Epithelial dysfunction associated with the development of colitis in conventionally housed mdr1a−/− mice

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Resta-Lenert, Silvia, Jane Smitham, and Kim E. Barrett. Epithelial dysfunction associated with the development of colitis in conventionally housed mdr1a−/− mice. Am J Physiol Gastrointest Liver Physiol 289: G153–G162, 2005. First published March 17, 2005; doi:10.1152/ajpgi.00395.2004.—P-glycoprotein, the product of the multidrug resistance protein 1 (MDR1) gene, is a xenobiotic transporter that may contribute to the physiology of the intestinal barrier. Twenty-five percent of mdr1a-deficient (mdr1a−/−) mice spontaneously develop colitis at variable ages when maintained under specific pathogen-free conditions. We hypothesized that this disease would result from epithelial dysfunction and that conventional housing would increase incidence and severity of the colitis phenotype. Wild-type congenic FVB (+/+ ) mice were maintained under the same conditions as controls. Knockout and wild-type mice were matched for age and gender and observed for signs of colitis. Colonic tissues from both groups of mice were examined for macroscopic and microscopic injury and for basal ion transport and transepithelial resistance (TER). Translocation of bacteria across the intestine was assessed by culturing the spleen and mesenteric lymph nodes. Protein analysis was performed by Western blot analysis. All mdr1a−/− mice developed weight loss and signs of colitis, whereas wild-type mice never showed such signs. Within the mdr1a−/− group, males consistently developed severe colitis earlier than females. Knockout mice showed increased basal colonic ion transport (females, 162.7 ± 4.6 vs. 49.7 ± 3.8 μA/cm²; males, 172.6 ± 5.6 vs. 54.2 ± 3.1 μA/cm²; P < 0.01) and decreased TER (females, 25.4 ± 0.3 vs. 36.4 ± 0.8 Ω·cm²; males, 23.1 ± 1.0 vs. 38.3 ± 0.3 Ω·cm²; P < 0.01) compared with wild-type mice. Barrier dysfunction was accompanied by decreased phosphorylation of tight junction proteins. Expression of cyclooxygenase-2 and inducible nitric oxide synthase in intestinal tissues was increased in the mdr1a−/− group (P < 0.01) and correlated with disease severity. Bacterial translocation was greater both in incidence (P < 0.01) and severity (P < 0.001) for the knockout group. With respect to all indexes studied, mdr1a−/− males performed worse than females. Our data support the hypothesis that alterations in the intestinal barrier alone, in the absence of immune dysfunction, may rapidly lead to colitis in the setting of a normal colonic flora.

ULCERATIVE COLITIS (UC) and Crohn’s disease (CD) collectively comprise the inflammatory bowel diseases (IBD), chronic inflammatory syndromes causing profound alterations in gastrointestinal structure and function. It is generally agreed that IBD results from a genetically determined abnormality in the mucosal immune response to normally innocuous constituents of the mucosal microflora (3, 8, 10, 17, 27) with accompanying tissue injury.

Great advances in our knowledge of the pathogenesis of IBD have been obtained from the analysis of a wide variety of mouse models of intestinal inflammation, with characteristics similar to IBD in humans. These animal models have generally been recognized as resulting from a variety of pathogenetic mechanisms. In such animals, spontaneous colitis can occur as a result of a natural genetic abnormality or because of a genetic defect induced by either gene targeting or the introduction of a transgene or after exposure to an exogenous agent acting as a hapten. In some models, colitis has also been induced in healthy mice by passive transfer of dysregulated T cells from affected animals (1, 2, 11, 19, 24, 26).

The interface between luminal contents and the intestinal epithelium represents the greatest surface of the host exposed to and actively interacting with the environment. Intestinal barrier function regulates transport and host-defense mechanisms at the mucosal interface with the outside world, and its role in the pathophysiology of gastrointestinal diseases has widely been emphasized (15). Transcellular and paracellular fluxes, the major physiological components of intestinal epithelial barrier function, are controlled by pumps, ion channels, and tight junctions, adapting permeability to the intestinal physiological milieu. Several lines of evidence support the concept that impaired barrier function is the trigger to altered tolerance to xenobiotics and exogenous antigens, leading to the establishment of chronic inflammation (2, 7, 11, 15). Furthermore, there is evidence the multidrug resistance protein (MDR) plays a critical role in host-bacterial interactions in the gastrointestinal tract and in the maintenance of intestinal homeostasis. The MDR, or P-glycoprotein (P-gp), is a 170-kDa plasma membrane protein of the ATP binding cassette superfamily of transport proteins. P-gp is encoded by multidrug resistance genes located in a region of the human genome (7q21.1) where a gene involved in susceptibility to IBD may also be present (23, 25, 29). The physiological importance of P-gp in the gastrointestinal tract has been elucidated using genetically engineered mice lacking the mdr1a gene (6, 12, 13, 28). Under specific pathogen-free conditions, 25% of mdr1a-deficient (mdr1a−/−) mice developed spontaneous colitis (18), whereas challenge of these animals with Helicobacter strains accelerated the occurrence of chronic colitis (16). Recently, the human MDR1 gene has been shown to be highly polymorphic and subject to multiple mutations (25). The nucleotide polymorphism C3435T is of special interest, because it is associated with 25–50% lower expression of P-gp in the intestine and various other tissues. Moreover, it was proposed that individ-

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uals with the 3435TT genotype are at higher risk for developing UC because of reduced intestinal barrier function and may represent a significant subgroup of UC patients (23, 25, 29).

In this study, we sought to define whether spontaneous colitis occurring in mdr1a<sup>−/−</sup> mice is related to epithelial barrier and transport dysfunction. A secondary objective was to test the hypothesis that exposure to commensals and pathogens in a conventionally housed mouse colony would increase the incidence of the colitis phenotype in mdr1a<sup>−/−</sup> mice. We therefore characterized the role and impact of normal flora colonization on the development of spontaneous colitis in this mouse model of IBD, and we defined age and gender differences in disease onset and severity by clinical, histopathological, and immunological methods.

**MATERIALS AND METHODS**

**Animals.** Knockout (KO) parental mice were obtained from the pathogen-free colony of mdr1a<sup>−/−</sup> mice at Taconic (Germantown, NY). A breeding colony of such mdr1a<sup>−/−</sup> mice was then established at the University of California San Diego (UCSD) animal facility and maintained under conventional housing conditions. Thus all KO mice used in these studies, except for a small number of parental mice, were born and raised conventionally. Wild-type congenic FVB (+/+<sup>t</sup>) mice were identically housed. All studies were approved by the UCSD Committee on Investigations Involving Animal Subjects.

**Experimental protocol.** mdr1a<sup>−/−</sup> and mdr1a<sup>+/+</sup> mice were matched for age and gender and observed for colitis (changes in weight, stool frequency and consistency, presence of blood in stools, prolapsed rectum, perianal abscess, wasting, and withdrawal). Mice were weighed three times weekly, and a clinical assessment was performed at the same time. Mice were evaluated for hemorrhage from the gastrointestinal tract using the fecal occult blood test (Hemoccult SENSIA, Beckman Coulter, CA; reaction time, 2 min, yes/no response). Moreover, several clinical parameters were selected for the evaluation of the severity of illness in the KO mice. Specifically, as indicated in Table 1, severity of diarrhea, blood in the stools, abdominal swelling, and perianal ulcer were combined to provide a measure of colitis severity. Scores of 1–4, 5–7, and 8–10 defined mild, moderate, and severe disease, respectively. At 5, 8, 12, and 21 wk of age, groups of KO and control mice were killed and small intestine, colon, spleen, and mesenteric lymph nodes were plated on nonselective and selective media. Briefly, the probes were added to the mucosal reservoirs, and 0.22-ml samples were taken from serosal reservoirs at 20-min intervals up to 180 min and replaced with fresh buffer. FITC-dextran and HRP concentrations were measured as previously described (21). Bacterial translocation was assessed by serially diluted samples of the probe as a standard. HRP activity was determined by adding 100 μl of substrate reagent (TMB One-Step Substrate System, S1600, DAKO, Carpinteria, CA) to serially diluted samples for 15 min and stopping the reaction with 100 μl 2N H<sub>2</sub>SO<sub>4</sub>. The concentration of HRP was determined spectrofluorimetrically (450 nm excitation and 450 nm emission).

**Bacterial translocation.** Homogenates of mesenteric lymph nodes and spleen were plated on nonselective and selective media. Briefly, the organs were homogenized by sonication for 10 min in PBS, and serial dilutions to 1<sup>10</sup> were prepared in PBS. An inoculum of 500 μl from each dilution was plated onto blood agar to count total anaerobic bacteria and onto standard trypticae soy agar to count total aerobic bacteria. All microbiological media were obtained from BBL (Cockeysville, MD). Plates were incubated for up to 72 h at 37°C under aerobic or anaerobic conditions, and the number of colony-forming units (CFU) was counted. Bacterial translocation was assessed by CFU counts and adjusted by tissue sample weight.

**Protein analysis.** Portions of small intestinal and colonic tissue homogenates were tested for occludin, zonula occludens-1 (ZO-1), claudin-1, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) expression by Western blot analysis as described in our previous studies (20). Briefly, aliquots of tissue homogenates were electrophoresed through 4.5–15% gradient polyacrylamide gels (Bio-Rad, Hercules, CA), and the proteins were then transferred onto blotting membranes (Polyscreen polyvinylidene difluoride, New England Nuclear, Boston, MA). After overnight blocking (PBS/Tween supplemented with 1% nonfat dry milk), blots were sequentially incubated with primary (all from Santa Cruz Biotechnology, Santa Cruz, CA) and secondary antibodies (HRP-conjugated anti-mouse IgG from Upstate Biotechnology, Lake Placid, NY) for 60 min at room temperature. Proteins were visualized by chemiluminescence [enhanced chemiluminescence (ECL Plus), Amersham, Piscataway, NJ] followed by exposure of the blots to X-OMAT film (Eastman Kodak, Rochester, NY). Quantitative results were obtained by scanning the resulting images and analyzing them densitometrically using National Institutes of Health Image software.

### Table 1. Criteria for evaluating severity of illness

<table>
<thead>
<tr>
<th>Variable</th>
<th>Severity of Illness (score)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild (0)</td>
</tr>
<tr>
<td>Diarrhea score&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Blood in stools&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Weight change&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Abdominal swelling&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Perianal ulcer&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Mild = frequent loose stools; moderate = continuous and abundant loose stools with mucus; severe = continuous and abundant loose stools with incontinence (anal leakage).<br>
<sup>b</sup> Blood in stools: intermittent, frequent, continuous.<br>
<sup>c</sup> Blood in stools: intermittent, frequent, continuous.<br>
<sup>d</sup> Abdominal swelling: absent, intermittent or continuous.<br>
<sup>e</sup> Perianal ulcer: present (Y) or not present (N).
ANOVA).
Under conventional housing, mdr1a
facility and mice born and grown in our breeding colony. For
were found between parental KO animals born at the vendor’s
of colitis under conventional housing conditions induced early
and after onset of diarrhea). All together, the KO mice were
wild-type mice. Figure 1 shows data for 12-wk-old mice that
findings in animals with (KO females, and matched wild-type (WT) mice. For the KO mice, the results combine
female mdr1a
Asterisks denote values significantly different from corresponding values for
KO females, n = 14; KO males, n = 13) diarrhea. All data are considered significant.

Statistical analysis. Results are presented as means + SE. Statistical comparisons were made by Student’s t-test, χ²-test, ANOVA, or
Meier-Kaplan log rank test as appropriate. P values of 0.05 or lower were considered significant.

RESULTS

Clinical parameters in mdr1a<sup>−/−</sup> and mdr1a<sup>+/+</sup> mice. Our initial studies focused on defining the main clinical end-points in mdr1a<sup>−/−</sup> and wild-type mice. No significant differences were found between parental KO animals born at the vendor’s facility and mice born and grown in our breeding colony. For this reason, all the findings for the KO mice were combined. Under conventional housing, mdr1a<sup>−/−</sup> mice showed significantly decreased body weight compared with age-matched wild-type mice. Figure 1 shows data for 12-wk-old mice that are representative of all clinical conditions observed (before and after onset of diarrhea). All together, the KO mice were smaller than their wild-type counterparts, and the progression of colitis under conventional housing conditions induced early weight loss and/or lack of weight gain.

![Fig. 1. Body weight in male and female 12-wk-old mdr1a<sup>−/−</sup> [knockout (KO)] and matched wild-type (WT) mice. For the KO mice, the results combine findings in animals with (KO females, n = 28; KO males, n = 29) and without (KO females, n = 14; KO males, n = 13) diarrhea. All data are expressed as means ± SE. KO n = 84, WT n = 26 (13 males and 13 females). Symbols denote values significantly different from WT (⁎P < 0.05, ⁎⁎P < 0.01 by ANOVA).](image)

Pathological parameters in mdr1<sup>−/−</sup> and mdr1<sup>+/+</sup> mice. By macroscopic pathological examination, mdr1a<sup>−/−</sup> mice from 5 wk on began to show consistent signs of moderately swollen, distended colons or true megacolon (defined as a massive enlargement of the colon), enlarged mesenteric lymph nodes and spleens, perforated intestinal loops with signs of bleeding, and, in the most severe cases, mucosal fragility, i.e., the excised tissues could easily be damaged by

Mdr1a KO, but not control FVB mice, consistently developed diarrhea when conventionally housed. In Fig. 2, we show that there was also a difference between male and female mdr1a<sup>−/−</sup> mice with respect to the time of the onset of diarrheal signs. The majority of female KO mice started to show loose stools at 9–16 wk, whereas diarrheal signs began in most males between the 5th and 12th wk. The incidence of disease among the KO mice was 100%. Table 3 shows the distribution and prevalence of other clinical signs that were observed and recorded in the course of this study. Overall, the male KO mice showed a significantly more severe and worsening disease picture than did females. When evaluated for severity of illness (Table 4), the majority of the KO males scored higher, i.e., showed more severe clinical findings, than their female counterparts. Furthermore, mdr1a<sup>−/−</sup> mice had a markedly shorter life expectancy compared with age- and sex-matched wild-type controls (Fig. 3). These data show that, in the absence of P-gp, exposure to conventional bacterial colonization induced progressive and severe colonic inflammation, with males more severely affected than females.

![Table 3. Distribution and prevalence of clinical signs among mdr1a<sup>−/−</sup> mice after the onset of diarrhea<sup>b</sup>](image)

<table>
<thead>
<tr>
<th>Clinical Sign</th>
<th>F (n = 42)</th>
<th>M (n = 42)</th>
<th>P+</th>
<th>Total (n = 84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal occult blood</td>
<td>10 ± 24&lt;sup&gt;⁎&lt;/sup&gt;</td>
<td>22 ± 52</td>
<td>0.05</td>
<td>32 ± 38</td>
</tr>
<tr>
<td>Abdominal swelling</td>
<td>5 ± 12</td>
<td>12 ± 28</td>
<td>0.03</td>
<td>17 ± 20</td>
</tr>
<tr>
<td>Withdrawal</td>
<td>2 ± 4</td>
<td>11 ± 26</td>
<td>0.05</td>
<td>13 ± 15</td>
</tr>
<tr>
<td>Perianal ulcer</td>
<td>2 ± 4</td>
<td>8 ± 19</td>
<td>0.03</td>
<td>10 ± 12</td>
</tr>
<tr>
<td>Oral-nasal ulceration</td>
<td>2 ± 4</td>
<td>5 ± 12</td>
<td>ns</td>
<td>7 ± 8</td>
</tr>
<tr>
<td>Perianal abscess</td>
<td>1 ± 2</td>
<td>5 ± 12</td>
<td>ns</td>
<td>6 ± 7</td>
</tr>
<tr>
<td>Wasting</td>
<td>1 ± 2</td>
<td>5 ± 12</td>
<td>ns</td>
<td>6 ± 7</td>
</tr>
<tr>
<td>Prolapsed rectum</td>
<td>1 ± 2</td>
<td>4 ± 9</td>
<td>ns</td>
<td>5 ± 6</td>
</tr>
<tr>
<td>Alopecia</td>
<td>0</td>
<td>3 ± 7</td>
<td>ns</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>Fistulization&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0</td>
<td>1 ± 2</td>
<td>ns</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Age 5–20 wk; †prevalence (%); *statistical analysis was by χ²-test to assess differences between male (M) and female (F) mice; ns, not significant; *fistulization and/or intestinal rupture were occasional findings at autopsy.

![Table 4. Severity of illness scores in 12-wk-old mdr1a<sup>−/−</sup> mice<sup>c</sup>](image)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (n = 42)</td>
<td>28 (67)</td>
<td>12 (28)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Male (n = 42)</td>
<td>3 (7)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>20 (48)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>19 (45)&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total (n = 84)</td>
<td>31 (37)</td>
<td>32 (38)</td>
<td>21 (25)</td>
</tr>
</tbody>
</table>

Values represent numbers of animals, with percentage of group in parentheses. *See text for definitions. *P < 0.05, †P < 0.01 by χ²-test compared with females.
even gentle manipulation. None of these signs were observed in tissues harvested from control FVB mice or in mdr1a<sup>−/−</sup> mice at weaning. In Fig. 4, we show the histological appearance of colonic tissue from 12-wk-old KO mice compared with wild-type FVB mice. The mdr1a<sup>−/−</sup> mice consistently showed increased neutrophilic infiltration, thickening of the lamina propria, loss of goblet cells, deep mucosal ulcerations, and crypt abscesses. Small intestinal samples from mdr1a<sup>−/−</sup> mice showed flattening of villi and some thickening of the lamina propria, with modest infiltration by inflammatory cells (not shown). Furthermore, the spleen (Fig. 5) and lymph nodes (not shown) of affected mice showed an increased trabecular infiltrate with neutrophils and substantial disarray of the tissue structure. Moreover, Peyer’s patches of the KO mice contained increased numbers of activated plasma cells (Fig. 5). Whereas mdr1a<sup>−/−</sup> mice at weaning (3–4 wk old) did not show significant histological alterations (not shown), both male and female KO mice showed patchy losses of goblet cells and thickening of the lamina propria as early as 5–6 wk of age (not shown) regardless of the presence of clinical signs of colitis. Table 5 shows that the male KO mice had more severe histopathological findings than their female counterparts. The only cases of perforation were observed among the mdr1a<sup>−/−</sup> male mice.Taken together, these observations strongly support our hypothesis that colonization of the gut with a normal bacterial flora is responsible for the early and severe development of chronic inflammation flora in the absence of P-gp expression in the gastrointestinal tract. Moreover, the appearance of this inflammation is strongly reminiscent of the clinical and histopathological features of human IBD.

Electrophysiological parameters in mdr1a<sup>−/−</sup> and mdr1a<sup>+/+</sup> mice. We next examined whether mdr1a<sup>−/−</sup> mice displayed alterations in epithelial transport or barrier function that might account for the abundant diarrhea seen in these animals. Colonic sections from KO mice and wild-type FVB animals were mounted in Ussing chambers, and TER was calculated using Ohm’s law. As shown in Fig. 6, tissues from mdr1a<sup>−/−</sup> mice showed significantly decreased TER compared with those from wild-type animals. Moreover, no significant difference in TER was observed between mice killed before or after the onset of diarrhea (not shown). This suggests that barrier dysfunction precedes, and may cause, the onset of disease in this colitis model. Furthermore, when we tested colonic tissues of mdr1a<sup>−/−</sup> and wild-type mice for permeability to FITC-dex-
tran and HRP, we observed a significant difference between the two groups of mice (Fig. 7). Table 6 shows that epithelial permeability was significantly abnormal already at weaning, i.e., before the onset of diarrhea, but became worse as the colitis progressed. There was no significant difference in permeability between male and female mice from the two groups once disease was established.

Because diarrhea may also result from an increase in intestinal ion secretion, we then investigated whether this was true in our model of colitis. Baseline ion transport, measured as short-circuit current ($I_{sc}$) in Ussing chambers was comparable among mdr1a$^{-/-}$ and wild-type mice at weaning, i.e., before the onset of diarrhea in the KO animals (Fig. 8A). In contrast, $I_{sc}$ was significantly increased in mdr1a$^{-/-}$ mice with established colitis compared with findings in wild-type mice (Fig. 8B). When ion transport was stimulated in Ussing chambers by addition of either 8Br-cAMP or carbachol, tissues from the colitic mice showed significantly diminished responses compared with those in tissues from FVB wild-type mice (Fig. 9). A decreased response to secretagogues has been reported in several other colitis models (1, 11, 26). When stimulated ion transport was measured in mice at weaning, with no clinical signs of colitis, no significant differences were observed between wild-type and KO mice (not shown). These data show that barrier dysfunction and early histological changes precede the onset of clinical signs and ion-transport dysfunction representative of established colitis.

Table 5. Histology score by study group

<table>
<thead>
<tr>
<th>Score</th>
<th>KO Female</th>
<th>KO Male</th>
<th>WT Female</th>
<th>WT Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10 (100)$^a$</td>
<td>10 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>17 (40)</td>
<td></td>
<td>13 (31)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25 (60)</td>
<td>25 (59)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>4 (10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values represent number of animals, with percentage of groups in parentheses. $^a$See Table 2 for scoring definitions. KO, knockout; WT, wild-type. $^a$(%)
by alterations in tight junction proteins, we investigated the expression of tight junction proteins in colonic tissue homogenates by Western blot analysis. Although the levels of occludin, ZO-1, and claudin-1 were essentially similar in KO mice vs. FVB normal controls, the levels of phosphorylated occludin were significantly diminished at both 4 and 12 wk of age in the KO compared with wild-type mice (Fig. 10). Similarly, there was a significant reduction in the level of phosphorylation of ZO-1 at 12 wk in KO vs. wild-type mice. Thus barrier dysfunction in KO mice may depend, at least in part, on altered function of tight junction proteins as reflected by their phosphorylation. Interestingly, there was no apparent difference in the expression of tight junction proteins between male and female mice.

Microbiological parameters in mdr1a−/− and mdr1a+/+ mice. It is well known that barrier dysfunction may facilitate passage of bacteria from the colonic lumen to the lamina propria and from there to the draining lymphatics, lymph nodes, and spleen. We thus investigated whether any bacterial translocation could be found in the mesenteric lymph nodes and spleens of mdr1a−/− mice or in matched wild-type controls. Table 7 shows that when we measured bacterial translocation by CFU counts in the spleen and lymph nodes, the KO mice had much higher numbers of bacteria compared with the FVB wild-type animals.Gram-negative bacteria were the prevalent strains isolated. Very few, if any, bacteria were found in

Table 6. Permeability to FITC-dextran and HRP in mdr1a−/− and wild-type mice of various ages

<table>
<thead>
<tr>
<th>Mdr1a−/−, age, wk</th>
<th>Probe</th>
<th>Wild-Type^ (n = 35)</th>
<th>4+ (n = 14)</th>
<th>5–8 (n = 16)</th>
<th>9–12 (n = 20)</th>
<th>13–16 (n = 20)</th>
<th>17–20 (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC-dextran, ng/ml</td>
<td>107±25</td>
<td>190±17*</td>
<td>255±27†</td>
<td>290±15†</td>
<td>287±20†</td>
<td>276±23†</td>
<td></td>
</tr>
<tr>
<td>HRP, pg/ml</td>
<td>131±20</td>
<td>280±42*</td>
<td>696±55†</td>
<td>789±64†</td>
<td>799±65†</td>
<td>798±58†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. ^No statistical difference in permeability measurements was found among wild-type mice of any age tested. *P < 0.05, †P < 0.01; (mdr1a−/− vs. wild-type by ANOVA). HRP, horseradish peroxidase.
the spleen and lymph nodes of normal mice. Furthermore, bacterial translocation was correlated with age, especially among the KO males (Fig. 11) and with a histology score of 3 or higher (Table 8). Moreover, increased bacterial translocation was found among animals that had shown the highest severity of illness scores (Fig. 12). However, whereas bacterial translocation clearly increased as colitis progressed, as might be expected in this setting of mucosal injury, >50% of both male and female animals had measurable bacterial translocation to lymphoid tissues at 4 wk of age, before the onset of clinical, macroscopic, or histological signs of disease (Fig. 11). Male KO mice had bacteria cultured from their spleens in greater number and more frequently than seen in their female counterparts. Overall, these data are consistent with the decreased TER and increased permeability we observed in mdr1a−/− mice and suggest that barrier dysfunction precedes the development of illness.

Proinflammatory markers in mdr1a−/− and mdr1a+/+ mice.

We and others have previously shown that acute and chronic inflammation associated with responses to bacterial antigens are accompanied by upregulation of inflammatory markers, such as COX-2 and iNOS. When we tested colonic tissue homogenates for COX-2 and iNOS protein expression by Western blot analysis, the levels of both enzymes were significantly upregulated in KO mice vs. FVB normal controls (Fig. 13). However, no significant gender difference or relationship to the duration of clinical signs was found in the expression of these markers among KO mice.

DISCUSSION

IBD is considered to result from abnormal mucosal immune responses to microbial antigens that would normally be harmless and tolerated. Evidence in support of this concept has been derived in great part from multiple models of mucosal inflammation in the gastrointestinal tract (1–5, 8, 9, 11). For example, genetically targeted mice of numerous
microscopic evidence of profound alterations in gut structure. Chronic diarrhea, shortened lifespan, and macroscopic and chronic inflammation of the gut, with consequent weight loss, a “normal” microbiota uniformly induced signs of severe analysis was by controls.† Bacterial translocation was tested in spleen and lymph node homogenates and measured as colony forming units (CFU)/g of tissue. * 

mdr1a to process antigens appropriately, colitis can ensue. In 15). When this barrier function is impaired due to a failure thereby contribute greatly to the homeostasis of the gut (4, 15). Moreover, intestinal epithelial cells act as a physical and physiological barrier to xenobiotics and responses. Moreover, intestinal epithelial cells act as a physical and physiological barrier to xenobiotics and thereby contribute greatly to the homeostasis of the gut (4, 15). When this barrier function is impaired due to a failure to process antigens appropriately, colitis can ensue. In mdr1a−/− mice, it is likely that the epithelium is no longer able to expel xenobiotics. As a consequence of this defect, the mucosa is burdened with an overwhelming exposure to bacterial and other antigens. As we have described here, these mice, once exposed to bacterial colonization from an open environment since birth, develop colitis uniformly.

Our studies showed that exposure of the mdr1a−/− mice to a “normal” microbiota uniformly induced signs of severe chronic inflammation of the gut, with consequent weight loss, chronic diarrhea, shortened lifespan, and macroscopic and microscopic evidence of profound alterations in gut structure.

These observations were complemented by evidence for a severe loss of barrier function and altered ion transport, both factors that likely contributed to the chronic diarrhea. None of these findings were seen in FVB mice housed under identical conditions, highlighting the importance of the genetic background. In previous studies of this model of colitis (16, 18), exposure of mdr1a−/− mice to specific pathogen-free conditions or to challenge with specific strains of bacteria only resulted in full-blown disease in 20–25% of cases and at ages significantly older than our mice. We conclude that the more complex flora to which mice were likely exposed in our conditions of conventional housing may have driven all animals that were genetically predisposed to colitis to develop disease, and at earlier time points. However, this conclusion must be qualified, because it rests, in part, on a comparison with historical data obtained in specific pathogen-free conditions by other investigators.

Our studies also uncovered an intriguing gender bias in the development of colitis in mdr1a−/− mice. Thus, whereas all female mice eventually developed disease, they did so at older ages and with less severity than their male counterparts. This finding may suggest that physiological influences that differ between male and female animals, such as sex steroids, could influence the outcome of the disease. In this regard, it is of interest that we noted that female animals seemed somewhat protected from the most severe forms of disease by pregnancy (data not shown). Certainly, the gender differences we have defined are deserving of additional study to define their mechanisms, and such experiments are ongoing.

The precise reason(s) why a lack of P-gp predisposes to colitis is unknown, although disease in such animals is known to result from an epithelial, rather than immune system, defect (6, 12, 18, 23, 28). In support of this concept, Panwala et al. (18) showed that whereas irradiated mdr1a−/− mice reconstituted with bone marrow from wild-type mice still develop colitis, irradiated wild-type mice 

Table 7. Bacterial translocation to lymphoid tissues in mdr1a−/− and wild-type mice

<table>
<thead>
<tr>
<th>Group/Tissue</th>
<th>Mdr1a−/− Females (n = 32)</th>
<th>Mdr1a−/− Males (n = 30)</th>
<th>Wild-type Females (n = 15)</th>
<th>Wild-type Males (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>5.8 ± 0.3†</td>
<td>79</td>
<td>8.7 ± 0.6†</td>
<td>81</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>3.8 ± 0.5†</td>
<td>68</td>
<td>7.1 ± 0.4†</td>
<td>66</td>
</tr>
</tbody>
</table>

Values are means ± SE. Studies were performed in mice aged 5–25 wk. All mdr1a−/− mice had colitis. None of the wild-type age-matched controls were ill. Bacterial translocation was tested in spleen and lymph node homogenates and measured as colony forming units (CFU)/g of tissue. *P < 0.001 vs. wild-type controls. †P < 0.05 vs. mdr1a−/− females.

Our studies showed that exposure of the mdr1a−/− mice to a “normal” microbiota uniformly induced signs of severe chronic inflammation of the gut, with consequent weight loss, chronic diarrhea, shortened lifespan, and macroscopic and microscopic evidence of profound alterations in gut structure.

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Table 8. Prevalence of bacterial translocation (%) among wild-type and mdr1a−/− mice by histology score

<table>
<thead>
<tr>
<th>Animals</th>
<th>Histology Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Female, WT</td>
<td>5</td>
</tr>
<tr>
<td>mdr1a−/−</td>
<td>8</td>
</tr>
<tr>
<td>Male, WT</td>
<td>5</td>
</tr>
<tr>
<td>mdr1a−/−</td>
<td>8</td>
</tr>
</tbody>
</table>

* n = 20, b n = 50, c n = 25, d n = 54.
reconstituted with bone marrow from mdr1a<sup>−/−</sup> mice did not develop any evidence of disease. Moreover, our data showed that barrier dysfunction and early histological changes precede the onset of clinical and electrophysiological signs of established colitis. These data clearly demonstrate that the colitis in this model is not the result of an immune cell deficiency but that the abnormality resides in the epithelium lining the gut. In turn, the role of the immune system is likely to generate an aggressive physiological response to the imbalance induced by the barrier dysfunction inherent in the mdr1a<sup>−/−</sup> genotype. In fact, under normal circumstances, T cells from the gut lamina propria are tolerant to their own microbiota but will respond to another individual’s microbiota. It is intriguing to speculate that the mucosal immune system in mdr1a<sup>−/−</sup> mice has lost tolerance to self microbiota because of excessive exposure secondary to lack of housekeeping by the mdr1a pump. Indeed, the mdr1a pump is well known to be capable of effluxing xenobiotics, such as bacterial toxins, from a variety of cell types. Presumably, in the gut, such efflux returns putative bacterial toxins to the lumen for excretion.

A subgroup of UC patients displays genetic defects in the MDR gene (25, 29), and these too may lack effective clearance of bacterial products. It should, however, be acknowledged that controversy exists as to the precise contribution of MDR polymorphisms in human IBD (23, 29).

In conclusion, colitis in mdr1a<sup>−/−</sup> mice is more severe, earlier in onset, and of higher prevalence in conventionally housed animals than reported in those housed under SPF conditions (18). Furthermore, our studies have revealed a significant gender bias in the development and progression of colitis in this model. Most importantly, our data support the hypothesis that alterations in the intestinal barrier alone, in the absence of immune dysfunction, may rapidly lead to colitis in the setting of a normal colonic microbiota.

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A preliminary account of this work was presented at the annual meeting of the American Gastroenterological Association (2003) and has been published in abstract form (22).

GRANTS
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![Graph](image1.png)

**Fig. 12.** Incidence of bacterial translocation to lymphoid tissues of mdr1a<sup>−/−</sup> mice according to severity of illness. Forty mdr1a<sup>−/−</sup> mice, 20 males and 20 females, were scored for severity of illness at the time of death (age 12 wk), and bacterial translocation was evaluated as described in MATERIALS AND METHODS. Results are expressed as %mice that showed bacterial translocation to their lymphoid tissue for each severity of illness group. Statistical analysis was by χ²-test (*P < 0.05; n = 40; mdr1a<sup>−/−</sup> males vs. mdr1a<sup>−/−</sup> females).

![Graph](image2.png)

**Fig. 13.** Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) protein expression in colonic tissue of 12-wk-old mdr1a<sup>−/−</sup> and matched WT mice. Colonic tissue homogenates from mdr1a<sup>−/−</sup> and age-matched WT mice were tested for COX-2 (A) and iNOS (B) expression by Western blot analyses. The left of each panel shows representative blots, whereas the right shows the corresponding densitometric analysis for 8 such studies. All blots were stripped and reprobed for actin to assess equal protein loading. Statistical analysis was by ANOVA (***P < 0.01; n = 8; mdr1a<sup>−/−</sup> vs. wt). PF, parental female; PM, parental male; FF, F1, 2 female (i.e., based at UCSD); FM, F1, 2 male (i.e., born at UCSD); WF, WT female; WM, WT males.
REFERENCES