Susceptibility of Inbred Mouse Strains to Infection with Serpula (Treponema) hyodysenteriae

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Several inbred strains of mice were inoculated with Serpula (Treponema) hyodysenteriae B204 to determine susceptibility to infection. Challenge doses of 10³ or 10⁶ spirochetes induced cecal lesions in C3H/HeJ mice and other C3H strains of mice. However, more than a 100-fold difference existed between the dose required to induce lesions in 50% of the infected C3H/HeJ mice (8.3 x 10⁵) and that required to induce them in 50% of the infected C3H/HeN mice (5 x 10⁵). C3H/HeJ mice lack a spleenocyte mitogenic response to Escherichia coli lipopolysaccharide but exhibited a mitogenic response comparable to those of other C3H strains of mice when stimulated with S. hyodysenteriae endotoxin (butanol-water extract). Different inbred strains exhibited different susceptibilities to infection, with the strain C3H/HeN being the most susceptible on the basis of colonization and development of macroscopic cecal lesions. The ity gene had no apparent effect on susceptibility of mice challenged with S. hyodysenteriae. The involvement of the H-2 haplotype with susceptibility is unclear, but the mice bearing H-2² were more susceptible than mice with the H-2³, H-2⁴, or H-2² haplotype. These data support the hypothesis that the host’s responsiveness to lipopolysaccharide influences the susceptibility to infection with S. hyodysenteriae. However, differences in susceptibility between inbred mice exist independent of the lps locus, suggesting that there are other inherent differences between mouse strains that affect susceptibility to infection by S. hyodysenteriae.

Swine dysentery is a mucohemorrhagic diarrheal disease of pigs caused by Serpula (Treponema) hyodysenteriae (7, 33). Affected pigs become gaunt and dehydrated as a result of nonreabsorption of fluids and colonic ion transport abnormalities (2). Affected cecal and colonic mucosae exhibit mucohemorrhagic exudation and fibrinonecrotic membrane formation (10). Histologic lesions include mucosal epithelial erosions, inflammatory cell infiltration of the lamina propria, and coagulative necrosis of the superficial mucosa (1, 10). Potential virulence determinants of S. hyodysenteriae include an endotoxinlike moiety (21, 22) and a β-hemolysin (18, 31). In vivo models (other than pigs) used to examine the virulence of S. hyodysenteriae have included guinea pigs (14), mice (12, 13, 20, 35), and chicks (36). Because of availability of reagents and the ability to adoptively transfer cells, we have utilized inbred mice as a model to study the pathogenesis of S. hyodysenteriae.

Ceca from mice infected with S. hyodysenteriae exhibit histopathological lesions similar to those of pigs with swine dysentery (12). The endotoxin of S. hyodysenteriae is biologically active (5, 6, 21) and was suggested as a virulence determinant by work which showed that lipopolysaccharide (LPS)-hyporesponsive C3H/HeJ mice did not develop macroscopic cecal lesions following S. hyodysenteriae infection, whereas LPS-responsive C3HeB/FeJ mice did develop cecitis (22). The host responsiveness to endotoxin from bacteria of the family Enterobacteriaceae has been shown to be important in resistance to mucosal infections with Escherichia coli (37). The lps gene has been shown to control the host’s sensitivity to lipid A, B-cell mitogenesis, and the induction of tumor necrosis factor (3, 30, 40). Another murine genetic locus, which determines susceptibility to gram-negative bacterial infections, has been identified as ity (26, 27). The role of the ity gene has been shown to be control of the proliferation of Salmonella typhimurium within the spleen and liver (26, 27). In contrast to a previous report (22), we have shown that C3H/HeJ mice developed mucosal lesions following S. hyodysenteriae infection which are comparable to those observed in other LPS-responsive C3H strains of mice. The present work was undertaken to define murine susceptibility to S. hyodysenteriae infection in terms of: (i) the influence of lps and ity, (ii) the influence of the murine H-2 haplotype, and (iii) determination of the number of spirochetes required to produce disease in 50% of the infected mice, or 50% infective dose (ID₅₀), for different inbred mouse strains.

MATERIALS AND METHODS

Mice. C3H/HeSn (lps⁺/lps⁺ ity⁺), C3HeB/FeJ (lps⁺/lps⁺ ity⁺), DBA/1J (lps⁺/lps⁺), and BALB/cByJ (lps⁺/lps⁺ ity⁺) mice were purchased from Jackson Laboratories, Bar Harbor, Maine. C57BL/6J (lps⁻/lps⁻ ity⁺) mice were kindly provided by C. J. Warner, Department of Biochemistry, Iowa State University, Ames. C3H/HeN (lps⁺/lps⁺ ity⁻) and C3H/HeJ (lps⁺/lps⁻ ity⁻) mice were obtained from breeding colonies that were originally procured from Harlan Sprague Dawley, Indianapolis, Ind., and Jackson Laboratories, respectively. Mice were maintained on Mouse Lab Chow no. 5010 (Purina Mills, Inc., St. Louis, Mo.) under controlled conditions in the Laboratory Animal Resource Facility of the College of Veterinary Medicine, Iowa State University, Ames. Mice were 6 to 10 weeks of age at the time of experimentation.

Bacterial strains. S. hyodysenteriae B204 (serotype 2) with less than 20 in vitro passages was grown anaerobically at 37°C in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% horse serum (HyClone Laboratories, Logan, Utah), 0.5% yeast extract...
(BBL), and 1% VPI salt solutions as previously described (38). Highly motile (motility, >90%) log-phase cultures were obtained by inoculating warm (37°C) broth with a 33% inoculum from an overnight culture and incubating the inoculated broth for approximately 5 h. Bacteria were enumerated by using a Petroff-Hauser counting chamber and diluted with warm complete medium prior to inoculation of mice.

Mitogenic response determination. C3H/HeSn, C3H/HeJ, C3HeB/FeJ, and C3H/HeN splenocytes (5 x 10^5 per well) were stimulated with 0.2, 1.0, 5.0, or 25.0 µg of E. coli LPS per ml or 1.0, 5.0, or 25.0 µg of S. hyodysenteriae endotoxin per ml (5), as previously described (39).

Infection procedure. Mice were administered two doses of bacteria 24 h apart by gastric intubation. Feed was removed 6 h prior to the first challenge and withheld for 36 h. The standard infective challenge utilized to determine susceptibility of various mouse strains was 10^7 bacteria in 1 ml of culture broth. In dose titration studies, spirochetes were diluted to the appropriate concentration and delivered in 1 ml of culture medium.

Necropsy procedure. Mice were killed at 10 or 15 days postinfection (p.i.). Criteria used to determine the presence of disease were (i) macroscopic cecal lesions, (ii) reisolation of S. hyodysenteriae from cecal contents, and (iii) high levels of S. hyodysenteriae CFU per gram of cecal tissue. Macroscopic cecal lesions were scored as follows: cecal organ atrophy and excess intraluminal mucus, 3; atrophy and excess intraluminal mucus localized in the cecal apex only, 2; excess cecal mucus with no evidence of atrophy, 1; and no gross lesions, 0. Cecal apices were fixed in neutral buffered formalin, sectioned, and stained with hematoxylin-eosin.

Isolation of S. hyodysenteriae. Cecal swabs were streaked onto citrated ovine blood agar plates containing antibiotics as previously described (16). Plates were incubated anaerobically at 37°C for 96 h. Strongly β-hemolytic colonies were confirmed to consist of large spirochetes by dark-field microscopy.

Plate count technique. Cecal were removed aseptically and weighed in sterile WhirlPak bags (Baxter Co., Minneapolis, Minn.), suspended 1:100 (wt/vol) in phosphate-buffered saline (NaCl, 136.9 mM; Na_2HPO_4, 8.1 mM; KH_2PO_4, 1.5 mM; KCl, 2.7 mM [pH 7.2]), and homogenized in a stomacher laboratory blender (A. J. Seward Co., London, United Kingdom). This suspension was directly examined by dark-field microscopy for the presence of large spirochetes. Appropriate dilutions of the homogenate were added to 5 ml of molten (45°C) Trypticase soy agar tubes supplemented with 5% ovine blood and antibiotics, vortexed, and poured into 60-mm-diameter petri dishes (no. 25010; Corning Glass Works, Corning, N.Y.). Petri plates were incubated anaerobically at 37°C for 96 h. The zones of β-hemolysis were enumerated, and the number of S. hyodysenteriae CFU per gram of cecal tissue was calculated.

Statistical evaluation. Student's t distribution (32) was used to determine significance. The ID_{50} was estimated by the method of Reed and Muench (28).

RESULTS

Evaluation of susceptibility of C3H mouse strains. The macroscopic cecal scores of C3H mice challenged with 1 x 10^7 S. hyodysenteriae are represented in Fig. 1, and corresponding colonization values are expressed in Fig. 2. All mice—C3H/HeN, C3H/HeJ, C3H/HeSn, and C3H/He/FeJ—necropsied at 10 days p.i. were culture positive for S. hyodysenteriae, and their susceptibilities to disease were similar. At 15 days p.i., C3H/HeN mice appeared to be the most susceptible mice (as evidenced by the greatest development of macroscopic lesions) but all strains exhibited signs of infection by S. hyodysenteriae (Fig. 1). At 15 days p.i., the lesion scores appeared more severe in C3H/HeN mice than in the other C3H strains (Fig. 1) but the numbers of S. hyodysenteriae recovered were similar for all strains examined (Fig. 2). To determine whether the lower lesion scores associated with the C3H/HeJ at 15 days p.i. were associated with enhanced resolution of the lesions, C3H/HeJ and C3H/HeN mice were killed at various times over a 70-day period (Fig. 3). Following infection, S. hyodysenteriae was isolated from both C3H/HeJ and C3H/HeN mice at all time points (Fig. 3), and both strains of mice presented cecal lesions, except for the C3H/HeJ mice at 60 days p.i.

Mitogenesis. As expected, C3H/HeJ splenocytes were hypo-responsive to E. coli LPS-induced mitogenesis (Fig. 4A) in comparison with spleen cells from other C3H strains

![FIG. 1. Susceptibility of C3H strains of mice following challenge with 1 x 10^7 S. hyodysenteriae B204. Cecal scores range from 3 (severe lesions) to 0 (no macroscopic lesions), as described in Materials and Methods. Results are expressed as the mean ± standard error of the mean. At 10 days p.i., n = 4, 4, 5, and 5 for C3H/HeN, C3H/HeJ, C3HeB/FeJ, and C3H/HeSn mice, respectively. At 15 days p.i., n = 5, 5, 5, and 4 for C3H/HeN, C3H/HeJ, C3HeB/FeJ, and C3H/HeSn mice, respectively.](http://iai.asm.org/)

![FIG. 2. Cecal colonization of C3H strains estimated on the basis of S. hyodysenteriae CFU recovered. Mice were challenged with 1 x 10^7 bacteria, and cecal contents were cultured as described in Materials and Methods. Results are expressed as the log_{10} of the mean number of CFU per gram of cecal tissue. Group sizes are the same as those described for Fig. 1.](http://iai.asm.org/)
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lesions following infection with *S. hyodysenteriae* B204. Mice were infected with $1 \times 10^5$ *S. hyodysenteriae* as described in Materials and Methods. Cecal scores were determined as described in Materials and Methods and range from 3 (severe lesions) to 0 (no macroscopic lesions). Results are expressed as the mean ± standard error of the mean. Percentages reflect the proportion of each respective group that were culture positive for *S. hyodysenteriae*. $n = 5$ for all groups. *+, no C3H/HeJ mice exhibited macroscopic lesions at 60 days p.i.

of mice. However, *S. hyodysenteriae* endotoxin (butanol-water extract [5]) induced a mitogenic response from C3H/HeJ spleen cells similar to that of spleen cells from LPS-responsive mice (Fig. 4B). This indicated that C3H/HeJ mice were able to respond mitogenically to spirochete cell wall components.

**Titration of infective dose for C3H/HeN, C3H/HeJ, BALB/cByJ, C57BL/6J, and DBA/1J mice.** Mice were challenged with increasing doses of *S. hyodysenteriae* B204 ranging from $1 \times 10^2$ to $1 \times 10^8$ organisms. Mice were killed at 10 or 15 days p.i. and evaluated for lesion development. Cecal scores are presented in Fig. 5. At the infective dose of $1 \times 10^8$ organisms, the two C3H strains developed similar macroscopic cecal lesions. As the challenge dose was decreased, fewer of the C3H/HeJ mice developed macroscopic lesions. The differences in the cecal scores between C3H/HeJ and C3H/HeN mice (Fig. 5) were significantly different ($P < 0.05$) at the challenge dose of $1 \times 10^5$ organisms.

The proportion of each group of mice that was culture positive for *S. hyodysenteriae* when killed is shown in Table 1. The percentages of C3H/HeN, C3H/HeJ, and DBA/1J mice that were culture positive for *S. hyodysenteriae* were similar (i.e., 71 to 100%) for infective doses of $1 \times 10^6$ or $1 \times 10^7$ organisms. However, cecal samples from DBA/1J mice produced fewer positive cultures than those from the C3H mice at doses of $1 \times 10^5$ and $1 \times 10^4$ *S. hyodysenteriae*. At a challenge dose of $1 \times 10^5$ organisms, C3H/HeJ mice developed less-severe macroscopic lesions than did C3H/HeN mice ($P < 0.05$, Fig. 5) and fewer *S. hyodysenteriae*...
TABLE 1. Percentage of mice culture positive after challenge with various doses of S. hyodysenteriae

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>% of mice positive after challenge with an infective dose of:</th>
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<tbody>
<tr>
<td></td>
<td>10^4</td>
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<tr>
<td>C3H/HeN</td>
<td>93 (14)</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>86 (7)</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>29 (7)</td>
</tr>
<tr>
<td>DBA/1J</td>
<td>100 (6)</td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>0 (8)</td>
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* The percentage of mice in each challenge group that were culture positive for S. hyodysenteriae B204 at the time the mice were killed. For C3H/HeN mice infected with 1 × 10^8 organisms, seven of the mice were killed at 10 days p.i. and seven were killed at 15 days p.i. All C3H/HeN mice receiving <1 × 10^8 organisms were killed at 15 days p.i. Seven C3H/HeJ mice challenged with either 1 × 10^6 or 1 × 10^7 organisms were killed at 10 or 15 days p.i. C3H/HeJ mice receiving 1 × 10^8 organisms were killed at 15 days p.i. Numbers in parentheses indicate total group size regardless of the time the mice were killed.

* Mice were challenged with decreasing doses of S. hyodysenteriae B204 as described in Materials and Methods.

were recovered from cecal samples in comparison with recovery from cecal samples from C3H/HeN mice (Fig. 6). In addition, examination of mice at 15 days p.i. demonstrated that C3H/HeJ mice developed significantly fewer (P < 0.05) macroscopic cecal lesions than C3H/HeN mice infected in a similar manner following challenge with 1 × 10^4 or fewer organisms (data not shown). Cecal lesions were noted in C3H/HeN mice following infection with as few as 100 spirochetes; however, 1 × 10^6 organisms failed to induce macroscopic lesions in C3H/HeJ mice killed at 15 days p.i. (data not shown). C57BL/6J mice were culture negative after inoculation with 1 × 10^6 and 1 × 10^7 organisms, and BALB/cByJ mice were culture negative at all challenge doses examined (Table 1). The data in Fig. 6 and 7A indicate the number of S. hyodysenteriae CFU recovered from the ceca of infected mice. The number of S. hyodysenteriae recovered from C3H/HeJ mice following infection with 1 × 10^4 to 1 × 10^6 organisms was significantly different (P < 0.05) from that recovered from C3H/HeN mice infected with similar levels (Fig. 6). Compared with the C3H/HeN mice, the numbers of S. hyodysenteriae CFU recovered from DBA/1J and C57BL/6J mice were at least 100- to 1,000-fold less at all challenge doses (Fig. 7A). In addition, cecal lesions in C57BL/6J and DBA/1J mice were much less frequent than those observed in C3H mice (Fig. 7B). BALB/cByJ mice failed to develop lesions regardless of the number of spirochetes in the challenge inoculum (data not shown).

By using a cecal score of 3 as evidence of murine susceptibility, the ID_{50} of S. hyodysenteriae was calculated to be 5 × 10^3 for C3H/HeN mice and 8.3 × 10^3 for C3H/HeJ mice. Other inbred mouse strains (BALB/cByJ, DBA/1J, and C57BL/6J) exhibited negligible susceptibility to the development of cecal lesions, and this prohibited calculation of ID_{50}.

DISCUSSION

The difference in susceptibility between C3H/HeN and C3H/HeJ mice following infection with S. hyodysenteriae suggests a role for endotoxin or the host's responsiveness to endotoxin in the development of cecal lesions. In agreement with results of previous work (22), we observed differences in susceptibility to infection with S. hyodysenteriae between the LPS-hyporesponsive C3H/HeJ (8, 40) mice and LPS-responsive mice. In contrast to the previous report (22), lesion development in C3H/HeN mice was similar to that observed for C3H/HeN mice when the mice were challenged with doses of ≥1 × 10^6 spirochetes (Fig. 5). C3H/HeJ mice were less susceptible to infection at a challenge dose of 1 × 10^6 and 1 × 10^7 spirochetes than were C3H/HeN mice, which were susceptible to lesion development at a challenge dose of 100 organisms (data not shown). In addition, differences in susceptibility to infection by S. hyodysenteriae existed between the other mouse strains tested, independent of lps genotype.

The reason(s) for the increased susceptibility to infection with S. hyodysenteriae of the C3H/HeN mice in comparison with C3H/HeJ mice is unknown. Previous work shows that C3H/HeN mice are less susceptible to infection with Salmonella typhimurium (23, 24, 29) and Klebsiella pneumoniae (25) than are the LPS-hyporesponsive C3H/HeJ mice. The increase in susceptibility to Salmonella infections of the C3H/HeJ mice has been attributed to the inability to activate macrophages via the lipid A moiety of bacterial LPS (30). Conversely, C3H/HeJ mice have been shown to be less susceptible to infection with Neisseria gonorrhoeae, to develop a greater peritoneal leukocyte response, and to clear gonococci from the circulation faster than C3H/HeN mice (34). These observations indicate that the host's responses play an active role in elimination of the pathogen but that an elevated response may result in a prolonged infection or development of more-severe lesions.
Most pathogenicity studies comparing \( lps^{d}/lps^{d} \) and \( lps^{d}/lps^{d} \) mice have involved invasive microorganisms that were administered either intravenously or intraperitoneally (23–25, 29). Recently, Svanborg-Edén et al. (37) evaluated both local and disseminated infection in C3H and C57BL mice and described the effect of the \( lps \) and \( ity \) genes on susceptibility to disease at a mucosal surface. Disseminated infection (i.e., intravenous \( Salmonella typhimurium \) challenge) was shown to be influenced by \( lps \) and \( ity \) genes, with the influence of the \( lps \) gene being greater in determining susceptibility. Local infection (i.e., intravesicular \( E. coli \) challenge) was not influenced by the \( ity \) genotype, but mice with the \( lps^{d}/lps^{d} \) genotype did exhibit impaired clearance (37). The effector mechanism(s) involved in clearance of the mucosal infections appeared to require polymorphonuclear leukocyte infiltration and acute inflammation (37). This mucosal inflammatory response was absent in the C3H/HeJ mouse (37). Treatment of C3H/HeN mice with indomethacin resulted in the development of mucosal infection and subsequent disease, indicating the importance of the host’s inflammatory response as a means of mucosal bacterial resistance (19, 37). The lesions associated with swine dysentery are not induced by a massive invasion of tissue by microorganisms (1, 10). It is possible that the continued presence of \( S. hyodysenteriae \) in the intestinal lumen stimulates a persistent inflammatory response that results in the presence of chronic lesions. This was suggested by the fact that the intestinal lesions were not resolved within 70 days following infection of either C3H/HeJ or C3H/HeN mice (Fig. 3). Indeed, a threshold level of colonizing \( S. hyodysenteriae \) appears to be necessary to cause macroscopic lesion development in mice; a dose of approximately \( 1 \times 10^5 \) spirochetes per gram of cecal tissue or more was necessary before severe cecitis (cecalt score of 3) was observed (S. K. Nibbelink, personal observation). C3H/HeJ mice may require a higher threshold stimulus (i.e., number of CFU per gram of cecum) to initiate pathogenesis; thus the higher \( ID_{90} \) in comparison with C3H/HeN mice. A higher stimulus threshold might also explain why C3H/HeJ mice have a lower incidence of cecal lesions (i.e., 60 days p.i., Fig. 3) than C3H/HeN mice infected at a similar level, even though the frequency of \( S. hyodysenteriae \) reisolation is comparable (Fig. 3).

Previous work failed to detect neutrophil infiltration into the cecal lamina propria of C3H/HeJ mice following challenge with \( S. hyodysenteriae \) (22). Our results indicate that C3H/HeJ mice, when challenged with \( \approx 1 \times 10^7 \) \( S. hyodysenteriae \), exhibit neutrophil accumulation in the lamina propria and submucosa similar to that exhibited by C3H/HeN mice (Fig. 8).

Since mucosal inflammation caused by \( E. coli \) LPS appears to be triggered at the mucosal epithelial surface (19), it is possible that the pathogenesis of swine dysentery also involves interaction of LPS or endotoxin with the colonial epithelium. \( S. hyodysenteriae \) has been shown to associate closely with porcine mucin in vitro and porcine colonic mucosal tissue in vivo (15).

This research suggests a multifaceted pathogenesis whereby the degree of responsiveness to LPS (whether it be spirochete endotoxin or LPS from the indigenous flora) corresponds to the relative susceptibility of the host to disease. However, alternate pathways for the induction of inflammation at the mucosal level might exist in C3H/HeJ mice, such as the induction of interleukin-6 resulting from tissue trauma (4). Also, C3H/HeJ macrophages have been shown to be activated when first primed with gamma interferon and then exposed to bacterial endotoxin (butanol-water extract) (9). It must be remembered that C3H/HeJ mice are LPS hyporesponders and that they may, therefore, be capable of a limited LPS- or endotoxin-induced inflammatory response. The ability of C3H/HeJ mice to respond to bacterial endotoxin has been demonstrated (30) (Fig. 4).

However, preliminary observations indicate that C3H/HeJ (in comparison with C3H/HeN) peritoneal exudate cells produce less tumor necrosis factor and interleukin-1 after stimulation with \( S. hyodysenteriae \) endotoxin in vitro (37a). This observation suggests that the mucosal inflammatory response(s) induced by the spirochete cell components in the C3H/HeJ mice is less severe than in C3H/HeN mice.

Others have observed different susceptibilities of mouse strains upon infection with \( S. hyodysenteriae \) (35), theorizing...
that differences in the intestinal microflora affect susceptibility or colonization. We have observed that manipulation of the intestinal microflora (by oral antibiotic treatment or diet changes) does affect susceptibility of mice to infection with *S. hyodysenteriae*. However, all mice used during these studies were maintained under similar environmental conditions and with similar feeding and care regimens, thereby minimizing differences in gut microflora (11). We have found the mouse model to be optimized by challenge with *S. hyodysenteriae* in log phase, by using only highly motile (≥90% of organisms motile by dark-field microscopy) cultures. We have not determined the difference between challenge with log-phase versus challenge with late-log- or stationary-phase cultures, but Lee and Falkow (17) have

FIG. 8. Murine ceca after challenge with *S. hyodysenteriae* (10 days p.i.). Small numbers of neutrophils were observed in multiple foci within cecal lamina propria of both C3H/HeN (A) and C3H/HeJ (B) mice. Magnification, ×315. Arrows indicate areas of inflammation.
shown that Salmonella pathogenicity is greatly increased in log-phase cultures as opposed to stationary-phase cultures. These results suggest the importance of host responsiveness to LPS in the development of an intestinal inflammatory disease. However, the response to LPS is not the only determinant in the development of the intestinal lesions, because C3H/HeJ mice are susceptible to lesion development at doses in excess of $1 \times 10^8$ bacteria. Involvement of the $H-2$ haplotype in susceptibility to S. hyodysenteriae is unclear at this time; however, all C3H strains ($H-2^\alpha$) were more susceptible than strains BALB/cByJ ($H-2^\beta$), DBA/1J ($H-2^\delta$), and C57BL/6J ($H-2^\beta$).

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