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A Novel Model of Inflammatory Bowel Disease: Mice Deficient for the Multiple Drug Resistance Gene, *mdr1a*, Spontaneously Develop Colitis

Chetan M. Panwala,* Jon C. Jones,† and Joanne L. Viney[1]*

The murine multiple drug resistance (*mdr*) gene, *mdr1a*, encodes a 170-kDa transmembrane protein that is expressed in many tissues including intestinal epithelial cells, a subset of lymphoid cells and hematopoietic cells. We report that *mdr1a* knockout (*mdr1a*$^{-/-}$) mice are susceptible to developing a severe, spontaneous intestinal inflammation when maintained under specific pathogen-free animal facility conditions. The intestinal inflammation seen in *mdr1a*$^{-/-}$ mice has a pathology similar to that of human inflammatory bowel disease (IBD) and is defined by dysregulated epithelial cell growth and leukocytic infiltration into the lamina propria of the large intestine. Treating *mdr1a*$^{-/-}$ mice with oral antibiotics can both prevent the development of disease and resolve active inflammation. Lymphoid cells isolated from mice with active colitis are functionally reactive to intestinal bacterial Ags, providing evidence that there is enhanced immunologic responsiveness to the normal bacterial flora during IBD. This study is the first description of spontaneous colitis in a gene knockout mouse with an apparently intact immune system. This novel model of spontaneous colitis may provide new insight into the pathogenesis of IBD, the nature of dysregulated immune reactivity to intestinal bacterial Ags, and the potential functional role of *mdr* genes expressed in the cells and tissues of the colonic microenvironment. *The Journal of Immunology,* **1998, 161: 5733–5744.**

The inflammatory bowel diseases (IBD)[2], Crohn's disease and ulcerative colitis, are syndromes characterized by chronic inflammation of the gastrointestinal tract. The etiology of these disorders remains unknown despite many years of investigation. The current hypotheses suggest that persistent intestinal inflammation may be the result of either enhanced or aberrant immunologic responsiveness to normal constituents of the gut lumen (1, 2) or an overall autoimmune dysregulation and imbalance (3–11). The recent establishment of various animal models relevant for studying the pathogenesis of intestinal inflammation has provided some insight into potential disease mechanisms. In particular, the discovery that many animals with altered T cell populations or cytokine deficiencies spontaneously develop colitis has implicated an immune imbalance as one of the critical factors in the manifestation of IBD (3–11). Although the pathologic changes associated with IBD and the extensive lymphoid infiltrates into the inflammatory lesion suggest that there is some immunologic dysregulation, it is likely that many of the immunologic changes associated with IBD are probably not the primary cause of the disease process, but may be due to secondary nonspecific inflammation.

There is increasing evidence that the intestinal microflora is an important cofactor in the pathogenesis of intestinal inflammation. The development of spontaneous colitis in many mouse models can be prevented if mice are generated and maintained in a germ-free environment or treated with oral antibiotics, highlighting the central role for bacterial colonization in the initiation and/or perpetuation of experimental IBD (4, 12–16). There is also evidence that human patients with IBD suffer from adverse and enhanced reactivity to their autologous resident intestinal flora (1, 2, 17). Considering that the cells of the mucosal immune system are protected from the large antigenic load in the gut lumen by a single layer of epithelial cells, it seems reasonable to assume that epithelial cells play a major role in providing a barrier that regulates contact between bacteria and immune cells. In support of this, transgenic mice expressing a mutant cadherin on their epithelial cells in the small intestine spontaneously develop IBD, presumably due to breaches occurring within the epithelial cell barrier (18). Thus, proteins expressed in epithelial cells may in some way contribute, directly or indirectly, to maintaining the protective barrier.

Intestinal epithelial cells and some lymphocyte subsets have been shown to express multiple drug resistance (*MDR*) genes (19–24). These genes belong to a family of transporters known as ATP binding cassette transporters, which are characterized by their ability to pump small amphiphilic and hydrophobic molecules across membranes in an ATP-dependent manner (25, 26). *MDR* genes were first identified by their ability to confer resistance to chemotherapeutic agents in tumors and neoplastic cell lines (27). Three *MDR* genes have been identified in rodents and two in humans, each of which has a restricted pattern of tissue expression and, most likely, a different function. Although it is well known that some of these gene products (*MDR*1 in humans and *mdr1a* and *mdr3* in mice) can actively pump toxic drugs out of cells, their natural in vivo role has not been fully elucidated (28, 29). In mice, *mdr1a* is expressed on many tissues including intestinal epithelial cells, CD8$^+$ T cells, a subset of CD4$^+$ T cells, hematopoietic cells, and cells at the blood-brain barrier (19, 21, 23, 30, 31). The function of *mdr1a* in each of these different cell types and tissues is unknown.

Departments of *Molecular Immunology and †Immunobiology, Immunex Corporation, Seattle, WA 98101

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[1] Address correspondence and reprint requests to Dr. J. L. Viney, Department of Molecular Immunology, Immunex Corporation, 51 University Street, Seattle, WA 98101. E-mail address: jviney@immunex.com

[2] Abbreviations used in this paper: IBD, inflammatory bowel disease; mdr, multiple drug resistance; SPF, specific pathogen free; PE, phycoerythrin; IEL, intraepithelial lymphocyte; LPL, lamina propria lymphocyte; MLN, mesenteric lymph node.

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Recently, mice with a targeted deletion of the *mdr1a* gene were generated (32). Analysis of these knockout mice has revealed that they have an increased sensitivity to certain drugs, but do not appear to show any constitutive abnormalities (31–34). In the current study we observe that *mdr1a*$^{-/-}$ mice housed under specific pathogen-free (SPF) conditions develop spontaneous intestinal inflammation. We describe the nature of the inflammatory cell infiltrate, describe a role of the intestinal microflora in the initiation and perpetuation of colitis in these mice, and provide evidence that the development of colitis appears to arise as a result of a defect in the intestinal epithelial barrier.

Materials and Methods

Mice

FVB control mice and *mdr1a*$^{-/-}$ mice were initially purchased from Taconic Farms (Germantown, NY) and then bred and maintained in the SPF facility at Immunex (Seattle, WA) in accordance with approved ethical guidelines. *mdr1a*$^{-/-}$ mice had been backcrossed onto the FVB strain for at least seven generations at Taconic Farms before shipping. Control and knockout mice used for experiments were age matched and received treatment simultaneously. Mice were monitored on alternate days for the presence of diarrhea and mucous discharge from the anus. All mice were bred and maintained under SPF conditions in Thoren isolation racks under positive pressure and fed autoclaved food and water ad libitum. The quality of the SPF facility is monitored through the Charles River Health Monitoring Plus program (Charles River, Wilmington MA) using the Charles River tracking profile. Sentinel mice receiving bedding from donor cages are tested for viral, parasite, and bacterial infection. There were no viruses, parasites, or pathogenic bacteria detected in our colony throughout the course of the study. Of particular note, we did not detect any evidence of *Helicobacter* infection (*Helicobacter bilis*, *Helicobacter hepaticus*, or *Helicobacter* spp.) in any mice.

Treatment of mice with oral antibiotics

Streptomycin sulfate (1.25% w/v), neomycin (1% w/v), bacitracin (1% w/v), and amphotericin (0.25% w/v) were dissolved in autoclaved drinking water (pH 2.5–2.8) supplemented with saccharine (1% w/v) and then filtered through a 0.22 μm Nalgene filter (Nalgene, Milwaukee, WI). As a control, mice received saccharine alone (1% w/v) in the drinking water. All reagents were obtained from Sigma (St. Louis, MO). Regular drinking water was replaced with antibiotic or saccharine drinking water during treatment.

Antibodies

The following primary Abs were used for flow cytometric analysis and immunoperoxidase histochemistry: anti-CD4 (rat IgG; L3T4), anti-CD8α (rat IgG; 53-6.7), anti-TCR$\alpha\beta$ (hamster IgG; H57-597), anti-TCR$\gamma\delta$ (hamster IgG; GL3), anti-GR1 (rat IgG; RB6–8C5), anti-B220 (CD45R) (rat IgG; RA3–6B2), and anti-CD3ϵ (hamster IgG; 500A2; Immunex). The primary Abs were conjugated either with biotin or R-phycoerythrin (PE). All Abs were obtained from PharMingen (San Diego, CA) except where indicated. The following secondary Abs were used for immunoperoxidase histochemistry: biotinylated sheep anti-rat IgG (Amersham, Arlington Heights, IL) and biotinylated goat anti-hamster IgG (Caltag, South San Francisco, CA).

Flow cytometric analysis of isolated cells

Isolated cells were incubated with the appropriate PE- or biotin-conjugated primary Ab for 30 min on ice in PBS supplemented with 5% BSA and 0.02% sodium azide essentially as described previously (35). Stained cells were washed two times, incubated with streptavidin-allophycocyanin (Molecular Probes, Eugene, OR), and then fixed in 1% paraformaldahyde before analysis. Stained cells were analyzed on a Becton Dickinson FACScan flow cytometer (Immunocytochemistry Systems, San Jose, CA) using Becton Dickinson Cellquest software.

Rhodamine efflux assay

Lymphocytes were isolated from mice and incubated with 0.4 μg/ml rhodamine 123 (Sigma) for 15 min at 37°C in complete RPMI 1640 supplemented with 10% FCS. For two-color analysis, cells were simultaneously stained with PE-conjugated primary Abs. The stained, rhodamine-loaded cells were then washed two times and incubated in complete medium without rhodamine 123 for an additional 2 h at 37°C. Cells were then washed two times in complete medium and analyzed on a Becton Dickinson FACScan flow cytometer (Immunocytochemistry Systems) using Becton Dickinson Cellquest software.

Immunoperoxidase histochemistry analysis of frozen sections

Large intestines were removed, embedded in OCT compound (Tissue-Tek, Torrence, CA), frozen in a dry ice/ethanol bath, and stored at −80°C until use. Frozen sections (5 μm) of intestine were cut, fixed for 15 min in acetone, and stored at −80°C until staining. Peroxidase staining was performed using the Vectastain ABC system (Vector Laboratories, Burlingame, CA) according to specified directions as described previously (35). Briefly, tissue sections were preincubated in 5% goat serum, 5% bovine serum, and 1% mouse serum for 30 min at room temperature and then incubated with biotin-conjugated primary Ab for 1 h at room temperature. Slides were washed three times in Tris-buffered saline, further incubated with a biotin-conjugated secondary Ab for 30 min at room temperature, and washed again three times in Tris-buffered saline. Sections were then incubated with avidin peroxidase (Vectastain ABC system), washed, and finally incubated with diaminobenzidine substrate (Sigma) before counterstaining in hematoxylin. Positive cells were identified by the presence of a brown reaction product.

Immunofluorescence analysis of frozen sections

Tissues were frozen in liquid nitrogen as described above, and 5-μm cryosections were cut, air dried, and acetone fixed before staining. Tissue sections from both *mdr1a*$^{-/-}$ and control FVB mice were stained with either 20 μg/ml FITC-conjugated anti-MDR mAb (C219; Signet Laboratories, Dedham, MA) or FITC-conjugated IgG2a isotype control mAb (PharMingen) in the presence of 2 mM TOTO-3 (Molecular Probes) and 30% mouse serum. Sections were visualized using a confocal laser scanning microscope (Molecular Dynamics, Sunnyvale, CA).

Intraepithelial lymphocyte (IEL) and lamina propria lymphocyte (LPL) cell isolations

IEL were isolated according to a modification of published procedures (35, 36). Briefly, the small and large intestines were removed and washed in Ca^{2+}/Mg^{2+}-free HBSS (Life Technologies, Bethesda, MD). Peyer's patches were removed, and the intestines were opened longitudinally, cut into 0.5-cm pieces, and placed in 1 mM EDTA in Ca^{2+}/Mg^{2+}-free HBSS at 37°C for three sequential 15-min incubations with intermittent vortexing to remove epithelial cells and IEL. Supernatants were collected and pooled for IEL purification. The remaining tissues were then washed in PBS and incubated in trypsin-EDTA two times to remove any residual epithelial cells, and the denuded tissues were digested with 90 U/ml collagenase type VIII (Sigma) in RPMI 1640/10% FCS for three sequential 20-min incubations at 37°C with intermittent vortex mixing to release LPL. Supernatants were collected and pooled for LPL purification. IEL and LPL were purified by passing cells over a prewet glass wool column before spinning them through a 40%/80% discontinuous Percoll gradient (Pharmacia, Uppsala, Sweden) as previously described (35). The cells at the 40%/80% interface were collected, washed, and resuspended in complete medium for analysis.

Intestinal bacterial Ag preparation

Intestinal bacterial Ags were prepared from fecal contents harvested from the cecum according to a modification of published procedures (37). Briefly, individual preparations were processed from three different mouse sources: 1) an FVB control mouse, 2) an *mdr1a*$^{-/-}$ mouse with no signs of inflammation, and 3) an *mdr1a*$^{-/-}$ mouse with active colitis. The cecal contents from the individual mice were placed in a 2-ml bead shaker tube half-filled with 2-μm zirconium beads suspended in PBS. The mixture was vortexed in a bead beater (Biospec Products, Bartlesville, OK) for 30 s at 5000 rpm and placed on ice. The intestinal bacterial slurry was then microfuged at 14,000 $\times$ *g*, and the supernatant was harvested and filtered through a 0.22 μm filter. The relative protein content in each sample was estimated using a Bradford assay (Bio-Rad, Hercules, CA).

Proliferation assay

Cell suspensions were prepared by gently mashing mesenteric lymph nodes (MLN) between ground glass slides and passing them through nylon mesh. MLN cells were cultured at a density of 2×10^5 cells/well in 96-well flat-bottom plates in complete RPMI supplemented with 10% FCS in a final volume of 200 μl in a humidified 6% CO_2 incubator at 37°C for 48 h. Cells were cultured either in the presence of titrating amounts of intestinal bacterial Ag preparations, in the presence of 1 μg/ml Con A, or in

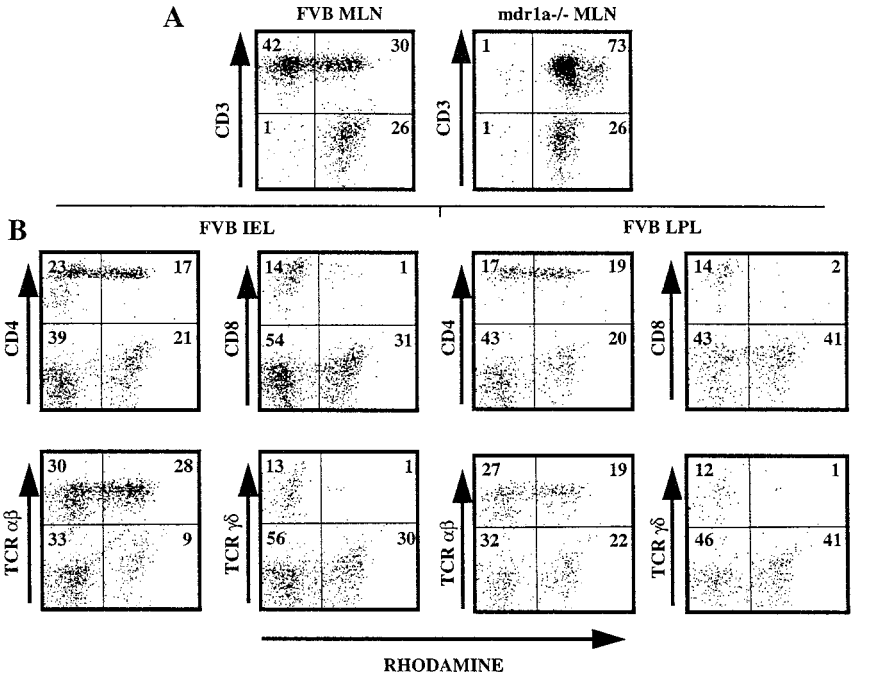

FIGURE 1. Mucosal lymphocytes from FVB mice, but not *mdr1a*$^{-/-}$ mice, exhibit *mdr1a* functional activity. MLN cells (*A*) and mucosal IEL and LPL (*B*) were loaded with rhodamine 123, simultaneously stained with PE-conjugated mAb (to CD3, CD4, CD8, TCR$\alpha\beta$, or TCR$\gamma\delta$), and then allowed to efflux rhodamine for 2 h. Cells were then analyzed by flow cytometry, and *mdr1a* activity was determined by decreased rhodamine intensity. Axes show $\log_{10}$ fluorescence intensity measured at 525 nm for rhodamine 123 and 575 nm for PE after excitation with a 488-nm argon laser. The FACS profiles represent data from one of two separate experiments.

anti-CD3 mAb-coated wells (1 μg/ml). Proliferation was assessed by addition of 1 μCi/well [^{3}H]TdR 18 h before harvesting using a Matrix-96 cell harvester (Inotech, Lansing, MI). The amount of radioactivity incorporated into DNA was measured using a direct β counter (Packard, Meriden, CT). All cultures were performed in triplicate. Data are reported as mean cpm $\pm$ 1 SD of triplicate wells.

Serum Ab titers

Mice were exsanguinated, and the serum was collected. Polyclonal serum Ab titers were determined by isotype-specific ELISAs for IgA, IgE, IgG1, IgG2a, IgG2b, IgG3, and IgM as previously described (38). Briefly, 96-well Nunc Maxisorp plates (Nunc, Naperville, IL) were coated overnight at 4°C with isotype-specific anti-mouse Ig Abs (0.2 mg/well), blocked with PBS/10% FCS for 2 h at room temperature, and washed with PBS/0.1% Tween-20. Serum samples and control samples were serially diluted in PBS/10% FCS starting at 1:100. Plates were incubated for 2 h at room temperature, washed, and incubated with the appropriate peroxidase-conjugated specific anti-isotype detecting Ab for an additional 2 h at room temperature. Plates were washed again, and enzyme activity was detected using TMB microwell peroxidase substrate reagents (Kirkegaard & Perry Laboratories, Gaithersburg, MD) and 2 N H_2SO_4. The amount of reaction product was assessed on an ELISA plate reader at OD 450 nm using the Deltasoft program (DeltaPoint, Monterey, CA). The affinity-purified Abs used for determining isotype-specific serum Ab titers by ELISA were obtained from Southern Biotechnology Associates (Birmingham, AL), except for anti-IgE, which was obtained from Serotec (Oxford, U.K.), and anti-IgG2a, which was obtained from PharMingen. Ig concentrations were determined by comparing test sample dilution curves with known concentrations of isotype controls. Results are indicated as mean concentration $\pm$ 1 SD of two separate mice for each group.

Bone marrow chimeric mice

mdr1a$^{-/-}$ mice and FVB mice were irradiated with 10 Gy using a ^{137}Cs source, rested for 4–6 h, and then reconstituted by i.v. injection with 2 $\times$ 10^6 bone marrow cells collected from the femurs of appropriate donors.

Bacterial identification

Analysis of cecal and colonic bacteria (Phoenix Laboratories, Everett, WA). Mice were killed under sterile conditions, and aerobe/anaerobe bacterial culture swabs (Medical Wire and Equipment, Corsham, Wilts, U.K.) were used to collect cecal and colonic contents. Samples were shipped to Phoenix Laboratories for identification of aerobic and anaerobic bacterial outgrowth. At Phoenix Laboratories, swabs were streaked and bacterial species were identified based on nutrient requirements, growth, morphology, and staining properties.

Analysis of colonic bacteria (Microcheck, Northfield, VT). Mice were killed under sterile conditions, and colonic washout samples were harvested and shipped to Microcheck for bacterial identification by microbial cellular fatty acid analysis. Samples were subcultured at Microcheck, and microbial fatty acid profiles were determined by high-resolution gas chromatography. Identification of bacteria in individual samples was determined by comparing the fatty acid profiles of the unknown sample with known profiles from microbial fatty acid libraries.

Results

mdr1a *is functionally expressed by mucosal lymphocytes and is on the apical surface of colonic epithelial cells*

Efflux of rhodamine 123 from preloaded lymphocytes has been used to demonstrate the presence of *mdr* activity in peripheral T cells (19, 30). However, the assay does not distinguish whether rhodamine efflux is due to *mdr1a* activity, *mdr3* activity, or perhaps even another unidentified transporter. We therefore analyzed rhodamine 123 efflux in lymphocytes from normal FVB mice and *mdr1a*$^{-/-}$ mice to determine whether rhodamine 123 efflux was associated with a functional *mdr1a* gene and to identify which mucosal and peripheral lymphocyte subsets might exhibit the pump activity. *mdr1a*-associated functional activity was detected in MLN cells from normal FVB mice but not from *mdr1a*$^{-/-}$ mice, as determined by ability to efflux rhodamine 123 (Fig. 1*A*). These data indicate that a functional *mdr1a* gene product is necessary for rhodamine 123 efflux. To determine which subsets of mucosal lymphocytes have the *mdr1a* activity, we performed the rhodamine efflux assay on IEL and LPL isolated from the large intestines of FVB mice. We simultaneously stained these cells with mAbs against CD3, CD4, CD8, TCR$\alpha\beta$, and TCR$\gamma\delta$ (Fig. 1*B*). The individual CD3, CD4, CD8, TCR$\alpha\beta$, and TCR$\gamma\delta$ subsets within the IEL and LPL compartments had similar efflux profiles. Virtually all of the CD8$^+$ cells and TCR$\gamma\delta^+$ cells appeared to exhibit *mdr* activity and efflux rhodamine 123, whereas only half of the CD4$^+$ cells and half of the TCR$\alpha\beta^+$ cells were able to efflux the dye. None of the mucosal lymphocyte populations from the *mdr1a*$^{-/-}$ mice were able to efflux the rhodamine 123 (data not shown), further suggesting that *mdr1a* is necessary for rhodamine 123 efflux activity in mucosal lymphocytes.

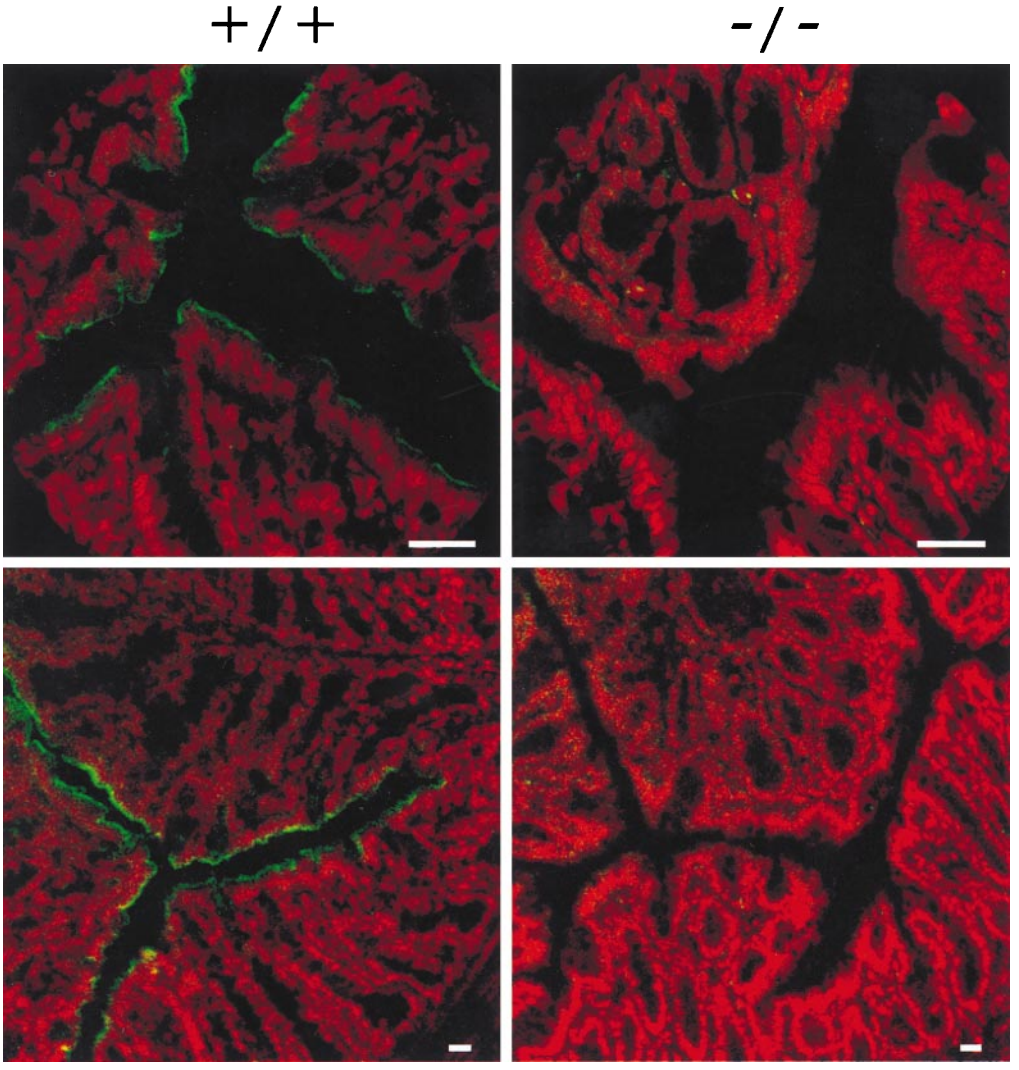

FIGURE 2. *mdr1a* is expressed on the apical surface of the intestinal epithelial cells in the colon. Frozen sections of large intestine from FVB control mice (+/+) and *mdr1a*-deficient mice (−/−) were stained for *mdr1a* expression with anti-*mdr* FITC mAb C219 (green) and counterstained with TOTO-3 (red). The scale bars represent 20 μm. The staining shown is representative of two mice analyzed. Similar staining was seen in two separate experiments.

Immunofluorescent staining was used to localize *mdr1a* expression in the colon. Fig. 2 shows *mdr1a* staining (green) on transverse sections of colons from FVB control (+/+) and *mdr1a*-deficient (−/−) mice. The sections were counterstained with TOTO-3 (red) to visualize gut architecture. *mdr1a* staining appears to be restricted to the apical surface of epithelial cells in FVB mice. No *mdr1a* staining was apparent on sections from *mdr1a*$^{-/-}$ mice or on sections from FVB mice stained with an isotype control (data not shown). We were also unable to detect any *mdr1a* staining on LPL or on IEL in FVB mice, despite functional evidence that these cells express *mdr1a* (see above). It is unclear whether this was due to low expression levels of *mdr1a* on mucosal lymphocytes or to the relatively low frequency of lymphocytes within each section.

Clinical characteristics and incidence of colitis in mdr1a$^{-/-}$ *mice*

Approximately 20–25% of the 169 *mdr1a* knockout mice included in this part of the study appeared to develop loose stools and anal mucous discharge by 1 year of age (Table I). Histologic analysis of mice with clinical signs of colitis confirmed the presence of active intestinal inflammation that was primarily restricted to the large intestine. The difference in incidence between males (20%) and females (26%) was not significant. The average age at onset was approximately 20 wk, although the first signs of colitis development could be seen in mice ranging between 8 and 36 wk of age. Although some mice with colitis developed a wasting-type disease, the majority of colitic animals were maintained for up to 3 mo without signs of cachexia. Disease was nontransmissible, since *mdr1a*$^{-/-}$ mice both with and without colitis were housed in the same cage. We observed no clinical or histologic signs of colitis in

Table I. *Incidence of colitis*[a] in mdrla$^{-/-}$ *mice*[b]

	Age at Disease Onset (wk)						
	5–10	11–15	16–20	21–25	26–30	31–35	Total
Male	1[c]	6	1	3		5	16/82[d]
Female	4	4	4	6	2	3	23/87

[a] Colitis was determined by the presence of loose stools and mucous discharge from the anus.
[b] Incidence of colitis was monitored in *mdrla*$^{-/-}$ mice housed under SPF conditions.
[c] Number of mice with colitis.
[d] There was no significant difference in incidence of colitis between males and females at a 95% confidence interval (z = .092).

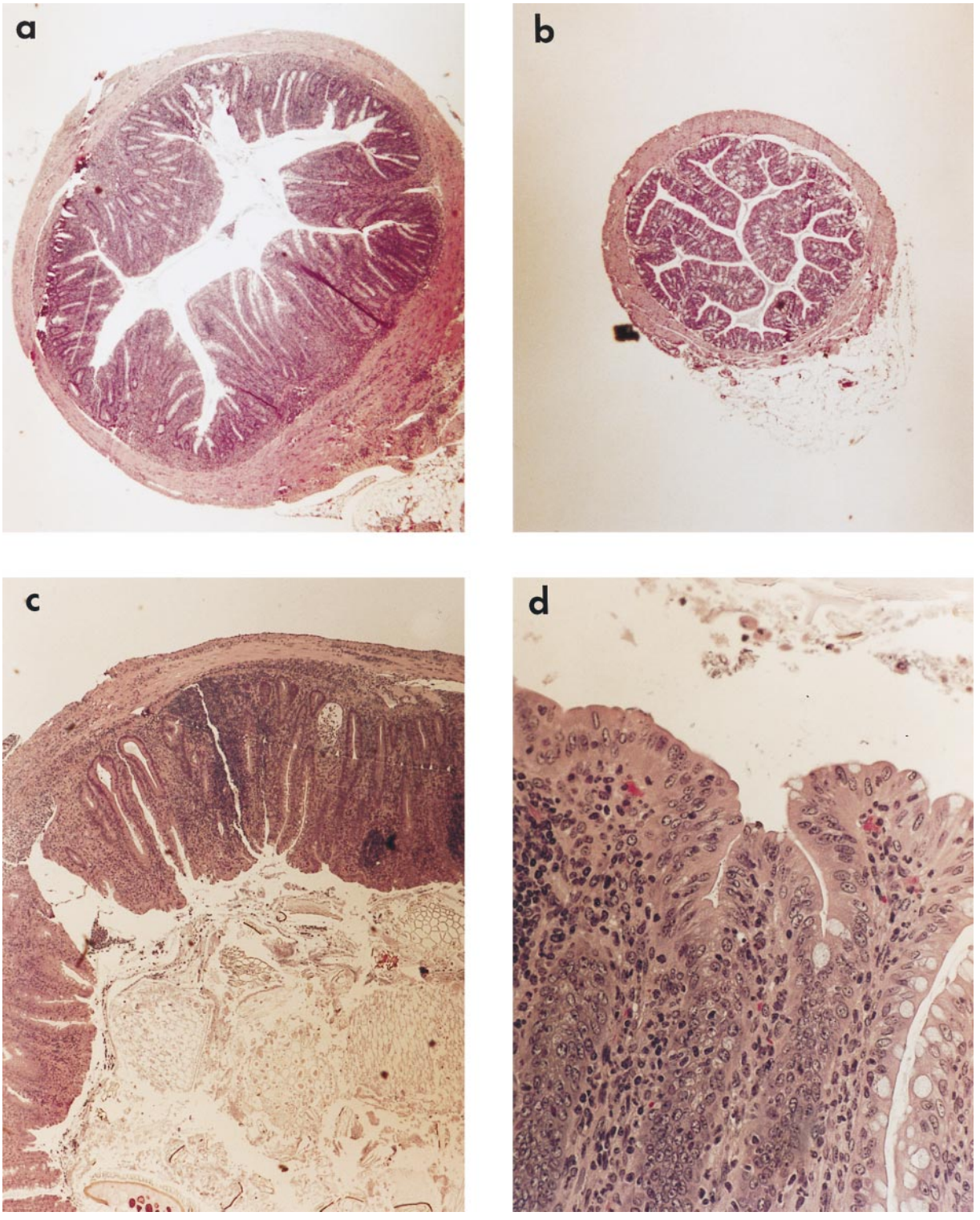

FIGURE 3. *mdr1a*$^{-/-}$ mice develop severe spontaneous inflammation of the large intestine. Frozen sections stained with hematoxylin and eosin revealed a massive thickening of the colonic tissues when *mdr1a*$^{-/-}$ mice with established colitis (*a*) were compared with *mdr1a*$^{-/-}$ mice without colitis (*b*); original magnification, ×63.5. *c*, Crypt abscesses and ulcers extending through the mucosa to the muscularis were prevalent in many sections of intestine from *mdr1a*$^{-/-}$ mice with active colitis (original magnification, ×250). *d*, Evidence of dysregulated epithelial cell growth in colitic mice (original magnification, ×500). *a–d* are from individual mice and are representative of multiple tissue sections analyzed from >15 mice per group.

any FVB control mice, heterozygous *mdr1a*$^{+/-}$ mice, or other immunocompetent mouse strains of any age housed within the facility.

Histopathologic changes associated with colitis in mdr1a$^{-/-}$ *mice*

The severe inflammation of the large intestine that develops in *mdr1a*$^{-/-}$ mice bears resemblance to the human IBD, ulcerative colitis. Histopathologic analysis of multiple colitic *mdr1a*$^{-/-}$ animals revealed inflammation spread along the entire length of the colon. The intestinal inflammation was characterized by a massive thickening of the mucosa and evidence of inflammatory cell infiltrates into the lamina propria in *mdr1a*$^{-/-}$ mice with active colitis (Fig. 3*a*) compared with *mdr1a*$^{-/-}$ mice without colitis (Fig. 3*b*).

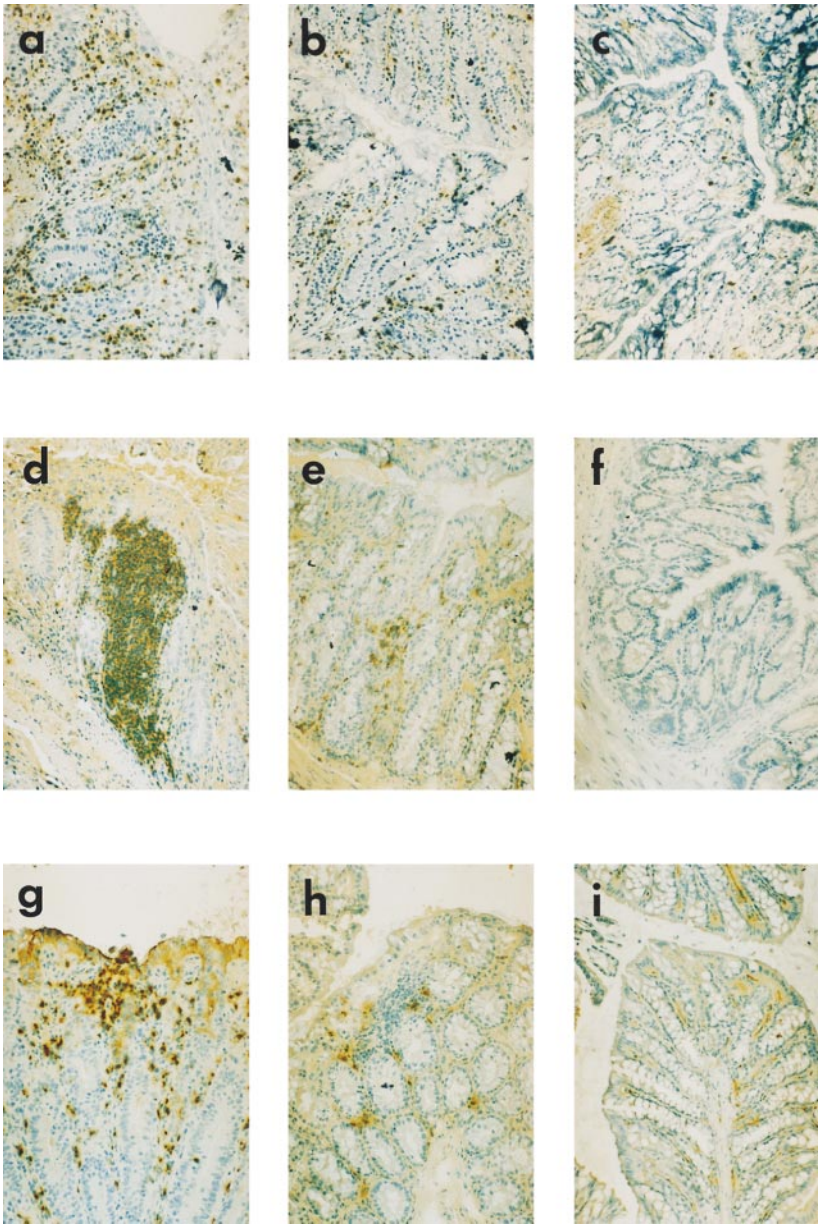

FIGURE 4. *mdr1a*$^{-/-}$ mice with established colitis have extensive T cell, B cell, and granulocyte infiltrates into the colonic lamina propria in areas of intestinal inflammation. Frozen sections of colon from *mdr1a*$^{-/-}$ mice with active colitis (*a*, *d*, and *g*), *mdr1a*$^{-/-}$ mice with early/intermediate colitis (*b*, *e*, and *h*) and control FVB mice (*c*, *f*, and *i*) were stained by the immunoperoxidase technique for expression of CD3 (*a–c*), B220 (*d–f*), and Gr1 (*g–i*). Positive staining is indicated by the presence of a brown reaction product. Note a dramatic increase in the number of CD3$^+$ T cells in the lamina propria of *mdr1a*$^{-/-}$ mice with active colitis (*a*) compared with early/intermediate colitic *mdr1a*$^{-/-}$ (*b*) and FVB (*c*) mice. B220 staining revealed the presence of B cells in follicular clusters in *mdr1a*$^{-/-}$ mice with active colitis (*d*). *mdr1a*$^{-/-}$ mice with early/intermediate colitis (*e*) did not have defined B cell clusters but had more lamina propria B cells than FVB mice (*f*). Increased frequencies of Gr1$^+$ cells could be seen aggregating in areas of inflammation in *mdr1a*$^{-/-}$ mice (*g*), particularly where breaches in the epithelium were apparent. Few Gr1$^+$ cells were present in the lamina propria of *mdr1a*$^{-/-}$ mice with early/intermediate colitis (*h*) or FVB mice (*i*). Sections are representative of data from two colitic mice, one early/intermediate colitic animal, and five control animals. At least three regions of intestine from the proximal to the distal end of each individual colon was analyzed.

Occasional crypt abscesses and ulcerations extending through the mucosa to the muscular layer were also often noted in the colitic animals (Fig. 3*c*). Crypt length was greatly increased in colitic animals, with approximately three- to fivefold more epithelial cells per crypt in colitic mice (Fig. 3*a*) compared with noncolitic mice (Fig. 3*b*). There also appeared to be some evidence of dysregulated epithelial cell growth in colitic *mdr1a*$^{-/-}$ mice in areas of inflammation (Fig. 3*d*).

Immunohistochemical analysis of the gastrointestinal tissues of animals with prevalent colitis or animals with early stages of colitis was used to further characterize the lymphocytic infiltrate into the mucosa of the large intestine compared with control FVB mice (Fig. 4). There was an increased number of infiltrating CD3$^+$ T cells scattered diffusely throughout the lamina propria of inflamed colonic tissue from mice with active colitis (Fig. 4*a*) compared with colonic tissue from control FVB mice (Fig. 4*c*). A less dramatic CD3$^+$ T cell infiltrate was observed in mice with early colitis (Fig. 4*b*).

FACS analysis of the large-intestine IEL and LPL isolated from colitic animals revealed that the majority of the infiltrating T cells were CD4$^+$TCR$\alpha\beta^+$ (Table II). Although there was an apparent decrease in the relative proportion of CD8$^+$ T cells in the lamina propria of colitic animals, the absolute number of CD8$^+$TCR$\alpha\beta^+$ T cells was not diminished, probably owing to the fourfold increase in the total number of LPL isolated from colitic animals (data not shown). There was no apparent increase in TCR$\gamma\delta^+$ T cells in the inflamed tissue (data not shown).

In addition to T cell infiltrates, immunohistochemical analysis also revealed the presence of numerous B220$^+$ B cell clusters/follicles within the lamina propria of animals with active colitis (Fig. 4*d*) compared with control FVB animals (Fig. 4*f*). Whereas the T cells were diffusely scattered throughout the lamina propria, the B cell infiltrates appeared to be localized to follicular clusters within the mucosa. The follicular clusters varied in size and were only observed in the colon. In contrast to animals with fully developed colitis, animals with intermediate colitis mostly had individual B220$^+$ cells scattered

Table II. *Mucosal T cell subsets isolated from colitic and control intestines*[a]

	IEL		LPL	
	Colitic *mdrla*$^{-/-}$ mice	Control FVB mice	Colitic *mdrla*$^{-/-}$ mice	Control FVB mice
% CD4$^+$/CD8$^-$	42	10	53	15
% CD8$^+$/CD4$^-$	30	36	7	30
% CD4$^+$/CD8$^+$	9	2	4	3
% TCR$\alpha\beta^+$	94	50	90	49

[a] IEL and LPL were isolated from large intestines from *mdrla*$^{-/-}$ mice with active colitis and from age-matched control FVB mice and stained for CD4, CD8, and TCR$\alpha\beta$ expression. Numbers are expressed as a percent of total mononuclear cells analyzed.

throughout the lamina propria and the few clusters that were present were reduced in size (Fig. 4*e*).

Gr1$^+$ cellular infiltrates were also prominent throughout the lamina propria of the large intestine in mice with active colitis (Fig. 4*g*) compared with control FVB mice, which appeared devoid of Gr1$^+$ cells (Fig. 4*i*). The Gr1$^+$ infiltrates were uniformly present throughout the lamina propria, although an increased number of cells did appear to be migrating across the epithelium into the gut lumen in regions where the epithelialbarrier may have been compromised (Fig. 4*g*). Mice with

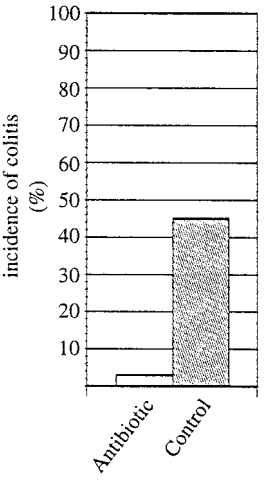

FIGURE 5. Treating *mdr1a*$^{-/-}$ mice with oral antibiotics can prevent the onset of colitis. Seventy *mdr1a*$^{-/-}$ mice with no visible signs of colitis were selected for the study. Regular drinking water was replaced with antibiotics and saccharine dissolved in drinking water ($n = 31$) or saccharine alone dissolved in drinking water ($n = 39$). Mice were monitored on alternate days for clinical signs of colitis for up to 16 wk. Mice were then killed and analyzed for histopathologic signs of colitis. Data represent the percentage of mice with clinical or histopathologic evidence of colitis.

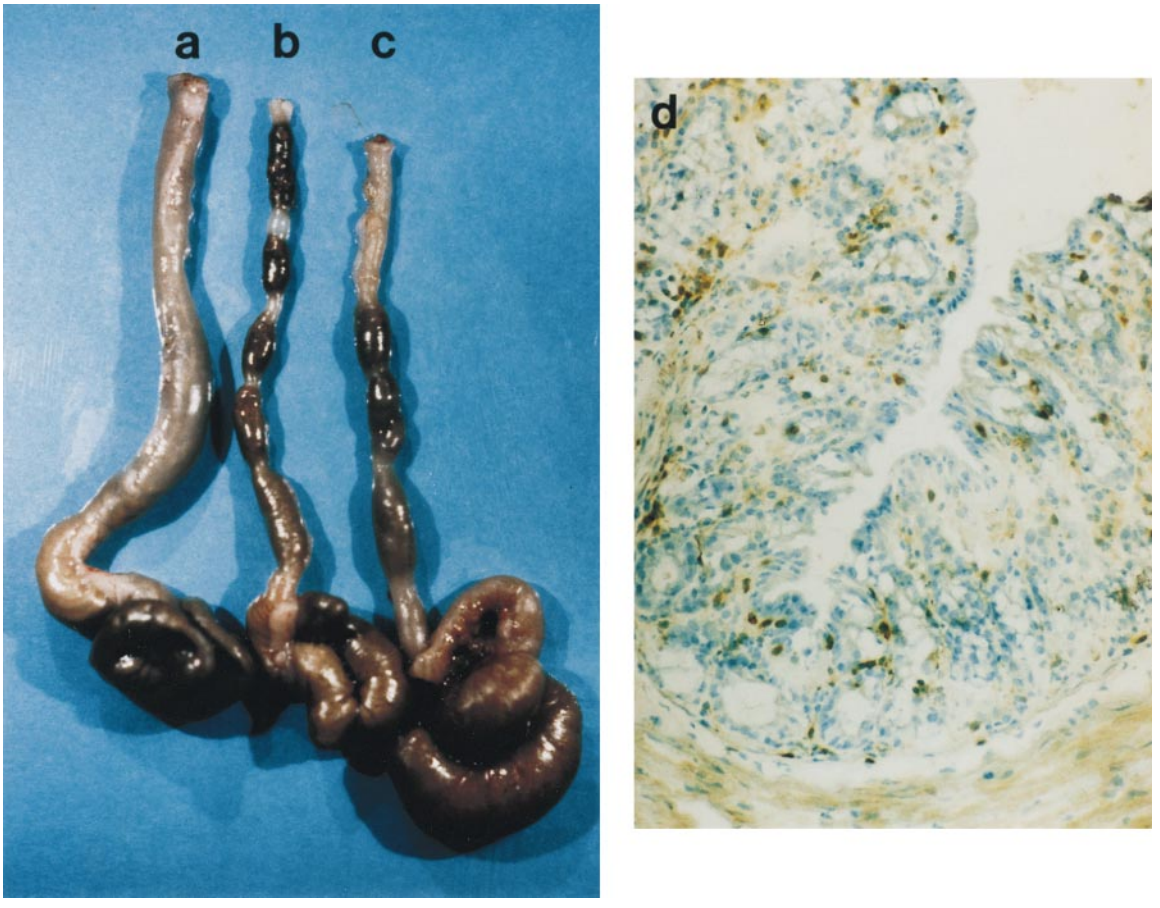

FIGURE 6. Therapeutic antibiotic treatment can reverse the gut inflammation associated with established colitis in *mdr1a*$^{-/-}$ mice. Mice with active signs of colitis (loose stools and anal discharge) were placed on antibiotic treatment for 10 wk ($n = 3$). At the end of the treatment period, colons were removed from untreated *mdr1a*$^{-/-}$ mice with active colitis (*a*), control FVB mice (*b*), and *mdr1a*$^{-/-}$ mice therapeutically treated with antibiotics (*c*). Note that the colon from the antibiotic-treated mouse does not appear inflamed and shows little residual evidence of thickening. *d*, Immunohistochemical analysis of colonic tissues from mice treated with antibiotics showed persistence of CD3$^+$ T cells despite significant reduction in inflammation.

Table III. *Identification of bacterial species*[a]

Mice	Aerobic Bacteria	Frequency[b]
FVB	*Lactobacillus* sp.	8/8
	Strep species, α-hemolytic	1/8
		1/8
	Enterococcus sp.	2/8
		1/8
	Bacillus	2/8
mdrla$^{-/-}$	*Lactobacillus* sp.	6/8
(no colitis)	*E. coli*	5/8
		1/8
	Bacillus sp.	1/8
	β hemolytic bacillus	1/8
		2/8
	Enterococcus	1/8
		2/8
	Proteus mirabilis	3/8
mdrla$^{-/-}$	*Proteus mirabilis*	2/6
(active colitis)	*Enterococcus*	1/6
		1/6
	Lactobacillus sp.	4/6
	Staphylococcus sp.	2/6
	Bacillus sp.	1/6

[a] Samples from cecum and colon were analyzed for identification of bacteria by two different methods (see *Materials and Methods*). All of the mice had a mixed anaerobic flora with heavy growth of *Bacteroides* sp.
[b] Frequency of mice with identified bacteria.

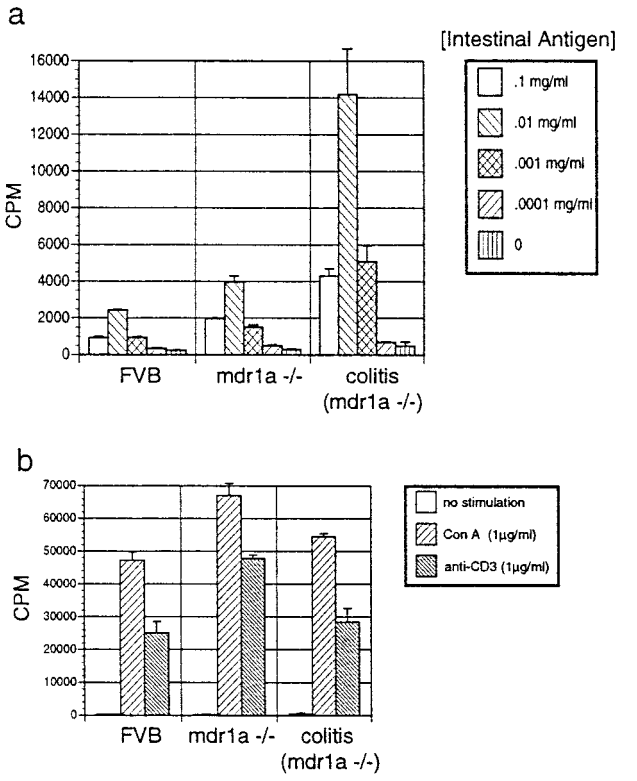

FIGURE 7. Lymphocytes from colitic *mdr1a*$^{-/-}$ mice have enhanced proliferative reactivity to bacterial Ags. Cells were prepared from the MLN of *mdr1a*$^{-/-}$ mice with active colitis (*mdr1a*$^{-/-}$, colitis), *mdr1a*$^{-/-}$ mice with no signs of colitis (*mdr1a*$^{-/-}$), and age-matched FVB mice (FVB) and assayed for proliferative reactivity to intestinal bacterial Ag preparations (*a*) or Con A and anti-CD3 mAb (*b*) as described in *Materials and Methods*. *a*, Cells from *mdr1a*$^{-/-}$ mice with active colitis demonstrated enhanced proliferative reactivity to the bacterial Ags ($p < 0.05$, Student's *t* test) compared with other mice. Cells from *mdr1a*$^{-/-}$ mice without colitis did not differ significantly from cells from FVB mice. *b*, Cells from all mice responded similarly following stimulation with Con A or anti-CD3. Values are expressed as mean cpm $\pm$ 1 SD of triplicate wells. The data shown are from one experiment and are representative of three similar experiments.

early colitis had relatively few Gr1$^+$ infiltrating cells, and these were restricted to small focal regions in the lamina propria (Fig. 4*h*).

Prophylactic treatment with oral antibiotics can prevent spontaneous colitis in mdr1a$^{-/-}$ *mice*

It has been well established in a majority of mouse models that spontaneous colitis can be prevented if mice are housed under germfree conditions, suggesting that the presence of the gut flora is necessary for the development of colitis (4, 12, 14). It has also been previously demonstrated in another model of spontaneous colitis that treating mice with antibiotics can prevent inflammation (13). In the first part of the study, we treated *mdr1a*$^{-/-}$ mice with broad-spectrum oral antibiotics to eliminate the commensal gut flora and then monitored for the development of colitis. Control mice were given water treated with saccharine alone. None of the mice had detectable colitis at the beginning of the study.

The incidence of colitis in *mdr1a*$^{-/-}$ mice treated with antibiotics was greatly reduced compared with *mdr1a*$^{-/-}$ mice treated with saccharine alone (Fig. 5). In the 16-wk treatment, 14 of 31 saccharine-treated *mdr1a*$^{-/-}$ mice developed colitis (loose stools and mucous discharge), whereas only 1 of 39 antibiotic-treated *mdr1a*$^{-/-}$ mice developed any signs of inflammation (no clinical signs, histopathologic evidence only). The one antibiotic-treated *mdr1a*$^{-/-}$ mouse that showed signs of colitis had only one minor focal region of inflammatory cell accumulation (data not shown) and did not show any other overt signs of intestinal disease.

Therapeutic antibiotic treatment can reverse ongoing active inflammation in mdr1a$^{-/-}$ *mice*

To ascertain whether the intestinal inflammation in mice with active colitis could be reversed by antibiotic treatment, mice with active colitis (as determined by evidence of loose stools and mucous discharge from the anus) were therapeutically treated with antibiotics in the drinking water. Within 3 wk of continuous antibiotic therapy, the previously colitic mice had no residual visible signs of anal discharge or loose stools. To determine whether the pathology associated with colitis could also be reversed along with the gross symptoms, mice treated with antibiotics for 10 wk were killed and the colons analyzed histopathologically. As expected, the colons of untreated colitic animals were grossly thickened (Fig. 6*a*), compared with control animals (Fig. 6*b*). Interestingly, the gross thickening of the colon associated with active IBD was significantly reduced in mice that had received therapeutic antibiotic treatment (Fig. 6*c*). On further histopathologic examination of these intestines, we observed little evidence of inflammation, and the general gut architecture appeared similar to that of control mice (data not shown). Immunohistochemical analysis revealed that the mice treated with antibiotics had no evidence of persisting Gr1$^+$ infiltrates or B cell follicles (data not shown). However, despite the apparent reduction in inflammation arising from antibiotic treatment, there still appeared to be an increased number of CD3$^+$ T cells in the lamina propria of these animals (Fig. 6*d*) compared with control mice (Fig. 4*c*).

Analysis of bacterial flora in colitic and control mice

Although the commensal bacterial flora resident in the intestinal lumen of the gut appears to play a major role in the induction of colitis, little is known about the role of individual bacteria in the

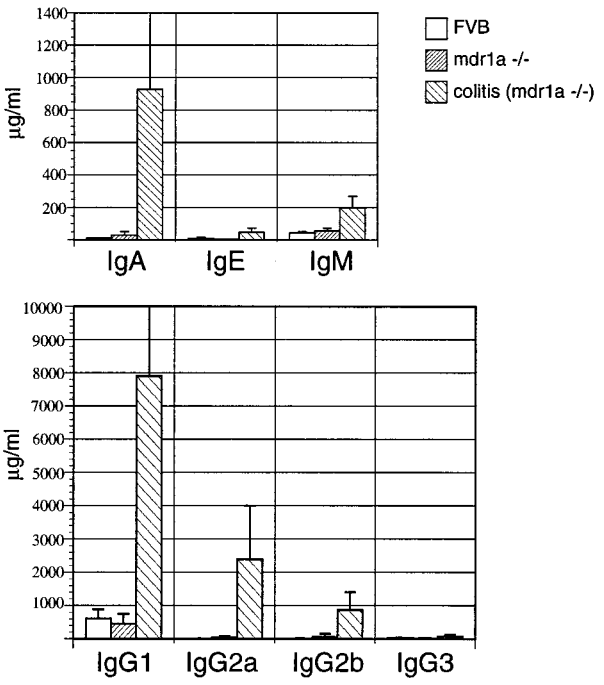

FIGURE 8. Serum Ab titers are elevated in *mdr1a*$^{-/-}$ mice with active colitis. Isotype-specific ELISAs were performed on serum from *mdr1a*$^{-/-}$ mice with active colitis (*mdr1a*$^{-/-}$, colitis), *mdr1a*$^{-/-}$ mice without active colitis (*mdr1a*$^{-/-}$), and control FVB animals (FVB). Colitic mice had an increase in serum Ab titers of all isotypes compared with noncolitic and control mice. Serum Ab titers were similar in noncolitic and control mice. Values are expressed as μg/ml for each isotype. Data represent the mean plus the SD of two separate animals analyzed for each experimental group.

initiation and perpetuation of intestinal inflammation. In this study we analyzed the bacterial flora present in the cecum and colon of *mdr1a*$^{-/-}$ mice with colitis, *mdr1a*$^{-/-}$ mice with no evidence of active colitis, and control FVB mice. Table III lists the major bacterial species recovered from the animals. All of the mice appeared to have a gut bacterial profile devoid of any pathogenic organisms.

Recently, it has been shown that *Helicobacter* infection in immunocompromised mice can give rise to a pathology resembling colitis (39–41). To ensure that colitis in the *mdr1a*$^{-/-}$ mice was not due to *Helicobacter* infection, samples from colitic, noncolitic, and control mice were analyzed by PCR for the presence of *H. bilis*, *H. hepaticus*, and *Helicobacter* spp. All mice were negative (data not shown).

In vitro proliferative reactivity of cells from the gut-associated lymphoid tissue following stimulation with intestinal bacterial Ags

Since bacterial colonization of the intestine appears to be crucial for the initiation of spontaneous colitis in *mdr1a*$^{-/-}$ mice, we analyzed the in vitro proliferative response of cells isolated from the MLNs following stimulation with intestinal Ags (Fig. 7). Intestinal Ags were prepared from cecal contents obtained from FVB control mice, *mdr1a*$^{-/-}$ mice with no overt signs of colitis, and *mdr1a*$^{-/-}$ mice with active colitis. MLN were removed from the same animals, and cell suspensions were tested for proliferative reactivity against each of the intestinal bacterial Ag preparations.

We observed a significant increase in proliferation in response to bacterial Ags when MLN from *mdr1a*$^{-/-}$ mice with active colitis were compared with those of other mice ($p < 0.05$). In contrast, there was no statistically significant difference in the response to bacterial Ag stimulation when MLN from FVB mice were compared with MLN from noncolitic *mdr1a*$^{-/-}$ mice ($p > 0.1$). Reactivity profiles of each source of MLN were similar regardless of the source of bacterial Ags (data not shown). No significant differences were noted in the proliferative responses to Con A or anti-CD3 between any of the three groups of mice analyzed in these experiments ($p > 0.1$). The data shown are from one experiment and are representative of three similar experiments.

Table IV. *Incidence of colitis in bone marrow chimeric mice*[a]

Host	BM Donor	% of Mice Developing Colitis
mdr1a$^{-/-}$	FVB	25 ($n = 12$)
FVB	*mdrla*$^{-/-}$	0 ($n = 40$)
mdr1a$^{-/-}$	*mdrla*$^{-/-}$	100 ($n = 15$)
FVB	FVB	0 ($n = 20$)

[a] Bone marrow chimeric mice were generated as described in *Materials and Methods.* Mice were observed for both clinical and histopathological signs of colitis.

mdr1a$^{-/-}$ *mice with active colitis have increased serum Ab titers*

The increased frequency of B cell clusters in the lamina propria of colitic animals suggests that there may be a dysregulated humoral response in these animals. To test this, we measured the serum Ab titers of colitic, noncolitic, and control animals. Each of the Ig isotype levels was assessed by ELISA. All of the serum Ig isotype levels were elevated in colitic animals compared with control FVB mice or *mdr1a*$^{-/-}$ mice without colitis, suggesting polyclonal activation of B cells associated with colitis. Serum IgA and IgG1 levels appeared most elevated in the mice with active colitis, although IgG2a, IgG2b, IgM, and IgE levels were also increased over control mice (Fig. 8).

Spontaneous colitis correlates with a lack of mdr1a *expression on intestinal epithelial cells*

Given that *mdr1a* is expressed on both lymphocytes and epithelial cells in the large intestine, we sought to determine whether the spontaneous development of colitis was the result of an *mdr1a* defect within the lymphoid or within the epithelial cell population. We generated irradiation bone marrow chimeras in which irradiated FVB animals were reconstituted with bone marrow from *mdr1a*$^{-/-}$ or FVB donors, or irradiated *mdr1a*$^{-/-}$ mice were reconstituted with bone marrow from FVB or *mdr1a*$^{-/-}$ donors. As controls, additional groups of mice were generated in which FVB mice were reconstituted with FVB bone marrow and *mdr1a*$^{-/-}$ mice were reconstituted with *mdr1a*$^{-/-}$ bone marrow. The incidences of colitis in the groups are listed in Table IV. *mdr1a*$^{-/-}$ mice that were reconstituted with wild-type bone marrow developed signs of colitis within 12 wk after reconstitution. After being maintained for 52 wk, 25% of the mice had developed active colitis. In contrast, two groups of FVB mice, which were reconstituted with *mdr1a*$^{-/-}$ bone marrow, were maintained for up to 38 wk without any overt signs of colitis. As expected, the control FVB mice reconstituted with FVB bone marrow did not develop colitis. Surprisingly, 100% of the *mdr*$^{-/-}$ mice reconstituted with *mdr1a*$^{-/-}$ bone marrow developed colitis. The reason for the increased incidence in the latter group compared with unmanipulated *mdr1a*$^{-/-}$ mice is unclear, although it is possible that radiation damage to the mucosa sufficiently enhanced susceptibility to developing colitis.

Discussion

Mucosal immunology has advanced in recent years with the discovery of several different mouse models of IBD. Most of the mouse models described involve a disruption or modification of the immune system. Spontaneous colitis occurs in mice genetically deficient for TCR$\alpha\beta^+$ cells (5) or the cytokines IL-2 (6) or IL-10 (4) and in mice deficient for the G$\alpha 2_i$ signaling protein (42). In addition, IBD occurs in human CD3ϵ-transgenic mice reconstituted with normal bone marrow (7), in HLA-B27 transgenic rats (43), and in immunodeficient SCID mice reconstituted with $CD4^+CD45RB^{high}$ cells (9, 12, 44). The great diversity of these models implies that there are complex mechanisms regulating the various components of the mucosal immune system that create and maintain a homeostatic balance. Disruption of this balance in any number of ways can obviously lead to the development of an adverse inflammatory response.

Here we describe a novel model of spontaneous colitis occurring in *mdr1a*$^{-/-}$ mice that is characterized by a broad inflammatory response that appears restricted to the entire length of the colon. The histopathology of the inflammation is most consistent with features associated with ulcerative colitis in humans and is characterized by dysregulated epithelial cell growth, ulcerations, crypt abscesses, and lymphocytic infiltration into the mucosa. Given the large number and diverse nature of the IBD models, it is not surprising that there is some controversy regarding the precise nature of the cells responsible for initiating and perpetuating IBD. The diffuse lymphocytic infiltrates in the *mdr1a*$^{-/-}$ mice are primarily $CD4^+$TCR$\alpha\beta^+$ T cells and $Gr1^+$ cells. In addition, follicular B cell clusters are prominent throughout the mucosa.

mdr1a$^{-/-}$ mice require a commensal bacterial flora for the development of colitis. Colitis can be prevented in *mdr1a*$^{-/-}$ animals by oral treatment with broad-spectrum antibiotics to eliminate intestinal bacteria. The requirement for bacterial flora in the induction of colitis in *mdr1a*$^{-/-}$ mice is consistent with many of the other IBD models. IBD does not develop in the TCRα or IL-10 knockout animals maintained under germfree conditions (4, 14), and IBD can be prevented in the $CD4^+CD45RB^{high}$ SCID transfer model if the animals have a reduced number of enteric flora or are treated with broad-spectrum antibiotics (12, 13). In addition to preventing the initiation of IBD, we show here that antibiotic treatment can therapeutically reverse colitis in *mdr1a*$^{-/-}$ mice with active inflammation. This indicates that intestinal flora is not only necessary for initiating inflammation but is also required for sustaining the inflammatory response. Interestingly, despite the reversal of inflammation, a large number of $CD3^+$ T cells remain present in the mucosa. The functional state of these T cells is unclear.

In the course of this study, we observed a relatively high incidence of colitis in *mdr1a*$^{-/-}$ mice treated with saccharine compared with unmanipulated knockout mice in the colony. We have no definitive explanation for this, since there is no *a priori* reason why saccharine should make animals more susceptible to developing colitis. However, it is possible that changes do occur in the commensal flora when saccharine is included in the drinking water, perhaps permitting outgrowth of certain bacterial species. Despite the increased incidence of colitis in the saccharine-treated mice, the addition of antibiotics was sufficient to eliminate the problem and prevent both the development of colitis and reverse ongoing intestinal inflammation.

It is possible that an individual bacterial species that colonizes the intestine might be responsible for initiating inflammation in these murine IBD models. To determine whether there might be a specific bacterial pathogen associated with disease in the *mdr1a*$^{-/-}$ mice, we analyzed the bacterial composition of the resident cecal and colonic flora in colitic and noncolitic *mdr1a*$^{-/-}$ mice and control FVB mice. We were not able to identify a single bacterial species that could be solely responsible for triggering intestinal disease. There did appear to be variations in the relative abundance of certain bacterial species in the different samples analyzed, and while it remains possible that an excess of a singular species might be responsible for the initiation of colitis, this may simply reflect a selective advantage for certain bacteria in the local microenvironment during intestinal inflammation.

Another potential explanation for the requirement of intestinal flora in the induction of IBD is that cross-reactive epitopes may exist between intestinal flora and enterocytes, such that the enterocytes become the target of an autoimmune inflammatory response. In support of this, IBD in TCRα knockout mice is accompanied by the appearance of autoAbs, including those directed against epithelial cell proteins (8, 45). *mdr1a*$^{-/-}$ mice with active colitis have large B cell follicles in the lamina propria. These mice also have a massive increase in serum Ab titers of all isotypes, suggesting a polyclonal activation of B cells; however, it is unclear whether the Ab response is directed against intestinal tissues. We are currently breeding the *mdr1a*$^{-/-}$ mice with B cell-deficient mice to determine whether B cell activation is a critical component in the initiation of colitis.

Since data from many of the other IBD models indicate that T cells are a major component in the induction of IBD, and much of the intestinal damage in IBD appears to be the result of T cell-mediated injury (46, 47), we decided to analyze the functional activity of T cells isolated from *mdr1a*$^{-/-}$ mice with active colitis. Lymphocytes isolated from animals with active inflammation appeared to be significantly more responsive to intestinal bacterial Ags compared with control animals. Lymphocytes isolated from *mdr1a*$^{-/-}$ mice without active inflammation had a similar proliferative reactivity compared with control FVB animals. This indicates that the *mdr1a* defect does not inherently cause hyperresponsiveness to bacterial Ags. Rather, it appears that lymphocytes become hyperresponsive to bacterial stimulation only after the onset of inflammation. The finding that bacterial Ags prepared from animals with colitis and animals without colitis were equally potent in stimulating proliferation further suggests that colitis is probably not caused by a single pathogenic organism.

A central question in the *mdr1a*$^{-/-}$ mouse IBD model is, where does the defect that leads to colitis reside? *mdr* genes are expressed on a number of immune and nonimmune cell types in mice and humans (19–21, 23). Thus, it is possible that *mdr1a* may have several different functional activities depending on the site of expression. We show here that *mdr1a* is expressed on murine peripheral and mucosal T lymphocytes including all TCR$\gamma\delta^+$ and $CD8^+$ cells and a proportion of TCR$\alpha\beta^+$ and $CD4^+$ cells. Despite the lack of *mdr1a* expression on immune cells in the *mdr1a*$^{-/-}$ mice, the immune system does not appear to have any gross phenotypic or functional defects. It is known that *mdr1a* is also expressed on the apical surface of colonic epithelial cells, where it has been shown to actively pump orally administered toxins into the lumen of the gut (48). There is also evidence that *mdr1a* may play a role in Cl^- channel regulation in epithelial cells (49–51). Thus, we thought it possible that *mdr1a*$^{-/-}$ mice have functional defects within the intestinal epithelial barrier that might ultimately lead to colitis. To assess whether immune or epithelial cell defects were responsible for the susceptibility to colitis in *mdr1a*$^{-/-}$ mice, we generated a series of chimeric mice. Irradiated *mdr1a*$^{-/-}$ mice reconstituted with wild-type bone marrow spontaneously developed colitis. In contrast, irradiated wild-type mice reconstituted with *mdr1a*-deficient bone marrow cells did not develop colitis.

These data clearly suggest that the defect that leads to the initiation of colitis in *mdr1a*-deficient mice is concordant with defective *mdr1a* expression on a radiation-resistant element within the host, most likely the epithelial cells lining the gut. These data also suggest that the colitis in *mdr1a*$^{-/-}$ mice is not likely to be associated with immune cell deficiencies.

The precise mechanistic defect causing colitis in the *mdr1a*$^{-/-}$ mice is unclear. One possibility is that bacterial products/toxins become sequestrated within epithelial cells, since small amphiphilic, hydrophobic molecules are less likely to be secreted in the absence of a functional *mdr* pump. It is not unreasonable to assume that a buildup of bacterial products within *mdr1a*-defective epithelial cells could subsequently initiate an inflammatory response, either by direct toxicity to the epithelial cell or indirectly by altering the nature of the Ag-presenting capability of the epithelial cell. The demonstrated requirement for bacterial flora in the initiation and progression of the inflammation, and the association of disease with defective *mdr1a* expression in epithelial cells, suggests that one of these scenarios might be true. We are currently attempting to ascertain the precise function of *mdr1a* in colonic epithelial cells.

With all of the different immune models of colitis, it has become apparent that the intestinal microenvironment is an exquisitely regulated system and that perturbation of individual components within this environment can lead to inflammation. The model described here has many similarities with existing murine models of spontaneous colitis, including an inflamed pathology restricted to the colon and a dependence on intestinal flora. In contrast to most existing IBD models, the immune system of *mdr1a*$^{-/-}$ mice does not have any gross abnormalities. Furthermore, this model reveals that disruption of a nonimmune component of the mucosal environment can also be responsible for the initiation of colitis. The *mdr1a* knockout mouse model of spontaneous colitis should provide us with insight into the role of the epithelial barrier in preventing aberrant immune responses to commensal bacterial, and possibly food, Ags. Moreover, it predicts a possible in vivo functional role for *mdr1a* in the maintenance of the intestinal epithelial barrier.

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