

Differential susceptibility of inbred mouse strains to dextran sulfate sodium-induced colitis

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Mähler, Michael, Ian J. Bristol, Edward H. Leiter, Aletha E. Workman, Edward H. Birkenmeier, Charles O. Elson, and John P. Sundberg. Differential susceptibility of inbred mouse strains to dextran sulfate sodium-induced colitis. *Am. J. Physiol.* 274 (Gastrointest. Liver Physiol. 37): G544–G551, 1998.—Dextran sulfate sodium (DSS)-induced murine colitis represents an experimental model for human inflammatory bowel disease. The aim of this study was to screen various inbred strains of mice for genetically determined differences in susceptibility to DSS-induced colitis. Mice of strains C3H/HeJ, C3H/HeJBir, C57BL/6J, DBA/2J, NOD/LtJ, NOD/LtSz-*Prkdc*^{scid}/*Prkdc*^{scid}, 129/SvPas, NON/LtJ, and NON.NOD-*H2*^{g7} were fed 3.5% DSS in drinking water for 5 days and necropsied 16 days later. Cecae and colons were scored for histological lesions based on severity, ulceration, hyperplasia, and area involved. Image analysis was used to quantitate the proportion of cecum ulcerated. Histological examination revealed significant differences among inbred strains for all parameters scored. In both cecum and colon, C3H/HeJ and a recently selected substrain, C3H/HeJBir, were highly DSS susceptible. NOD/LtJ, an autoimmune-prone strain, and NOD/LtSz-*Prkdc*^{scid}/*Prkdc*^{scid}, a stock with multiple defects in innate and adoptive immunity, were also highly DSS susceptible. NON/LtJ, a strain closely related to NOD, was quite DSS resistant. The major histocompatibility (MHC) haplotype of NOD mice (*H2*^{g7}), a major component of the NOD autoimmune susceptibility, was not crucial in determining DSS susceptibility, since NON mice congenic for this MHC haplotype retained resistance. C57BL/6J, 129/SvPas, and DBA/2J mice showed various degrees of susceptibility, depending upon the anatomical site. A greater male susceptibility to DSS-induced colonic but not cecal lesions was observed. In summary, this study demonstrates major differences in genetic susceptibility to DSS-induced colitis among inbred strains of mice. Knowledge of these strain differences in genetic responsiveness to acute inflammatory stress in the large intestine will permit design of genetic crosses to elucidate the genes involved.

animal model; inflammatory bowel disease; histological analysis; genetic predisposition; dextran sulfate sodium; *Helicobacter*

THE INFLAMMATORY BOWEL DISEASES (IBD) ulcerative colitis and Crohn's disease have a genetic component, as evidenced by increased prevalence in certain populations, increased prevalence among first-degree relatives of patients, and a high concordance rate for disease among identical twins (51). The pattern of inheritance is complex and is likely to be multigenic (51). Differences in disease expression among inbred strains of rodents with experimental IBD have also been identified, differences that are likely to be due to genetic influences (4, 11, 25, 28, 39).

One experimental animal model for human IBD is based on the oral administration of dextran sulfate sodium (DSS) to mice (8, 32), rats (46), hamsters (50), and guinea pigs (19). DSS can produce both acute and chronic ulcerative colitis with features somewhat similar to the symptomatic and histological findings in humans (8, 32). In addition to inducing colitis, the long-term administration of low-dose DSS produces colonic adenomas, adenocarcinomas, and papillomas in rats (16, 46) and hamsters (50). Differences in the clinical course, severity, and location of pathological changes of DSS-induced intestinal inflammation exist among animal species. The guinea pig is more susceptible to DSS-induced colitis than any other laboratory animal tested (19). In hamster (50) and guinea pig models (19), the right colon was more severely affected than the left. In Wistar rats, the right colon was also more severely damaged (46), whereas the opposite side was more damaged in Fischer 344 rats (10). In BALB/c and CBA/J mice, the severity of lesions predominated in the left colon (32), whereas, in Swiss-Webster mice, the midcolon was most severely affected (8). Although not yet proven experimentally, these findings may be due to genetic differences between the rat or mouse strains utilized in each study. The pathogenesis of DSS-induced colitis is unclear. Toxic effects on colonic epithelium (9, 32), alterations of luminal bacterial flora (32, 50), and activation of macrophage inflammatory responses (31, 32) have been suggested as possible mechanisms by which DSS induces colitis. Pathogenic contributions from the adaptive immune system appear to be secondary in this model, since T and B lymphocyte-deficient severe combined immunodeficiency (*Prkdc*^{scid}/*Prkdc*^{scid}) mice also develop DSS-induced lesions (1, 9).

Identification of differences in the response to DSS among inbred strains of mice could provide the basis for investigations of the genes determining susceptibility or resistance to colitis. We report here a quantitative histological analysis of DSS-induced colitis in nine mouse strains using a standardized protocol. The results demonstrate major differences in DSS responsiveness among strains, due either to genetic differences in the ability of the mucosa to withstand inflammatory damage, differences in the ability to limit the inflammatory response, or both.

METHODS

Mice. Clinically healthy mice of both sexes, 6–9 wk old, were used for the induction of experimental colitis. These mice were obtained either from Animal Resources or research colonies at The Jackson Laboratory (Bar Harbor,

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ME) and consisted of the inbred strains C3H/HeJ, C3H/HeJBir, C57BL/6J, DBA/2J, 129/SvPas, NOD/LtJ, and NON/LtJ. In addition, the NON.NOD-*H2^{g7}* congenic stock (generations N₂₁F₁₉ and N₂₂F₁₁) was used (42). The *H2^{g7}* major histocompatibility complex (MHC) haplotype is a major component for susceptibility of NOD mice to autoimmune diabetes mellitus (22). The NON.NOD-*H2^{g7}* congenic stock (designated NON.*H2^{g7}*) was tested to analyze the contribution of this unique MHC haplotype to DSS-induced colitis susceptibility. The NOD/LtSz-*Prkdc^{scid}/Prkdc^{scid}* congenic stock (generation N₁₀F₂₄) homozygous for the severe combined immunodeficiency allele at the protein kinase, DNA activated, catalytic polypeptide locus (43) was also tested. This stock (hereafter designated NOD-*scid*) lacks functional lymphoid cells and has multiple defects in innate immunity (43). The NOD-*scid* mice were maintained in a pressurized, individually vented caging system and received sulfamethoxazole-trimethoprim (Goldline Laboratories, Ft. Lauderdale, FL) on three consecutive days per week as previously described (43) to protect the mice from infection with *Pneumocystis carinii*. All other mice used in this study were maintained under standard laboratory conditions with a maximum of five mice per cage (330 cm² floor area). Mice were allowed free access to feed (diet 911A; Emory Morse, Guilford, CT) and to drinking water (pH 2.8–3.2) acidified with hydrochloric acid to limit growth of *Pseudomonas* species. The possibility of strain differences in willingness to drink DSS-supplemented water was assessed by monitoring DSS-supplemented water consumption of singly caged 5- to 6-wk-old mice over the 5-day treatment period. Routine animal health monitoring was conducted on representative mice from each of the above-mentioned colonies. All mice used in this study came from areas where mice were seronegative for common murine viruses and *Mycoplasma* species, free of significant bacterial pathogens on culture of trachea or intestine, and free of ectoparasites and intestinal helminths. Mice of strains C3H/HeJBir, C3H/HeJ, 129/SvPas, and C57BL/6J were positive for *Helicobacter hepaticus*, *H. bilis*, and/or *H. muridarum*, whereas those of strains NOD/LtJ, NON/LtJ, and DBA/2J tested *Helicobacter* negative. Occasional mice were positive for *Pasteurella pneumotropica* or trichomonads. To test the role of *Helicobacter* species in determining DSS responsiveness, near-term litters of C3H/HeJBir mice were hysterectomy rederived into a *Helicobacter*-free facility at The Jackson Laboratory. A small colony was established and was confirmed to be free of *Helicobacter* species by microbiological tests that included polymerase chain reaction amplification of cecal and fecal DNA, microaerophilic culture of cecum, and histology (Steiner's stain) of cecum and/or colon (14). This study was approved by the Animal Care and Use Committee of The Jackson Laboratory following the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Induction of colitis. Experimental colitis was induced by giving 3.5% (wt/vol) DSS (mol wt 36,100–45,500; TdB Consultancy, Uppsala, Sweden) in acidified drinking water *ad libitum* for 5 days. DSS administration was then stopped, and mice received acidified drinking water alone for 16 days until necropsy on day 21. This dose was empirically established to induce moderate to severe colitis while minimizing the mortality (5). None of the mice in this study died before termination of the experiment at day 21.

Histological evaluation of colitis. Mice were euthanized by CO₂ asphyxiation on day 21. The large intestine was collected, and the cecum was separated from the colon. Intestinal specimens were gently inflated with Fekete's acid-alcohol-Formalin fixative (13) by intraluminal injection. The entire

colon, including the rectum, was prepared as an intestinal roll by placing it on an index card and twisting it in concentric centrifugal circles around a central toothpick in the plane of the card (29). Samples were immersed in fixative overnight and then transferred to 70% ethanol. Tissues were processed, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin.

Two sections of each cecum and one section of each colon/rectum were coded with an accession number and reviewed by a pathologist (J. P. Sundberg) without access to the code. Each section was scored for lesions based on severity, ulceration, hyperplasia, and area involved. The severity of lesions was graded as follows: 0, normal; 1, mild; 2, moderate; and 3, severe. Mild lesions were small, focal, or widely separated multifocal areas of inflammation and/or fibrosis limited to the lamina propria. Moderate lesions were either multifocal or represented by locally extensive areas of inflammation and/or fibrosis extending into the submucosa. Severe lesions were ulcers that covered large areas (1 mm or more) of the mucosa. Ulceration was recorded as follows: 0, not present and 1, present. Ulcers were areas of the mucosa in which the epithelial lining was missing. Ulcers were covered by an exudate of neutrophils and necrotic debris (44). Hyperplasia was graded as follows: 0, normal; 1, mild; 2, moderate; and 3, severe. Mild hyperplasia consisted of lining epithelium that appeared to be morphologically normal; however, the thickness (length of crypts) was two or more times that of adjacent (or control) mucosa. Moderate hyperplasia was characterized by the following: the lining epithelium was two to three times normal thickness, cells were hyperchromatic, numbers of goblet cells were decreased, and scattered individual crypts developed an arborizing pattern. Severe hyperplastic regions exhibited markedly thickened epithelium (4 or more times normal), marked hyperchromasia of cells, few to no goblet cells, a high mitotic index of cells within the crypts, and numerous crypts with arborizing patterns. Nests of crypts extended down to and compressed the muscularis mucosa, occasionally extending into the submucosa. The most severe examples resembled dysplasia. Pseudopolyps rose high above the level of adjacent mucosa associated with various degrees of fibrosis and crypt loss in the adjacent lamina propria. The area involved by the disease process was estimated as follows: 0, normal; 1, 1–25%; 2, 26–50%; 3, 51–75%; and 4, 76–100% of surface area examined. The percent area of involvement was assessed separately for the cecum, proximal colon, middle colon, and distal colon/rectum. The average area involved in the colon was calculated as the sum of the three colon segments divided by three. A total score was determined separately for the cecum and colon by adding the scores of severity, ulceration, hyperplasia, and area involved; the average area involved was used for the calculation of the total colon score. Of the scores obtained from the two sections per cecum, only those of the more severely affected section were considered.

In an attempt to quantitate the extent of cecal ulceration, computerized image analysis was performed on the more severely affected section of each cecum with ulceration by using a Quantimet 600HR image processing and analysis system (Leica Imaging Systems, Cambridge, UK). Images of each cecal ulcer were captured via a DMRX upright light microscope (Leica, Deerfield, IL) coupled to a DEI-470 CCD video camera system (Optronics Engineering, Goleta, CA), digitized using the Q600HR workstation, and displayed on a monitor. The size of each ulcer (µm) was measured as the distance between the two edges at the ulcer base by tracing with the computer mouse (Fig. 1A). The luminal perimeter of each cecum with ulceration was similarly determined by

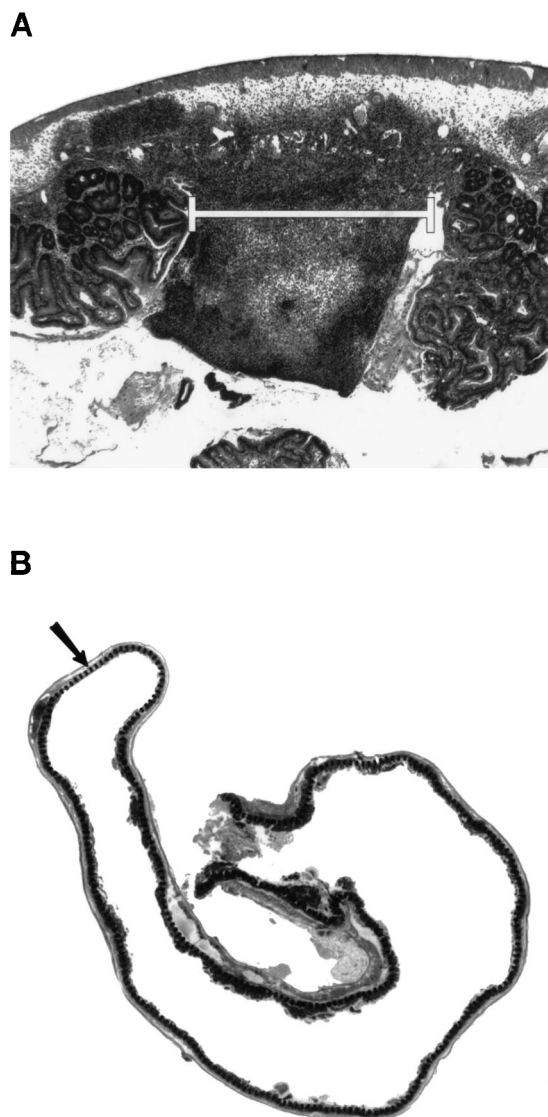


Fig. 1. Histological section of an ulcer [A; hematoxylin and eosin (H&E) stain; magnification $\times 35$] and a cecum [B; H&E stain; magnification $\times 8$]. Ulcer size is defined as distance between the two edges at its base (bar). Luminal perimeter of the cecum is determined as linear measurement of the mucosal layer (line). For each cecum, the extent of ulceration was calculated as ulcer size divided by the luminal perimeter.

means of a Wild M10 stereomicroscope (Leica) coupled to a Megaplus 1.4 digital camera (Kodak, Rochester, NY) and by using the Q600HR image analysis system. The luminal perimeter (μm) was evaluated as linear measurement of the mucosal layer (Fig. 1B). For each cecum, the extent of ulceration was then calculated as ulcer size (or sum of ulcer sizes) divided by the perimeter.

Data analysis. Statistical analyses were performed with StatView 4.5 software (Abacus Concepts, Berkeley, CA) for MacIntosh. Contingency chi square analysis was used to evaluate differences in the frequency of ulceration between individual strains and between sexes. A Bonferroni correction for multiple comparisons was then applied. All other data were analyzed first by analysis of variance (ANOVA) to detect major effects among strains and between sexes for each parameter. Post hoc comparisons were conducted by Bonferroni/Dunn test, with a significance level of $P < 0.05$.

RESULTS

General findings. Almost all mice developed loose stools or diarrhea during feeding of DSS. Gross evidence of blood was frequently detected in the stools. Most mice gradually recovered clinically while receiving only water until necropsy. Histological examination revealed various degrees of typhlitis and colitis in mice of all strains. Characteristic findings included mucosal ulceration, infiltration with mononuclear and polymorphonuclear leukocytes in the lamina propria and submucosa, crypt distortion, hyperplastic epithelium, and ulcers healing by reepithelialization.

Comparison of histological lesions in the cecum. Histological scoring data obtained from the ceca are presented in Tables 1 and 2. Strains C3H/HeJBir, C3H/HeJ, and NOD/LtJ had a significantly higher frequency of cecal ulceration than strain 129/SvPas (Table 1). C3H/HeJBir and C3H/HeJ were significantly higher than NON.*H2^{g7}*, with C3H/HeJBir exhibiting a significantly greater frequency of ulceration than NON/LtJ as well. Interestingly, no ulcer formation was observed in the cecum for mice of strains 129/SvPas and NON.*H2^{g7}*. There was no statistically significant difference in the frequency of ulceration between sexes (data not shown).

Two-factor ANOVA of data for each of the other histological parameters in the cecum revealed significant differences among strains (Table 2). C3H/HeJBir was the most susceptible strain to DSS-induced lesions in the cecum, as indicated by the highest value for each parameter, whereas NON/LtJ, NON.*H2^{g7}*, and 129/SvPas were the most resistant strains, showing consistently low values. Based on morphometric data (extent of ulceration) and on total score obtained from the cecum, decreasing susceptibility was observed in strains C3H/HeJBir, C3H/HeJ, NOD-*scid*, NOD/LtJ, DBA/2J, C57BL/6J, NON.*H2^{g7}*, NON/LtJ, and 129/SvPas (Table 2). There was no significant sex effect or significant interactions between strain and sex.

Comparison of histological lesions in the colon. Histological scoring data obtained from the colons are presented in Tables 1, 3, and 4. Strains C3H/HeJBir, C3H/HeJ, NOD/LtJ, C57BL/6J, and 129/SvPas had a

Table 1. Frequency of ulceration in the cecum and colon in nine strains of mice after DSS administration

Strain	Cecum	Colon
C3H/HeJBir	10/12 (83) ^{a,b,c}	10/12 (83) ^d
C3H/HeJ	13/17 (77) ^{a,b}	15/17 (88) ^{d,e}
NOD/LtJ	9/12 (75) ^a	9/12 (75) ^d
NOD- <i>scid</i>	11/16 (69)	9/16 (56)
DBA/2J	5/12 (42)	0/12 (0)
C57BL/6J	4/13 (31)	9/13 (69) ^d
NON/LtJ	2/12 (17)	3/12 (25)
NON. <i>H2^{g7}</i>	0/7 (0)	1/7 (14)
129/SvPas	0/8 (0)	6/8 (75) ^d

Data are number of mice with ulceration/number of total mice per strain (%). $P < 0.0014$ was considered significant based on a Bonferroni correction. DSS, dextran sulfate sodium. ^a $P < 0.0014$ vs. 129/SvPas; ^b $P < 0.0014$ vs. NON.*H2^{g7}*; ^c $P < 0.0014$ vs. NON; ^d $P < 0.0014$ vs. DBA/2J; and ^e $P < 0.0014$ vs. NON and NON.*H2^{g7}*.

Table 2. *Histological scores for the cecum in nine strains of mice after DSS administration*

Strain	M/F	Extent of Ulceration	Severity	Hyperplasia	Area Involved	Total Score
C3H/HeJBir	7/5	0.083 ± 0.022^b	2.33 ± 0.26^c	2.42 ± 0.19^c	3.00 ± 0.30^e	8.58 ± 0.74^d
C3H/HeJ	12/5	0.037 ± 0.015 ^{a,b}	1.82 ± 0.21 ^{b,c}	2.29 ± 0.17 ^c	2.29 ± 0.21 ^{d,e}	7.18 ± 0.56 ^d
NOD- <i>scid</i>	9/7	0.008 ± 0.002 ^a	2.06 ± 0.14 ^c	2.25 ± 0.87 ^c	2.06 ± 0.17 ^{c,d}	7.06 ± 0.44 ^d
NOD/LtJ	6/6	0.034 ± 0.010 ^{a,b}	2.17 ± 0.27 ^c	2.25 ± 0.25 ^c	1.50 ± 0.15 ^{b,c,d}	6.67 ± 0.69 ^{c,d}
DBA/2J	6/6	0.017 ± 0.008 ^a	1.42 ± 0.19 ^{b,c}	1.33 ± 0.14 ^b	1.08 ± 0.08 ^{a,b}	4.25 ± 0.35 ^{b,c}
C57BL/6J	8/5	0.006 ± 0.005 ^a	0.92 ± 0.31 ^{a,b}	1.62 ± 0.24 ^{b,c}	1.31 ± 0.18 ^{b,c}	4.15 ± 0.75 ^{b,c}
NON. <i>H2g7</i>	4/3	0.000 ± 0.000^a	0.71 ± 0.18 ^{a,b}	0.71 ± 0.18 ^{a,b}	0.86 ± 0.26 ^{a,b}	2.29 ± 0.61 ^{a,b}
NON/LtJ	6/6	0.001 ± 0.001 ^a	0.17 ± 0.11 ^a	1.08 ± 0.31 ^b	0.67 ± 0.14 ^{a,b}	2.08 ± 0.58 ^{a,b}
129/SvPas	4/4	0.000 ± 0.000^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.13 ± 0.13^a	0.13 ± 0.13^a
ANOVA						
Strain		<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001
Sex		NS	NS	NS	NS	NS
Strain × sex		NS	NS	NS	NS	NS

Data are expressed as means ± SE. Maximal and minimal values for each measure are in bold, and strains are rank ordered by total score. M, males; F, females; ANOVA, analysis of variance. Values that do not share a superscript are significantly different at *P* < 0.0014 based on Bonferroni/Dunn test. NS, not significant (*P* ≥ 0.05).

significantly higher frequency of ulceration in the colon than DBA/2J mice (Table 1). The frequency of ulceration in C3H/HeJ was also significantly higher than in NON/LtJ and NON.*H2g7*. Remarkably, none of the DBA/2J mice showed any ulcer formation in the colon. There was no statistically significant difference in the frequency of ulceration between sexes (data not shown).

In each strain, the area involved by the disease process increased from the proximal to the distal colon (Table 3). Similarly, the significance of the difference among strains increased from the proximal to the distal colon. C3H/HeJBir, C3H/HeJ, and NOD/LtJ were strains with a high extent of involvement in the distal colon. C57BL/6J, NOD-*scid*, and 129/SvPas were intermediate, whereas NON.*H2g7*, NON/LtJ, and DBA/2J represented strains with a lower extent of distal involvement. Sex had no significant effect; strain and sex did not interact.

Severity of lesions, hyperplasia, and total score in the colon differed significantly among strains (*P* < 0.0001). A significant male gender bias for severity (*P* = 0.0375), hyperplasia (*P* = 0.0104), and total score (*P* = 0.0184) was noted. Therefore, we decided to analyze these data separately for female and male mice by one-factor

ANOVA, as displayed in Table 4. Significant strain differences for all three parameters were found in both female and male mice. As shown by the total score, strains C3H/HeJBir, C3H/HeJ, NOD/LtJ, NOD-*scid*, C57BL/6J, and 129/SvPas were highly susceptible to DSS-induced lesions in the colon, whereas NON.*H2g7*, NON/LtJ, and, in particular, DBA/2J proved to be less susceptible strains (Table 4).

The inbred strain differences in DSS susceptibility did not correlate with differences in consumption of DSS-supplemented water. Mean consumption (±SE) over the 5-day DSS treatment period by susceptible C3H/HeJ mice (3 of each sex) was 15.3 ± 0.6 ml, whereas the relatively resistant C57BL/6J mice (3 per sex) consumed 17.1 ± 0.9 ml. Similarly, susceptible NOD/Lt mice (3 females, 5 males) consumed 19.8 ± 0.7 ml over 5 days versus 22.4 ± 0.6 ml consumed by the resistant NON/Lt mice (8 males).

It has recently been suggested that *H. hepaticus*, a bacterium that colonizes the lower intestinal tract and liver of mice, may play a role in development of IBD in immunodeficient mice (49). Our animal health monitoring revealed the presence of *Helicobacter* species in mice of strains C3H/HeJBir, C3H/HeJ, 129/SvPas, and

Table 3. *Histological scores of the area involved by the disease process in the colon of nine mouse strains after DSS administration*

Strain	M/F	Proximal Colon	Middle Colon	Distal Colon	Average Colon
C3H/HeJBir	7/5	0.42 ± 0.15	1.17 ± 0.11 ^{a,b}	3.58 ± 0.26^c	1.72 ± 0.09^b
NOD/LtJ	6/6	0.00 ± 0.00	1.67 ± 0.19 ^b	3.17 ± 0.30 ^{b,c}	1.61 ± 0.11 ^b
C3H/HeJ	12/5	0.31 ± 0.12	1.06 ± 0.14 ^{a,b}	3.24 ± 0.24 ^c	1.52 ± 0.12 ^b
C57BL/6J	8/5	0.08 ± 0.08	1.77 ± 0.28^b	2.62 ± 0.33 ^{b,c}	1.49 ± 0.19 ^b
NOD- <i>scid</i>	9/7	0.25 ± 0.11	1.06 ± 0.14 ^{a,b}	2.88 ± 0.24 ^{b,c}	1.40 ± 0.09 ^b
129/SvPas	4/4	0.00 ± 0.00	1.25 ± 0.16 ^{a,b}	2.38 ± 0.26 ^{a,c}	1.21 ± 0.13 ^{a,b}
NON. <i>H2g7</i>	4/3	0.00 ± 0.00	1.43 ± 0.30 ^{a,b}	1.71 ± 0.47 ^{a,b}	1.05 ± 0.22 ^{a,b}
NON/LtJ	6/6	0.00 ± 0.00	0.75 ± 0.18^a	1.08 ± 0.08^a	0.61 ± 0.07^a
DBA/2J	6/6	0.00 ± 0.00	0.75 ± 0.22^a	1.33 ± 0.26 ^a	0.69 ± 0.13 ^a
ANOVA					
Strain		<i>P</i> = 0.0158	<i>P</i> = 0.0003	<i>P</i> < 0.0001	<i>P</i> < 0.0001
Sex		NS	NS	NS	NS
Strain × sex		NS	NS	NS	NS

Data are expressed as means ± SE. Maximal and minimal values for each measure are in bold, and strains are rank ordered by total colon score (Table 4). Post hoc analysis of proximal colon did not reveal significant differences between individual strain means. Values that do not share a superscript are significantly different at *P* < 0.0014 based on Bonferroni/Dunn test. NS, not significant (*P* ≥ 0.05).

Table 4. *Histological scores of severity and hyperplasia and total score for the colon in nine mouse strains after DSS administration*

Strain	M/F	Severity		Hyperplasia		Total Score	
		Female	Male	Female	Male	Female	Male
C3H/HeJBir	7/5	2.14 ± 0.40 ^{a,b}	2.80 ± 0.20^d	2.29 ± 0.36 ^{b,c}	3.00 ± 0.00^c	6.76 ± 1.01 ^c	8.67 ± 0.18^d
NOD/LtJ	6/6	2.00 ± 0.26 ^{a,b}	2.17 ± 0.31 ^{b,d}	2.67 ± 0.21^c	3.00 ± 0.00^c	7.28 ± 0.67^c	7.28 ± 0.61 ^{c,d}
C3H/HeJ	12/5	2.25 ± 0.28^b	2.60 ± 0.25 ^{c,d}	2.50 ± 0.23 ^c	2.20 ± 0.20 ^{a,c}	7.19 ± 0.58 ^c	7.00 ± 0.71 ^{b,d}
C57BL/6J	8/5	1.63 ± 0.32 ^{a,b}	2.60 ± 0.25 ^{c,d}	2.25 ± 0.25 ^{b,c}	2.80 ± 0.20 ^{b,c}	5.75 ± 0.80 ^{b,c}	8.07 ± 0.61 ^{c,d}
NOD- <i>scid</i>	9/7	2.22 ± 0.22 ^{a,b}	2.29 ± 0.29 ^{b,d}	2.33 ± 0.17 ^c	2.29 ± 0.29 ^{a,c}	6.59 ± 0.46 ^c	6.43 ± 0.86 ^{b,d}
129/SvPas	4/4	2.25 ± 0.25 ^{a,b}	2.25 ± 0.25 ^{a,d}	1.75 ± 0.25 ^{a,c}	1.50 ± 0.29 ^{a,b}	6.00 ± 0.71 ^{b,c}	5.67 ± 0.69 ^{a,d}
NON- <i>H2g7</i>	4/3	1.00 ± 0.00 ^{a,b}	1.33 ± 0.33 ^{a,b,c}	1.00 ± 0.00 ^{a,b}	1.33 ± 0.33^{a,b}	2.92 ± 0.29 ^{a,b}	4.22 ± 1.39 ^{a,b,c}
NON/LtJ	6/6	1.33 ± 0.21 ^{a,b}	1.00 ± 0.00^a	1.00 ± 0.00 ^a	1.83 ± 0.31 ^{a,c}	2.83 ± 0.22 ^{a,b}	4.06 ± 0.48 ^{a,b}
DBA/2J	6/6	0.83 ± 0.31^a	1.33 ± 0.21 ^{a,b}	0.50 ± 0.22^a	1.33 ± 0.42^a	2.00 ± 0.54^a	3.39 ± 0.58^a
ANOVA		<i>P</i> = 0.0062	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> = 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001

Data are expressed as means ± SE. Maximal and minimal values for each measure are in bold, and strains are rank ordered by the average total score of females and males per strain (not shown). Values that do not share a superscript are significantly different at *P* < 0.0014 based on Bonferroni/Dunn test.

C57BL/6J raised in a lower-level SPF status research mouse room, whereas strains NOD/LtJ, NON/LtJ, and DBA/2J obtained from a high SPF-status barrier facility tested negative (see METHODS). To test a potential contribution of *H. hepaticus* to DSS susceptibility, we treated rederived *Helicobacter*-free C3H/HeJBir mice with DSS. C3H/HeJBir mice from *Helicobacter*-positive and -negative colonies showed equal sensitivity to DSS (Table 5). Vehicle (acidified water) controls were free of cecal ulceration regardless of *Helicobacter* status (Table 5). Hence, differential colonization by this potential opportunistic pathogen was unlikely to be the basis for the strain differences found.

DISCUSSION

Our data clearly demonstrate differences in susceptibility to DSS-induced intestinal inflammation among inbred strains of mice. In both cecum and colon, C3H/HeJBir, C3H/HeJ, NOD/LtJ, and NOD-*scid* proved to be highly susceptible to DSS-induced lesions, whereas NON/LtJ and NON-*H2g7* were resistant. Although the C3H/HeJBir substrain was originally selected in an effort to fix juvenile-onset colitis that sporadically appeared in a large parental C3H/HeJ colony (45), the 41 C3H/HeJBir mice sampled between January 1996 and January 1997 failed to express this phenotype, regardless of the *Helicobacter* status. We have not

identified the specific changes in husbandry conditions that have led to the change in the frequency of colitis in C3H/HeJBir mice compared with what we previously observed (45). Given the apparent disappearance of overt disease and subclinical histopathological lesions, the high sensitivity of both C3H/HeJBir and C3H/HeJ mice to DSS-induced colitis is unlikely to reflect a high baseline produced by underlying spontaneously developing disease. The present data further show that strain differences in susceptibility to DSS-induced lesions vary between anatomical sites. Compared with the other strains tested, DBA/2J showed intermediate susceptibility in the cecum while being most resistant in the colon. In contrast, strains C57BL/6J and 129/SvPas were relatively and strongly resistant in the cecum, respectively, but both were susceptible in the colon. Because these genetically distinct strains of mice were raised in a standardized environment, we infer that the differences observed are primarily the result of genetic variation.

In the present study, the histopathological phenotype was determined 16 days after the cessation of DSS. This experimental design does not allow distinction as to whether strain differences in DSS susceptibility represent genetically controlled differences in response to acute inflammatory damage, to the healing of this damage, or both. In previous studies, we fed 5% DSS in drinking water for 5 days to 6- to 8-wk-old male mice of strains C57BL/6J, C3H/HeJ, and C3H/HeJBir (5). Clinical symptoms and histopathological response by day 7 or earlier revealed increasing susceptibility in strains C57BL/6J, C3H/HeJ, and C3H/HeJBir. Because the same pattern of strain susceptibility was observed on experimental day 21 in the present study, differential susceptibility was assumed to reflect strain differences in the acute inflammatory response to DSS to a greater extent than differences in wound healing. DSS exerts toxic effects on the intestinal mucosa, with onset over the course of feeding (12). However, because we did not quantify differences in ulceration healing rates, we cannot be certain that differential strain sensitivity to ulceration was limited to the acute phase. We did eliminate the possibility that differential susceptibility

Table 5. *Frequency of ulceration in the cecum and colon of C3H/HeJBir mice raised in a Helicobacter-positive or -negative vivarium administered DSS or acidified water (vehicle)*

Treatment	Cecum		Colon	
	<i>Helicobacter</i> +	<i>Helicobacter</i> -†	<i>Helicobacter</i> +	<i>Helicobacter</i> -†
Vehicle*	0/17 (0)	0/24 (0)	0/17 (0)	0/24 (0)
DSS	10/12 (83)	7/8 (88)	10/12 (83)	8/8 (100)

Data are number of mice with ulceration/number of total mice per group (%). *41 C3H/HeJBir mice were randomly sampled and examined histologically over a period of 1 yr between the ages of 4–6 wk to determine baseline ulceration before DSS administration. †C3H/HeJBir housed in a *Helicobacter*-positive vivarium were hysterectomy rederived into a *Helicobacter*-negative vivarium.

between strains was produced by genetically determined aversive behavior with regard to consumption of DSS-supplemented water. We found that the differential responsiveness of strains C3H/HeJ versus C57BL/6J and NOD/LtJ (young, prediabetic) versus NON/LtJ was evident despite comparable levels of DSS-supplemented water consumption.

Genetic differences in susceptibility among inbred rodents have also been described in other induced models of IBD (11, 25, 39). Interestingly, a pattern of susceptibility versus resistance similar to that found here has also been seen in some of these models. For example, C3H/HeJ mice are more susceptible to trinitrobenzene sulfonic acid-induced colitis than mice of the strains DBA/2J and C57BL/6J (11). Similarly, in rats, the Lewis strain is susceptible to chronic intestinal and systemic inflammation induced by both peptidoglycan-polysaccharide polymers (25) and indomethacin (39), whereas the Fischer 344 strain is resistant to both. Moreover, genetic host susceptibility has been demonstrated in several genetically engineered models of IBD (4, 15, 17, 28, 38). The phenotype of disease is highly dependent on the genetic background of mice with disrupted genes encoding T cell receptor- α (28), interleukin (IL)-2 (38), IL-10 (4), and G protein subunit $G_{i\alpha 2}$ (17). Based on these reports, it appears that strains 129, BALB/c, and C3H/He confer disease susceptibility, whereas strain C57BL/6 confers resistance (17, 45). In the present study, the 129/SvPas strain was not ranked among the most susceptible strains for susceptibility to DSS-elicited lesions in the distal colon and, in fact, was resistant to lesions in the cecum. However, it should be noted that the DSS susceptibility is independent of the presence of T lymphocytes, as reflected by the high sensitivity of NOD/LtSz-*scid/scid* mice. The genetic factors controlling DSS susceptibility in mice are unknown. Earlier studies published by our laboratory indicate that susceptibility to spontaneous colitis in C3H/HeJBir mice was not inherited in a simple Mendelian fashion and was probably a quantitative trait (5). A complex mode of inheritance has also been suggested for DSS susceptibility by segregation analysis utilizing crosses between C3H/HeJ and C57BL/6J (5). The generic pattern of susceptibility versus resistance to several forms of colitis among inbred strains of mice suggests one of the following attractive hypotheses: either an identical subset of genes is involved in various mouse models of IBD, or different genes controlling common pathways are involved.

The C3H/HeJBir substrain was the most susceptible of any strain in the cecum, scoring 30–50% higher than the progenitor C3H/HeJ strain in extent of ulceration, severity, and area involved. However, these differences did not reach statistical significance with the number of animals tested. To date, despite extensive testing (including biochemical, immunological, and molecular genetic markers) performed at The Jackson Laboratory, no genetic difference between the two C3H substrains has been detected that could explain their difference in susceptibility to spontaneous colitis. Ni et al. (30) have recently reported differences in expression of inte-

grin- β_7 and other cell adhesion molecules as well as in cytotoxic, proliferative, and aggregative responses of lymphocytes between spontaneously colitic C3H/HeJBir and normal C3H/HeJ mice. Furthermore, a strong serum immunoglobulin G_{2a} and CD4⁺ TH-1 cell reactivity toward antigens of the enteric bacterial flora has previously been observed in C3H/HeJBir but not C3H/HeJ mice (6, 7). These immunological differences apparently play no significant role in determining susceptibility to acute DSS-induced colitis. Both substrains share a common *Lps*^d allele on Chromosome 4 encoding a defective response to bacterial lipopolysaccharide (45). This mutation exerts its effect on functions of macrophages, B cells, T cells, and fibroblasts (37, 48). No evidence is available to show that the *Lps* gene is a major determinant in susceptibility of C3H substrains to DSS-induced colitis. Axelsson et al. (3) did not find a significantly altered mortality rate between DSS-treated C3H/HeJ and C3H/HeN mice. Similarly, Lange et al. (21) found no differences in DSS-induced acute histopathological changes in a 2-cm portion of the distal colon in normal (*Lps*ⁿ) strain C3H/HeN versus hyporesponsive (*Lps*^d) C3H/HeJ substrains. These authors did observe differential survivorship between C57BL/6J (*Lps*ⁿ) versus C57BL/10ScN (*Lps*^d) after 10 days of treatment with 3% DSS. However, the differential response cannot be assumed to be a consequence of the *Lps* difference, since B6 and B10 represent distinct inbred strains that differ at numerous loci in addition to *Lps* (26, 44).

The NOD/LtJ strain was also found to be highly susceptible to DSS and, in contrast to the hyporesponsiveness to LPS exhibited by strains C3H/HeJ and C3H/HeJBir, presumably carries the wild-type *Lps*ⁿ allele, since NOD/LtJ mice are acutely sensitive to endotoxin administration (unpublished observations). NOD mice are susceptible to spontaneous autoimmune insulin-dependent diabetes mellitus (IDDM) as they age and show a large number of dysregulated immune functions (22, 23, 34, 42). These include defects in the differentiation and function of antigen-presenting cells, defects in the structural genes encoding Fc γ receptors I and II, subnormal secretion of monokines by peripheral macrophages in response to LPS stimulation, subnormal secretion of IL-2 and IL-4 by splenic and thymic T lymphocytes, depressed natural killer (NK) cell activity, depressed thymocyte responses to mitogenic stimulation, and T cell accumulation in lymphoid organs (41). As a result, NOD mice are subject to leukocytic infiltrates in a variety of organs, including an aging-associated typhilitis in the large bowel (22). NOD-*scid* mice, lacking both functional T and B lymphocytes, as well as exhibiting markedly reduced NK cell activity (43), were as sensitive to DSS as standard NOD mice. Thus T lymphocytes, B lymphocytes, and probably NK cells are not factors determining the high susceptibility of NOD mice to DSS-induced colitis. Interestingly, the related NON/LtJ strain (20), which is resistant to IDDM (35), is also resistant to DSS-induced lesions. Although macrophage functions in NON/Lt appear to be normal (42), this strain exhibits some unusual

immunological features in that it becomes T lymphocytopenic with age (35). A major genetic component of IDDM susceptibility of NOD mice is contributed by the unique *H2^{g7}* MHC haplotype on Chromosome 17 (22). Genes within the MHC are probably not involved in the genetic control of DSS susceptibility, since NON.*H2^{g7}* congenic mice were as resistant as standard NON mice. Similarly, NOD mice congenic for the MHC of NON retained sensitivity to DSS (unpublished observations). We have recently confirmed a genetic contribution to DSS susceptibility by studying NOD/Lt mice congenic for a segment of Chromosome 9 from NON/Lt and for a segment of Chromosome 2 from C57BL/6J (24). Both congenic NOD/Lt stocks were less susceptible to DSS than the standard NOD/Lt strain.

As observed by others (32, 47), we found that DSS-induced lesions generally predominated in the distal colon, although a portion of the midcolon was consistently involved. In contrast, Cooper et al. (8) and Minochi et al. (27) found the most severe changes in the midcolon. Similar discrepancies have been reported for rats (10, 46). This variance may in part be due to differences in inbred strains used. Interestingly, in the guinea pig, DSS-induced lesions are most severe in the cecum (19). This finding has been attributed to 1) the selective uptake of DSS in the cecum of guinea pigs, suggesting a relative weak point in the intestinal barrier in this region (18) and 2) the distribution of macrophages in the large intestine of guinea pigs, with the highest numbers in the cecum (18, 19). It is speculative whether similar peculiarities in barrier integrity or distribution of cells mediating inflammatory responses account for the regional differences in DSS-induced lesions among inbred strains of mice or rats. Other factors that could contribute to the variance among different studies are endogenous bacterial flora (40), degree of sulfate content and molecular weight of DSS (2), experimental protocol, and potentially other undefined environmental factors. One should also consider the preparation of tissues for histological examination. Longitudinal sections, such as those obtained by intestinal loops (29, 45), may better reflect the actual damage than cross sections because of the patchy nature of DSS-induced lesions (8). Finally, with regard to the DSS model of induced colitis, there is the inherent variability associated with potential inter- and intra-strain differences in the rates of mucosal repair of DSS-elicited injury, an issue that was not addressed in the present study.

Our finding that male mice were more severely affected in the colon than females indicates a possible male gender bias for susceptibility to DSS-induced colitis in mice. This is a novel observation and needs further evaluation. However, male mice have been found to have a higher incidence of clinical signs of IBD in the C3H/HeJBir substrain (45) and in a colony of T cell receptor- α mutant mice (H. R. Gaskins, personal communication). In humans, the male-to-female ratio is greater than one in ulcerative colitis but less than one in Crohn's disease (36). It should be noted that, in the present study, the male sex bias was limited to

colon and was not observed for colonic ulceration, location of inflammation, or in cecal inflammation.

In summary, there is a genetically determined variance in susceptibility to DSS-induced colitis among inbred strains of mice. The strain differences described here will be useful for the design of genetic mapping studies in mice to identify genes that determine susceptibility to DSS-induced colitis and possibly to other forms of experimental colitis. Identification of such genes may predict pathways for therapeutic intervention in human IBD and may also identify the location of homologous human genes (33).

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REFERENCES

1. Axelsson, L., E. Landstrom, T. Goldschmidt, A. Grönberg, and A. Bylund-Fellenius. Dextran sodium sulfate (DSS) induced experimental colitis in immunodeficient mice: effects in CD4⁺-cell depleted, athymic and NK-cell depleted SCID mice. *Inflamm. Res.* 45: 181–191, 1996.
2. Axelsson, L., E. Landstrom, C. Lundberg, and A. Bylund-Fellenius. The degree of sulfate content and the molecular weight of dextran sulfate and carrageenan are important for the induction of colitis in mice (Abstract). *Gastroenterology* 110: A858, 1996.
3. Axelsson, L.-G., T. Midtvedt, and A.-C. Bylund-Fellenius. The role of intestinal bacteria, bacterial translocation and endotoxin in dextran sodium sulphate-induced colitis in the mouse. *Microbial Ecol. Health Dis.* 9: 225–237, 1996.
4. Berg, D. J., N. Davidson, R. Kühn, W. Müller, S. Menon, G. Holland, L. Thompson-Snipes, M. W. Leach, and D. Rennick. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4⁺ TH1-like responses. *J. Clin. Invest.* 98: 1010–1020, 1996.
5. Birkenmeier, E. H., A. Torrey, and J. P. Sundberg. Chromosomal location of modifier genes determining sensitivity of mice to dextran sulphate sodium. In: *Inflammatory Bowel Disease*, edited by G. Tytgat, J. Bartelsman, and S. van Deventer. Boston: Kluwer, 1995, p. 401–407.
6. Brandwein, S. L., R. P. McCabe, Y. Cong, K. B. Waites, B. U. Ridwan, P. A. Dean, T. Ohkusa, E. H. Birkenmeier, J. P. Sundberg, and C. O. Elson. Spontaneously colitic C3H/HeJBir mice demonstrate selective antibody reactivity to antigens of the enteric bacterial flora. *J. Immunol.* 159: 44–52, 1997.
7. Cong, Y., S. L. Brandwein, A. Lazenby, R. P. McCabe, E. H. Birkenmeier, J. P. Sundberg, and C. O. Elson. Th1 CD4⁺ T cell reactivity to enteric bacterial antigens in colitic C3H/HeJBir mice (Abstract). *Gastroenterology* 110: A887, 1996.
8. Cooper, H. S., S. N. Murthy, R. S. Shah, and D. J. Sedergran. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab. Invest.* 69: 238–249, 1993.
9. Dieleman, L. A., B. U. Ridwan, G. S. Tennyson, K. W. Beagley, R. P. Bucy, and C. O. Elson. Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. *Gastroenterology* 107: 1643–1652, 1994.
10. Domek, M. J., F. Iwata, E. I. Blackman, J. Kao, M. Baker, A. Vidrich, and F. W. Leung. Anti-neutrophil serum attenuates dextran sulfate sodium-induced colonic damage in the rat. *Scand. J. Gastroenterol.* 30: 1089–1094, 1995.
11. Elson, C. O., K. W. Beagley, A. T. Sharmanov, K. Fujihashi, H. Kiyono, G. S. Tennyson, Y. Cong, C. A. Black, B. W.

- Ridwan, and J. R. McGhee. Hapten-induced model of murine inflammatory bowel disease: mucosal immune responses and protection by tolerance. *J. Immunol.* 157: 2174–2185, 1996.
12. Elson, C. O., R. B. Sartor, G. S. Tennyson, and R. H. Riddell. Experimental models of inflammatory bowel disease. *Gastroenterology* 109: 1344–1367, 1995.
 13. Fekete, E. A comparative morphologic study of the mammary gland in a high and low tumor strain of mice. *Am. J. Pathol.* 14: 557–578, 1938.
 14. Fox, J., and A. Lee. The role of *Helicobacter* species in newly recognized gastrointestinal tract diseases of animals. *Lab. Anim. Sci.* 47: 222–255, 1997.
 15. Hammer, R. E., J. A. Richardson, W. A. Simmons, A. L. White, M. Breban, and J. D. Taurog. High prevalence of colorectal cancer in HLA-B27 transgenic F344 rats with chronic inflammatory bowel disease. *J. Invest. Med.* 43: 262–268, 1995.
 16. Hirono, I., K. Kuhara, S. Hosaka, S. Tomizawa, and L. Goldberg. Induction of intestinal tumors in rats by dextran sulfate sodium. *J. Natl. Cancer Inst.* 66: 579–583, 1981.
 17. Hörnquist, C. E., X. Lu, P. M. Rogers-Fani, U. Rudolph, S. Shappell, L. Birnbaumer, and G. R. Harriman. Gai2-deficient mice with colitis exhibit a local increase in memory CD4⁺ T cells and pro-inflammatory Th1-type cytokines. *J. Immunol.* 158: 1068–1077, 1997.
 18. Hoshi, O., T. Iwanaga, and M. A. Fujino. Selective uptake of intraluminal dextran sulfate sodium and senna by macrophages in the cecal mucosa of the guinea pig. *J. Gastroenterol.* 31: 189–198, 1996.
 19. Iwanaga, T., O. Hoshi, H. Han, and T. Fujita. Morphological analysis of acute ulcerative colitis experimentally induced by dextran sulfate sodium in the guinea pig: some possible mechanisms of cecal ulceration. *J. Gastroenterol.* 29: 430–438, 1994.
 20. Kikutani, H., and S. Makino. The murine autoimmune diabetes model: NOD and related strains. In: *Advances in Immunology*, edited by F. J. Dixon. New York: Academic, 1992, p. 285–322.
 21. Lange, S., D. S. Delbro, E. Jennische, and I. Mattsby-Baltzer. The role of the *Lps* gene in experimental ulcerative colitis in mice. *APMIS* 104: 823–833, 1996.
 22. Leiter, E. H. Multifactorial control of autoimmune insulin dependent diabetes in NOD mice: lessons for IBD. *Can. J. Gastroenterol.* 9: 153–159, 1995.
 23. Luan, J.-J., R. C. Monteiro, C. Sautes, G. Fluteau, L. Eloy, W. H. Fridman, J.-F. Bach, and H.-J. Garchon. Defective FcγRII gene expression in macrophages of NOD mice: genetic linkage with upregulation of IgG1 and IgG2b in serum. *J. Immunol.* 157: 4707–4716, 1996.
 24. Mähler, M., J. P. Sundberg, E. H. Birkenmeier, I. J. Bristol, C. O. Elson, and E. H. Leiter. Chromosomal location of genes determining susceptibility of mice to dextran sulfate sodium (DSS)-induced colitis (Abstract). *Gastroenterology* 112: A1031, 1997.
 25. McCall, R. D., S. Haskill, E. M. Zimmermann, P. K. Lund, R. C. Thompson, and R. B. Sartor. Tissue interleukin 1 and interleukin-1 receptor antagonist expression in enterocolitis in resistant and susceptible rats. *Gastroenterology* 106: 960–972, 1994.
 26. McClive, P. J., D. Huang, and G. Morahan. C57BL/6 and C57BL/10 inbred mouse strains differ at multiple loci on chromosome 4. *Immunogenetics* 39: 286–288, 1994.
 27. Minocha, A., C. Thomas, and R. Omar. Lack of crucial role of mast cells in pathogenesis of experimental colitis in mice. *Dig. Dis. Sci.* 40: 1757–1762, 1995.
 28. Mombaerts, P., E. Mizoguchi, M. J. Grusby, L. H. Glimcher, A. K. Bhan, and S. Tonegawa. Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. *Cell* 75: 275–282, 1993.
 29. Moolenbeek, C., and E. J. Ruitenberg. The “Swiss roll”: a simple technique for histological studies of the rodent intestine. *Lab. Anim.* 15: 57–59, 1981.
 30. Ni, J., S.-F. Chen, and D. Hollander. Immunological abnormality in C3H/HeJ mice with heritable inflammatory bowel disease. *Cell. Immunol.* 169: 7–15, 1996.
 31. Ohkusa, T., I. Okayasu, S. Tokoi, A. Araki, and Y. Ozaki. Changes in bacterial phagocytosis of macrophages in experimental ulcerative colitis. *Digestion* 56: 159–164, 1995.
 32. Okayasu, I., S. Hatakeyama, M. Yamada, T. Ohkusa, Y. Inagaki, and R. Nakaya. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* 98: 694–702, 1990.
 33. Paigen, K. A miracle enough: the power of mice. *Nature Med.* 1: 215–220, 1995.
 34. Prins, J.-B., J. A. Todd, N. R. Rodrigues, S. Ghosh, P. M. Hogarth, L. S. Wicker, E. Gaffney, P. L. Podolin, P. A. Fischer, A. Sirotna, and L. B. Peterson. Linkage on chromosome 3 of autoimmune diabetes and defective Fc receptor for IgG in NOD mice. *Science* 260: 695–698, 1993.
 35. Prochazka, M., E. H. Leiter, D. V. Serreze, and D. L. Coleman. Three recessive loci required for insulin-dependent diabetes in nonobese diabetic mice. *Science* 237: 286–289, 1987.
 36. Russel, M. G., and R. W. Stockbrügger. Epidemiology of inflammatory bowel disease: an update. *Scand. J. Gastroenterol.* 31: 417–427, 1996.
 37. Ryan, J. L., and K. P. W. J. McAdam. Genetic non-responsiveness of murine fibroblasts to bacterial endotoxin. *Nature* 269: 153–155, 1977.
 38. Sadlack, B., J. Löhler, H. Schorle, G. Klebb, H. Haber, E. Sickel, R. J. Noelle, and I. Horak. Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of CD4⁺ T cells. *Eur. J. Immunol.* 25: 3053–3059, 1995.
 39. Sartor, R. B., D. E. Bender, and L. C. Holt. Susceptibility of inbred rat strains to intestinal inflammation induced by indomethacin (Abstract). *Gastroenterology* 102: A690, 1992.
 40. Schuh, J. C. L., and J. L. Viney. Endogenous bacterial flora modulate experimentally-induced colitis in mice (Abstract). *Vet. Pathol.* 33: 610A, 1996.
 41. Serreze, D., and E. Leiter. Insulin dependent diabetes mellitus (IDDM) in NOD mice and BB rats: origins in hematopoietic stem cell defects and implications for therapy. In: *Lessons from Animal Diabetes* (5th ed.), edited by E. Shafir. London: Smith-Gordon, 1995, p. 59–73.
 42. Serreze, D. V., H. R. Gaskins, and E. H. Leiter. Defects in the differentiation and function of antigen presenting cells in NOD/Lt mice. *J. Immunol.* 150: 2534–2543, 1993.
 43. Shultz, L. D., P. A. Schweitzer, S. W. Christianson, B. Gott, I. B. Schweitzer, B. Tennent, S. McKenna, L. Mobraaten, T. V. Rajan, D. L. Greiner, and E. H. Leiter. Multiple defects in innate and adaptive immunological function in NOD/LtSz-scid mice. *J. Immunol.* 154: 180–191, 1995.
 44. Slingsby, J., M. Hogarth, E. Simpson, M. Walport, and B. Morley. New microsatellite polymorphisms identified between C57BL/6, C57BL/10, and C57BL/KsJ inbred mouse strains. *Immunogenetics* 43: 72–75, 1995.
 45. Sundberg, J. P., C. O. Elson, H. G. Bedigian, and E. H. Birkenmeier. Spontaneous, heritable colitis in a new substrain of C3H/HeJ mice. *Gastroenterology* 107: 1726–1735, 1994.
 46. Tamaru, T., H. Kobayashi, S. Kishimoto, G. Kajiyama, F. Shimamoto, and W. R. Brown. Histochemical study of colonic cancer in experimental colitis of rats. *Dig. Dis. Sci.* 38: 529–537, 1993.
 47. Tokoi, S., T. Ohkusa, I. Okayasu, and K. Nakamura. Population changes in immunoglobulin-containing mononuclear cells in dextran sulfate sodium-induced colitis. *J. Gastroenterol.* 31: 182–188, 1996.
 48. Vetvicka, V. C3H/HeJ mice. In: *Immunological Disorders in Mice*, edited by B. Rihova and V. Vetvicka. Boca Raton, FL: CRC, 1991, p. 153–171.
 49. Ward, J. M., M. R. Anver, D. C. Haines, J. M. Melhorn, P. L. Gorelick, L. Yan, and J. G. Fox. Inflammatory large bowel disease in immunodeficient mice naturally infected with *Helicobacter hepaticus*. *Lab. Anim. Sci.* 46: 15–20, 1996.
 50. Yamada, M., T. Ohkusa, and I. Okayasu. Occurrence of dysplasia and adenocarcinoma after experimental chronic ulcerative colitis in hamsters induced by dextran sulphate sodium. *Gut* 33: 1521–1527, 1992.
 51. Yang, H., and J. Rotter. Genetic aspects of idiopathic IBD. In: *Inflammatory Bowel Disease* (4th ed.), edited by J. Kirsner and R. Shorter. Baltimore, MD: Williams and Wilkins, 1995, p. 301.