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Acute colitis induced by dextran sulfate sodium progresses to chronicity in C57BL/6 but not in BALB/c mice: correlation between symptoms and inflammation

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Melgar, Silvia, Agneta Karlsson, and Erik Michaëlsson. Acute colitis induced by dextran sulfate sodium progresses to chronicity in C57BL/6 but not in BALB/c mice: correlation between symptoms and inflammation. Am J Physiol Gastrointest Liver Physiol 288: G1328–G1338, 2005. First published January 6, 2005; doi:10.1152/ajpgi.00467.2004—Exposure to dextran sulfate sodium (DSS) induces acute colitis, which is normally resolved after DSS removal. To study chronicity, mice are typically subjected to three to five cycles of weekly DSS exposures, each followed by a 1- to 2-wk rest period. Here, we describe a novel and convenient way of inducing chronic, progressive colitis by a single exposure to DSS. C57BL/6 mice exposed to DSS for 5 days developed acute colitis that progressed to severe chronic inflammation. The plasma haptoglobin levels remained high during the chronic phase, showing that the inflammation was active. Surprisingly, the mice regained their original weight along with the progression of colitis, and the only apparent symptom was loose feces. Histopathological changes 4 wk after DSS removal were dense infiltrates of mononuclear cells, irregular epithelial structure, and persistent deposits of collagen. A progressive production of the cytokines IL-1β, IL-12 p70, and IL-17 correlated with the extensive cellular infiltration, whereas high IFN-γ production was mainly found late in the chronic phase. Similar to C57BL/6 mice, BALB/c mice exposed to 5 days of DSS developed acute colitis as previously described. The acute colitis was accompanied by elevated plasma levels of haptoglobin and increased colonic levels of IL-1α/β, IL-6, IL-18, and granulocyte colony-stimulating factor. However, soon after DSS removal, BALB/c mice recovered and were symptom free within 2 wk and completely recovered 4 wk after DSS removal in terms of histopathology, haptoglobin levels, and local cytokine production. In summary, these data stress the effect of genetic background on the outcome of DSS provocation. We believe that the present protocol to induce chronic colitis in C57BL/6 mice offers a robust model for validating future therapies for treatment of inflammatory bowel disease.

ULCERATIVE COLITIS (UC) and Crohn’s disease (CD) are the major chronic inflammatory bowel diseases (IBD) affecting the gastrointestinal tract in humans. IBD patients show flares of remission and relapses, with symptoms of bloody diarrhea, abdominal pain, and rectal bleeding. The etiology is unknown, but the pathogenesis is likely dependent on the interaction between local immune reactions and environmental factors in genetically susceptible individuals (44).

Several animal models have been developed to understand the pathology of IBD, but no specific model exists for either disease. Gut inflammation can be induced using chemical compounds [e.g., dextran sulfate sodium (DSS)] or by genetic targeting of specific genes (e.g., regulatory cytokines). The majority of the knockout (KO) mice develop colitis with histopathology resembling UC (e.g., IL-2 KO mice) or entero-colitis (e.g., IL-10 KO mice) (4). In 1990, Okayasu and colleagues (39) described a model in which mice receiving DSS orally developed acute and chronic colitis resembling UC. During the acute phase, the mice developed colonic mucosal inflammation with ulcerations, body weight loss, and bloody diarrhea (39). Administration of three to five cycles of DSS to the mice induced chronic inflammation, characterized by severe infiltration of mononuclear cells (MNC) and regenerative changes in the epithelium (39). The mechanism by which DSS induces colitis is not clear. Proposed mechanisms have been toxic effects on the epithelium and increased exposure to luminal antigens by destruction of mucin content or altered macrophage function due to ingestion of DSS (26, 37, 39). The resulting inflammation has been shown to include polymorphonuclear cells, macrophages, and B and T cells. Interestingly, Dieleman and colleagues (12) demonstrated that severe combined immunodeficient mice, without T and B cells, were able to develop DSS-induced colitis on the basis of clinical symptoms and histology, suggesting that adaptive immunity is not involved in acute colitis. In contrast, T cells producing IFN-γ and IL-4 were observed in Swiss Webster mice with chronic inflammation induced by DSS (11).

Differences in susceptibility to DSS-induced colitis due to genetic background are well established. Similar to IBD in humans, multiple loci affect the disease (31, 32).

In the present study, we used two mouse strains, BALB/c and C57BL/6, to analyze the consequences of DSS exposure in a careful kinetic study of symptoms, histology, and local and systemic levels of inflammatory mediators for up to 4 wk after DSS removal.

MATERIALS AND METHODS

Mice. Specific pathogen-free female BALB/c and C57BL/6 mice, 7–12 wk old, weighing 18–22 g, were obtained from Harlan. The mice were kept at the animal house facilities at AstraZeneca with 50% humidity and 12:12-h light-dark cycles and fed a standard pellet diet (R3 pellets, Lactamin) and tap water ad libitum. Mice were acclimated for ≥2 wk before entering the study. The Local Animal Research Board Committee (Göteborg) approved these studies.

Induction of colitis. DSS (45 kDa; TdB Consultancy, Uppsala, Sweden) was added to tap water at a concentration of 3% for C57BL/6 mice and 5% for BALB/c mice. Fresh DSS solution was prepared daily. Inasmuch as initial studies revealed that C57BL/6 mice exposed...
to 5% DSS became too diseased (data not shown), a lower concentration (3%) was used. When C57BL/6 mice were exposed to DSS for 7 days, instead of 5 days, and then studied for recovery, the symptoms and histopathological changes were aggravated, leading to high mortality (data not shown). Therefore, we chose to study the 5-day exposure. Mice were divided into groups of three to nine per time point and received DSS as shown in Fig. 1. Briefly, mice receiving DSS for 1, 3, or 5 days were used to analyze the response during the acute phase. Mice were exposed to DSS for 5 days and killed 1, 2, 3, or 4 wk after DSS removal (days 12, 19, 26, and 33, respectively) to study the progression from the acute phase to chronic inflammation or recovery. Healthy control animals received tap water only. The clinical symptoms recorded in DSS-treated and control mice were body weight, stool consistency, fecal bleeding, and diarrhea.

**Tissue and plasma sampling.** Colonic tissue and plasma samples were collected at time points shown in Fig. 1. At each time point, mice were anesthetized by inhalation of isoflurane (Abbott Scandinavia, Solna, Sweden), blood was drawn by retroorbital puncture, and the mice were killed by cervical dislocation. Blood was collected in tubes containing EDTA, and plasma was frozen and kept at −80°C. The intestines were excised and carefully rinsed with saline. The colon was cut close to the ileocecal valve and rectum, and the length was measured. Sections (1 cm) of the distal and proximal colonic wall were cut out and opened longitudinally, fixed in zinc-formalin solution (pH 7.4; Histolab Products, Göteborg, Sweden), and embedded in paraffin for histological analysis. The remaining part of the colon was weighed and frozen in liquid nitrogen.

**Assessment of inflammation: symptoms and inflammatory score.** Clinical assessment of inflammation included daily monitoring of body weight and general health condition. At necropsy, the macroscopic appearance of the colon (inflammatory score), based on the degree of inflammation and the presence of edema and/or ulcers, stool consistency (diarrhea score), and visible fecal blood, was scored separately on a scale of 0–3 (Table 1).

**Histology.** Tissue sections (5 μm) of distal and proximal colonic wall were stained with hematoxylin-eosin to address the degree of inflammation and with Masson’s trichrome to visualize the connective tissue. The stained tissue was analyzed independently (by S. Melgar) and in a blinded fashion (by a pathologist, G. Böttcher) with a standard microscope (Zeiss). The pathophysiology of the tissue was characterized by the presence of ulcerations, inflammatory cells (neutrophils, macrophages, lymphocytes, and plasma cells), signs of edema, crypt loss, surface epithelial cell hyperplasia, goblet cell reduction, and signs of epithelial regeneration.

**Analysis of local and systemic inflammatory markers.** Levels of the acute-phase proteins haptoglobin (55) and serum amyloid A (SAA) (17) were determined using a Cobas Bio centrifugal analyzer with a commercial reagent kit for haptoglobin (TP 801, Tridelta Development) and a commercial solid-phase sandwich ELISA for SAA (Tridelta Development).

Production of IL-1β, IL-6, IL-12 p40, IL-12 p70, IL-17, IL-1β, IFN-γ, and granulocyte colony-stimulating factor (G-CSF) was analyzed on whole colonic tissue homogenates. Dissected tissues were homogenized (Ultra Turrax T8, Tamro, Sweden) in homogenization buffer containing phosphate sodium saline (PBS; Gibco, Invitrogen) supplemented with complete miniproteinase inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany) and 10% fetal calf serum (Gibco). The homogenized solution was centrifuged at 10 min at 18,000 rpm, and the supernatant was separated into aliquots and frozen at −80°C until analysis. Production of IL-1β, IL-6, IL-12 p40, IL-12 p70, IL-17, and G-CSF was analyzed using the xMAP technology developed by Luminex (Austin, TX) (8). The concentration was determined using Bioplex Multiplex Suspension Array System kits, according to the manufacturer’s instructions (Bio-Rad Laboratories, Hercules, CA) and, subsequently, analyzed with five-parameter curve fitting using the Bioplex Manager 3.0 software (Bio-Rad). The homogenization buffer was used to generate the standard curve as a background to be subtracted from the standards as well as the samples and finally to dilute the samples 1:2 before analysis. IL-18, IFN-γ, and IL-1β were analyzed by ELISA according to the manufacturer’s instructions (R & D Systems). IL-1β was analyzed by ELISA and xMAP technology to compare the two techniques, and similar results were obtained (data not shown). The protein values are expressed as picograms per 100 mg of colonic tissue.

**Statistics.** Values are means ± SE. Mann-Whitney’s U-test was used for statistical analysis. *P* < 0.05 was considered significant. Spearman’s ρ test (Graph Pad Software) was used for correlation analysis.

**RESULTS**

**BALB/c and C57BL/6 mice display similar clinical symptoms and histopathological changes during acute colitis.** Clinical symptoms on exposure to DSS for 5 days were loss of body weight, loose feces/watery diarrhea, and fecal blood, consistent with the previous report (39). The symptoms were aggravated in both strains as disease progressed in the acute phase (Fig. 2, A–C). Body weight loss was observed from day 4 in C57BL/6 mice and from day 6 in BALB/c mice (Fig. 2A).

### Table 1. Assessment of inflammation by means of clinical and macroscopic score

<table>
<thead>
<tr>
<th>Score</th>
<th>Diarrhea Score</th>
<th>Visible Fecal Blood</th>
<th>Inflammatory Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal pellets</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Slightly loose feces</td>
<td>Slightly bloody</td>
<td>Slight inflammation</td>
</tr>
<tr>
<td>2</td>
<td>Loose feces</td>
<td>Bloody</td>
<td>Moderate inflammation and/or edema</td>
</tr>
<tr>
<td>3</td>
<td>Watery diarrhea</td>
<td>Blood in whole colon</td>
<td>Heavy inflammation and/or ulcers and/or edema</td>
</tr>
</tbody>
</table>

Score reflects degree of diarrhea, visible fecal blood, and inflammatory score on the day of termination and is characterized on a scale of 0–3. Inflammatory score reflects macroscopical appearance of the colon (inflammatory score) judged by degree of inflammation and presence of ulcers and/or edema in the tissue.

![Fig. 1. Overview of induction of acute, chronic, and recovery phases in BALB/c and C57BL/6 mice.](http://www.ajpgi.org)
Peak levels of diarrhea and visible fecal blood were found on day 5 in both strains (Fig. 2, B and C). Macroscopically, the inflammation was generally located in the distal colon in both strains. The inflammatory score increased as the inflammation progressed and was somewhat higher in C57BL/6 than in BALB/c mice at day 5 (Fig. 2D). Furthermore, the length of the colon decreased as the disease developed and was significantly shorter in both strains at day 5 (~20% in C57BL/6 mice and 30% in BALB/c mice; Table 2). At this time, the colonic wet weight had increased by ~50% in both strains (Fig. 3A). Plasma levels of haptoglobin were gradually elevated as the acute inflammation progressed (Fig. 3B) and correlated well with the macroscopic inflammatory score \( (r = 0.82 \text{ and } 0.85 \text{ for C57BL/6 and BALB/c mice, respectively, } P < 0.0001) \) and with colonic wet weight \( (r = 0.78 \text{ and } 0.86 \text{ for C57BL/6 and BALB/c mice, respectively, } P < 0.0001) \). Similar results were obtained for SAA (data not shown). Other features found at day 5 in C57BL/6 mice were enlargement of the mesenteric lymph nodes, decrease in size of the cecum, and occasional blood in the small intestinal lumen, stomach, and/or cecum (data not shown). Extraintestinal features found in BALB/c mice at day 5 were significantly enlarged spleen and mesenteric lymph nodes (Table 2).

Histopathological analysis was carried out using hematoxylin-and-eosin-stained sections of the proximal and distal colon (Fig. 4). At day 3, both strains showed minimal changes in the surface epithelium, although a slight infiltration of inflammatory cells to the mucosa was observed (data not shown). The changes were more pronounced in both strains at day 5, with loss of crypts and reduction of goblet cells, signs of surface epithelial regeneration, focal ulcerations, moderate infiltration of inflammatory cells to the mucosa, and edema in the submucosa (Fig. 4, b and d). The number of neutrophils was only slightly increased and remained elevated during the acute inflammation in C57BL/6 mice, whereas there was a marked increase in neutrophils in BALB/c mice (data not shown). In general, similar histopathological changes were observed in the proximal and distal colon of DSS-treated C57BL/6 and BALB/c mice.

Table 2. Colon length and spleen weight in C57BL/6 and BALB/c during acute, chronic, and recovery phases of colitis

<table>
<thead>
<tr>
<th>Days</th>
<th>C57BL/6</th>
<th>BALB/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.5±0.4</td>
<td>9.9±0.5</td>
</tr>
<tr>
<td>5</td>
<td>6.0±0.7</td>
<td>8.3±0.4</td>
</tr>
<tr>
<td>10</td>
<td>4.7±0.3</td>
<td>7.0±0.4</td>
</tr>
<tr>
<td>12</td>
<td>4.3±0.1</td>
<td>9.6±0.5</td>
</tr>
<tr>
<td>19</td>
<td>5.6±0.6</td>
<td>9.3±0.9</td>
</tr>
<tr>
<td>26</td>
<td>5.0±0.2</td>
<td>9.4±0.8</td>
</tr>
<tr>
<td>33</td>
<td>5.1±0.1</td>
<td>9.1±1.1</td>
</tr>
</tbody>
</table>

Values are means ± SD, based on records of 3–9 mice per time point as delineated in Fig. 1. C57BL/6 and BALB/c mice received dextran sulfate sodium (DSS) for 1, 3, or 5 days in the acute phase and 5 days of DSS followed by 7 (day 12), 14 (day 19), 21 (day 26), and 28 (day 35) days of water in chronic and recovery phases. Day 0 represents control/healthy mice (i.e., mice receiving only tap water throughout the study). Statistical values using Mann-Whitney U-test comparing mice at different time points with healthy mice (day 0): *P < 0.05; †P < 0.001; ‡P < 0.0001.
Overall, the clinical symptoms and histopathological changes found in both strains are in agreement with previously described features in murine DSS-induced acute colitis (39).

Acute colitis completely resolves in BALB/c mice, whereas C57BL/6 mice develop severe chronic inflammation with mild clinical symptoms. To determine the fate of inflammation after 5 days of DSS exposure, mice were monitored for an additional 4 wk. BALB/c mice regained their body weight 2–3 days after DSS removal (Fig. 2A), and no signs of diarrhea/loose stools remained at day 26 (Fig. 2B). No fecal blood could be found subsequent to DSS exposure (Fig. 2C). Furthermore, the inflammatory score was significantly decreased at day 19 and remained low thereafter (Fig. 2D). In addition, colonic wet weight, plasma haptoglobin, and SAA returned to normal levels at day 19 (Fig. 3, A and B; data not shown).

The symptomatic resolution of colitis in BALB/c mice correlated well with the histological changes (Fig. 4, h–j). At 1 wk after DSS removal (day 12), we observed a reduced number of inflammatory cells, signs of regeneration of crypts and goblet cells, and epithelial restitution (Fig. 4h). At 3–4 wk after DSS removal, the epithelial architecture was fully recovered, showing crypt and goblet cell regeneration and few inflammatory cells in the mucosa (Fig. 4j; see Fig. 6f).

C57BL/6 mice continued to lose body weight after DSS removal (Fig. 2A). Not until 1 wk after DSS, at day 12, did the mice start to gain weight, reaching their original weight after an additional 2 wk (day 26). At days 12–19, visible fecal blood could no longer be detected, whereas the diarrhea score remained high (Fig. 2, B and C). At later times, the only apparent sign of disease in C57BL/6 mice was loose stools (Fig. 2B), which was still present 5 wk after DSS removal (data not shown).

On the basis of the symptomatic parameters, it appeared that the two mouse strains behaved similarly on a qualitative level, although C57BL/6 mice displayed more severe disease. On necropsy, however, major qualitative differences were evident. The inflammatory score in C57BL/6 mice remained at the same high level as observed at day 5 until termination of the experiment (day 26; Fig. 2D). The colon was significantly shortened at days 12 and 26 (Table 2), and the colonic wet weight increased progressively until day 26, after which it remained high (Fig. 3A). The plasma levels of haptoglobin remained high during the chronic phase (days 26 and 33), indicating ongoing systemic inflammation (Fig. 3B). As observed during DSS exposure, there was also a good correlation between haptoglobin and inflammatory score ($r = 0.93, P < 0.0001$) and colonic wet weight ($r = 0.90, P < 0.0001$) during the subsequent 4 wk. SAA levels also remained high ($>190 \, \mu g/ml$) during these late time points (data not shown).

As the inflammation progressed in C57BL/6 mice, the colon became stiff and the midportion often tightly adhered to the mesentery (data not shown). Other features found in C57BL/6 mice after day 26 were increased size of the spleen and enlarged mesenteric lymph nodes (Table 2).

In contrast to the symptoms, the microscopic progression of colitis in C57BL/6 mice involved a gradual infiltration of macrophages, lymphocytes, and plasma cells into the mucosa and submucosa as the inflammation progressed (Fig. 4, e–g). The number of neutrophils was constant during the entire period, although aggregates of neutrophils were found in conjunction with ulcerations (data not shown). Furthermore, small formations of tightly packed MNC were observed at day 12 in the lamina propria, which seemed to enlarge as the inflammation progressed (Fig. 4, e–g). The highest number of inflammatory cells was found at day 26 (Fig. 4g). Transmural inflammation was occasionally detected, whereas no transmural ulcerations were found at any time (data not shown). Marked loss of crypts, goblet cells, and large ulcerations were mainly denoted at day 12 (Fig. 4e), and regeneration of crypts and reepithelialization were observed at day 19 (Fig. 4f). At 3–4 wk after DSS removal, the regenerated epithelial structure was irregular and contained areas with ulcerations (Fig. 4g; see Fig. 6c). Overall, similar histopathological changes were observed in the proximal and distal colon, although there was less focal inflammation in the proximal colon (data not shown).

Thus the acute disease in C57BL/6 mice does not resolve after DSS removal. Instead, it progresses to a severe, chronic colitis, including systemic manifestations, such as high plasma levels of acute-phase proteins. Paradoxically, symptoms of diarrhea and weight loss do not correlate with the severity of the inflammation. Rather, these symptoms correlate better with the condition of the epithelium (Fig. 5). In BALB/c mice, on the other hand, the acute inflammation and the acute symptoms are self-limited and start to resolve directly after DSS removal. Interestingly, we have found that the time of resolution in BALB/c mice is the same, despite a prolonged (9 days) DSS exposure (data not shown).

Masson’s trichrome staining demonstrates increased deposits of collagen in mucosa and submucosa of acute and chronic inflamed colonic tissue. One common feature of the inflamed colons, during the acute or chronic phase, was a marked...
rigidity. To investigate whether this feature was related to infiltration/inflammation or to fibrosis, distal colonic tissue from both strains was stained with Masson’s trichrome during the acute, recovery, and chronic phases to analyze production of collagen as a marker of fibrosis (Fig. 6). Collagen deposition in the mucosa and submucosa of both strains increased as the disease developed into the acute phase (day 5; Fig. 6, b and e). Furthermore, collagen deposition in the mucosa and submucosa of C57BL/6 mice remained elevated at days 26 and 33, most likely manifest in the stiffness observed in the chronic inflamed colons (Fig. 6c). Moreover, areas containing several bundles of collagen were found in focal regions of the serosa in the chronic C57BL/6 mice. In contrast, Masson’s trichrome staining appeared normal in BALB/c mice at day 33 (Fig. 6f).
Further supporting the total recovery of the colonic tissue in BALB/c mice.

Progressive upregulation of IL-1β, IL-12 p70, and IL-17 follows chronic development in C57BL/6 mice. To investigate the degree of activation of the infiltrating cells during development of colitis, we analyzed the production of selected cytokines in colonic tissue homogenates. The chosen cytokines have been implicated in the pathogenesis of IBD, i.e., IL-1α/β, IL-6, IL-12, IL-17, IL-18, IFN-γ, and G-CSF (14, 16, 21, 24, 35, 43). We chose to measure these mediators at the protein, rather than the mRNA, level and without ex vivo stimulation of the cells/tissue, inasmuch as we consider this the most relevant condition for mimicking conditions in vivo.

As the symptoms were aggravated into the acute phase (at day 5), production of IL-1α, IL-1β, IL-6, IL-18, and G-CSF was elevated in BALB/c mice (Fig. 7A, Table 3). IL-1β production correlated well with plasma haptoglobin levels (r = 0.75, P < 0.0001), inflammatory score (r = 0.80, P < 0.0001), and colonic weight (r = 0.66, P = 0.0007). Furthermore, IFN-γ levels were very low, resembling those in healthy mice (Fig. 7E), whereas IL-12 p70 or IL-17 production was not detected in healthy or diseased mice (Fig. 7, B and D). Thus the highly elevated levels of IL-1, IL-6, IL-18, and G-CSF coincided with the peak of infiltrating cells at day 5. Interestingly, production of IL-12 p40 was increased in the acute phase, but the highest peak was assayed 1 wk after DSS removal, at day 12 (Fig. 7C). In contrast, the other cytokines were rapidly downregulated at day 12 and normalized 3−4 wk after DSS removal (Fig. 7, Table 3). The decreased cytokine production coincided with the resolution of disease in terms of clinical symptoms, haptoglobin levels, and histopathology (Figs. 2–4 and 7).

In contrast, in C57BL/6 mice, the cytokine response of IL-1α, IL-1β, IL-12 p40, IL-12 p70, and IL-17 was progressively increased after DSS removal (Fig. 7, A−D, Table 3). This production coincided with the increasing number of infiltrating cells (Fig. 4, e−g). IL-1β production in the chronic phase (days 26−33) was well correlated with haptoglobin levels (r = 0.92, P < 0.0001), inflammatory score (r = 0.88, P < 0.0001), and colonic weight (r = 0.86, P < 0.0001). IL-6 and G-CSF were highly elevated at days 5 and 12 and intermediate thereafter (Table 3). IFN-γ production was heterogeneous, with relatively increased levels at day 5 and higher levels late in the chronic phase, at day 33 (Fig. 7E). IL-18 production was also heterogeneous, with high interindividual values displaying no significant differences between healthy and diseased mice (Table 3).

In summary, the cytokine production assayed in the acute phase of BALB/c mice and the progressive chronic inflammation in C57BL/6 mice indicate that the infiltrating cells are actively involved in disease development in both models.

DISCUSSION
In the present study, we have described a new approach, where a short 5-day exposure of DSS is sufficient to induce progressive chronic colitis in C57BL/6 mice. In contrast, BALB/c mice developed acute colitis during DSS exposure, which was completely resolved after DSS removal. The clinical symptoms correlated well with inflammation in the acute phase in both strains, whereas only mild symptoms were observed in the chronic phase of C57BL/6 mice.

The data presented here describe different phases of colitis development, which we have classified as acute, chronic, and recovery phases. The classification is based on clinical symptoms and histopathological changes (epithelial architecture, type of inflammatory infiltrate, and fibrosis). The acute phase (day 5) was characterized by clinical symptoms, i.e., body weight loss, visible fecal blood and diarrhea, high inflammatory score, high levels of acute-phase proteins, and histopathological changes (e.g., ulcerations, polymorphonuclear cells and MNC infiltration, and loss of epithelial structures). The chronic phase (day 26 and beyond) was characterized by recovered body weight, no/ few clinical symptoms, high inflammatory score, high levels of acute-phase proteins, and histopathological changes (e.g., irregular epithelium, dense infiltrates of MNC and plasma cells, and persistent fibrosis). Finally, recovery was accomplished when no clinical symptoms or systemic markers were found and the histological structure was recovered (i.e., healed epithelial structure with crypts and goblet cells and few inflammatory cells in the mucosa).

DSS is commonly administered in a dose range of 3−10% for 7−10 days to induce an acute inflammation, depending on the susceptibility of the mouse strain or the molecular weight of DSS (25, 31, 39). To induce “chronicity,” DSS is normally administered in three to five cycles with a 1- to 2-wk rest period between cycles (39). Our data show that one cycle of DSS is sufficient to induce chronicity in C57BL/6 mice. Possibly this is also true in Swiss Webster mice (7, 11). In contrast, BALB/c mice recovered quickly after DSS removal, in terms of clinical symptoms, histopathology, and inflammatory markers (present study; 12). We do not think that the concentration of DSS is the critical factor for development of chronicity, because C57BL/6 mice exposed to 2% DSS for 5 days developed chronic colitis, albeit with milder symptoms during the acute stage (unpublished observation). Moreover, BALB/c mice exposed to 5% DSS for up to 9 days partly recovered 1 wk after DSS removal (unpublished observation),
implying that neither 5 nor 9 days of DSS exposure is a critical factor for the rapid recovery in BALB/c mice. Thus this is the first time that DSS has been used as a priming agent to induce a progressive inflammatory response in C57BL/6 mice.

In the clinical setting, one way to diagnose and monitor IBD is by recording the patient’s clinical symptoms. Symptoms often related to UC are rectal bleeding or bloody diarrhea, whereas symptoms often related to CD are abdominal pain and weight loss (44). Correlation between clinical symptoms and endoscopy score in patients is controversial (6, 18, 19, 48). In the present experimental study, we found good correlation between clinical symptoms and local cytokine response during the acute phase, but not the chronic phase, where loose feces were the only clinical sign found. This is in contrast to the good correlations reported between clinical symptom occult blood and inflammation in BALB/c mice treated with cycles of DSS or in chronic inflamed Swiss Webster mice (7, 39). The discrepancies may depend on the type of analysis (i.e., hemoccult vs. gross bleeding) or strain differences.

Several subclinical markers are used to monitor disease activity in humans. One of these markers is serum C-reactive protein, which is generally elevated in 95% of patients with active CD. However, a weak correlation was reported between serum C-reactive protein levels and symptom values (51). We measured the acute-phase protein haptoglobin as a marker of systemic inflammation and found a good correlation with inflammatory score, colonic weight, and IL-1ß production in the acute phase of both strains and in the chronic phase of C57BL/6 mice. Moreover, haptoglobin levels decreased in parallel as clinical symptoms and inflammation resolved in BALB/c mice after DSS removal. In addition, SAA, another acute-phase protein proven to be a good marker for disease activity in IL-2 KO mice (9), was analyzed, yielding results similar to those of haptoglobin (data not shown). Thus we are of the opinion that haptoglobin can be used as a systemic marker to monitor disease activity in murine experimental colitis.

Histological features typical for chronic IBD compared with other mucosal inflammations are epithelial distortion, such as crypt branching and shortening and decreased crypt density (increased spacing between crypts), and severe infiltration of inflammatory cells to the intestinal wall. Furthermore, reduction in goblet cell number is typical for UC but not for CD (18, 19).
At the late phase after DSS removal in C57BL/6 mice, we observed irregular epithelial formation with crypt distortion and goblet cell depletion, equivalent to changes found in UC patients with ≥2 mo of inflammation (2, 53). Similar histopathological changes were reported in chronically inflamed Swiss Webster mice, BALB/c mice subjected to cycles of DSS, and rats exposed to peptidoglycan-polysaccharide or trinitrobenzene sulfonic acid (TNBS) (7, 11, 39, 54, 57). Another typical histological feature found in the inflamed intestinal wall of IBD patients is the severe infiltration of MNC and plasma cells into the basal lamina propria (18, 52). An increased number of inflammatory cells, e.g., MNC, and plasma cells was observed at day 12 in the lamina propria of C57BL/6 mice and was even more pronounced at day 26, thus reflecting the progression to chronicity, rather than the resolution of disease, in these mice. Notably, the number of neutrophils was constant during the chronic phase, indicating that the inflammation was active in these areas, further supporting the remaining high levels of haptoglobin and SAA at this stage. Other histological features such as granuloma and transmural ulcerations, typical for CD, or crypt abscesses and cryptiditis, found in UC (18), were not observed in the present model. In general, endoscopy and histology results are not well correlated in IBD (15, 18). We observed good a relation between inflammatory score and histology at the different phases, although the histological changes were generally patchy, and the inflammatory score was based on the appearance of the entire colon. One reason for the discrepancy observed in IBD patients may be related to the size and site of the biopsies, which may not reflect the whole inflammatory profile.

Fibrosis is a nonspecific feature of chronic inflammation and is observed in UC and CD (22, 34, 45). Fibrosis in CD is characterized by increased deposits of collagen due to altered collagen metabolism in all layers of the intestinal wall, but especially around and within smooth muscle (22). Moreover, excessive fibrosis due to severe collagen deposition may result in strictures in CD patients (22, 45). In contrast, fibrosis in UC is characterized by increased deposits of collagen in the submucosa and lamina propria (34). In the present models, we detected extensive deposition of collagen in the submucosa of acute and chronic inflamed colon and even occasionally in the muscle layers or in the serosa of the chronic inflamed colon, features resembling fibrosis in UC, rather than CD. A similar finding was recently reported in CD1 or BALB/c mice receiving weekly doses of TNBS (29), whereas certain rat strains injected with peptidoglycan-polysaccharide develop fibrosis and granulomatous enterocolitis resembling CD (57). One important factor for the observed differences in fibrosis induction between the different IBD models may again be related to the genetic background, as shown in experimental models of lung pathology (28).

It is well established that differences in genetic background are a major determinant for an inflammatory response, e.g., as observed after Leishmania major infections (46). BALB/c mice are more susceptible to L. major infections because of a defective Th1 response. In contrast, infected C57BL/6 mice effectively mount a Th1 response, leading to clearance of the infection and, eventually, healing (46). Analysis of cytokine production in the present models reveals differences in the inflammatory response between the two strains. A high production of IL-1α, IL-1β, IL-6, IL-18, and G-CSF was found in...
the acute phase of BALB/c mice, which coincided with the peak infiltration of inflammatory cells. Macrophages and epithelial cells are the main producers of these cytokines, suggesting that macrophages may be among the drivers of the acute response in BALB/c mice as has been proposed earlier (this study; 12, 39). However, other studies suggest that T cells also play an important role in DSS-induced acute colitis (20, 49). Furthermore, IFN-γ production during the acute phase was low in BALB/c, but higher in C57BL/6, mice compared with earlier study, where a high IFN-γ mRNA expression was found in BALB/c mice (13). Taken together, the data suggest that the acute response in BALB/c mice is most likely macrophage driven, although T cells may be observed as a secondary response.

The factor(s) that regulates the resolution of disease in BALB/c mice is not known. However, an increased production of IL-12 p40, without any concomitant elevation of IL-12 p70, was observed in the acute phase (day 5) and early in the recovery phase (day 12), suggesting that active IL-12 is not supporting the acute inflammation in BALB/c mice. IL-12 is an immunoregulatory cytokine involved in cell-mediated immune responses by inducing differentiation of Th1 cells and IFN-γ production (5). However, in murine systems, homodimers of p40 have been shown to bind to the IL-12 receptor and work as a natural inhibitor of IL-12 activity (23). Our data suggest that the increased IL-12 p40 levels may form homodimers, which antagonize IL-12 activity and, thus, inhibit proinflammatory responses in BALB/c mice (23). In addition, we have also seen an inverse correlation between IL-1β and PGE2, suggesting that PGE2 is also involved in disease resolution in BALB/c mice (unpublished observations).

In contrast to BALB/c mice, C57BL/6 mice progressively develop a chronic inflammation subsequent to the acute response. An important question is what factor(s) are the major drivers for this response. To this end, we observed differences in the cytokine response, especially in the progressive production of IL-1, active IL-12, and IL-17, which correlated with the dense infiltration of lymphocytes, plasma cells, and macrophages into the tissue.

IL-12 p70 heterodimers were produced in a progressive manner during chronic development in C57BL/6 mice, in contrast to BALB/c mice, suggesting that bioactive IL-12 favors chronic development in these mice. In other IBD models, e.g., TNBS-induced colitis and IL-10 KO mice, disease development was ameliorated by administration of anti-IL-12 antibodies (36, 47). In humans, the data on IL-12 expression are somewhat controversial. An increased expression and secretion of IL-12 were reported in CD, but not in UC, patients (35, 42), whereas in other studies, no significant differences in IL-12 expression were found between UC and CD patients (38, 40). A recent publication on anti-IL-12 treatment to CD patients provided some evidence on the role of IL-12 in IBD. However, more studies need to be conducted (33). In addition, the p40 subunit can also bind to p19 and form the recently described cytokine IL-23, the function of which is to promote proliferation of memory CD4+ T cells as well as induce production of IFN-γ and IL-17 (1, 41). IL-17, which was recently reported to be expressed in active UC and CD patients (16), is a T cell-derived cytokine that stimulates epithelial, endothelial, and fibroblast cells to produce, e.g., IL-6 and IL-8. Notably, IL-17 is regulated by IL-23, but not by IL-12 (1). In our models, IL-17 was progressively upregulated in C57BL/6, but not in BALB/c, mice, suggesting that activated T cells may be involved in chronic development. Taken together, the data favor a role for IL-12, as well as IL-23, in development of chronicity in C57BL/6 mice.

IL-1 has also been suggested to play an important role in initiation and perpetuation of colitis, because increased levels of IL-1ß are found in IBD patients with active disease as well as in experimental IBD models (10, 21, 30). Administration of anti-mouse IL-1ß antibody suppressed clinical symptoms in murine acute DSS colitis (3). In addition, IL-1ß-converting enzyme KO mice were protected from cycle-induced chronic colitis in terms of clinical symptoms, histological changes, spontaneous secretion of IL-1ß, and T cell activation (50). On the other hand, Kojouharoff et al. (27) showed that addition of anti-IL-1 reagents aggravated acute colitis and had no effect on cycle-induced chronic colitis in BALB/c mice. This discrepancy could be due to genetic background as well as the readout that was used.

Analysis of the cytokines IL-4, IL-5, and IL-10 by ELISA and multiplex technology revealed low levels (<30 pg/100 mg tissue) at all time points and in both mouse strains (data not shown). It is most likely that detection of these cytokines requires that the analysis be carried out on isolated cells or on tissue sections. Dieleman and colleagues (11) reported an elevation of IFN-γ and IL-4 in chronic induced colitis in Swiss Webster mice. Similarly, we measured an increase in IFN-γ production in the chronic inflamed intestine. In humans, IFN-γ levels have been preferentially reported in CD patients, whereas no IL-4 expression has been detected in CD or UC (14, 56). Thus the observed differences in resolution of disease...
and progression to chronicity between the two strains most likely reflect the genetic ability to mount different inflammatory responses and/or healing mechanisms as observed by the different cytokine profiles. The aim of future investigations is an in-depth analysis of the cell subsets that are involved in disease processes(s) as well as an investigation into the identity of the mediators that regulate cellular infiltration in these two distinct models of colitis.

In summary, we believe that the acute model of colitis in BALB/c mice may be useful in the study of normal mucosal healing and that the progression to chronicity observed in C57BL/6 mice offers a promising animal model for the pathological inflammatory changes observed in UC. Moreover, we are of the opinion that the chronic model is a powerful and convenient tool that can be used to validate future candidate treatments for IBD.

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GRANTS

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