS for 30 min at 37°C, with stirring, to release intraepithelial lymphocytes (IEL). The supernatant, containing IEL, was retained and laminia propria lymphocytes (LPL) were subsequently isolated from the remaining tissue fragments by incubation in 0.2 mg/ml collagenase/dispase (Boehringer Mannheim, Indianapolis, IN) for 30 min at 37°C with stirring. The IEL and LPL preparations were then washed (5 min, 350 *g*) and sieved through nylon gauze (pore size, 100 μm) followed by 70-μm Falcon cell filters (Becton Dickinson & Co., Franklin Lakes, NJ) to remove cell clumps and larger epithelial cells. Lymphocytes were enriched by centrifugation within 40% (vol/vol) Percoll (Pharmacia LKB, Uppsala, Sweden) overlaid on 75% (vol/vol) Percoll (20 min, 750 *g*). Before cell sorting, T cells were enriched by depletion of B cells, neutrophils, and macrophages. Cell preparations were incubated with B220, 8C5, and Mac-1 culture supernatants and Ab-stained cells were removed in a magnetic field using a combination of goat anti–rat IgG (Fc)- and goat anti–rat IgG (H+L)-coated magnetic beads (Advanced Magnetics, Cambridge, MA). The remaining cells were stained with CD4-FITC and CD8α-PE for 20 min on ice for subsequent cell sorting.

For flow cytometry, 10^6 lymphocytes were incubated with mAb as described above for 20 min on ice and washed in PBS/FCS (5 min, 350 *g*). Cells were examined using a FACScan® flow cytometer equipped with CellQuest software (Becton Dickinson & Co., San Jose, CA). Two-color cell sorting was performed using a FACStar Plus® (Becton Dickinson & Co.), sorted populations being >98% pure upon reanalysis. Unsorted LPL or IEL, or sorted LPL, were injected intravenously into 3–6-mo-old RAG-$2^{-/-}$ mice in 100 μl PBS/FCS containing 1,000 U penicillin and 1 mg/ml streptomycin (Sigma Chemical Co., St. Louis, MO).

Detection of Cytokine Expression by Flow Cytometry. Cytokine production by lymphocytes isolated from the colon of RAG-$2^{-/-}$ recipients of either IL-$10^{-/-}$ or WT LPL was assessed after in vitro stimulation with PMA and ionomycin according to Openshaw et al. (23). Lymphocytes were isolated and surface molecule staining was performed as described above. Intracellular IL-4 was detected in fixed and permeabilized cells using the Ab 11B11 conjugated to PE, whereas FITC-conjugated AN18 revealed the presence of IFN-γ. A TH1 cell line, CDC25, producing IFN-γ but not IL-4, and a TH2 clone, DC.11.10, producing IL-4 but not IFN-γ, were used as positive controls after in vitro stimulation (provided by Dr. A. O'Garra).

Results

The Frequency of Colonic Ig^+ Cells Is Enhanced and Serum Colon-reactive Abs Can Be Detected in IL-$10^{-/-}$ Mice. A possible role for B cells as a mediator of IBD in IL-$10^{-/-}$ mice was evaluated. An increased frequency of Ig^+ cells was detected by immunofluorescent staining of frozen colon sections in the lamina propria of IL-$10^{-/-}$ mice compared with WT mice (Fig. 1, *A* and *B*). The degree of diffuse staining, indicative of secreted Ig, was also enhanced in IL-$10^{-/-}$ mice. As IgG1 autoAbs have been implicated in the pathogenesis of ulcerative colitis in humans (8, 9), sera from IL-$10^{-/-}$ and WT mice were tested on frozen sections of monkey colon for colon-reactive IgG using a γ-specific secondary Ab. Table 1 indicates that IgG reactive with monkey colon were present in the sera of ~60% of IL-$10^{-/-}$ mice <6 mo of age. In contrast, such Ab were absent in the sera of WT mice in this age range (Table 1). The staining pattern of the sera Ab from IL-$10^{-/-}$ but not WT mice included both crypt and surface epithelium and lamina propria cells (Fig. 1, *C* and *D*). Reactivity with other monkey tissues, including kidney, salivary gland and esophagus, was not detected with either IL-$10^{-/-}$ or WT sera. In mice >6 mo of age, 75% of IL-$10^{-/-}$ mice had colon-reactive serum IgG, this frequency reflecting the increased severity of their disease with age (Table 2; data for $B^{+/+}$ IL-$10^{-/-}$ mice). Interestingly, 37% of WT mice >6 mo of age also developed colon-reactive IgG. Therefore, the functional significance of colon-reactive Abs in IL-$10^{-/-}$ mice required further clarification.

B Cell–deficient IL-$10^{-/-}$ Mice Develop IBD. To ascertain the functional significance of abnormal B cell responses in IBD, IL-$10^{-/-}$ mice were crossed with mice lacking mature B cells due to a deletion of the membrane exon of the Ig μ chain gene (21). The gastrointestinal tract and other major organs of $B^{-/-}$IL-$10^{-/-}$ and $B^{+/+}$IL-$10^{-/-}$ mice were assessed for pathological alterations (Table 2). Pathologic changes detected in the colon of $B^{-/-}$IL-$10^{-/-}$ mice at 3–6 mo of age were mild, similar to those of $B^{+/+}$IL-$10^{-/-}$ mice in this age range. These changes consisted of colitis characterized by multifocal epithelial hyperplasia and leukocyte infiltration into the lamina propria of the cecum and descending colon. At 6 mo of age, severe colitis was seen in the majority of $B^{-/-}$IL-$10^{-/-}$ mice examined, characterized by extensive leukocyte infiltration of the lamina propria and marked epithelial hyperplasia. Rectal prolapses were also observed and high peripheral blood leukocyte and platelet counts were detected (data not shown). These pathologic and clinicopathologic findings were comparable with those seen in age-matched $B^{+/+}$IL-$10^{-/-}$ mice (18; Table 2). Control $B^{-/-}$IL-$10^{+/+}$ and $B^{+/+}$IL-$10^{+/+}$ mice showed no clinical or microscopic indication of colitis at any time points tested. These data indicate that neither B cells nor Abs were necessary for the initiation and maintenance of IBD.

Adoptive Transfer of LPL or IEL from IL-$10^{-/-}$ Mice into RAG-$2^{-/-}$ Mice Results in IBD. A role for T cell mediation of IBD in IL-$10^{-/-}$ mice was then assessed using a cell transfer model in which LPL or IEL prepared from the entire gut of IL-$10^{-/-}$ or WT mice were adoptively transferred into RAG-$2^{-/-}$ immunodeficient hosts. Both the LPL and IEL preparations from IL-$10^{-/-}$ mice were comprised primarily of αβTCR$^+$ T cells, many of which were CD4$^+$CD8α$^-$ or CD4$^+$CD8α$^+$, as previously documented (18). In contrast, the WT LPL and IEL preparations were

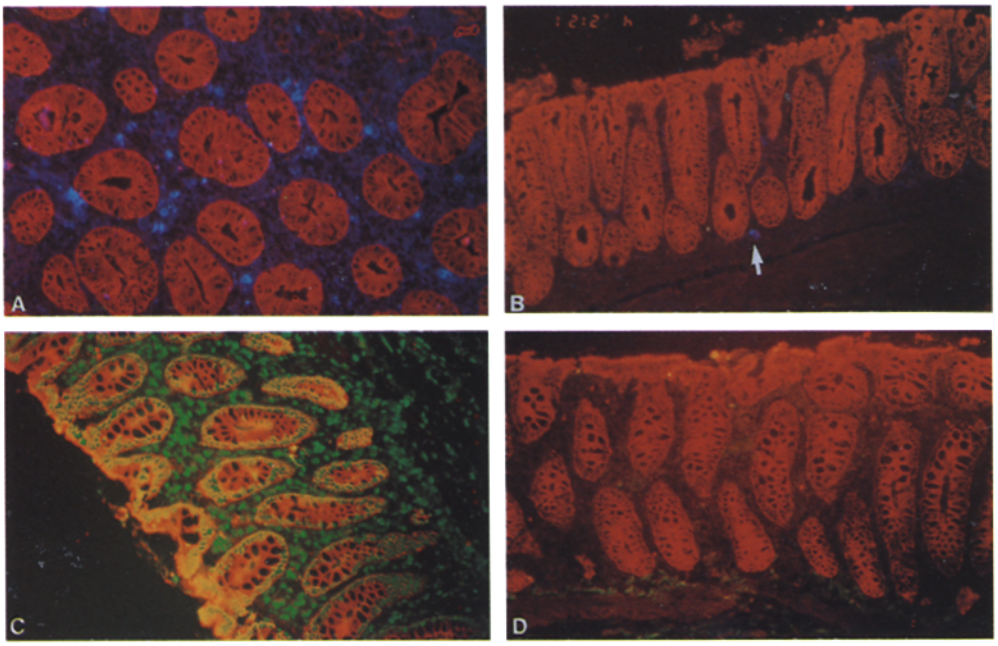

Figure 1. Distribution of Ig^+ cells and reactivity of serum Ab in the colon of IL-$10^{-/-}$ and WT mice. In all photomicrographs, rhodamine staining (*red*) of cytokeratin defines epithelial cells. (*A* and *B*) Frozen tissue sections of mid-colon from 8-mo-old animals were stained with an anti–mouse Ig-AMCA Ab. (*A*) Ig^+ cells were scattered throughout the lamina propria of the IL-$10^{-/-}$ colon, and diffuse staining, indicative of secreted Ig, was clearly evident. (*B*) Few Ig^+ cells (*arrow*) and relatively little diffuse staining was detected in the WT colon. (*C*) The serum from IL-$10^{-/-}$ mice stained (*FITC*) luminal surface and crypt epithelial cells (yellow labeling indicates coincident staining of serum Ab and anticytokeratin) and nonepithelial cells of the lamina propria in monkey colon tissue sections. (*D*) Serum from WT animals showed only a background reactivity (*FITC*) with monkey colon. ×110.

almost devoid of $CD4^+CD8\alpha^+$ cells, and the IEL fraction contained a high frequency of $\gamma\delta TCR^+$ and $CD8\alpha^+$ T cells, in accordance with previous studies (24). The composition of the transferred populations and the outcome of the cell transfers are summarized in Table 3.

RAG-$2^{-/-}$ recipients of the above cell fractions were monitored for 10 wk and then assessed for IBD. All recipients of IL-$10^{-/-}$ and WT LPL showed lymphoid reconstitution of the gut (Fig. 2). However, only recipients of IL-$10^{-/-}$ LPL developed severe inflammation of the colon (Table 3). Recipients of the highest number of IL-$10^{-/-}$ LPL (2×10^6) were severely affected by 2 wk post-cell transfer, one individual suffering from necrotizing colitis. Other organs and tissues were examined, including the stomach, small intestine, heart, liver, lung, and kidney, none of which was significantly affected. The inflammatory lesions induced in the colon of RAG-$2^{-/-}$ recipients of IL-$10^{-/-}$ LPL were multifocal, consisting of a mucosal and often submucosal infiltration of lymphocytes, plasma cells, and neutrophils. Epithelial hyperplasia was observed, giant cells were scattered throughout the lamina propria, and

Table 1. *Incidence of Colon-reactive Ab in IL-$10^{-/-}$ and WT Mice*

Mouse strain	Age	Anticolon Abs
	mo	
IL-$10^{-/-}$*	3–6	7/11‡
	>6	3/4
IL-$10^{+/+}$	3–6	0/6
	>6	3/8§

*12/15 IL-$10^{-/-}$ mice showed rectal prolapse.
‡Serum positive at both 1:10 and 1:100 dilutions.
§WT mice aged 7, 8, and 12 mo, all appeared healthy.

Table 2. *Incidence and Severity of IBD in B Cell–deficient IL-$10^{-/-}$ Mice*

		Colitis*		
Mouse strain	Age	Normal	Mild	Severe
	mo			
$B^{-/-}$ IL-$10^{-/-}$	3–6	0/20	20/20	0/20
	>6	0/8	1/8	7/8
$B^{+/+}$ IL-$10^{-/-}$	3–6	2/20	14/20	4/20
	>6	0/15	6/15	9/15
$B^{-/-}$ IL-$10^{+/+}$	3–6	8/8	0/8	0/8
	>6	N/A‡	N/A	N/A
$B^{+/+}$ IL-$10^{+/+}$	3–6	10/10	0/10	0/10
	>6	8/8	0/8	0/8

*Colon/caecum pathology graded as follows: normal: indistinguishable from normal or unmanipulated control mice; mild: low degree of leukocyte infiltration and slight epithelial hyperplasia; severe: extensive leukocyte infiltration, often transmural, marked epithelial hyperplasia, loss of goblet cells, crypt abcesses and ulceration.
‡Mice not available.

Table 3. *Colitis in RAG-2$^{-/-}$ Mice after Reconstitution with LPL or IEL*

Reconstitution			Colitis*		
			Normal	Mild	Severe
LPL‡	1×10^5	IL-10$^{-/-}$	0/4	0/4	4/4
		IL-10$^{+/+}$	3/4	1/4	0/4
	5×10^5	IL-10$^{-/-}$	0/4	1/4	3/4
		IL-10$^{+/+}$	4/4	0/4	0/4
	2×10^6	IL-10$^{-/-}$	0/6	2/6	4/6
		IL-10$^{+/+}$	4/4	0/4	0/4
IEL§	1×10^5	IL-10$^{-/-}$	2/4	1/4	1/4
		IL-10$^{+/+}$	4/4	0/4	0/4
	5×10^5	IL-10$^{-/-}$	1/4	2/4	1/4
		IL-10$^{+/+}$	4/4	0/4	0/4

*Colon pathology assessed 10 wk post-cell transfer except for recipients of 2×10^6 LPL which were assessed at 2 wk after transfer due to the severity of colon inflammation. Degree of colon pathology defined in legend to Table 2.

‡Phenotype: IL-10$^{-/-}$: 55% αβTCR$^+$, 7% γδTCR$^+$, 23% CD4$^+$ CD8α$^-$, 20% CD4$^+$CD8α$^+$, 13% CD4$^-$CD8α$^+$; WT:40% αβTCR$^+$, 7% γδTCR$^+$, 25% CD4$^+$CD8α$^-$, <1% CD4$^+$CD8α$^+$, 13% CD4$^-$ CD8α$^+$.

§Phenotype: IL-10$^{-/-}$: 75% αβTCR$^+$, 17% γδTCR$^+$, 11% CD4$^+$ CD8α$^-$, 33% CD4$^+$CD8α$^+$, 35% CD4$^-$CD8α$^+$; WT: 52% αβTCR$^+$, 27% γδTCR$^+$, <1% CD4$^+$CD8α$^-$, <1% CD4$^+$CD8α$^+$, 55% CD4$^-$CD8α$^+$ (phenotypic data indicate lymphoid composition and are representative of three independent experiments).

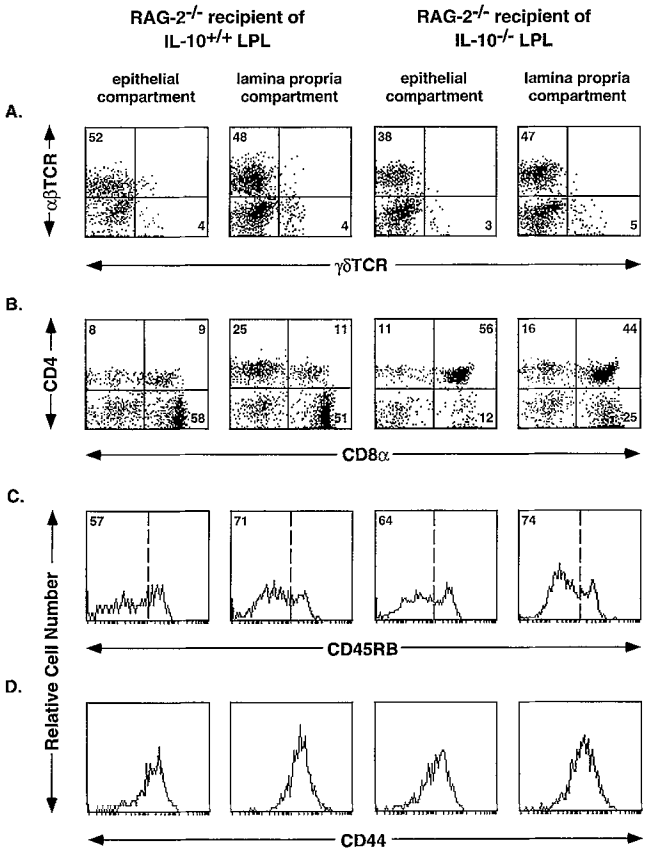

Figure 2. FACS® analysis of gut lymphocytes isolated from the epithelial and lamina propria compartments of RAG-2$^{-/-}$ mice transplanted with WT or IL-10$^{-/-}$ LPL. RAG-2$^{-/-}$ mice were injected with 5×10^5 WT or IL-10$^{-/-}$ LPL, the lymphoid populations were recovered and analyzed 10 wk after transfer. (*A*) The expression of αβTCR versus γδTCR on lymphoid-gated cells isolated from the host RAG-2$^{-/-}$ epithelial and lamina propria compartments. (*B*) The distribution of CD4 versus CD8 on gated CD3$^+$ T cells. Expression of (*C*) CD45RB and (*D*) CD44 on gated CD4$^+$ lymphocytes.

crypt abscesses were common. These pathological changes were similar to those documented for IL-10$^{-/-}$ mice (16, 17, 18), however, the lesions in IL-10$^{-/-}$ mice were more localized to discrete areas of the organ, and giant cells and crypt abscesses, although present, were less common. Recipients of WT LPL showed no evidence of colitis but for one mouse with minimal disease. IL-10$^{-/-}$ IEL, but not WT IEL, also induced colitis in recipient mice, although it was not as severe as that induced by IL-10$^{-/-}$ LPL. All other organs examined were unaffected.

Phenotypic Characteristics of Inflammatory Lymphocytes. The phenotype of the IL-10$^{-/-}$ or WT cells infiltrating the colons of RAG-2$^{-/-}$ mice 10 wk post-cell transfer of LPL were analyzed. In all recipients, T cells expressing the αβTCR were recovered from both the epithelium and the lamina propria of RAG-2$^{-/-}$ recipients, whereas very few γδTCR$^+$ cells were detected (Fig. 2). Although all CD4/CD8α-defined T cell populations could be detected in recipients of either IL-10$^{-/-}$ or WT LPL, a striking increase in the frequency of CD4$^+$CD8α$^+$ T cells in recipients of IL-10$^{-/-}$ LPL was evident. Considering that cell recovery in recipients of IL-10$^{-/-}$ LPL was threefold greater than in recipients of WT cells, a 12-fold absolute increase in CD4$^+$CD8α$^+$ had occurred in the former, indicating a preferential expansion of these cells. Another difference noted was the high frequency of CD4$^-$CD8α$^+$ T cells found in the gut of WT LPL recipients (Fig. 2). However, calculations revealed that the absolute number of these cells was not greatly different from recipients of IL-10$^{-/-}$ LPL.

The CD4$^+$ cells that homed to the host gut, regardless of their source, expressed an activated/memory phenotype, being predominantly CD45RB$^{-/low}$ and CD44$^+$ (Fig. 2). This finding was not surprising considering that activated/memory cells traffic more efficiently from the blood to peripheral tissues than do naive cells (25). Interestingly, regardless of whether LPL (Fig. 2) or IEL (data not shown) were transferred from WT or IL-10$^{-/-}$ mice, lymphocytes could be recovered from both the epithelial and lamina propria compartments of recipient mice. This distribution of cells was also clearly observed by immunofluorescent staining of tissue sections (data not shown). This is likely to result from significant cell traffic occurring back and forth between the epithelial and lamina propria compartments (24).

When IEL were transferred, similar results were obtained

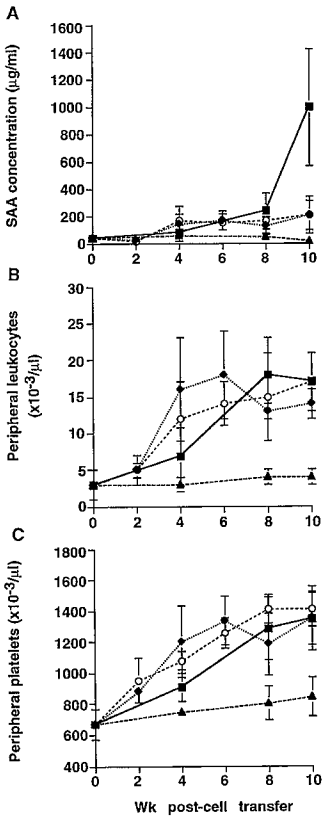

Figure 3. Development of IBD in RAG-$2^{-/-}$ recipients of sorted IL-$10^{-/-}$ LPL. RAG-$2^{-/-}$ mice were injected with sorted LPL isolated from IL-$10^{-/-}$ colon: (*square*) 5×10^5 $CD4^+CD8\alpha^-$; (*diamond*) 5×10^4 $CD4^+CD8\alpha^-$; (*open circle*) 10^5 $CD4^+CD8\alpha^+$; and (*triangle*) 3×10^5 $CD4^-CD8\alpha^+$. (*A*) SAA, (*B*) peripheral leukocyte, and (*C*) platelet counts were measured every 2 wk for 10 wk. The statistical significance between values for each group was assessed using an unpaired Student's *t* test. From 4–6 wk post-cell transfer, the values for recipients of any CD4-expressing population did not differ statistically from one another but were significantly greater than those of $CD4^-CD8\alpha^+$ LPL recipients. Data represent the mean of two independent experiments involving multiple mice per group.

(data not shown). The phenotype of lymphocytes recovered from the colon epithelial and lamina propria compartments of RAG-$2^{-/-}$ recipients of either WT or IL-$10^{-/-}$ IEL was comparable with that after transfer of LPL. Furthermore, IEL could also reconstitute both the epithelial and lamina propria compartments of the RAG-$2^{-/-}$ mouse colon.

RAG-$2^{-/-}$ Recipients of $CD4^+CD8\alpha^-$ or $CD4^+CD8\alpha^+$ IL-$10^{-/-}$ LPL Develop IBD. To determine which T cell subsets could mediate colitis, $CD4^+CD8\alpha^-$, $CD4^+CD8\alpha^+$, and $CD4^-CD8\alpha^+$ T cells were sorted from the lamina propria of colitis-affected IL-$10^{-/-}$ mice and transferred into RAG-$2^{-/-}$ hosts. Characteristics indicative of an inflammatory response, such as SAA levels and peripheral blood leukocyte and platelet counts, were monitored periodically in recipient mice for 10 wk to assess the kinetics of IBD development (Fig. 3). Recipients of either $CD4^+CD8\alpha^-$ or $CD4^+CD8\alpha^+$ IL-$10^{-/-}$ LPL showed steadily increasing SAA levels, leukocyte and platelet numbers over the 10-wk period. These values were statistically comparable, suggesting that both of these subsets could induce signs of inflammation at an equivalent rate. The SAA, leukocyte, and platelet values of recipients of IL-$10^{-/-}$ $CD4^-CD8\alpha^+$ LPL were not different from values for unmanipulated RAG-$2^{-/-}$ mice (data not shown).

A histological assessment of transplanted RAG-$2^{-/-}$ recipients confirmed that both $CD4^+CD8\alpha^-$ and $CD4^+CD8\alpha^+$ LPL from IL-$10^{-/-}$ mice could induce IBD (Table 4, Fig. 4). Recipients of $CD4^+CD8\alpha^-$ LPL, irrespective of the cell number administered (10-fold range), developed severe colitis that was comparable with that in donor IL-$10^{-/-}$ mice (Fig. 4, *A* and *B*). The colitis featured diffuse inflammation with a mucosal and submucosal infiltration of lymphocytes and neutrophils, numerous multinucleated giant cells, crypt abscesses, and epithelial hyperplasia. This inflammation was similar to that induced by unseparated IL-$10^{-/-}$ LPL. In contrast, recipients of WT $CD4^+CD8\alpha^-$ LPL showed only a limited leukocyte reconstitution in the

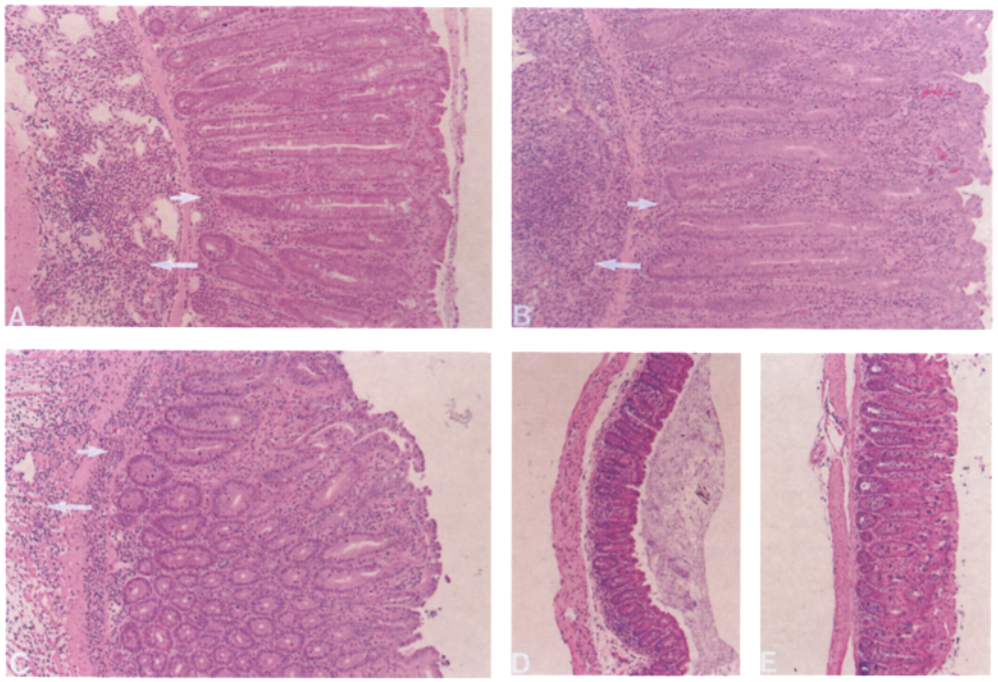

Figure 4. IBD can be induced in RAG-$2^{-/-}$ recipients of $CD4^+CD8\alpha^-$ or $CD4^+CD8\alpha^+$ IL-$10^{-/-}$ LPL. (*A–D*) Colon sections stained with H & E. Colon inflammation is characterized by extensive mucosal (*short arrow*) and submucosal (*long arrow*) mononuclear cell infiltration and epithelial cell hyperplasia. (*A*) Donor IL-$10^{-/-}$ mouse; (*B*) RAG-$2^{-/-}$ recipient of IL-$10^{-/-}$ $CD4^+CD8\alpha^-$ LPL; (*C*) RAG-$2^{-/-}$ recipient of IL-$10^{-/-}$ $CD4^+CD8\alpha^+$ LPL; (*D*) RAG-$2^{-/-}$ recipient of $CD4^-CD8\alpha^+$ LPL; and (*E*) unmanipulated RAG-$2^{-/-}$ colon. ×65.

Table 4. *Colitis in RAG-2$^{-/-}$ Mice after Reconstituiton with CD4/CD8-defined LPL*

Phenotype of LPL inoculum			Colitis*		
			Normal	Mild	Severe
CD4$^+$CD8α$^-$	IL-10$^{-/-}$	5 × 10^5	0/5	1/5	4/5
		5 × 10^4	0/9	3/9	6/9
	IL-10$^{+/+}$	1 × 10^5	2/3	1/3	0/3
CD4$^+$CD8α$^+$	IL-10$^{-/-}$	1 × 10^5	0/5	5/5	0/5
		5 × 10^4	0/8	8/8	0/8
	IL-10$^{+/+}$	ND‡			
CD4$^-$CD8α$^+$	IL-10$^{-/-}$	3 × 10^5	4/4	0/4	0/4
		1 × 10^5	3/3	0/3	0/3
	IL-10$^{+/+}$	1 × 10^5	4/4	0/4	0/4

*Colon pathology (defined in legend to Table 2) assessed 10–12 wk post-cell transfer.
‡Not done as extremely low number of CD4$^+$CD8α$^+$ T cells present in IL-10$^{+/+}$ lamina propria.

colon. One WT animal showed a multifocal inflammation that was less severe than recipients of IL-10$^{-/-}$ cells. None of the recipients showed significant pathology in any other organs examined. RAG-2$^{-/-}$ recipients of IL-10$^{-/-}$ CD4$^+$-CD8α$^+$ LPL also suffered from colitis (Fig. 4 *C*), although it was not as severe as that induced by an equivalent number of CD4$^+$CD8α$^-$ LPL (5 × 10^4 cells, Table 4). In contrast, colitis did not develop in recipients of either IL-10$^{-/-}$ or WT CD4$^-$CD8α$^+$ LPL. The colons of these mice were microscopically identical to unmanipulated RAG-2$^{-/-}$ mice (Fig. 4, *D* and *E*), and flow cytometry revealed a lack of lymphoid reconstitution (data not shown).

Immunohistological studies demonstrated the distribution of CD4$^+$ and CD8α$^+$ T cells in the colon of diseased RAG-2$^{-/-}$ recipients of IL-10$^{-/-}$ T cells. When CD4$^+$-CD8α$^-$ LPL were transferred into RAG-2$^{-/-}$ mice, CD4$^+$ (Fig. 5 *A*) and CD8α$^+$ (Fig. 5 *B*) cells were clearly evident in both the epithelial and lamina propria compartments of the host colon. Neither CD4$^+$ nor CD8$^+$ lymphocytes were detected in the colon of unmanipulated RAG-2$^{-/-}$ mice (data not shown). Flow cytometric analysis of lymphocytes recovered from the colon of RAG-2$^{-/-}$ recipients of IL-10$^{-/-}$ CD4$^+$CD8α$^-$ LPL revealed that all lymphocytes expressing CD8α coexpressed CD4. Although the proportion of transplanted CD4$^+$CD8α$^-$ LPL that acquired CD8α expression in the RAG-2$^{-/-}$ host was variable between experiments (20–80%), the majority of cells consistently remained CD45RB$^{-/low}$ and CD44$^+$ (data not shown).

Cytokine Production by IL-10$^{-/-}$ LPL Subsets Recovered from RAG-2$^{-/-}$ Recipients. Lymphocytes were isolated from the colon of RAG-2$^{-/-}$ recipients of either WT or IL-10$^{-/-}$ LPL . The ability of these cells to produce IFN-γ and IL-4 was assessed by flow cytometry after a brief in vitro stimulation. The results clearly show that IL-10$^{-/-}$ αβTCR$^+$ T cells recovered from RAG-2$^{-/-}$ mice with colitis produced IFN-γ, but very little IL-4 (Fig. 6). Interestingly, WT αβTCR$^+$ T cells isolated from recipients that did not have colitis also produced IFN-γ but not IL-4. As three- to fourfold more lymphocytes were recovered from recipients of IL-10$^{-/-}$ LPL as compared with WT LPL, we presumed that the former possessed significantly more T cells capable of producing IFN-γ.

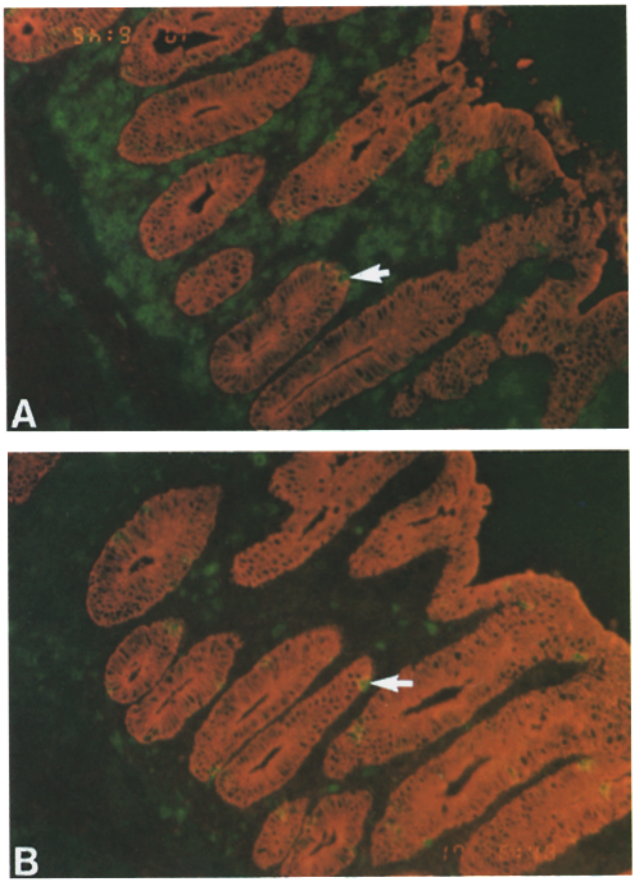

Figure 5. (*A* and *B*) Colon of RAG-2$^{-/-}$ recipient of IL-10$^{-/-}$ CD4$^+$CD8α$^-$ LPL. Frozen tissue sections stained with rhodamine-conjugated anticytokeratin to reveal epithelial cells (red) and either FITC-conjugated anti-CD4 (*A*) or anti-CD8α (*B*). (*Arrows*) IEL. ×110.

This hypothesis was confirmed by further analysis of T cell subsets isolated from the lamina propria of RAG-2$^{-/-}$ mice reconstituted with WT or IL-10$^{-/-}$ LPL. A larger number of CD4$^+$CD8α$^-$ and CD4$^+$CD8α$^+$ lymphocytes were present in recipients of IL-10$^{-/-}$ LPL that were capable of producing IFN-γ (Table 5). This represented a 7- and 22-fold increase, respectively, in the number of IFN-γ–producing cells present among the CD4$^+$CD8α$^-$ and CD4$^+$-CD8α$^+$ IL-10$^{-/-}$ LPL compared to that of WT cells.

Perhaps the most striking finding was that we could detect ~120 pg/ml of IFN-γ in the sera of RAG-2$^{-/-}$ recipients of IL-10$^{-/-}$ LPL, whereas this cytokine was undetectable in the sera of recipients without colitis.

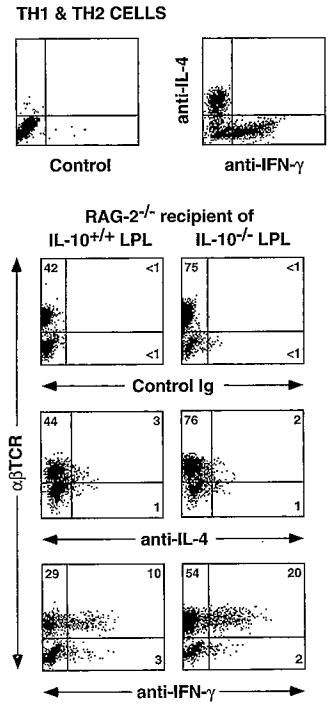

Figure 6. Cytokine production by LPL recovered from the colon of transplanted RAG-$2^{-/-}$ mice. A TH1 cell line and a TH2 clone were mixed after stimulation and included as positive controls for cytokine staining (*top*). Lymphocytes were isolated from the colons of RAG-$2^{-/-}$ mice 8 wk post-cell transfer of either 10^6 WT or IL-$10^{-/-}$ LPL and stained for IFN-γ and IL-4 production after 4 h of in vitro stimulation (*bottom*). The results are representative of two experiments.

Discussion

IL-$10^{-/-}$ mice develop chronic enterocolitis that is characterized by extensive epithelial hyperplasia, intense mononuclear infiltration of the mucosa, and a high incidence of colorectal carcinoma (16, 17, 18). Our aim was to ascertain which cell types are primarily involved in mediating IBD in the IL-$10^{-/-}$ mouse model.

A pathogenic role for intestinal B cells and Abs has been implicated by a number of recent findings. Serum IgA and IgG1 levels are elevated in IL-$10^{-/-}$ mice (16), and serum IgG from IL-$10^{-/-}$ mice has been shown to react with enteric bacterial antigens by Western blotting (Elson, C., and S. Brandwein, University of Alabama, Birmingham, AL, personal communication). These findings are consistent with our demonstration herein that the frequency of Ig^+ cells in the colon lamina propria was increased in IL-$10^{-/-}$ mice. Given that human IBD patients may possess potentially pathogenic autoAbs that cross-react with enteric flora (1–3, 6, 7), we examined the cross-reactivity of IL-$10^{-/-}$ serum Ig with various monkey tissues. IgG reacting specifically with colon epithelial and nonepithelial cells was present in the majority of IL-$10^{-/-}$ sera tested. To ascertain the role of these Abs, we generated a B cell–deficient strain of IL-$10^{-/-}$ mice. The finding that IBD develops in $B^{-/-}$IL-$10^{-/-}$ mice indicated that neither B cells nor their Ab products were necessary for the initiation or perpetuation of IBD in the IL-$10^{-/-}$ mouse model. Therefore, our data would suggest that colon-reactive Abs in IL-$10^{-/-}$ mice are simply an epiphenomenon related to inflammation of the colon.

The inflammatory infiltrate in the colon of colitis-affected IL-$10^{-/-}$ mice also included abnormally high numbers of activated/memory $\alpha\beta TCR^+$ T cells expressing either CD4 alone or in combination with CD8α (17, 18). These cells were capable of producing large amounts of IFN-γ but little or no IL-4 after in vitro stimulation, suggesting a pathogenic role for TH1-like T cells in the IL-$10^{-/-}$ mouse model of IBD. The present study has clearly demonstrated that IL-$10^{-/-}$ LPL can induce colitis upon transfer to immunodeficient RAG-$2^{-/-}$ recipients whereas WT cells had no such effect. The inflammation induced in RAG-$2^{-/-}$ mice by IL-$10^{-/-}$ LPL was characterized by extensive mononuclear infiltration of the colonic and cecal mucosa and submucosa, epithelial cell hyperplasia, and crypt abscesses. This profile was similar to the major features of IBD observed in donor IL-$10^{-/-}$ mice (16–18). IEL isolated from IL-$10^{-/-}$ mice also appeared to have the capacity to induce colitis in RAG-$2^{-/-}$ recipients, albeit less efficiently than

Table 5. *Cytokine Production by T Cell Subsets Recovered from the Lamina Propria of RAG-$2^{-/-}$ Mice Transplanted with IL-$10^{+/+}$ or IL-$10^{-/-}$ LPL*

T cell subset recovered from host RAG-$2^{-/-}$ lamina propria	Source of donor lymphocytes: IL-$10^{+/+}$ LPL: Total no. cells ($\times 10^{-4}$)	IL-$10^{+/+}$ LPL: No. of cells ($\times 10^{-4}$) producing: IL-4	IL-$10^{+/+}$ LPL: No. of cells ($\times 10^{-4}$) producing: IFN-γ	IL-$10^{-/-}$ LPL: Total no. cells ($\times 10^{-4}$)	IL-$10^{-/-}$ LPL: No. of cells ($\times 10^{-4}$) producing: IL-4	IL-$10^{-/-}$ LPL: No. of cells ($\times 10^{-4}$) producing: IFN-γ
$CD4^+CD8\alpha^-$	20.4*	1.4‡	3.6	93.9*	1.5‡	24.9
$CD4^+CD8\alpha^+$	1.4	0.2	0.2	15.1	0.3	4.5

*Average number of CD4/CD8-defined lymphocytes recovered per colon from a pool of four mice.

‡Data indicate total number of T cells in each defined subset producing IL-4 or IFN-γ. LPL were recovered from the colons of RAG-$2^{-/-}$ mice 8 wk after transfer of either 10^6 IL-$10^{+/+}$ WT or IL-$10^{-/-}$ LPL and stained for IFN-γ and IL-4 production after 4 h of in vitro stimulation. Total colon lymphocyte recovery from individual RAG-$2^{-/-}$ recipients of IL-$10^{+/+}$ LPL was (6.0 ± 6.9) × 10^5 and recovery from RAG-$2^{-/-}$ recipients of IL-$10^{-/-}$ LPL was (16.8 ± 7.2) × 10^5. The results are representative of two to four experiments.

IL-10$^{-/-}$ LPL. However, given that LPL can traffic between the epithelial and lamina propria compartments (24, and Figs. 2 and 5), we suspect that colitis-causing effector cells present within the IL-10$^{-/-}$ IEL may have originated from the lamina propria.

Analysis of the infiltrating lymphocytes after transfer of LPL revealed that the expansion of IL-10$^{-/-}$ cells in RAG-2$^{-/-}$ recipients was three- to fourfold greater than that of WT cells. The phenotypic profile of donor lymphocytes recovered from the epithelial or lamina propria compartments of either groups of recipients was similar. The majority of lymphocytes were $\alpha\beta$TCR$^+$ and those expressing CD4 were mainly activated/memory cells, being CD45RB$^{-/low}$ and CD44$^+$. The most striking difference between the experimental groups was a large increase in the absolute number of CD4$^+$CD8α^+ lymphocytes found in both the lamina propria and the epithelial compartments of RAG-2$^{-/-}$ recipients of IL-10$^{-/-}$ cells. This very high incidence of CD4$^+$CD8α^+ lymphocytes implicated a role for this T cell subset in the disease process.

To determine which populations of IL-10$^{-/-}$ T cells could mediate IBD, we adoptively transferred purified CD4$^+$CD8α^-, CD4$^+$CD8α^+, or CD4$^-$CD8α^+ LPL into RAG-2$^{-/-}$ recipients. The ability of CD4$^+$CD8α^- and CD4$^+$CD8α^+ T cell subsets to induce colitis was confirmed by histological analysis of colon tissues. The kinetics of disease development, as measured by SAA, peripheral leukocyte, and platelet values were statistically comparable, indicating that these two populations of T cells had similar functional abilities with respect to IBD induction. Colitis was not induced by IL-10$^{-/-}$ CD4$^-$CD8α^+ LPL as these cells failed to reconstitute the gut. Other reports have documented the inability to establish a population of CD8$^+$ T cells in the gut and other organs of *scid* mice (26, 27). CD4$^-$CD8α^+ T cells could be detected in the gut of RAG-2$^{-/-}$ mice after reconstitution with unseparated LPL or IEL, suggesting that CD8$^+$ T cells do not home well to gut tissues and/or do not proliferate upon arrival in the absence of help from other T cell subsets. Hence, a role for CD8$^+$ T cells in the pathogenesis of IBD in IL-10$^{-/-}$ mice cannot be excluded, although they were not required for transfer of the disease to immunodeficient hosts (this study; 13, 14, 28, 29).

We have demonstrated herein that CD4$^+$CD8α^+ T cells isolated from the lamina propria of IL-10$^{-/-}$ colon were capable of inducing IBD in RAG-2$^{-/-}$ mice. This result was surprising given that CD4$^+$CD8α^+ T cells are normally present in the epithelial compartment of the small intestine and are nonpathogenic (24). Recent studies have demonstrated that they derive from CD4$^+$CD8α^- T cells (30, 31). CD4$^+$CD8α^+ T cells are conspicuously absent in the small intestine of germ-free rodents (32, 33), implying that the acquisition of CD8α by CD4$^+$ T cells is driven by a response to enteric flora. In support of this premise, human CD4$^+$ T cells will synthesize and express CD8α after mitogen-induced activation (34, 35). Compared with conventional CD4$^+$CD8α^- lymphocytes, CD4$^+$CD8α^+ IEL exhibit poor proliferative responses to in vitro stimulation with nominal antigen, superantigens, or anti-TCR mAbs (25, 36). This low proliferative potential may explain the failure of CD4$^+$CD8α^+ T cells purified from normal mice to reconstitute the tissues of *scid* mice (30). Other in vitro studies have shown that CD4$^+$CD8α^+ IEL can become cytotoxic upon TCR triggering (36) and can produce various cytokines including IFN-γ (37). Presently, the in vivo role of CD4$^+$CD8α^+ T cells in mucosal immunity is not known. Herein, we have demonstrated that CD4$^+$CD8α^+ T cells derive from CD4$^+$CD8α^- LPL during a pathogenic response and they can mediate IBD in immunodeficient recipients in the absence of other T cell subsets. However, their pathogenic potential was not as great as CD4$^+$CD8α^- LPL in this transfer model. Given that CD4$^+$CD8a$^+$ cells may have a low proliferative potential (25, 26), the expansion of effector cells upon transfer to RAG-2$^{-/-}$ mice may be less efficient than that of CD4$^+$CD8α^- LPL. Alternatively, CD4$^+$CD8α^+ LPL may not home to the gut as well as CD4$^+$CD8α^- LPL. Regardless, CD4$^+$CD8α^+ T cells are clearly an effector of IBD in IL-10$^{-/-}$ mice and, therefore, have a pathogenic potential that has not been heretofore identified for this T cell subset.

To assess the pathogenic function of transplanted IL-10$^{-/-}$ cells, T cells recovered from RAG-2$^{-/-}$ recipients of IL-10$^{-/-}$ or WT LPL were analyzed for cytokine production. No differences between IL-10$^{-/-}$ and WT LPL were observed as both populations included cells that could be induced to produce IFN-γ in vitro, whereas few cells from either subset produced IL-4. This implied that IL-10$^{-/-}$ T cells reconstituting RAG-2$^{-/-}$ recipients were not abnormally skewed towards a TH1 phenotype, although in terms of absolute numbers, TH1-like cells were more abundant in the diseased recipients. A more relevant finding was that IFN-γ could be detected in the sera of RAG-2$^{-/-}$ recipients with colitis. Apparently, a TH1 response in the colon becomes pathogenic when it results in the excessive production of cytokines such as IFN-γ.

It is clear that IL-10 plays a fundamental role in the controlled development of TH1 mucosal responses in vivo. Exaggerated IFN-γ production was readily detected in the original donor IL-10$^{-/-}$ mice (18), but interestingly, IL-10$^{-/-}$ T cells continued to produce large amounts of IFN-γ after reconstituting RAG-2$^{-/-}$ tissues that contained macrophages and epithelial cells capable of producing IL-10 (19, 20). This observation suggests that the most critical source of IL-10 is the T cells themselves. Recently, a murine model of IBD has been described where C.B-17 *scid* mice develop severe colitis after reconstitution with the CD45RB^{hi} fraction of CD4$^+$ spleen cells from normal BALB/c mice (13, 14, 28). The CD45RB^{low}CD4$^+$ splenic T cells had no such effect and, when coinjected with the CD45RB^{hi} fraction, were able to prevent colitis development. It was found that colitis induction by CD45RB^{hi}CD4$^+$ T cells was due to their elevated IFN-γ and TNF production (13, 28). It has been proposed that CD45RB^{low}CD4$^+$ T cells contain a regulatory subset that inhibits the in vivo TH1 responses of CD45RB^{hi}CD4$^+$ cells. This regulation may involve IL-10 as systemic treatment of *scid* recipients

of $CD45RB^{hi}CD4^+$ T cells with IL-10 prevented the development of colitis (28).

As $IL\text{-}10^{-/-}$ mice and $CD45RB^{hi}CD4^+$ T cell-reconstituted *scid* mice are models of IBD involving a pathogenic $CD4^+$ TH1 response, a comparison of these systems is highly informative. The induction of colitis by $IL\text{-}10^{-/-}$ $CD4^+$ LPL did not depend upon their separation into $CD45RB^{hi}$ and $CD45RB^{low}$ fractions. Furthermore, the majority of $IL\text{-}10^{-/-}$ $CD4^+$ LPL used in these reconstitution studies were $CD45RB^{low}$, possessing the phenotype but not the function of regulatory $CD45RB^{low}$ splenic T cells from normal mice. An obvious explanation for this is that regulatory cells are present within the $IL\text{-}10^{-/-}$ $CD45RB^{low}CD4^+$ LPL population but are ineffective because of their inability to produce IL-10. However, this conclusion may be oversimplified when other data are taken into consideration. For example, IL-10 treatment of weanling $IL\text{-}10^{-/-}$ mice prevented the induction of IBD, whereas delayed administration of IL-10 was unable to eliminate established disease (18). This finding is contrary to the concept that regulatory cells need only to produce IL-10 in order to control TH1 responses. It is possible that IL-10 may play a critical role in the generation of regulatory cells which mediate their control independently of their own production of IL-10. Relevant to this, in the $CD45RB^{hi}$ transfer model, IL-10 production by $CD45RB^{low}$ cells that provide protection is as low as that of $CD45RB^{hi}$ cells inducing colitis (13).

In conclusion, we have clearly established that TH1-like $\alpha\beta TCR^+CD4^+CD8\alpha^-$ and $CD4^+CD8\alpha^+$ T cells mediate IBD in $IL\text{-}10^{-/-}$ mice. As IBD in numerous murine models is proposed to result from a perturbation of T cell–dependent regulation in the gut and a subsequent breakdown of oral tolerance (38, 39), we are employing the $IL\text{-}10^{-/-}$ and the $CD45RB^{hi}CD4^+$ T cell transfer models of IBD to define the regulatory circuits that ensure the integrity of the mucosal immune response.

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