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Resistance to Mycoplasmal Lung Disease in Mice Is a Complex Genetic Trait

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Mouse strains differ markedly in resistance to *Mycoplasma pulmonis* infection, and investigation of these differences holds much promise for understanding the mechanisms of antimycoplasmal host defenses. To determine the potential genetic diversity of resistance to disease in murine respiratory mycoplasmosis (MRM) and to select disease-resistant and nonresistant mouse strains for further genetic analysis, we screened 17 inbred mouse strains of various *Bcg* and *H-2* genotypes for resistance to *M. pulmonis*. Mice were inoculated intranasally with 10^4 CFU of *M. pulmonis* UAB CT and evaluated at 21 days postinfection for severities of the four histologic lung lesions characteristic of MRM: alveolar exudate, airway exudate, airway epithelial hyperplasia, and lymphoid infiltrate. On the basis of these assessments of MRM severity, one group of mouse strains was found to be extremely resistant to disease (C57BR/cdJ, C57BL/6NCr, C57BL/10ScNCr, and C57BL/6J). The remaining strains of mice (C57L/J, SJL/NCr, BALB/cAnNCr, A/JCr, C3H/HeJ, SWR/J, AKR/NCr, CBA/NCr, C58/J, DBA/2NCr, C3H/HeNCr, C3HeB/FeJ, and C3H/HeJCr) developed disease of widely varying severities. Furthermore, strains in the group with more disease varied in pattern of lesion severity. While the severities of all four lesions were correlated in most mouse strains, this was not always true. DBA/2NCr mice had one of the highest scores for alveolar exudate, only a moderate score for airway exudate, and significantly lower scores for both airway epithelial hyperplasia and lymphoid infiltrate than all other strains susceptible to lung disease. DBA/2NCr mice had one of the highest mortality rates. We concluded that resistance to MRM is a complex trait. The observed differences in lung disease severity could not be explained by known differences at the *Bcg* or *H-2* locus in the strains of mice we studied.

Pneumonia is a major cause of death and disability, and *Mycoplasma pneumoniae* is a leading cause of pneumonia worldwide (1, 14, 18, 19, 20, 21). In the United States, *M. pneumoniae* accounts for 20 to 30% of all cases of pneumonia in the general population, up to 45% of cases of pneumonia in military recruits, and 56% of cases of pneumonia in university students, for a total of 8 to 15 million cases per year (1, 7, 14, 21, 22). Despite extensive investigations of antimycoplasmal host defenses (5, 12, 15, 23, 36, 40), the mechanisms of protective immunity in respiratory mycoplasmosis remain poorly understood.

Murine respiratory mycoplasmosis (MRM) due to *Mycoplasma pulmonis* is an excellent animal model of human respiratory mycoplasmal disease with clinical and pathologic features similar to those in the human disease (5, 11, 12, 22, 36). Like the disease caused by *M. pneumoniae* respiratory infection in humans, MRM is associated with rhinitis, otitis media, laryngotracheitis, and bronchopneumonia. The lung disease in both hosts is characterized by the following histologic lesions: (i) neutrophilic exudate in airway lumina (airway exudate), (ii) hyperplasia of airway epithelium (airway epithelial hyperplasia), (iii) peribronchial and -vascular lymphoid hyperplasia/infiltration (lymphoid infiltrate), and (iv) mixed neutrophilic and histiocytic exudate in alveoli (alveolar exudate) (5, 6).

Certain mouse strains (C57BL/6N and C3H/HeN) are known to differ markedly in resistance to *M. pulmonis* infection (13, 24). C57BL/6N mice are much more resistant to MRM than C3H/HeN mice, as evidenced by the fact that C57BL/6N mice have a 100-fold-higher 50% gross pneumonia dose, 50% microscopic lesion dose, and 50% lethal dose (13, 31, 32). In addition, C57BL/6N mice mount a lower humoral response but continue to be more resistant than C3H/HeN mice to mycoplasmal lung disease and mycoplasma infection (as evidenced by 2- to 5-log-fewer organisms in their lungs) for at least 2 months postinfection (p.i.) (4). Previous studies (13) of inbred mice of three different major histocompatibility (*H-2*) haplotypes suggested that the resistance of mice to MRM might be the result of differences in *H-2* genotype. Lai et al. (24, 25) suggested that pulmonary mycoplasmal killing is due to a single gene not associated with *H-2* on chromosome 17 but possibly linked to chromosome 4. It is not known whether the *Bcg* locus (chromosome 1) influences resistance of mice to MRM. However, since the *Bcg* locus is known to control the early, innate phase of macrophage killing of some bacteria (37–39, 41) and there is strong evidence that alveolar macrophages are important in the early phase of MRM resistance (10–12, 32), *Bcg* is a logical candidate for a role in antimycoplasmal defense.

To evaluate the possible effects of different host genotypes on MRM severity, 17 inbred mouse strains spanning most of the genealogic tree of *Mus musculus* (with various *Bcg* and *H-2* genotypes) were evaluated for resistance to MRM at 21 days p.i. by quantitative assessment of four characteristic histologic lung lesions. Although mycoplasmal lung disease severity generally correlates directly with numbers of *M. pulmonis* present in the lungs (4, 13, 24), numbers of organisms alone do not always reflect severity of disease. Both C57BL/6N and C3H/

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HeN mice still harbor large numbers of *M. pulmonis* in their lungs ($\geq 10^2$ CFU) at 63 days postinoculation (4). Furthermore, the host immunological response may contribute more to pulmonary disease and illness than does direct tissue injury by the organism. Evidence for this includes studies showing that severe combined immunodeficient (SCID) mice, unlike immunocompetent mice, have minimal lung lesions after experimental induction of MRM (3, 16).

We reasoned that pathological parameters would be useful in directly monitoring severity of lung disease and relating it to genotype. Also, evaluation of four histopathological lesions involving different cell types might help to identify specific genes or sets of genes that affect mycoplasmal lung disease. Such a large screening of mouse strains for MRM susceptibility had never been done. On the basis of our results, we concluded that resistance to MRM is a complex trait (26) controlled by genetic factors other than *Bcg* or *H-2*. In addition, we selected C57BL/6, C3H/He, and DBA/2 as strains likely to be particularly instructive in further genetic analysis of antimycoplasmal resistance.

MATERIALS AND METHODS

Animals. Pathogen-free male mice of 17 inbred strains (A/JCr [*H-2^a Bcg^r*], AKR/NCr [*H-2^k Bcg^r*], BALB/cAnNCr [*H-2^d Bcg^s*], CBA/NCr [*H-2^k Bcg^r*], C3HeB/FeJ [*H-2^k Bcg^r*], C3H/HeJ [*H-2^k Bcg^r*], C3H/HeJCr [*H-2^k Bcg^r*], C3H/HeNCr [*H-2^k Bcg^r*], C57BL/6J [*H-2^b Bcg^s*], C57BL/6NCr [*H-2^b Bcg^s*], C57BL/10ScNCr [*H-2^b Bcg^s*], C57BR/cdJ [*H-2^k Bcg^r*], C57BL/J [*H-2^b Bcg^r*], C58/J [*H-2^k Bcg^r*], DBA/2NCr [*H-2^d Bcg^r*], SJL/NCr [*H-2^s Bcg^r*], and SWR/J [*H-2^q Bcg^r*]) were obtained from either the Frederick Cancer Research and Development Center, National Cancer Institute, Frederick, Md., or the Jackson Laboratory, Bar Harbor, Maine. The mice were shipped to the University of Alabama at Birmingham (UAB) in bacteriologic-filter-protected cartons. All mice were monitored at UAB for the presence of murine pathogens by using a comprehensive battery of virus serologies, bacterial cultures, endo- and ectoparasite examinations, and histopathology of all major organs, as described previously (17). All mice were consistently negative for pathogens by these tests. All uninfected control mice were negative for lesions, bacterial pathogens, and serum antibodies to *M. pulmonis* at termination of the experiments. Mice were maintained in autoclaved Microisolator cages (Lab Products, Maywood, N.J.), supplied with sterile hardwood chip bedding (PJ Murphy Forest Products, Rochelle Park, N.J.), and provided food (Agway, Inc., Syracuse, N.Y.) and water ad libitum. For inoculation and euthanasia, mice were anesthetized with ketamine (8.7 mg/100 g of body weight; Aveco, Fort Dodge, Iowa) and xylazine (1.3 mg/100 g of body weight; Haver, Shawnee, Kans.) given by intramuscular injection.

***M. pulmonis* infections.** The UAB CT strain of *M. pulmonis* was used in all experiments (8). Stock cultures of UAB CT were grown in mycoplasma broth (broth A) and frozen in 1-ml aliquots at −70°C as described previously (16). Thawed ampoules containing 2×10^7 CFU of *M. pulmonis* per ml were diluted in broth A to 1×10^4 CFU/50 μl at the time of inoculation. CFUs in the inoculum were confirmed by enumeration after standard dilution, inoculation of agar plates, and incubation for 7 days at 37°C in room air with 95% relative humidity. At 8 to 10 weeks of age, groups of 7 to 12 mice of each strain were given intranasally 10^4 CFU of *M. pulmonis* in 50 μl of inoculum. Control mice received the same volume of broth A alone.

Quantitative culture of lungs. Representative mice from each group were euthanatized 21 days p.i. Lungs were removed aseptically and individually minced and sonicated for 30 s in broth A. Ten-fold dilutions in broth A were prepared in 24-well plates, and 25 μl of each dilution was plated onto mycoplasma agar. CFUs in lungs from each animal were determined after incubation for 7 days as described previously (8).

Assessment of lung lesion severity. Lungs were fixed in 95% ethanol, embedded in paraffin, sectioned at a thickness of 5 μm, and stained with hematoxylin and eosin for light microscopy. Each lung lobe was sectioned separately, randomly coded, and subjectively scored by two observers for lesion severity (scale of 0 to 4) on the basis of the characteristic lesions of MRM as described previously (33). Scores refer to (i) mixed neutrophilic and histiocytic exudate in alveoli (hereafter referred to as alveolar exudate), (ii) neutrophilic exudate in airway lumina (airway exudate), (iii) peribronchial and -vascular lymphoid hyperplasia or infiltration (lymphoid infiltrate), and (iv) hyperplasia of airway mucosal epithelium (airway epithelial hyperplasia) (33). Scores for each lesion were weighted according to the percentage each lobe contributes to total lung weight in arriving at a total lesion score for each set of lungs. For each of the four lesions, a lesion index was calculated by dividing the observed lesion score by the maximum lesion score possible. Thus, the maximum lesion index possible for any lesion was 1.0 (13, 32, 33).

Statistics. Lesion scores were analyzed by using the Kruskal-Wallis one-way analysis of variance and the Mann-Whitney U test for statistical significance (43). Correlations were determined by Pearson's rank correlation (43). Survival probability analysis was performed by the Kaplan-Meier method (27). Probabilities of 0.05 or less were considered significant.

RESULTS

Lung disease severity in mouse strains. Mice of 17 inbred strains were infected experimentally with 10^4 CFU of *M. pulmonis* and the severity of their lung lesions was evaluated histologically 21 days p.i. All lesions observed were those characteristic of MRM, but major differences in lesion severity were found in different strains of mice. Inasmuch as natural and experimental (intranasal inoculation) *M. pulmonis* infection and disease spread centrifugally in the respiratory tract (29), the presence of cellular exudates in alveoli is indicative of maximum disease severity. For this reason, we present the data on severity of alveolar exudate first (Fig. 1A) and list mice in the same order in subsequent depictions of data for the other lesions (Fig. 1B to D).

On the basis of severity of alveolar exudate (Fig. 1A), the mouse strains could be separated into two disease groups. One group of mice (C57BR/cdJ, C57BL/6NCr, C57BL/10ScNCr, and C57BL/6J) was extremely resistant to disease as evidenced by low lesion indices (0 to 0.05). The remaining group of mice (C57L/J, SJL/NCr, BALB/cAnNCr, A/JCr, C3H/HeJ, SWR/J, AKR/NCr, CBA/NCr, C58/J, DBA/2NCr, C3H/HeNCr, C3HeB/FeJ, and C3H/HeJCr) included strains with a wide range of lesion indices (0.25 to 0.70). The same distribution of mouse strains into resistant and susceptible groups was found when mice were grouped on the basis of lesion scores for airway exudate (Fig. 1B), lymphoid infiltrate (Fig. 1C), and airway epithelial hyperplasia (Fig. 1D). Collectively, the resistant strains usually had lesion indices of 0.05 or less for all lesion types, while lesion indices among susceptible strains ranged from 0.25 to 0.70, i.e., their scores were 5 to 14 times those of the resistant phenotype. Among the resistant strains, the lesion scores did not differ significantly ($P > 0.05$). However, the lesion scores for all resistant strains differed significantly ($P < 0.05$) from the lesion scores for all susceptible strains. Furthermore, among the susceptible mouse strains there were some with fatalities, all occurring during the third week of infection: 75% for C3HeB/FeJ, 50% for C3H/HeJCr, 50% for DBA/2NCr, 43% for SJL/NCr, 38% for CBA/NCr, 14% for SWR/J, 13% for C3H/HeNCr, and 8% for C57L/J.

Within the group of susceptible mouse strains, there was a continuum of gradually increasing severity of alveolar exudate (Fig. 1A), and among the mice of most strains there was a high degree of correlation (r) between scores for the four lesion types ($r > 0.8$, $P < 0.05$), with a few exceptions. The score for alveolar exudate in C3H/HeJ mice correlated poorly ($r < 0.3$) with the much higher scores in that strain for lymphoid infiltrate and airway epithelial hyperplasia (Fig. 1). The severity of alveolar exudate in DBA/2NCr mice was very high, comparable to that in C3H/He and C58/J mice, the most susceptible strains on the basis of scores for all four lesions. However, in DBA/2NCr mice the high scores for alveolar exudate ($r < 0.1$) and airway exudate ($r < 0.4$) did not correlate with the low scores for airway epithelial hyperplasia and lymphoid infiltrate. In fact, DBA/2NCr mice had lower scores for airway epithelial hyperplasia ($P < 0.05$) and lymphoid infiltrate ($P < 0.05$) than all other susceptible mice. Thus, DBA/2NCr mice had a unique pattern of lung disease for susceptible mouse strains.

Relative resistances of C57BL/6NCr, C3H/HeNCr, and DBA/2NCr mice. Although the severity of the four characteristic MRM lesions correlated in most of the mouse strains, DBA/2NCr mice had a variable pattern of lung lesion devel-

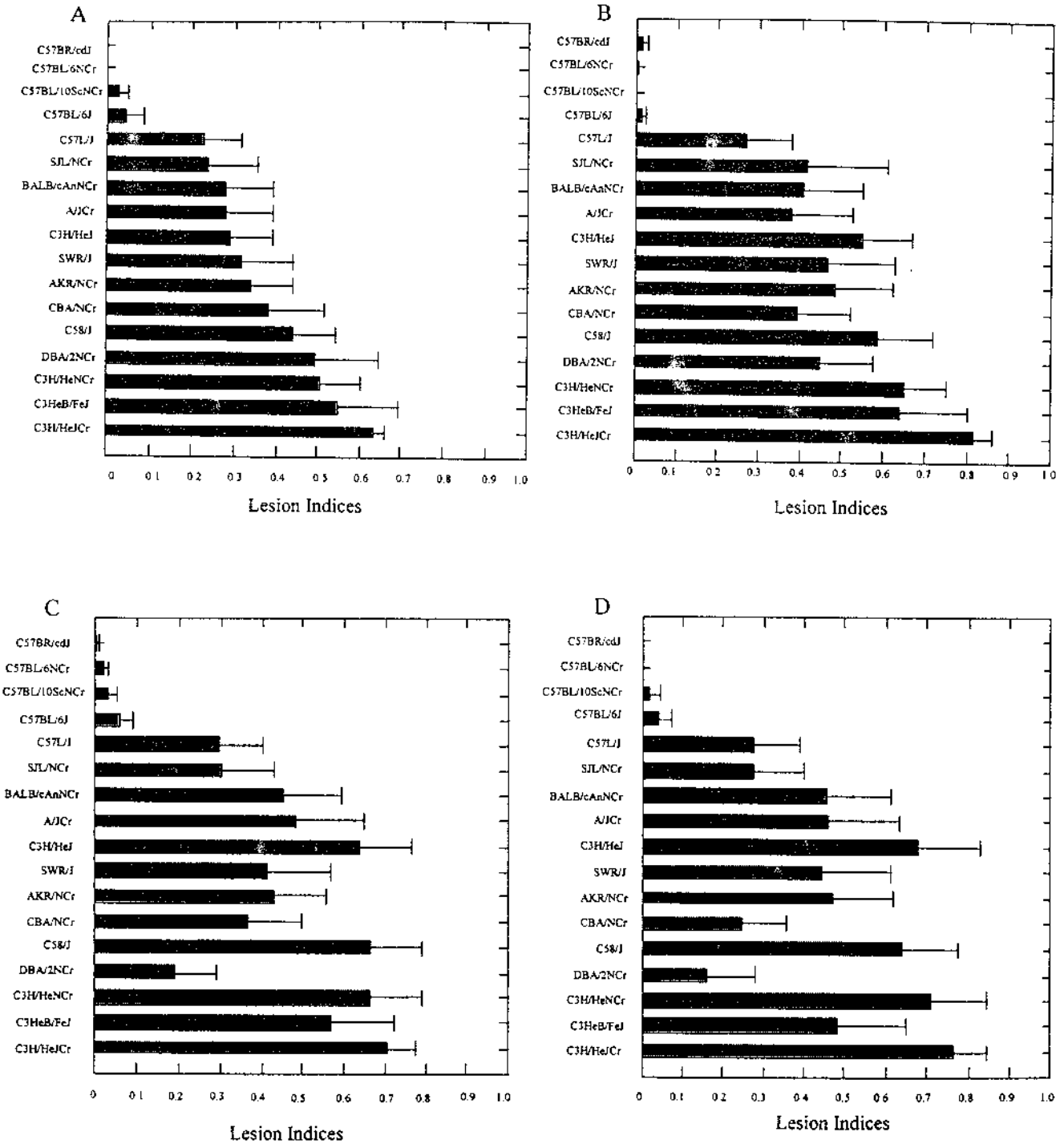

FIG. 1. Severities of lung lesions in 17 strains of mice. Mice (n = 7 to 12) were infected with 10^4 CFU of *M. pulmonis*, and lesion severity in lungs was determined 21 days after infection. Shown are means and standard errors of lung lesion index scores for alveolar exudate (A), airway exudate (B), lymphoid infiltrate (C), and airway epithelial hyperplasia (D).

opment. As DBA/2 is the parental strain with the largest recombinant inbred strain set (BXD/Ty) and would be useful in further genetic analysis, additional experiments were done to further compare the relative resistances of C57BL/6NCr, C3H/HeNCr, and DBA/2NCr mice to MRM. Groups of mice of each strain were infected with 10^4 CFU of *M. pulmonis* UAB CT and killed on day 14 p.i. rather than day 21 p.i. (as in Fig. 1). Both C3H/HeNCr and DBA/2NCr mice had significantly ($P < 0.05$) higher scores for all lung lesions than C57BL/6NCr mice (Fig. 2). DBA/2NCr mice had significantly ($P < 0.05$) less severe airway epithelial hyperplasia and lymphoid infiltrate than C3H/HeNCr mice, while there were no significant differences between the two strains in scores for alveolar exudate and airway exudate (confirming the lesion pattern for DBA/

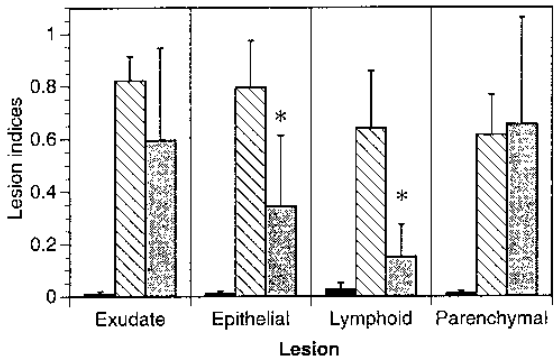

FIG. 2. Severities of lung lesions in C57BL/6NCr (■), C3H/HeNCr (▨), and DBA/2NCr (▤) mice. Mice (six per strain) were infected with 10^4 CFU of *M. pulmonis*, and lesion severity in lungs was determined 14 days after infection. Data are means and standard deviations of lung lesion index scores. The asterisk indicates significant differences between DBA/2N and C3H/N mice ($P \leq 0.05$).

2NCr in Fig. 1). In additional animals in the same experiment, the numbers of mycoplasmas cultured from lungs of DBA/2NCr ($2.3 \times 10^6 \pm 5.5 \times 10^6$ CFU, $n = 6$) and C3H/HeNCr ($9.8 \times 10^5 \pm 1.7 \times 10^6$ CFU, $n = 5$) mice did not differ significantly. Both DBA/2NCr and C3H/HeNCr mice had $\geq 9.8 \times 10^5$ CFU more organisms in their lungs than C57BL/6NCr mice, which had less than 10 CFU ($P < 0.05$).

To further compare the resistances of C57BL/6NCr, C3H/HeNCr, and DBA/2NCr mice to MRM, mice were intranasally infected with *M. pulmonis* at a range of doses (10^3 to 10^7 CFU) and the survival of the mice over a 21-day period was determined. DBA/2NCr mice had a much higher mortality rate than either of the other mouse strains (Fig. 3). In fact, survival analysis indicated that at a dose of 10^7 CFU, 100% of DBA/2NCr, 12.5% of C3H/HeNCr, and 0% of C57BL/6NCr mice died by 21 days p.i. In addition, the probability of survival for DBA/2NCr mice was shown to be inversely related to increasing dose of the organism, with 100% mortality observed at 10^7 CFU (Fig. 4).

***Bcg* and *H-2* status of mouse strains with different phenotypes.** Among the MRM-resistant mouse strains, C57BL/6NCr, C57BL/10ScNCr, and C57BL/6J mice were Bcg^s and only C57BR/cdJ mice were Bcg^r. Both the Bcg^r and Bcg^s genotypes were represented in the MRM-susceptible mouse strains. Bcg^r strains included A/JCr, AKR/NCr, CBA/NCr, C3HeB/FeJ, C3H/HeJ, C3H/HeJCr, C3H/HeNCr, C57L/J, C58/J, DBA/2NCr, SJL/NCr, and SWR/J; Bcg^s strains included BALB/cAnNCr. Similarly, the MRM-resistant mouse strains were of two different *H-2* haplotypes, $H\text{-}2^b$ (C57BL/6NCr, C57BL/10ScNCr, and C57BL/6J) and $H\text{-}2^k$ (C57BR/cdJ). MRM-susceptible mice were of all six haplotypes represented in this study: $H\text{-}2^a$ (A/JCr), $H\text{-}2^b$ (C57L/J), $H\text{-}2^d$ (DBA/2NCr and BALB/cAnNCr), $H\text{-}2^k$ (CBA/NCr, AKR/NCr, C3H/HeJ, C3HeB/FeJ, C58/J, C3H/HeNCr, and C3H/HeJCr), $H\text{-}2^q$ (SWR/J), and $H\text{-}2^s$ (SJL/NCr). Thus, both *H-2* haplotypes seen in the MRM-resistant strains, $H\text{-}2^b$ and $H\text{-}2^k$, were represented in the MRM-susceptible strains.

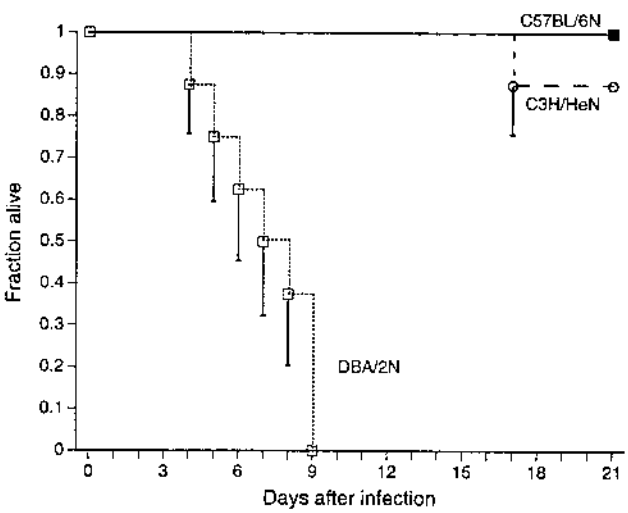

FIG. 3. Survival of mice infected with 10^7 CFU of *M. pulmonis*. The survival of C57BL/6N, C3H/HeN, and DBA/2NCr mice (eight per strain) was monitored for 21 days after infection. Data are the survival probabilities and standard errors as determined by Kaplan-Meier analysis.

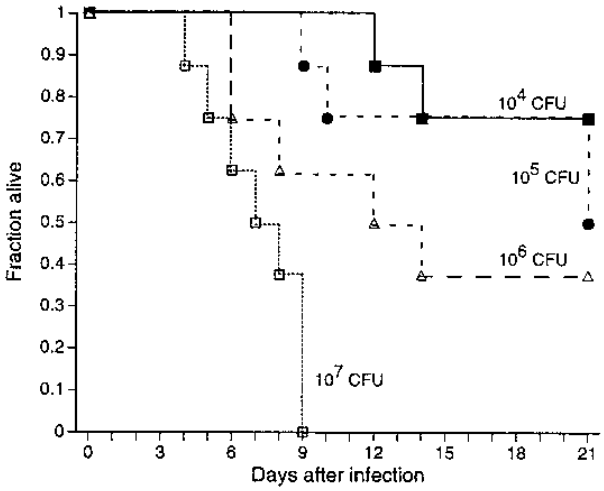

FIG. 4. Survival of DBA/2NCr mice infected with different doses of *M. pulmonis*. The survival of DBA/2NCr mice ($n = 8$) was monitored to 21 days after infection. Data are the percentages of animals alive each day postinoculation.

DISCUSSION

Mycoplasma infections are important causes of respiratory disease, but the host factors that control resistance are not well understood for any mycoplasma disease. To begin defining the host factors that control resistance to mycoplasmal disease, we took advantage of a model, *M. pulmonis* infection in mice, that has histopathologic features very similar to those of mycoplasma respiratory disease in humans (5, 6, 9, 22, 28). Mouse strains differ markedly in resistance to *M. pulmonis* infection, and investigation of these differences should prove useful in understanding the mechanisms of antimycoplasmal host defenses (5, 11, 12, 22, 36). Of particular interest is the fact that differences in the genetic backgrounds of the mouse hosts can have a major effect on early resistance to infection and progression of disease (4, 13, 24, 31). C57BL/6N mice are more resistant to MRM than C3H/HeN mice (13, 31). Davis et al. (13) studied MRM resistance in four strains of mice, C57BL/6 ($H\text{-}2^b$ Bcg^s), C3H/HeN ($H\text{-}2^k$ Bcg^r), C3B6 F1 ($H\text{-}2^{k/b}$ $Bcg^{r/s}$), CBA ($H\text{-}2^k$ Bcg^r), and B10.D2 ($H\text{-}2^d$ Bcg^s) and observed that $H\text{-}2^k$ mice had 50% lethal doses and 50% gross pneumonia doses 100-fold lower than those of $H\text{-}2^b$ mice and 50-fold lower than those of $H\text{-}2^{k/b}$ mice. These results suggested that *H-2* haplotype might contribute to MRM resistance in inbred mice. In contrast to these results, Lai et al. (23) examined the killing

of *M. pulmonis* in the respiratory tracts of mice with varied *H-2* haplotypes (A.By [$H\text{-}2^b$], BALB.B [$H\text{-}2^b$], C57BL/6 [$H\text{-}2^b$], C3H/Bi [$H\text{-}2^k$], C3H/HeJ [$H\text{-}2^k$], B10.BR [$H\text{-}2^k$], B6C3F1 [$H\text{-}2^{b/k}$], and B6C3F2 [$H\text{-}2^{b/b}$, $H\text{-}2^{b/k}$, or $H\text{-}2^{k/k}$]) and mice of selected recombinant inbred strains (BXH [C57BL/6 × C3H] and CXB [BALB/c × C57BL/6]) and concluded that resistance was a single gene effect not associated with *H-2* haplotype but potentially linked to an unidentified locus on chromosome 4.

As in previous studies (4, 13, 24, 25, 31), we found that C57BL strains of mice were resistant to MRM whereas C3H strains developed severe pulmonary lesions of all four types characteristic of MRM. It should be noted that genealogically C57BL and C57BR, the resistant strains, and C58/J, a susceptible strain, represent somewhat independent lineages among mice that are only distantly related to our other susceptible strains (2, 30). Also, C3H mice were derived along with CBA mice from a mating of DBA mice to progenitors of BALB/c and A strain mice (30). Thus, mice of C3H strains would be expected to share more host defense genes with DBA, CBA, A, and BALB/c mice than with C57BL and C57BR mice. It is of interest, however, that C58/J mice, alleged to be the closest relative of C57BL immunologically (2), are susceptible to MRM. Although C57BL, C57BR, and C58/J mice are genealogically closely related, it has been estimated that they may differ at 20 to 30% of their loci (2).

There is extensive literature attesting to the genetic susceptibility of mice to infectious diseases such as tuberculosis, leishmaniasis, salmonellosis, and listeriosis (37–39). Recently, the gene product of *Bcg* (*Nramp*) has been shown to be involved in macrophage activation essential in early killing of and resistance to *Mycobacterium tuberculosis* and, potentially, to other macrophage-regulated diseases. As other in vitro and in vivo evidence has shown macrophages to be important in the killing of *M. pulmonis* in mice (10–12, 32), *Bcg* appeared to be a prime candidate for a role in MRM resistance.

Our results demonstrated that neither *H-2* nor *Bcg* is likely to have a significant role in prevention of *M. pulmonis* disease, as mice of the disease-resistant phenotype varied in their *H-2* haplotypes and *Bcg* alleles and many of them shared *H-2* and *Bcg* genotypes with mice of the disease-susceptible phenotype. Both the Bcg^s and Bcg^r genotypes were represented among mouse strains of the disease severity phenotypes used by us. Similarly, the MRM-resistant mouse strains were of two different *H-2* haplotypes and the MRM-susceptible mice were of all six *H-2* haplotypes represented in the study. Also of interest is the lack of a difference in disease severity between C3H/HeNCr (Lps^n) and C3H/HeJ (Lps^d) mice, indicating that *Lps* does not play a significant role in the observed variations in MRM.

Our data did not support the hypothesis that severity of *M. pulmonis* disease is controlled by a single gene. However, our results do not directly contradict previous reports of MRM killing being a monogenic effect (24, 25), as killing and disease severity very well may be regulated by different genes or sets of genes. On the basis of severity of lesions at four different locations in the lungs, our data clearly identified two major phenotypic groupings of mice, a highly resistant group and a susceptible group characterized by highly variable disease severity and differing lesion patterns. While the severities of all four characteristic lesions of MRM usually correlated in each strain of mouse in the susceptible group, there was a major exception. DBA/2NCr mice had severe alveolar exudation and airway exudation, but in contrast to all other susceptible strains, they had significantly lower scores for both lymphoid infiltration and epithelial hyperplasia. Furthermore, among the susceptible mouse strains there was a wide range of mortalities as found in our initial strain comparisons and in our demonstration of a significantly higher mortality rate in DBA/2NCr mice than in the susceptible C3H/HeNCr strain of mice. Thus, there were clear differences in severity of certain types of lesions and in mortality rates among susceptible strains of mice. These results indicate considerable heterogeneity of host responses among susceptible strains and strongly support the possibility that the severity of MRM is influenced by multiple genes.

Host genetic factors may influence resistance to *M. pulmonis* disease through altered mechanisms of host defense and/or inflammatory response. Previous studies suggest that the difference between C57BL/6N and C3H/HeN mice in severity of MRM is associated with innate host defenses active within hours after infection (3, 10, 11, 23, 31, 32). Pulmonary clearance is much greater in the resistant C57BL/6N mice than in the susceptible C3H/HeN mice, an effect thought to be due to killing of mycoplasmas by alveolar macrophages (10, 11). Other studies have demonstrated that the higher production of inflammatory cytokines (interleukin 6 and tumor necrosis factor alpha) by pulmonary macrophages in C3H/HeN than in C57BL/6N mice is associated with the development of more severe disease in the susceptible C3H/HeN mice (3, 17). We found that *M. pulmonis*-infected DBA/2NCr mice developed more severe alveolar and airway disease and had a lower survival rate than most other susceptible strains, but lymphoid infiltrate and airway epithelial hyperplasia were greatly reduced or delayed. Similar numbers of mycoplasmas were recovered from the lungs of C3H/HeNCr and DBA/2NCr mice, suggesting that killing of organisms was similarly impaired in the lungs of both of these mouse strains. Most likely, the decreased epithelial and lymphoid responses in DBA/2NCr mice are linked to factors, such as cytokines, that regulate inflammatory responses. Either the level of production of or the capacity to respond to these mediators could contribute to differences in lesion severity. Additional studies are needed to determine the role of cytokines and the different cellular responses in the pathogenesis of MRM.

In summary, our results demonstrated that resistance to MRM is a complex trait (26) controlled by multiple genes, and differences at the *Bcg* and *H-2* loci do not account for the differences in susceptibility that we observed. Furthermore, our findings suggest that immune responses and lesion development may be independently controlled by different genetic mechanisms, resulting in different lesion characteristics between inbred strains of mice. Three strains of mice (C57BL/6, C3H/He, and DBA/2) embody most of the differences seen and were chosen as models for future studies to identify and characterize the host factors involved in pathogenesis of mycoplasma respiratory disease. C3H/He mice developed severe pulmonary lesions, including prominent lymphoid infiltration and airway epithelial hyperplasia. Although DBA/2 mice also were highly susceptible to MRM and developed severe alveolar lesions, their disease was qualitatively different from that in C3H/He mice, as epithelial hyperplasia and lymphoid infiltration were minimal. In contrast to both C3H/He and DBA/2 mice, C57BL/6 mice were resistant to infection and subsequent lesion development. These susceptible (C3H/He and DBA/2) and resistant (C57BL/6) strains should prove useful in further genetic analysis of MRM resistance and disease pathogenesis.

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REFERENCES

1. **Anonymous.** 1987. Statistical compendium on adult lung diseases. American Lung Association, New York.
2. **Atchley, W. R., and W. M. Fitch.** 1991. Gene trees and the origins of inbred strains of mice. Science **254:**554–558.
3. **Cartner, S. C., J. W. Simecka, G. H. Cassell, and J. R. Lindsey.** Unpublished data.
4. **Cartner, S. C., J. W. Simecka, J. R. Lindsey, G. H. Cassell, and J. K. Davis.** 1995. Chronic respiratory mycoplasmosis in C3H/HeN and C57BL/6N mice: lesion severity and antibody response. Infect. Immun. **63:**4138–4142.
5. **Cassell, G. H.** 1982. Derrick Edward Award Lecture. The pathogenic potential of mycoplasmas: Mycoplasma pulmonis as a model. Rev. Infect. Dis. **4**(Suppl.)**:**S18–S34.
6. **Cassell, G. H., J. R. Lindsey, R. G. Overcash, and H. J. Baker.** 1973. Murine mycoplasma respiratory disease. Ann. N. Y. Acad. Sci. **225:**395–412.
7. **Clyde, W. A., Jr., and G. W. Fernald.** 1990. Studies on the etiology and epidemiology of pneumonia in university students. Zentralbl. Bakteriol. **20**(Suppl.)**:**717.
8. **Davidson, M. K., J. R. Lindsey, R. F. Parker, J. G. Tully, and G. H. Cassell.** 1988. Differences in virulence for mice among strains of *Mycoplasma pulmonis*. Infect. Immun. **56:**2156–2162.
9. **Davis, J. K., and G. H. Cassell.** 1982. Murine respiratory mycoplasmosis in LEW and F344 rats: strain differences in lesion severity. Vet. Pathol. **19:** 280–293.
10. **Davis, J. K., M. K. Davidson, and T. R. Schoeb.** 1991. Murine respiratory mycoplasmosis: a model to study effects of oxidants. Research report number 47. Health Effects Institute, Cambridge, Mass.
11. **Davis, J. K., M. K. Davidson, T. R. Schoeb, and J. R. Lindsey.** 1992. Decreased intrapulmonary killing of *Mycoplasma pulmonis* after short-term exposure to NO_2 is associated with damaged alveolar macrophages. Am. Rev. Respir. Dis. **145:**406–411.
12. **Davis, J. K., K. M. Delozier, D. K. Asa, F. C. Minion, and G. H. Cassell.** 1980. Interactions between murine alveolar macrophages and *Mycoplasma pulmonis* in vitro. Infect. Immun. **29:**590–599.
13. **Davis, J. K., R. F. Parker, H. White, D. Dziedzic, G. Taylor, M. K. Davidson, N. R. Cox, and G. H. Cassell.** 1985. Strain differences in susceptibility to murine respiratory mycoplasmosis in C57BL/6 and C3H/HeN mice. Infect. Immun. **50:**647–654.
14. **Denny, F. W., W. A. Clyde, Jr., and W. P. Glezen.** 1971. *Mycoplasma pneumoniae* disease: clinical spectrum, pathophysiology, epidemiology, and control. J. Infect. Dis. **123:**74–94.
15. **Denny, F. W., D. Taylor-Robinson, and A. C. Allison.** 1972. The role of thymus-dependent immunity in *Mycoplasma pulmonis* infections of mice. J. Med. Microbiol. **5:**327–336.
16. **Evengard, B., K. Sandstedt, G. Bolske, R. Feinstein, I. Riesenfelt-Orn, and C. I. E. Smith.** 1995. Intranasal inoculation of *Mycoplasma pulmonis* in mice with severe combined immunodeficiency (SCID) causes a wasting disease with grave arthritis. Clin. Exp. Immunol. **98:**388–394.
17. **Faulkner, C. B., J. W. Simecka, M. K. Davidson, J. K. Davis, T. R. Schoeb, J. R. Lindsey, and M. P. Everson.** 1995. Gene expression and production of tumor necrosis factor alpha, interleukin 1, interleukin 6, and gamma interferon in C3H/HeN and C57BL/6N mice in acute *Mycoplasma pulmonis* disease. Infect. Immun. **63:**4084–4090.
18. **Fernald, G. W., A. M. Collier, and W. A. Clyde, Jr.** 1975. Respiratory infections due to *Mycoplasma pneumoniae* in infants and children. Pediatrics **55:**327–335.
19. **Flournoy, D. J., and J. B. Jones.** 1987. The significance of infection as a cause of death in veterans. Mil. Med. **152:**587–589.
20. **Foy, H. M.** 1993. Infections caused by *Mycoplasma pneumoniae* and possible carrier state in different populations of patients. Clin. Infect. Dis. **17**(Suppl.)**:** S37–S46.
21. **Foy, H. M., G. E. Kenney, M. K. Cooney, and I. D. Allan.** 1979. Long-term epidemiology of infections with *Mycoplasma pneumoniae*. J. Infect. Dis. **139:**681–687.
22. **Krause, D. C., and D. Taylor-Robinson.** 1992. Mycoplasmas which infect humans, p. 417–444. *In* J. Maniloff, R. N. McElhaney, L. R. Finch, and J. B. Baseman (ed.), Mycoplasmas: molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.
23. **Lai, W. C., M. Bennett, S. P. Pakes, V. Kumar, D. Steuterman, I. Owusu, and A. Mikhael.** 1990. Resistance to *Mycoplasma pulmonis* mediated by activated natural killer cells. J. Infect. Dis. **161:**1269–1275.
24. **Lai, W. C., G. Linton, M. Bennett, and S. P. Pakes.** 1993. Genetic control of resistance to *Mycoplasma pulmonis* infection in mice. Infect. Immun. **61:** 4615–4621.
25. **Lai, W. C., S. P. Pakes, and M. Bennett.** 1994. Mapping of *Mycoplasma pulmonis* resistance genes to mouse chromosome 4, p. 649. Abstracts of the 10th International Congress of the International Organization for Mycoplasmology.
26. **Lander, E. S., and N. J. Schork.** 1994. Genetic dissection of complex traits. Science **265:**2037–2048.
27. **Lee, E. T.** 1980. Statistical methods for survival data analysis. Wadsworth, Belmont, Calif.
28. **Lindsey, J. R., and G. H. Cassell.** 1973. Experimental *Mycoplasma pulmonis* infection in pathogen-free mice. Models for studying mycoplasmosis of the respiratory tract. Am. J. Pathol. **72:**63–90.
29. **Lindsey, J. R., G. H. Cassell, and M. K. Davidson.** 1982. Mycoplasmal and other bacterial diseases of the respiratory system, p. 21–41. *In* H. L. Foster, J. D. Small, and J. G. Fox (ed.), The mouse in biomedical research, vol. II. Academic Press, New York.
30. **Morse, H. C., III (ed.).** 1978. Origins of inbred mice. Academic Press, New York.
31. **Parker, R. F., J. K. Davis, D. K. Blalock, R. B. Thorp, J. W. Simecka, and G. H. Cassell.** 1987. Pulmonary clearance of *Mycoplasma pulmonis* in C57BL/6N and C3H/HeN mice. Infect. Immun. **55:**2631–2635.
32. **Parker, R. F., J. K. Davis, G. H. Cassell, H. White, D. Dziedzic, D. K. Blalock, R. B. Thorp, and J. W. Simecka.** 1989. Short-term exposure to NO_2 enhances susceptibility to murine respiratory mycoplasmosis and decreases intrapulmonary killing of *M. pulmonis*. Am. Rev. Respir. Dis. **140:**502–512.
33. **Pinson, D. M., T. R. Schoeb, J. R. Lindsey, and J. K. Davis.** 1986. Evaluation by scoring and computerized morphometry of lesions of early *Mycoplasma pulmonis* infection and ammonia exposure in F344/N rats. Vet. Pathol. **23:** 550–555.
34. **Roifman, C. M., and P. A. Quinn.** 1990. Suppression of mycoplasmas with high dose intravenous immune serum globulin in patients with hypogammaglobulinemia and chronic lung disease. Zentralbl. Bakteriol. **20**(Suppl.)**:**812–813.
35. **Roifman, C. M., C. P. Rao, H. M. Lederman, S. Lavi, P. Quinn, and E. W. Gelfand.** 1986. Increased susceptibility to mycoplasma infection in patients with hypogammaglobulinemia. Am. J. Med. **80:**590–594.
36. **Simecka, J. W., J. K. Davis, M. K. Davidson, S. E. Ross, C. T. K.-H. Städtlander, and G. H. Cassell.** 1992. Mycoplasma diseases of animals, p. 391–415. *In* J. Maniloff, R. N. McElhaney, L. R. Finch, and J. B. Baseman (ed.), Mycoplasmas: molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.
37. **Skamene, E.** 1985. Genetic control of host resistance to infection and malignancy. Prog. Leukocyte Biol. **3:**111–559.
38. **Skamene, E.** 1994. The *Bcg* gene story. Immunobiology **191:**451–460.
39. **Skamene, E., and C. E. Pietrangeli.** 1991. Genetics of the immune response to infectious pathogens. Curr. Opin. Immunol. **3:**511–517.
40. **Taylor, G., and C. J. Howard.** 1981. Protection of mice against *Mycoplasma pulmonis* infection using purified mouse immunoglobulins: comparison between protective effect and biological properties of immunoglobulin classes. Immunology **43:**519–525.
41. **Vidal, S. M., D. Malo, K. Vogan, E. Skamene, and P. Gros.** 1993. Natural resistance to infection with intracellular parasites: isolation of a candidate for *Bcg*. Cell **73:**469–485.
42. **Webster, A. D. B., P. M. Furr, N. C. Hughes-Jones, B. D. Gorick, and D. Taylor-Robinson.** 1988. Critical dependence on antibody for defense against mycoplasmas. Clin. Exp. Immunol. **71:**383–387.
43. **Wilkinson, L.** 1989. SYSTAT: the system for statistics. SYSTAT, Inc., Evanston, Ill.

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