American Journal of Pathology, Vol. 148, No. 5, May 1996

Inflammatory Bowel Disease in C.B-17 *scid* Mice Reconstituted with the CD45RB^{high} Subset of CD4$^+$ T Cells

Michael W. Leach,* Andrew G. D. Bean,† Smita Mauze,† Robert L. Coffman,† and Fiona Powrie†

From the Schering-Plough Research Institute, Lafayette, New Jersey, and DNAX Research Institute of Molecular and Cellular Biology Inc.,† Palo Alto, California*

Chronic inflammation developed spontaneously in the large intestine of C.B-17 scid mice restored with the CD45RB^{high} subset of CD4$^+$ T cells obtained from normal BALB/c mice. The inflammation, which extended diffusely from the cecum to the rectum, was localized to the lamina propria of mildly affected mice but became transmural in severely affected mice. Immunohistochemical and flow cytometric analyses showed that the inflammatory infiltrate contained numerous macrophages accompanied by moderate numbers of activated CD4$^+$ lymphocytes. Some mice also had scattered multinucleated giant cells. Mucin depletion and epithelial hyperplasia resulting in glandular elongation and mucosal thickening were also consistently seen. Less frequent findings included ulceration with fibrosis, crypt abscesses, crypt loss, and granulomatous inflammation. Immunofluorescent analysis of inflamed large intestinal sections demonstrated increased epithelial expression of major histocompatibility class II antigens. The changes in the large intestine of these mice are similar to those seen in patients with idiopathic inflammatory bowel disease (Crohn's disease and ulcerative colitis). This murine model may be useful for studying mucosal immunoregulation as it relates to the pathogenesis and treatment of chronic inflammatory bowel diseases in the large intestine of human patients. (Am J Pathol 1996, 148:1503–1515)

A number of chronic gastrointestinal inflammatory diseases exist in human beings. Some of the most enigmatic are the idiopathic inflammatory bowel diseases (IBDs), which encompass at least two forms of intestinal inflammation, namely, Crohn's disease (CD) and ulcerative colitis (UC).[1] In CD, and to a lesser extent in UC, there is compelling evidence that T-cell-mediated immune reactions play a major role in the disease processes.[2–9] However, the exact role of the immune system in the initiation, perpetuation, and resolution of the inflammatory processes in CD and UC remains unclear.

To better understand IBD and to develop appropriate therapies, a number of animal models of intestinal inflammation have been investigated. However, none have proven to be entirely representative of either CD or UC. Many models have relied on administration of bacteria or chemical irritants to normal animals to induce intestinal inflammation.[10–13] The inflammatory responses in these models may therefore be considered to represent the appropriate immune responses of normal animals to the inciting noxious stimuli. Thus, although these investigations have provided important information about the mechanisms of intestinal damage and repair, they have provided little information about the possible cause(s) of the uncontrolled chronic inflammation characteristic of IBD. As current evidence suggests that a dysregulated immune reaction to normal flora may be important in the pathogenesis of IBD, examination of animals with spontaneously occurring chronic intestinal inflammation might provide a model with pathogenetic similarities to human IBD.[2,14] Such a model of a dysregulated inflammatory response should increase our understanding of how the inflammatory process in IBD is initiated and

DNAX Research Institute of Molecular and Cellular Biology is supported by the Schering-Plough Corporation.

Accepted for publication January 15, 1996.

Address reprint requests to Dr. Michael W. Leach, Schering-Plough Research Institute, PO Box 32, Route 94 South, Lafayette, NJ 07848-0032.

how it might be controlled by endogenous cells or pharmacological intervention.

During lymphocyte transfer experiments in genetically immunodeficient mice, we and others noted that chronic large intestinal inflammation spontaneously developed after transfer of CD45RB^{high} CD4^{+} T cells from BALB/c or C.B-17 mice to C.B-17 *scid* mice.[15–17] Previous reports of this model have only briefly characterized the pathological findings in the large intestine.[15–17] The purpose of the present report is to characterize the morphological features, immunohistological changes, and major histocompatibility complex (MHC) class II expression seen in this model and discuss the relevance of this model to human IBD.

Materials and Methods

Animals

Female BALB/c and C.B-17 (*scid/scid*) mice (Simonsen Laboratories, Gilroy, CA) were maintained in microisolator cages or contained in laminar flow hoods under specific-pathogen-free conditions. Sentinel mice were also maintained in the colony under identical conditions. Mice were 8 to 12 weeks of age at the initiation of experiments.

Cell Purification and Reconstitution of C.B-17 scid Mice

Lymphocyte purification and reconstitution were performed exactly as described.[16,17] Briefly, CD4^{+} CD45RB^{high} and CD45RB^{low} populations were purified from spleen cells from BALB/c mice by two-color sorting on a FACStar Plus (Becton Dickinson, Sunnyvale, CA). The CD45RB^{high} population was defined as the brightest staining 40 to 50% of CD4^{+} cells. The CD45RB^{high} CD4^{+} T cell subpopulation was at least 98% pure on reanalysis. C.B-17 *scid* mice were injected intravenously with 1×10^5 to 5×10^5 sorted CD45RB^{high} CD4^{+} T cells suspended in 100 μl of phosphate-buffered saline (PBS).

Histopathology

Between 4 and 12 weeks after intravenous injection of the CD45RB^{high} CD4^{+} T cells, *scid* mice were sacrificed by cervical dislocation and tissues were collected, fixed in 10% neutral buffered formalin, routinely processed, and stained with hematoxylin and eosin (H&E) for light microscopic examination. One sample from each of the following digestive tract tissues was examined: tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, ascending colon, transverse colon, descending colon, and rectum. Stomachs were sectioned to include nonglandular, fundic, and pyloric regions. Tongue, esophageal, and intestinal samples (except rectum) were sectioned transversely. Rectal samples were sectioned longitudinally. Other tissues examined included liver, pancreas, mandibular salivary gland, kidney, urinary bladder, thymus, lung, trachea, thyroid gland, and heart. Lymph nodes were not specifically collected but were in some cases present in the samples examined microscopically.

Tissues were evaluated by routine microscopic examination by one observer (M. W. Leach). The data represent the results from 16 separate experiments in which mice were injected with the CD45RB^{high} subset of CD4^{+} T cells. Normal female BALB/c mice were used as controls. The large intestinal samples were graded semiquantitatively as 0 (no change) to 4 (most severe) for the following inflammatory lesions: severity of chronic inflammation, crypt abscesses, and granulomatous inflammation; and for the following epithelial lesions: hyperplasia, mucin depletion, ulceration, and crypt loss. Results are expressed as the mean of the grades $\pm$ standard deviation of affected mice. In addition, the depth of the inflammatory process into the large intestinal wall was categorized as extending into the mucosa, the submucosa, or the tunica muscularis or as being transmural (extending to the serosa).

Immunohistochemistry

Immunohistochemical staining of frozen sections of transverse colon was performed by a peroxidase technique, using the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) and diaminobenzidine substrate kit (Vector), according to the manufacturer's instructions. The following monoclonal antibodies were used: M1/70, anti-mouse Mac-1 (ATCC TIB128); and RM4–4 and RM4–5, anti-mouse CD4 (PharMingen, San Diego, CA). Substitution of an irrelevant monoclonal antibody of the same isotype was used as a control. The immunohistochemical analysis was performed on four mice per experiment and was repeated in three separate experiments.

Flow Cytometric Analysis of Lamina Propria Lymphocytes

Lamina propria lymphocytes were purified from the transverse colon of normal BALB/c mice or mice

receiving the CD45RB^{high} subset of CD4$^+$ T cells, as previously described.[17] Briefly, lamina propria cells were isolated from pieces of transverse colon measuring 0.5 to 1 cm in length. Lamina propria cells were then layered on a Percoll (Pharmacia LKB, Upsala, Sweden) gradient (bottom layer, 2 ml of 75% Percoll; middle layer, 3 ml of 40% Percoll; top layer, 2 ml of 30% Percoll) and spun at 600 × *g* for 20 minutes at room temperature. Lymphoid cells were isolated from the 75% and 40% interface. The following monoclonal antibodies were used for fluorescence-activated cell sorting analysis: fluorescein isothiocyanate (FITC)-conjugated 16A, anti-mouse CD45RB (PharMingen); phycoerythrin-conjugated anti-mouse CD4 (Caltag, South San Francisco, CA), and FITC- and PE-conjugated isotype control monoclonal antibodies (PharMingen). Samples were analyzed on a FACScan using CellQuest software (Becton Dickinson). Data were pooled from four mice per experiment and represent the percentage of cells within the lymphoid gate, determined on forward and side-scatter analysis. The flow cytometric analysis was repeated in three separate experiments.

Immunofluorescence

Transverse colon samples from mice receiving the CD45RB^{high} subset of CD4$^+$ T cells were examined. Transverse colon samples from normal BALB/c mice and C.B-17 *scid* mice reconstituted with unseparated T cells (which do not develop disease[16]) served as controls. Five samples from each of two separate experiments were examined from each treatment group. Colons were removed, trimmed of excess fat, and washed extensively in PBS. The tissue was then immersed in Tissue-Tek embedding compound (Miles Scientific, Elkhart, IN) and snap frozen in liquid nitrogen. Four-micron-thick sections were cut and immediately air dried onto glass microscope slides.

Rat anti-mouse MTS6 (monoclonal anti-I-A and anti-I-E, undiluted; a kind gift from Dr. R. L. Boyd) was used to reveal MHC class II expression, whereas a rabbit wide-spectrum anti-cytokeratin (whole rabbit serum, 1:100 dilution; Dako, Santa Barbara, CA) was used to identify epithelial cells. Tissue sections were simultaneously stained with MTS6 and anti-cytokeratin using a two-step protocol. Sections were first rinsed in 1% (v/v) fetal calf serum in PBS and then incubated with 30 μl of MTS6 and rabbit anti-cytokeratin in a humidified staining box for 20 minutes at room temperature. Sections were then washed three times for five minutes each by immersion in PBS. Next, sections were incubated in a mixture of FITC-conjugated goat anti-rat IgG (H+L, 8 μg/ml; Caltag) and tetramethyl-rhodamine-isothiocyanate-conjugated goat anti-rabbit IgM and IgG (H+L, 25 μg/ml; Southern Biotechnology Associates, Birmingham, AL) for an additional 20 minutes, and washed again as described above. Sections were mounted in Vectashield mounting medium (Vector) under glass coverslips. FITC labeling was detected using a Zeiss Axioskop fluorescent microscope set on narrow-band blue excitation (440 to 500 nm), whereas tetramethyl-rhodamine-isothiocyanate labeling was visualized using narrow-band green excitation (510 to 565 nm). Photomicrographs were taken using Kodak 1600 ASA professional film.

Detection of Pathogens

Live mice, both experimental and sentinel animals, were sent to the Research Animal Diagnostic and Investigative Laboratory, College of Veterinary Medicine, University of Missouri, for sacrifice and complete necropsy examination. Cultures from the nasopharynx, cecum, peritoneum, and liver were performed, including culture for *Citrobacter freundii*. Parasitological examination of the cecal contents and perianal area were performed. Routine serological tests for murine viral and bacterial pathogens were also performed. Sequential sections from multiple organs, including the large intestine, were stained for infectious agents using Brown and Brenn, acid fast, Steiner silver, Grocott methenamine silver, Gomori's methenamine silver, or periodic acid-Schiff stains by the diagnostic laboratory and/or by the authors.

Results

Clinical Findings

C.B-17 *scid* mice developed progressive weight loss that began 3 to 5 weeks after injection of the CD45RB^{high} subset of CD4$^+$ T cells.[16] Mice had soft stools with increased mucus but did not have diarrhea or visible blood in the stool.

Histopathology

Tissues from 79 mice were examined. The number of each specific tissue examined is shown in Tables 1 and 4. Lesions were seen with the greatest incidence and severity in the large intestine and were broadly divided into inflammatory and epithelial lesions. All mice receiving the CD45RB^{high} subset of CD4$^+$ T cells developed inflammatory and/or epithelial le-

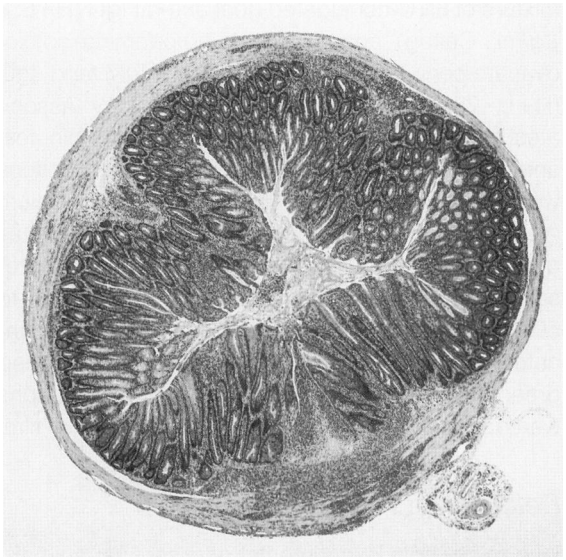

Figure 1. *Representative low magnification photomicrograph of inflammatory bowel disease in the colon of* scid *mice reconstituted with the CD45RB^{high} subset of CD4^{+} T cells. The entire circumference of the mucosa is thickened due to inflammation and epithelial hyperplasia. Note the ulcer at the bottom of the photomicrograph. H&E; magnification, ×20.*

sions in at least one of the five large intestinal samples (cecum, ascending colon, transverse colon, descending colon, and rectum) examined (data not shown). Furthermore, inflammatory and/or epithelial lesions were seen in every sample from 77% of the 60 mice from which all large intestinal samples were available, demonstrating the diffuse nature of the disease process in most animals.

At low magnification, the colon was enlarged and had a greatly thickened wall due to inflammation and epithelial hyperplasia, which involved the entire circumference of the bowel wall (Figure 1). Inflammatory lesions in the large intestine consisted of chronic inflammation, crypt abscesses, and granulomatous inflammation, as shown in Figure 2, and the incidence and severity of these changes are presented in Table 1. The chronic inflammation usually consisted of infiltrates of macrophages and lymphocytes accompanied by small numbers of neutrophils and eosinophils (Figure 2A), although in a few areas neutrophils predominated (Figure 2B). Initial lesions were characterized by a slight multifocal or diffuse increase in inflammatory cells in the lamina propria. As the severity of inflammation increased, intraepithelial lymphocytes (Figure 2A), neutrophils (Figure 2B), and crypt abscesses (Figure 2A) were seen. Additionally, inflammatory cells separated colonic glands (Figure 2, A and B), and occasional multinucleated giant cells with peripheral nuclei were observed (Figures 2A and 3A). Infrequently, the inflammation was granulomatous in small focal areas (Table 1), characterized by aggregates of epithelioid macrophages accompanied by fewer lymphocytes and neutrophils (Figure 2C). Granulomatous inflammation was often associated with lymphangitis and was usually in the mesentery attached to the large intestine but occasionally was observed in the submucosa or tunica muscularis. Multinucleated giant cells were not associated with these clusters of epithelioid macrophages. Other mesenteric inflammatory changes consisted of infiltration by lymphocytes, macrophages, and neutrophils.

Characteristic epithelial lesions (hyperplasia, mucin depletion, ulceration, and crypt loss) are shown in Figure 3, and the incidence and severity of these changes are presented in Table 2. The most striking epithelial lesion was hyperplasia, characterized by marked crypt elongation (crypt hypertrophy) (Figure 3A), villous transformation (Figure 3B), slight gland branching and gland tortuosity (Figure 3C), increased height of the epithelium lining glands (Figure 3D), increased mitotic figures (Figure 3D), increased epithelial basophilia, and cellular crowding (Figure 3D). Despite the extensive epithelial hyperplasia, morphological features suggesting neoplasia were not seen. Additional epithelial lesions consisted of mucin depletion (Figure 3A), ulceration (Figure 3C), and crypt loss (Figure 3E). Ulcers sometimes appeared to overly depleted foci of lymphoid tissue, although identification of lymphoid tissue in the intestinal tract of these *scid* mice was difficult. Ulcers were often associated with overlying neutrophils, forming a mountain peak appearance (Figure 3C), whereas underlying tissues contained numerous inflammatory cells and mild fibrosis. Other large intestinal lesions included luminal sloughed epithelial cells, luminal neutrophils (Figure 2B), individually necrotic cells within the epithelium in the deeper areas of the crypts (Figure 3D), submucosal edema seen primarily in the cecum (Figure 2C), and lymphangiectasia (Figure 2C).

As transmural inflammation is a feature of CD, the depth of inflammation in the intestinal wall was evaluated and is summarized in Table 3. Transmural inflammation developed most frequently in the transverse colon, was usually associated with more severe mucosal inflammation, and was often associated with overlying ulceration (Figure 3C). Transmural lesions were multifocal and did not involve the entire circumference of the intestinal wall.

Inflammatory lesions were also seen in other digestive tract tissues (Table 4). The stomach was most frequently affected with infiltrates of lymphocytes, neutrophils, and eosinophils located just

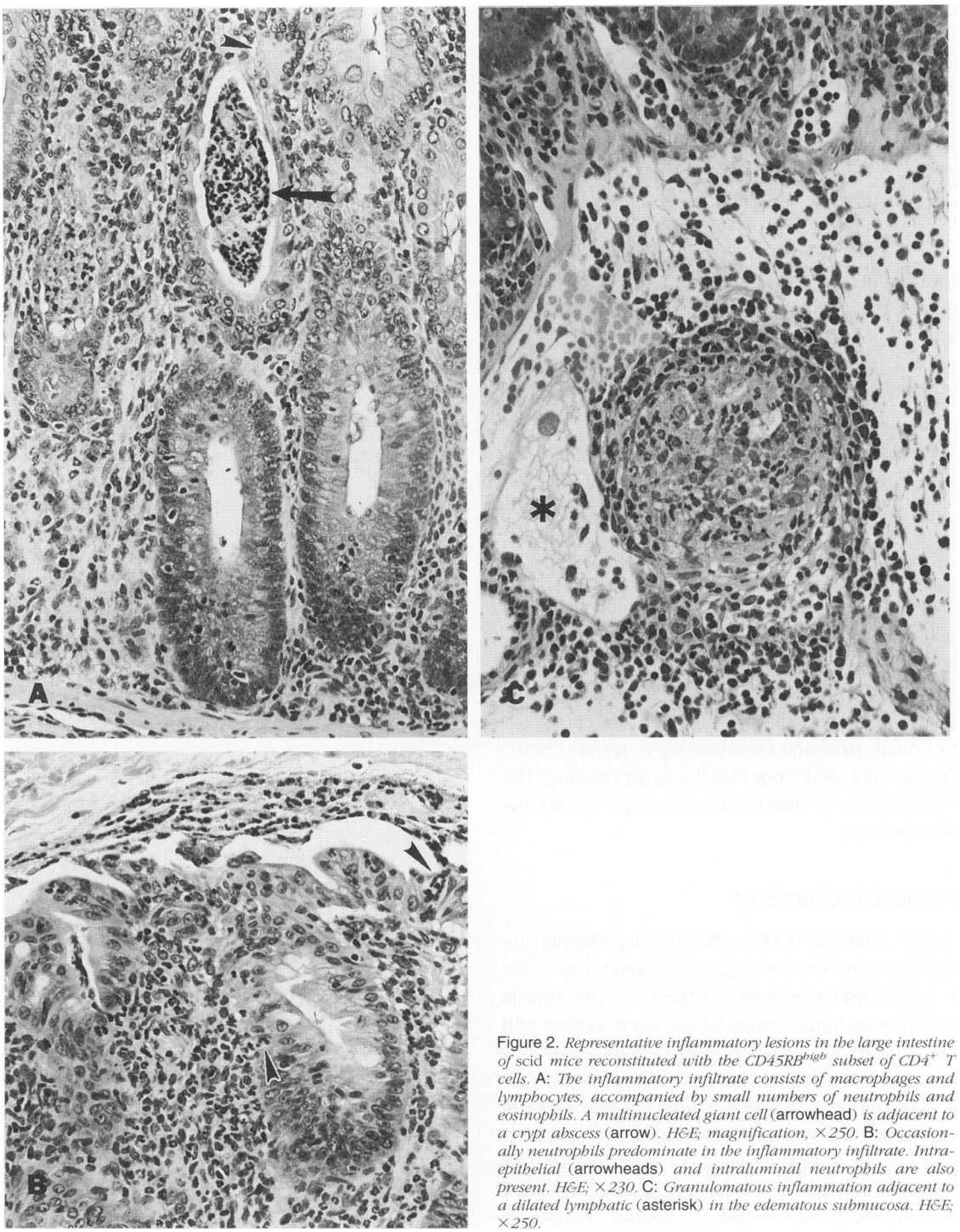

Figure 2. *Representative inflammatory lesions in the large intestine of* scid *mice reconstituted with the CD45RB^{high} subset of CD4$^+$ T cells.* A: *The inflammatory infiltrate consists of macrophages and lymphocytes, accompanied by small numbers of neutrophils and eosinophils. A multinucleated giant cell* (arrowhead) *is adjacent to a crypt abscess* (arrow). *H&E; magnification, ×250.* B: *Occasionally neutrophils predominate in the inflammatory infiltrate. Intraepithelial* (arrowheads) *and intraluminal neutrophils are also present. H&E; ×230.* C: *Granulomatous inflammation adjacent to a dilated lymphatic* (asterisk) *in the edematous submucosa. H&E; ×250.*

above the muscularis mucosa. A few mice had more severe gastritis associated with replacement of parietal and chief cells with less differentiated basophilic epithelial cells. Duodenitis was occasionally seen, consisting of a minimal to mild infiltrate of lymphocytes in the lamina propria and mild epithelial hyperplasia. Although cecal lesions were very common, no changes were noted in the ileum. Esophagitis was seen rarely and consisted of a mild epithelial and subepithelial infiltrate of lymphocytes, neutrophils, and eosinophils.

Inflammatory lesions were also seen outside of the digestive tract, most commonly in the liver (Table 4). Inflammation in the liver consisted of small numbers

Table 1. *Mean Severity and Incidence of Inflammatory Lesions in the Large Intestine of C.B-17* scid *Mice Receiving $CD4^+$ $CD45RB^{high}$ T Lymphocytes*

Tissue	n	Chronic inflammation	Crypt abscesses	Granulomatous inflammation
Cecum	76	1.7 ± 0.8 (83%)*	0.0 ± 0.0 (0%)	1.3 ± 0.5 (16%)
Ascending colon	75	2.1 ± 0.8 (93%)	1.0 ± 0.0 (4%)	1.6 ± 0.5 (11%)
Transverse colon	62	2.5 ± 0.8 (92%)	1.0 ± 0.0 (11%)	1.3 ± 0.6 (5%)
Descending colon	75	2.3 ± 0.8 (85%)	1.1 ± 0.3 (24%)	1.8 ± 0.8 (7%)
Rectum	74	2.5 ± 0.8 (91%)	1.1 ± 0.4 (41%)	1.6 ± 0.5 (9%)

Results are presented as mean severity ± SD of affected mice, with percentage of mice affected in parentheses.

of periportal lymphocytes and neutrophils, which only rarely involved bile ducts, and focal clusters of macrophages and lymphocytes randomly scattered throughout hepatic acini. The lung had minimal or mild perivascular accumulations of lymphoid cells, or multifocal clusters of macrophages and occasional multinucleated giant cells in alveoli. Inflammatory lesions in other organs were typically composed of infiltrates of lymphocytes and macrophages. Some infiltrates in the thyroid gland were particularly severe, resulting in unilateral destruction of the gland. Lymph nodes lacked corticomedullary distinction and follicles and were populated by a mixture of small (lymphocytic) and large mononuclear cells. Some mesenteric lymph nodes had prominent fibrosis.

Extramedullary hematopoiesis was seen in the livers of most mice and occasionally in lymph nodes, the mesentery, and the wall of the large intestine. The extramedullary hematopoiesis primarily involved myeloid elements.

Immunohistochemistry

To further characterize the inflammatory infiltrate, immunohistochemical staining of the colon from mice with colitis was performed (Figure 4). The results from three separate experiments were similar and demonstrated numerous Mac-1-immunoreactive cells and moderate numbers of CD4-immunoreactive cells present diffusely in the lamina propria. Mac-1 is a marker for macrophages and granulocytes.[18,19] As mononuclear cells were the primary cell type observed in the lamina propria, the colonic infiltrates were considered to contain numerous macrophages and moderate numbers of $CD4^+$ lymphocytes.

Flow Cytometry

Additional analysis of lamina propria lymphocytes isolated from the colon of mice with colitis was performed using flow cytometry. Results from three separate experiments were similar. Mice with colitis had a three-fold higher frequency of $CD4^+$ T cells in the lamina propria, compared with cells similarly isolated from control BALB/c mice, as shown in Figure 5. Additionally, 70% of the $CD4^+$ lymphocyte population in mice with colitis expressed low levels of the CD45RB antigen, suggesting that these cells were activated.[20,21] In contrast, the majority of $CD4^+$ T cells from the lamina propria of normal mice were $CD45RB^{high}$, a phenotype characteristic of naive resting T cells.[20,21]

Immunofluorescence

Immunofluorescent labeling of MHC class II antigens in the colon showed prominent expression, primarily in lamina propria cells, in both normal BALB/c mice and C.B-17 *scid* mice reconstituted with unseparated T cells (control mice, Figure 6, A–C). In contrast, MHC class II expression was prominent in the epithelium as well as in the lamina propria cells of mice reconstituted with the $CD45RB^{high}$ subset of $CD4^+$ T cells (Figure 6, D–F). Cytokeratin expression in control mice was primarily localized in the middle and basal crypt regions and in the surface epithelium (Figure 6B), whereas expression was more uniform throughout the epithelium of mice reconstituted with the $CD45RB^{high}$ subset of $CD4^+$ T cells (Figure 6E). Results from five colon samples from each of two separate experiments were similar.

Detection of Pathogens

No significant pathogens were detected in these mice. Sentinels did not develop inflammatory lesions.

Discussion

C.B-17 *scid* mice injected with $CD45RB^{high}$ $CD4^+$ T cells from BALB/c mice consistently developed chronic inflammatory and epithelial lesions that extended diffusely from the cecum to the rectum. The incidence and severity of inflammatory and epithelial

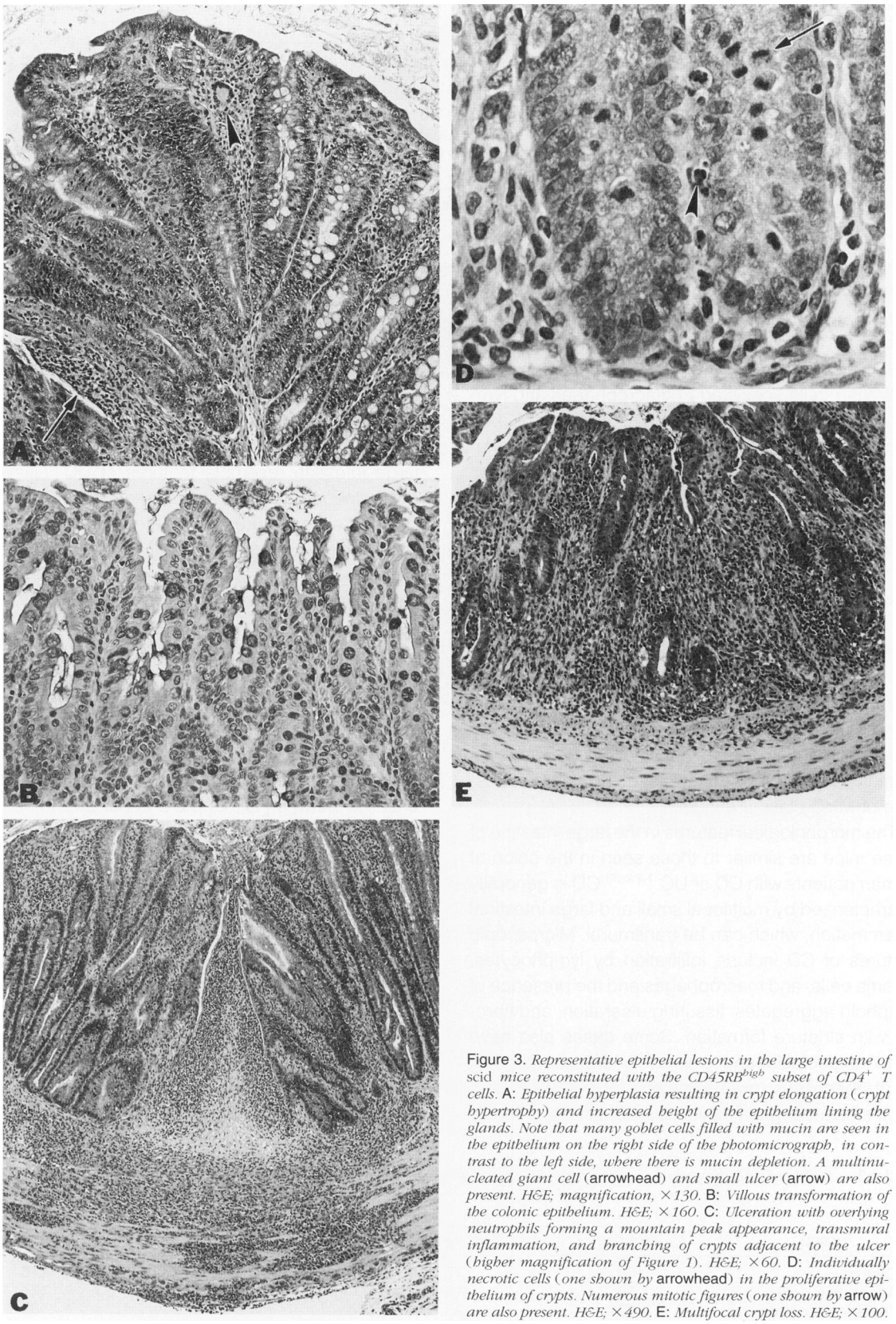

Figure 3. *Representative epithelial lesions in the large intestine of* scid *mice reconstituted with the CD45RB^{high} subset of CD4^{+} T cells.* A: *Epithelial hyperplasia resulting in crypt elongation (crypt hypertrophy) and increased height of the epithelium lining the glands. Note that many goblet cells filled with mucin are seen in the epithelium on the right side of the photomicrograph, in contrast to the left side, where there is mucin depletion. A multinucleated giant cell* (arrowhead) *and small ulcer* (arrow) *are also present. H&E; magnification, ×130.* B: *Villous transformation of the colonic epithelium. H&E; ×160.* C: *Ulceration with overlying neutrophils forming a mountain peak appearance, transmural inflammation, and branching of crypts adjacent to the ulcer (higher magnification of Figure 1). H&E; ×60.* D: *Individually necrotic cells (one shown by* arrowhead) *in the proliferative epithelium of crypts. Numerous mitotic figures (one shown by* arrow) *are also present. H&E; ×490.* E: *Multifocal crypt loss. H&E; ×100.*

Table 2. *Mean Severity and Incidence of Epithelial Lesions in the Large Intestine of C.B-17* scid *Mice Receiving $CD4^+$ $CD45RB^{high}$ T Lymphocytes*

Tissue	Epithelial hyperplasia	Mucin depletion	Ulceration	Crypt loss
Cecum	1.4 ± 0.5 (88%)	1.1 ± 0.4 (46%)	1.0 ± 0.0 (1%)	1.3 ± 0.5 (29%)
Ascending colon	2.0 ± 0.7 (91%)	2.0 ± 0.8 (88%)	1.4 ± 0.6 (29%)	1.2 ± 0.4 (43%)
Transverse colon	2.6 ± 0.6 (94%)	2.5 ± 0.9 (92%)	1.8 ± 0.8 (19%)	1.3 ± 0.5 (58%)
Descending colon	2.4 ± 0.8 (91%)	2.3 ± 0.9 (87%)	1.0 ± 0.0 (7%)	1.2 ± 0.5 (48%)
Rectum	2.4 ± 0.7 (91%)	2.3 ± 1.0 (88%)	2.0 ± 0.0 (1%)	1.2 ± 0.6 (65%)

Results are presented as mean severity ± SD of affected mice, with percentage of mice affected in parentheses.

lesions was generally greatest between the transverse colon and rectum, whereas the cecum was comparatively least affected. Inflammation was localized in the mucosa of mildly affected mice but was transmural in more severely affected animals. Infiltrating cells consisted of macrophages and lymphocytes, accompanied by small numbers of neutrophils, eosinophils, and in some mice rare multinucleated giant cells. Immunohistochemical staining of the colon showed large numbers of macrophages and moderate numbers of $CD4^+$ lymphocytes in the inflammatory infiltrate. Flow cytometric analysis of the colonic infiltrate confirmed the presence of increased numbers of $CD4^+$ lymphocytes, many with an activated phenotype. In addition to chronic inflammation, epithelial hyperplasia and mucin depletion was consistently seen. Ulceration with fibrosis, crypt abscesses, crypt loss, and granulomatous inflammation were observed less frequently. Immunofluorescent labeling of inflamed large intestinal sections demonstrated increased epithelial expression of MHC class II antigens.

The morphological features in the large intestine of these mice are similar to those seen in the colon of human patients with CD or UC.[1,22,23] CD is generally characterized by multifocal small and large intestinal inflammation, which can be transmural. Microscopic features of CD include infiltration by lymphocytes, plasma cells, and macrophages and the presence of lymphoid aggregates, fissuring ulceration, and fibrosis with stricture formation. Some cases also have lymphangiectasia and edema in the submucosa, crypt abscesses, granulomatous inflammation, and multinucleated giant cells. UC, which is primarily confined to the large intestinal mucosa, is typically characterized by diffuse inflammation extending proximally from the rectum, broad-based ulceration, mucin depletion, and crypt abscesses. The inflammatory infiltrate in UC includes plasma cells, lymphocytes, and neutrophils. Crypt elongation and branching may also be seen. The diffuse distribution of lesions in these mice and restriction primarily to the large intestine resemble features of UC. Likewise, crypt elongation and branching and extensive mucin depletion are features associated with UC. In contrast, the transmural nature of the inflammation in these mice is more similar to that seen in CD, as is the presence of many macrophages and lymphocytes, occasional multinucleated giant cells, rare crypt abscesses, ulcers with a mountain peak appearance, and submucosal lymphangiectasia and edema.[1,23] Thus, the lesions in the large intestine of these mice do not exactly mimic the features of either CD or UC. A mixture of these features is also seen in some human patients in which morphological differentiation between CD and UC is not possible.[22]

Epithelial hyperplasia was a significant feature in mice receiving $CD45RB^{high}$ $CD4^+$ T cells and has also been described in other murine models of intestinal inflammation.[10,24,25] Although increased thickness of the lamina propria is seen in both UC and CD due to inflammation and edema,[26] marked epithelial hyperplasia is not frequently described in patients with IBD. However, significantly increased intestinal epithelial cell proliferation is identified in UC patients when cell proliferation assays are used,[27,28] possibly due to T cell activation and resultant cytokine release.[29–31] Why the murine and

Table 3. *Depth of Inflammation in the Large Intestine of Affected C.B-17* scid *Mice Receiving $CD4^+$ $CD45RB^{high}$ T Cells*

Tissue	Depth of inflammation (%)			
	Mucosa	Submucosa	Tunica muscularis	Transmural
Cecum	60	17	6	16
Ascending colon	56	13	4	27
Transverse colon	32	35	0	33
Descending colon	56	33	0	11
Rectum	43	52	0	4

Table 4. *Incidence of Histopathological Inflammatory Lesions in C.B-17* scid *Mice Receiving $CD4^+$ $CD45RB^{high}$ T Cells*

Tissue	n	Incidence of inflammation (%)
Tongue	72	0
Esophagus	72	4
Stomach	69	84
Duodenum	70	20
Jejunum	72	0
Ileum	72	0
Liver	72	44
Pancreas	72	0
Salivary gland	72	1
Kidney	71	8
Urinary bladder	72	6
Thymus	72	0
Trachea	72	3
Lung	72	26
Thyroid gland	69	7
Heart	72	10

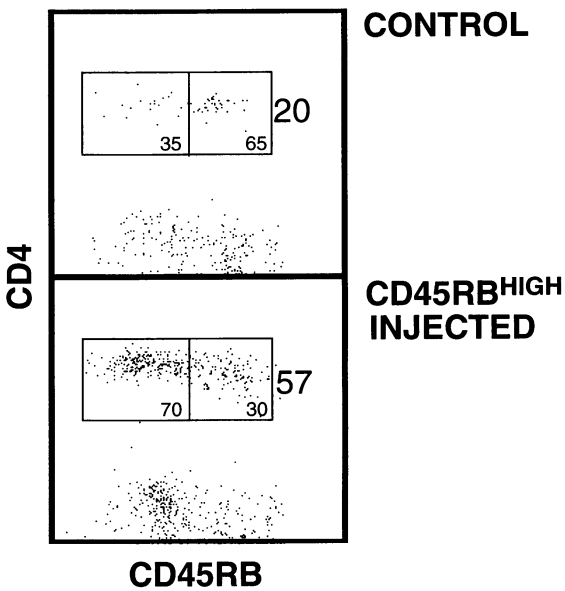

Figure 5. *Flow cytometric analysis of lamina propria lymphocytes from normal BALB/c mice (controls) or C.B-17* scid *mice restored with the $CD45RB^{high}$ subset of $CD4^+$ T cells obtained from normal BALB/c mice (mice with colitis). Compared with controls, mice injected with the $CD45RB^{high}$ subset have increased numbers of $CD4^+$ T cells, and these cells express low levels of the CD45RB antigen.*

human large intestinal epithelium appears to react differently to chronic inflammation is not known but could relate to fundamental differences in the cytokines produced during inflammation, the response of epithelial cells to the cytokines secreted, or the kinetics of large intestinal epithelial cell renewal. Cell renewal in the large intestine is longer in humans than in rodents.[32]

Increased MHC class II antigen expression was seen in the epithelium from areas of inflamed transverse colon. The demonstrated involvement of interferon-γ (from Th1 lymphocytes) and tumor necrosis factor-α in the pathogenesis of the murine disease may explain this finding.[16,17] Both interferon-γ and tumor necrosis factor-α have been shown to in-

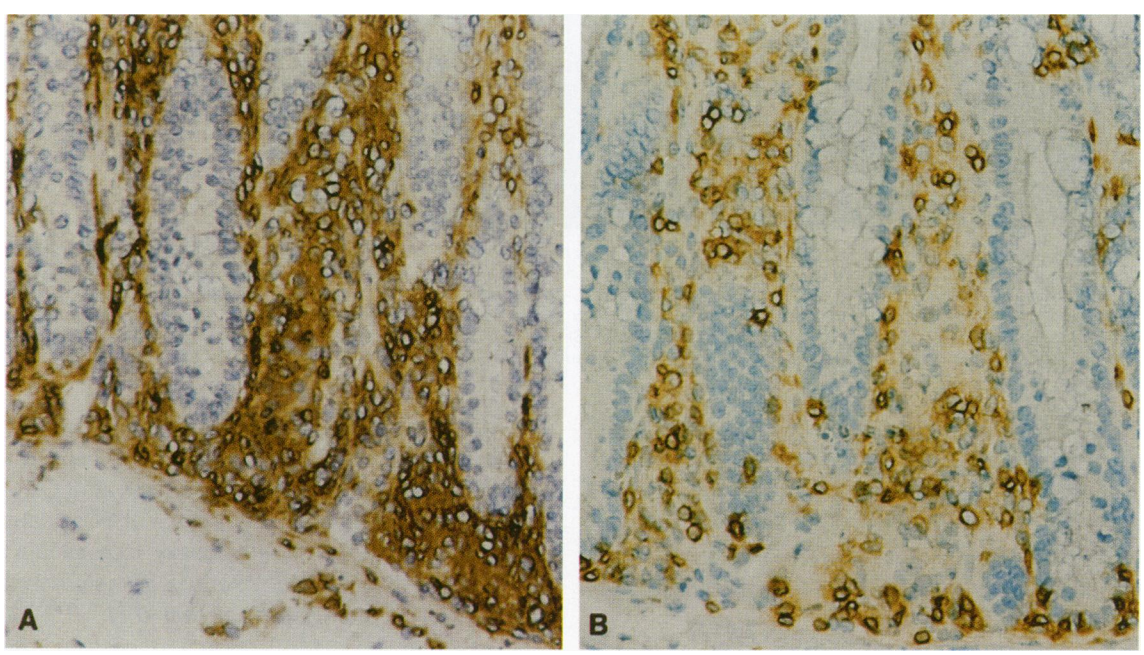

Figure 4. *Immunohistochemical analysis of the inflammatory infiltrate in the colon of* scid *mice reconstituted with the $CD45RB^{high}$ subset of $CD4^+$ T cells (immunoperoxidase, hematoxylin counterstain; magnification, ×230).* **A:** *Numerous mononuclear cells in the lamina propria have Mac-1 immunoreactivity.* **B:** *Smaller numbers of mononuclear cells in the lamina propria have CD4 immunoreactivity.*

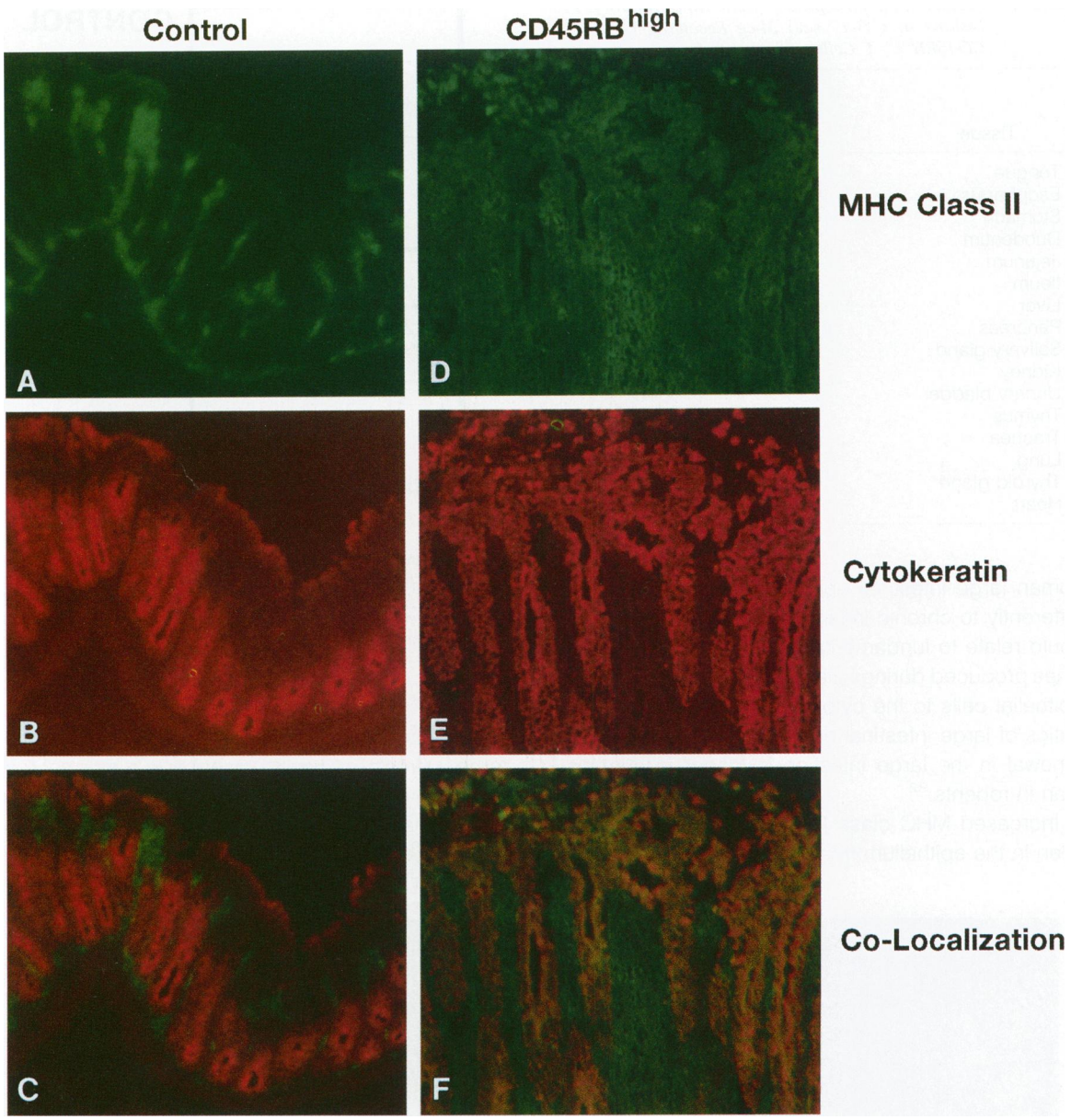

Figure 6. *Immunofluorescent analysis of MHC class II expression in the transverse colon (magnification, x150). Sections stained with both MTS6 (anti-I-A and anti-I-E) and anti-cytokeratin antibodies. Control mouse* (scid *mouse reconstituted with unsorted $CD4^+$ T cells;* **A** *to* **C**) *and* scid *mouse reconstituted with the $CD45RB^{high}$ subset of $CD4^+$ T cells* (**D** *to* **F**). *MHC class II expression (green label) is localized in the lamina propria in the control* (**A**), *in contrast to the reconstituted mouse in which MHC class II expression is located in both the lamina propria and epithelium* (**D**). *Epithelial cells (red label) are shown in control* (**B**) *and reconstituted* (**E**) *mice. Co-localization of MHC class II and cytokeratin in the control* (**C**) *and reconstituted mouse* (**F**). *Note the regions of overlapping reactivity in the epithelium of* **F**.

crease MHC class II expression in colonic epithelial cells and thus probably play a role in up-regulating MHC class II expression in the present model.[33] Compared with control epithelium, expression of cytokeratin in inflamed areas of colon was more diffuse. This finding may be related to the marked epithelial hyperplasia.

The immunopathological findings seen in these mice have some similarity to those found in patients with CD or UC. T-cell-mediated immune reactions appear to play a major role in the disease processes in IBD, especially CD.[2–9] Specifically, there is evidence that CD is associated with activated $CD4^+$ T cells,[7–9] and it has been proposed that CD is caused by a Th1 response.[34,35] Additionally, increased macrophage numbers and phenotypic heterogeneity have been reported in patients with IBD.[36,37] Finally, increased epithelial MHC class II expression is seen in human IBD.[4,38,39] Thus, the findings in the murine large intestine of many macrophages and activated

$CD4^+$ T cells, and increased epithelial expression of MHC class II, are similar to the findings in patients with IBD.

Gastrointestinal inflammation has also been reported in a number of other murine models with immune system alterations, including murine models of graft-*versus*-host disease and autoimmune disease, and in mice deficient in the T cell receptor, MHC class II antigens, transforming growth factor-β1, interleukin-10, and interleukin-2.[24,25,40–44] That such different alterations in the immune system all result in gastrointestinal inflammation highlights the importance of immune regulation in this organ, which is constantly exposed to numerous luminal antigens. The importance of gastrointestinal flora and luminal antigens has been further demonstrated in graft-*versus*-host disease as well as in interleukin-2- and interleukin-10-deficient mice, in which disease is decreased significantly or eliminated by decreasing or eliminating the bacterial flora.[41,42,45–47] A unifying hypothesis for the role of normal flora in the pathogenesis of these lesions is that local immune responses to normal antigens progress to excessive inflammation and tissue damage in animals with immunoregulatory defects of various types.[48] Similar events may also occur in IBD, for which a dysregulated immune response to luminal antigens has been suggested as a cause of IBD.[2,14,49,50] It has been shown that T cells in areas of inflamed CD mucosa have apparently lost their normal unresponsiveness to luminal antigens,[51] and autoantibodies to colonic epithelial antigens, possibly cross-reacting with bacterial antigens, have been reported in patients with UC and CD.[52,53]

In addition to the inflammation in the large intestine, inflammatory lesions were observed in a number of other tissues from these mice. Multi-organ inflammation can be seen in IBD patients, although the exact relationship of these lesions to IBD is unknown.[23] The extramedullary hematopoiesis seen in these mice, primarily myeloid in nature, was considered to be secondary to the ongoing inflammation, although a primary alteration in hematopoiesis cannot be excluded.

The inflammation in these mice did not appear to be the result of a contagious pathogen based on several findings. Pathogens were never cultured from sacrificed mice, and sentinel mice did not develop inflammatory lesions. Periodic comprehensive serology and histopathology revealed no indication of pathogens in sentinel or experimental mice. Finally, investigators performing similar experiments in completely separate laboratories report similar lesions.[15]

In summary, we describe a readily available, reproducible model of spontaneous large intestinal inflammation that develops in mice injected with $CD45RB^{high}$ $CD4^+$ T cells from normal mice. This model may provide insight into the etiology, pathogenesis, and treatment of chronic gastrointestinal inflammatory diseases.

Acknowledgments

The authors gratefully acknowledge the expert technical assistance of Ms. B. D. Hartman and the staff of the Histology Laboratory at the Schering-Plough Research Institute, especially Mr. J. Syed. Special thanks go to Dr. R. C. Johnson and Dr. D. W. Frank for critically reviewing the manuscript.

References

1. Lewin KJ, Riddell RH, Weinstein WM: Inflammatory bowel diseases. Gastrointestinal Pathology and Its Clinical Implications. Edited by KJ Lewin, RH Riddell, WM Weinstein. New York, Igaku-Shoin, 1992, pp 812–989
2. Braegger CP, MacDonald TT: Immune mechanisms in chronic inflammatory bowel disease. Ann Allergy 1994, 72:135–141
3. Breese E, Braegger CP, Corrigan CJ, Walker-Smith JA, MacDonald TT: Interleukin-2- and interferon-γ-secreting T cells in normal and diseased human intestinal mucosa. Immunology 1993, 78:127–131
4. Fais S, Capobianchi MR, Pallone F, DiMarco P, Boirivant M, Dianzani F, Torsoli A: Spontaneous release of interferon γ by intestinal lamina propria lymphocytes in Crohn's disease: kinetics of *in vitro* response to interferon γ inducers. Gut 1991, 32:403–407
5. Meuwissen SGM, Feltkamp-Vroom TM, de la Rivièra AB, von dem Borne AEG, Tytgat GN: Analysis of the lympho-plasmacytic infiltrate in Crohn's disease with special reference to identification of lymphocyte-subpopulations. Gut 1976, 17:770–780
6. Targan SR, Deem RL, Shanahan F: Role of mucosal T-cell-generated cytokines in epithelial cell injury. Immunol Res 1991, 10:472–478
7. Choy MY, Walker-Smith JA, Williams CB, MacDonald TT: Differential expression of CD25 (interleukin-2 receptor) on lamina propria T cells and macrophages in the intestinal lesions in Crohn's disease and ulcerative colitis. Gut 1990, 31:1365–1370
8. Schreiber S, MacDermott RP, Raedler A, Pinnau R, Bertovich MJ, Nash GS: Increased activation of isolated intestinal lamina propria mononuclear cells in inflammatory bowel disease. Gastroenterology 1991, 101:1020–1030
9. Haruta J-i, Kusugami K, Kuroiwa A, Ina K, Shinoda M, Morise K, Iokawa H, Morita M, Ishihara A, Sarai S:

Phenotypic and functional analysis of lamina propria mononuclear cells from colonoscopic biopsy specimens in patients with ulcerative colitis. Am J Gastroenterol 1992, 87:448–454
10. Cooper HS, Murthy SNS, Shah RS, Sedergran DJ: Clinicopathologic study of dextran sulphate sodium experimental murine colitis. Lab Invest 1993, 69:238–249
11. Rachmilewitz D, Simon PL, Schwartz LW, Griswold DE, Fondacaro JD, Wasserman MA: Inflammatory mediators of experimental colitis in rats. Gastroenterology 1989, 97:326–337
12. Oestreicher P, Nielsen ST, Rainsford KD: Inflammatory bowel disease induced by combined bacterial immunization and oral carrageenan in guinea pigs. Dig Dis Sci 1991, 36:461–470
13. Mitchell IC, Turk JL: An experimental animal model of granulomatous bowel disease. Gut 1989, 30:1371–1378
14. MacDonald TT: Inflammatory bowel disease in knockout mice. Curr Biol 1994, 4:261–263
15. Morrissey PJ, Charrier K, Braddy S, Liggitt D, Watson JD: $CD4^+$ 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 1993, 178:237–244
16. Powrie F, Leach MW, Mauze S, Caddle LB, Coffman RL: Phenotypically distinct subsets of $CD4^+$ T cells induce or protect from chronic intestinal inflammation in C. B-17 *scid* mice. Int Immunol 1993, 5:1461–1471
17. Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL: Inhibition of Th1 responses prevents inflammatory bowel disease in *scid* mice reconstituted with $CD45RB^{hi}$ $CD4^+$ T cells. Immunity 1994, 1:553–562
18. Springer T, Galfre G, Secher DS, Milstein C: Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. Eur J Immunol 1979, 9:301–306
19. Hynes RO: Integrins: a family of cell surface receptors. Cell 1987, 48:549–554
20. Birkeland ML, Johnson P, Trowbridge IS, Puré E: Changes in CD45 isoform expression accompany antigen-induced murine T-cell activation. Proc Natl Acad Sci USA 1989, 86:6734–6738
21. Bottomly K, Luqman M, Greenbaum L, Carding S, West J, Pasqualini T, Murphy DB: A monoclonal antibody to murine CD45R distinguishes CD4 T cell populations that produce different cytokines. Eur J Immunol 1989, 19:617
22. Tanaka M, Riddell RH: The pathological diagnosis and differential diagnosis of Crohn's disease. Hepato-gastroenterology 1990, 37:18–31
23. Fenoglio-Preiser CM, Lantz PE, Listrom MB, Davis M, Rilke FO (Eds): Idiopathic inflammatory bowel disease. Gastrointestinal Pathology: An Atlas and Text. New York, Raven Press, 1989, pp 427–484
24. Eigenbrodt ML, Eigenbrodt EH, Thiele DL: Histologic similarity of murine colonic graft-*versus*-host disease (GVHD) to human colonic GVHD and inflammatory bowel disease. Am J Pathol 1990, 137:1065–1076
25. Bucy RP, Xu XY, Li J, Huang Gq: Cyclosporin A-induced autoimmune disease in mice. J Immunol 1993, 151:1039–1050
26. Rosekrans PCM, Meijer CJLM, van der Wal AM, Cornelisse CJ, Lindeman J: Immunoglobulin containing cells in inflammatory bowel disease of the colon: a morphometric and immunohistochemical study. Gut 1980, 21:941–947
27. Serafini EP, Kirk AP, Chambers TJ: Rate and pattern of epithelial cell proliferation in ulcerative colitis. Gut 1981, 22:648–652
28. Franklin WA, McDonald GB, Stein HO, Gatter KC, Jewell DP, Clarke LC, Mason DY: Immunohistologic demonstration of abnormal colonic crypt cell kinetics in ulcerative colitis. Hum Pathol 1985, 16:1129–1132
29. MacDonald TT, Spencer J: Evidence that activated mucosal T cells play a role in the pathogenesis of enteropathy in human small intestine. J Exp Med 1988, 167:1341–1349
30. Evans CM, Phillips AD, Walker-Smith JA, MacDonald TT: Activation of lamina propria T cells induces crypt epithelial proliferation and goblet cell depletion in cultured human fetal colon. Gut 1992, 33:230–235
31. MacDonald TT: The role of activated T lymphocytes in gastrointestinal disease. Clin Exp Allergy 1990, 20: 247–252
32. Eastwood GL: Gastrointestinal epithelial renewal. Gastroenterology 1977, 72:962–975
33. Ishii N, Chiba M, Iizuka M, Horie Y, Masamune O: Induction of HLA-DR antigen expression on human colonic epithelium by tumor necrosis factor-α and interferon-γ. Scand J Gastroenterol 1994, 29:903–907
34. Karttunnen R, Breese EJ, Walker-Smith JA, MacDonald TT: Decreased mucosal interleukin-4 (IL-4) production in gut inflammation. J Clin Pathol 1994, 47:1015–1018
35. Autschbach F, Schürmann G, Qiao L, Merz H, Wallich R, Meuer SC: Cytokine messenger RNA expression and proliferation status of intestinal mononuclear cells in noninflamed gut and Crohn's disease. Virchows Arch 1995, 426:51–60
36. Allison MC, Cornwall S, Poulter LW, Dhillon AP, Pounder RE: Macrophage heterogeneity in normal colonic mucosa and in inflammatory bowel disease. Gut 1988, 29:1531–1538
37. Rugtveit J, Brandtzaeg P, Halstensen TS, Fausa O, Scott H: Increased macrophage subset in inflammatory bowel disease: apparent recruitment from peripheral blood monocytes. Gut 1994, 35:669–674
38. Trejdosiewicz LK, Badr-El-Din S, Smart CJ, Malizia G, Oakes DJ, Heatley RV, Losowsky MS: Colonic mucosal T lymphocytes in ulcerative colitis: Expression of CD7 antigen in relation to MHC class II (HLA-D) antigens. Dig Dis Sci 1989, 34:1449–1456
39. Hirata I, Berrebi G, Austin LL, Keren DF, Dobbins WO III: Immunohistological characterization of intraepithelial and lamina propria lymphocytes in control ileum

and colon and in inflammatory bowel disease. Dig Dis Sci 1986, 31:593–603
40. Mombaerts P, Mizoguchi E, Grusby MJ, Glimcher LH, Bhan AK, Tonegawa S: Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. Cell 1993, 75:275–282
41. Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W: Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993, 75:263–274
42. Sadlack B, Merz H, Schorle H, Schimpl A, Feller AC, Horak I: Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. Cell 1993, 75:253–261
43. Kulkarni AB, Ward JM, Yaswen L, Mackall CL, Bauer SR, Huh C-G, Gress RE, Karlsson S: Transforming growth factor-β1 null mice: an animal model for inflammatory disorders. Am J Pathol 1995, 146:264–275
44. Boivin GP, O'Toole BA, Orsmby IE, Diebold RJ, Eis MJ, Doetschman T, Kier AB: Onset and progression of pathological lesions in transforming growth factor-β1-deficient mice. Am J Pathol 1995, 146:276–288
45. Storb R, Prentice RL, Buckner CD, Clift RA, Appelbaum F, Deeg J, Doney K, Hansen JA, Mason M, Sanders JE, Singer J, Sullivan KM, Witherspoon RP, Thomas ED: Graft-*versus*-host disease and survival in patients with aplastic anemia treated by marrow grafts from HLA-identical siblings. N Engl J Med 1983, 308:302–307
46. van Bekkum DW, Roodenburg J, Heidt PJ, van der Waaij D: Mitigation of secondary disease of allogenic mouse radiation chimeras by modification of the intestinal microflora. J Natl Cancer Inst 1974, 52:401–404
47. Keast D: A simple index for the measurement of the runting syndrome and its use in the study of the influence of the gut flora in its production. Immunology 1968, 15:237–245
48. Powrie F: T cells in inflammatory bowel disease: Protective and pathogenic roles. Immunity 1995, 3:171–174
49. Posnett DN, Schmelkin I, Burton DA, August A, McGrath H, Mayer LF: T cell antigen receptor V gene usage: increases in Vβ8+ T cells in Crohn's disease. J Clin Invest 1990, 85:1770–1776
50. Aisenberg J, Ebert EC, Mayer L: T-cell activation in human intestinal mucosa: the role of superantigens. Gastroenterology 1993, 105:1421–1430
51. Pirzer U, Schönhaar A, Fleischer B, Hermann E, zum Büschenfelde K-HM: Reactivity of infiltrating T lymphocytes with microbial antigens in Crohn's disease. Lancet 1991, 338:1238–1239
52. Hibi T, Ohara M, Toda K, Hara A, Ogata H, Iwao Y, Watanabe N, Watanabe M, Hamada Y, Kobayashi K, Aiso S, Tsuchiya M: *In vitro* anticolon antibody production by mucosal or peripheral blood lymphocytes from patients with ulcerative colitis. Gut 1990, 31:1371–1376
53. Thayer WR, Brown M, Sangree MH, Katz J, Hersh T: *Escherichia coli* 0:14 and colon hemagglutinating antibodies in inflammatory bowel disease. Gastroenterology 1969, 57:311–318