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Chronic Respiratory Mycoplasmosis in C3H/HeN and C57BL/6N Mice: Lesion Severity and Antibody Response

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***Mycoplasma pneumoniae* is a leading, worldwide cause of death and disability due to pneumonia. *Mycoplasma pulmonis* infection in mice is an invaluable model for the study of host defenses against respiratory mycoplasmas in vivo. C3H/HeN mice are much more susceptible to acute inflammatory lung disease due to *M. pulmonis* than C57BL/6N mice, but little is known about the chronic disease in these mouse strains. We infected C3H/HeN and C57BL/6N mice with 10^4 CFU of *M. pulmonis* UAB CT and evaluated them at weekly intervals by quantitative mycoplasma culture of nasal passages, trachea, and lungs, assessment of lesion severity in nasal passages, trachea, and lungs, and determination of serum immunoglobulin classes and subclasses by enzyme-linked immunosorbent assay. We found that C3H/HeN mice had 2 to 5 logs more organisms in their lungs and far more severe lung disease than C57BL/6N mice through 63 days postinfection. Although both strains of mice developed the same classes of antibody, C3H/HeN mice had much greater anti-*M. pulmonis* immunoglobulin G (IgG) responses in the IgG1 and IgG2a subclasses than C57BL/6N mice. These results suggest that adaptive immunity does not effect resolution of chronic mycoplasma infection and disease in the lungs.**

Mycoplasma pneumoniae is a leading, worldwide cause of death and disability due to pneumonia (2, 12, 15–17). Although numerous investigations have addressed the question of host defense in mycoplasma infections of the respiratory tract (4, 9, 13, 31), none as yet has defined the essential mechanisms involved in protective immunity against these diseases. *Mycoplasma pulmonis* infection in mice (murine respiratory mycoplasmosis [MRM]) is an invaluable model for the study of host defenses against respiratory mycoplasmas in vivo (4).

Mice infected with *M. pulmonis* develop both acute and chronic lung diseases, with a corresponding bimodal distribution of deaths (6). The initial peak of deaths coincides with occurrence of maximum acute inflammation with pulmonary edema and hemorrhage. In contrast, the second peak of deaths is associated with lesions consisting of neutrophilic exudate in airways, hyperplasia and dysplasia of airway epithelium, submucosal lymphoid hyperplasia, and varying numbers of neutrophils and macrophages in alveoli (6, 21). Comparable lesions occur in humans infected with *M. pneumoniae* (4, 6, 8, 19).

Mouse strains differ in susceptibility to *M. pulmonis*, and studies of these differences give insights into the mechanisms of host defense and pathogenesis of respiratory mycoplasmosis. During the first 2 weeks of infection (acute disease), C3H/HeN mice are much more susceptible to the infection than C57BL/6N mice, as evidenced by the fact that C3H/HeN mice have a 100-fold-lower 50% gross pneumonia dose, 50% microscopic lesion dose, and 50% lethal dose (10, 24). However, it is not known whether the differences in susceptibility persist during the chronic disease (2 weeks to 2 months) or how antibody responses of the two mouse strains relate to progression of the chronic disease.

We infected C3H/HeN and C57BL/6N mice with 10^4 CFU of *M. pulmonis* UAB CT and, at weekly intervals to 63 days postinfection (p.i.), evaluated them by quantitative mycoplasma culture of nasal passages, trachea, and lungs; assessment of lesion severity in nasal passages, trachea, and lungs; and determination of serum immunoglobulin classes and subclasses by enzyme-linked immunosorbent assay (ELISA). We found that chronically infected C3H/HeN mice consistently had much greater numbers of CFU of *M. pulmonis* in their lungs, greater severity of lung lesions, and higher serum antibody responses. These results suggest that adaptive immunity is ineffective in eliminating pulmonary mycoplasma infection and disease and may actually enhance lung lesion severity. The results also provide further evidence that differences in nonspecific host defense may account for the disparate numbers of the organism and disease severity associated with both the acute and chronic lung diseases in these mouse strains.

MATERIALS AND METHODS

Animals. C3H/HeN and C57BL/6N mice, 3 to 6 weeks old, with equal numbers of males and females, were obtained from the National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Md., shipped to the University of Alabama at Birmingham in bacteriologically filtered cartons, and raised to 8 to 10 weeks of age. All mice were monitored for the presence of murine pathogens with a comprehensive battery of virus serologies, bacterial cultures, endo- and ectoparasite examinations, and histopathology of all major organs. Results have been consistently negative. Mice were maintained in sterile Microisolator cages (Lab Products, Maywood, N.J.), supplied with sterile hardwood chip bedding (PJ Murphy Forest Products, Rochelle Park, N.J.) and sterile food (Agway, Inc., Syracuse, N.Y.) and water ad libitum. All control animals were negative for lesions, bacterial pathogens, and serum antibodies to *M. pulmonis*. Mice were anesthetized for inoculation and euthanasia. Anesthesia was ketamine HCl (Aveco, Fort Dodge, Iowa; 8.7 mg/100 g of body weight) and xylazine (Haver, Shawnee, Kans.; 1.3 mg/100 g of body weight) by intramuscular injection.

M. pulmonis. The UAB CT strain of *M. pulmonis* was used in all experiments

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(7). Stock cultures of CT were grown as described previously in mycoplasma broth (broth A) and frozen in 1-ml aliquots at −70°C. Thawed ampoules containing 2×10^7 CFU of *M. pulmonis* per ml were diluted in broth A at the time of inoculation to 10^4 CFU/50 μl. CFU in the inoculum were quantitated by performing 10-fold dilutions and inoculation of agar plates. Inoculations were given intranasally in 50-μl volumes. Control mice received the same volume of broth A alone.

M. pulmonis cell lysate was used as the antigen for the ELISA determination of serum antibody (18). This lysate was prepared by propagating 15 ml of the stock culture described above in 500 ml of broth A for 24 h at 37°C. Cells were harvested by centrifugation at 10,000 × *g* for 15 min at 4°C. The organisms were washed three times with sterile phosphate-buffered saline (pH 7.3) and suspended in the same buffer to give a concentration of 5 mg of protein per ml as determined by a protein assay (Bio-Rad Laboratories, Richmond, Calif.). The organisms were then lysed by the addition of 0.05 M carbonate-bicarbonate buffer (CBB; pH 10.0) at 37°C in a 1:20 proportion (organisms/CBB). After 10 min of incubation, lysis was terminated by adding boric acid (2.2 g/100 ml of CBB). This lysate, which contained 200 μg of protein per ml (determined by the Bio-Rad protein assay), was diluted to 10 μg/ml in the ELISA coating buffer (0.1 M sodium bicarbonate).

Assessment of lesion severity. Nasal passages, tracheas, and lungs were fixed in 95% alcohol, and the nasal passages were demineralized in DeCalcifying Solution (Baxter Healthcare Corporation, McGraw Park, Ill.). Tissues were embedded in paraffin, sectioned at a 5-μm thickness, and stained with hematoxylin and eosin for light microscopy. All sections were coded randomly and scored subjectively (0 to 4) by two observers for lesion severity on the basis of the characteristic lesions of chronic respiratory mycoplasmosis: (i) exudate in airway lumena, (ii) hyperplasia-dysplasia of mucosal epithelium, (iii) peribronchial and perivascular lymphoid accumulation, and (iv) inflammatory cell infiltration into alveoli (parenchymal lesions) (26). All scores were weighted by the percentage each lobe contributes to the total lung weight in arriving at the lesion index for lungs. For each of the four lesions, the lesion index was calculated by dividing the observed lesion score by the maximum lesion score possible. Thus, the maximum lesion index possible was 1.0 (11, 21, 26).

Quantitative cultures. Nasal passages, tracheas, and lungs were removed aseptically and individually minced and sonicated for 30 s in broth A. Tenfold dilutions in broth A were prepared in 24-well plates, and 25 μl of each dilution was plated on mycoplasmal agar. The CFU in each animal's nasal passages, trachea, and lungs were determined after 7 days of incubation at 37°C in room air with 95% relative humidity.

ELISA for serum antibody levels. Antibody levels in serum samples were compared by ELISA as described previously (29). Briefly, serial dilutions (1:50 to 1:4,050) of each serum sample were added in duplicate to microtiter wells (Titertek) coated with an *M. pulmonis* UAB CT lysate at a concentration of 10 μg/ml. After overnight incubation, the unbound antibody was removed by washing the wells with 0.9% NaCl washing solution–0.05% Tween 20. Alkaline phosphatase (AP)-labelled secondary antibodies were added at dilutions determined to have minimal nonspecific binding to each well and allowed to incubate for 5 h. AP-conjugated goat-anti-mouse immunoglobulin A (IgA), IgM, IgG, IgG1, IgG2b, IgG3 (Southern Biotechnology Associates, Inc., Birmingham, Ala.), and AP-conjugated goat-anti-mouse IgG2a (Caltag, San Francisco, Calif.) were used to detect antigen-specific mouse antibodies. Washing was performed as described for the serum, and the AP substrate (*p*-nitrophenyl phosphate; Sigma Chemical Company, St. Louis, Mo.) was added to each well. After a 10-min incubation at 37°C, the optical densities at 405 nm were determined with a model 3550 microplate reader (Bio-Rad Laboratories, Richmond, Calif.). The relative antibody activities were determined with a diluted (1:10) standard serum made from pooled sera of *M. pulmonis*-infected C3H/HeN mice and given an arbitrary activity value of 1,000 ELISA units. All other serum antibody activities were compared with this standard.

Statistics. Lesion scores, CFU data, and antibody activities were analyzed by the Kruskal-Wallis one-way analysis of variance and the Mann-Whitney U Test for statistical significance. Regression analysis was used to determine the primary subclasses of the IgG humoral response. Probability (*P*) values of 0.05 or less were considered significant.

RESULTS

Disease severity in C3H/HeN versus C57BL/6N mice. To characterize the development of MRM in C3H/HeN and C57BL/6N mice, age- and sex-matched mice of each strain were infected with *M. pulmonis*, and histologic evaluation of disease severity was performed on equal numbers ($n = 6$) of each mouse strain at 7, 14, 21, 28, 35, 49, and 63 days p.i. The characteristic lesions of MRM were present in infected mice. By 7 days p.i., neutrophils were present in the airways. Epithelial hyperplasia was apparent in the upper airways at 7 days, but by 14 days, it was present throughout the entire respiratory tract. Lymphoid hyperplasia was similar in both strains, with mononuclear cells in the submucosa surrounding the airways. Parenchymal lesions consisting of alveoli filled with neutrophils and macrophages were present by 14 days p.i.

TABLE 1. Comparison of lung lesion index scores between C3H/HeN and C57BL/6N mice[a]

Day p.i.	Mouse strain[b]	Lung lesion index score[c] for: Exudate	Epithelial hyperplasia	Lymphoid hyperplasia	Parenchymal lesions
7	C3	0.32 (0.29)[d]	0.08 (0.09)	0.12 (0.10)[d]	0.08 (0.08)[d]
	B6	0.00 (0.00)	0.01 (0.02)	0.02 (0.04)	0.00 (0.00)
14	C3	0.53 (0.28)[d]	0.48 (0.20)[d]	0.47 (0.25)[d]	0.35 (0.17)[d]
	B6	0.05 (0.08)	0.01 (0.02)	0.06 (0.06)	0.01 (0.02)
21	C3	0.72 (0.07)[d]	0.73 (0.06)[d]	0.84 (0.04)[d]	0.57 (0.07)[d]
	B6	0.03 (0.03)	0.01 (0.03)	0.20 (0.08)	0.01 (0.02)
28	C3	0.70 (0.12)[d]	0.76 (0.18)[d]	0.78 (0.15)[d]	0.65 (0.19)[d]
	B6	0.00 (0.01)	0.00 (0.01)	0.16 (0.09)	0.00 (0.00)
35	C3	0.26 (0.23)	0.30 (0.29)[d]	0.36 (0.29)	0.24 (0.23)[d]
	B6	0.02 (0.02)	0.01 (0.03)	0.19 (0.07)	0.01 (0.03)
49	C3	0.42 (0.28)	0.44 (0.35)[d]	0.57 (0.32)	0.39 (0.29)[d]
	B6	0.10 (0.17)	0.10 (0.20)	0.30 (0.23)	0.09 (0.18)
63	C3	0.42 (0.29)[d]	0.41 (0.32)[d]	0.51 (0.33)	0.38 (0.30)[d]
	B6	0.00 (0.01)	0.00 (0.00)	0.17 (0.04)	0.00 (0.00)
C[e]	C3	0.00 (0.01)	0.00 (0.00)	0.02 (0.03)	0.00 (0.00)
	B6	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

[a] Mice ($n = 6$ per time point per strain) were inoculated with 10^4 CFU of *M. pulmonis*.
[b] C3, C3H/HeN; B6, C57BL/6N.
[c] Mean (standard deviation) of lesion index scores.
[d] Significant difference ($P < 0.05$) between C3 and B6 lesion scores.
[e] Scores for uninfected mice (C) for all time points were pooled. All scores for uninfected mice were significantly different from those for infected mice ($P < 0.05$).

Although infected C3H/HeN and C57BL/6N mice had similar lesion characteristics, they differed in severity of lung disease. C3H/HeN mice had greater lung parenchymal lesion index scores ($P \leq 0.05$) than C57BL/6N mice at all time points (Table 1). Other lung lesion index scores were significantly greater ($P \leq 0.05$) in C3H/HeN mice than in C57BL/6N mice at most time points (Table 1). These differences included increased exudate, epithelial hyperplasia, and lymphoid hyperplasia in the lungs. There was no difference observed between males and females in either strain.

Severities of nasal and tracheal lesions were similar between the two mouse strains; however, there were differences at individual time points (data not shown). C57BL/6N mice had significantly greater ($P \leq 0.05$) epithelial hyperplasia and lymphoid hyperplasia at 14, 21, and 28 days in nasal passages than C3H/HeN mice. C3H/HeN mice had significantly greater nasal airway exudate at 63 days than C57BL/6N mice. C3H/HeN mice had significantly greater tracheal lesion scores at several time points, but the pattern was not consistent. Again, there was no difference observed between males and females of either strain.

Quantification of organisms in C3H/HeN and C57BL/6N mice. To determine whether the burden of infection corresponded with disease severity, the numbers of organisms were determined in the lungs, tracheas, and nasal passages of mice ($n = 6$) at various times p.i. There were significant differences between the numbers of organisms recovered from the lungs of C3H/HeN and C57BL/6N mice (Table 2). C3H/HeN mice consistently had 10^2 to 10^5 more organisms per set of lungs ($P \leq 0.05$) than did C57BL/6N mice at 7, 14, 21, 35, 49, and 63 days p.i. Culture results for the nasal passages and trachea showed only sporadic differences. More organisms were recovered from the nasal passages of C57BL/6N mice than of C3H/HeN

TABLE 2. Numbers of *M. pulmonis* recovered from the respiratory tracts of C3H/HeN and C57BL/6N mice[a]

Day p.i.	Mouse strain[b]	Log CFU[c]		
		Nasal passages	Trachea	Lungs
7	C3	5.78 (0.55)[d]	6.06 (0.94)	5.43 (2.74)[d]
	B6	6.79 (0.47)	4.97 (0.42)	2.12 (1.41)
14	C3	6.02 (0.76)	6.88 (1.04)	7.09 (1.32)[d]
	B6	7.07 (0.69)	5.92 (0.61)	2.77 (2.22)
21	C3	6.79 (0.54)	6.66 (0.32)[d]	5.26 (2.58)[d]
	B6	6.80 (0.60)	5.93 (0.58)	2.43 (2.78)
28	C3	6.37 (0.63)	6.84 (0.48)	5.29 (3.10)
	B6	6.13 (0.73)	5.97 (1.36)	2.10 (2.71)
35	C3	7.36 (0.51)	6.92 (0.38)[d]	6.21 (1.80)[d]
	B6	6.73 (0.83)	5.67 (0.72)	2.84 (2.28)
49	C3	6.79 (0.47)	6.63 (0.47)	6.98 (0.55)[d]
	B6	6.18 (0.50)	4.92 (1.09)	3.77 (2.28)
63	C3	4.73 (3.67)	4.50 (3.49)	5.98 (1.28)[d]
	B6	2.79 (3.08)	2.78 (2.36)	0.48 (1.19)

[a] Mice (n = 6 per time point per strain) were inoculated intranasally with 10^4 CFU of *M. pulmonis*. Viable organisms were determined at the indicated times.
[b] C3, C3H/HeN; B6, C57BL/6N.
[c] Numbers represent means (standard deviations) of the logarithm of the CFU recovered from the indicated tissues.
[d] Significant difference ($P < 0.05$) between the two strains.

mice at 7 days p.i. and from the trachea of C3H/HeN mice than of C57BL/6N mice at 21 and 35 days p.i. ($P \leq 0.05$). The sex of the animals made no difference in the number of organisms recovered from respiratory tissues at the time points selected (data not shown). No *M. pulmonis* or other pathogen was recovered from uninfected (control) mice of either strain.

Development of antibody responses in infected C3H/HeN and C57BL/6N mice. *M. pulmonis*-specific serum antibody titers were determined at various times after inoculation (Table 3). IgM responses appeared 7 to 14 days p.i. Specific IgG activity was apparent by 14 days p.i., with the highest concentrations present at 63 days. IgA responses were not detected in serum until 21 days p.i.

Infected C3H/HeN and C57BL/6N differed in their antibody responses to *M. pulmonis*. C3H/HeN mice had significantly greater ($P \leq 0.05$) IgM responses at 7 days p.i. than C57BL/6N mice, but there was no difference in IgM level at any other time point. Although the means of the IgM responses appear different at 21 days p.i., they were not statistically different. A larger sample size may have aided interpretation at this time point. Also, there was no difference between the mouse strains in levels of specific IgA. In contrast, IgG responses to *M. pulmonis* were significantly higher in infected C3H/HeN mice than in C57BL/6N mice ($P \leq 0.05$) at all time points after 7 days p.i. The sex of the animals made no difference in the antibody activity.

To further evaluate the difference in IgG responses in the two mouse strains, we compared the levels of specific antibody in serum for each of the IgG subclasses. C3H/HeN mice had significantly higher titers of specific IgG1 and IgG2a ($P \leq 0.05$) than C57BL/6N mice at all time points after 7 days p.i. (Table 4). There was no difference in the IgG2b or IgG3 responses between the mouse strains. Furthermore, stepwise regression analysis showed that IgG1 and IgG2a in both C3H/HeN and C57BL/6N mice were the major predictors of total *M. pulmonis*-specific IgG responses ($P \leq 0.05$).

TABLE 3. Comparison of serum antibody responses in *M. pulmonis*-infected C3H/HeN and C57BL/6N mice[a]

Day p.i.	Mouse strain[b]	Relative antibody titer[c]		
		IgA	IgM	IgG
7	C3	1.0 (5.0)	50.1 (1.4)[d]	1.0 (6.3)
	B6	1.0 (3.2)	7.9 (1.5)	1.8 (4.0)
14	C3	1.2 (12.6)	398.1 (1.3)	50.1 (1.7)[d]
	B6	1.9 (6.6)	251.2 (1.6)	15.8 (1.4)
21	C3	20.5 (5.0)	1,000.0 (2.0)	200.0 (1.6)[d]
	B6	12.6 (10.0)	158.0 (6.3)	79.4 (1.3)
28	C3	79.4 (7.94)	316.2 (3.2)	398.1 (1.3)[d]
	B6	25.1 (20.0)	501.2 (1.2)	200.0 (1.2)
63	C3	125.9 (10.0)	199.5 (2.0)	1,000.0 (1.2)[d]
	B6	125.9 (4.0)	199.5 (2.5)	501.1 (1.2)

[a] Antibody activity of sera was determined by bioassay analysis of ELISA data and expressed as the relative titers within each class of antibody. In each group, n = 4 per strain per time point.
[b] C3, C3H/HeN; B6, C57BL/6N.
[c] Geometric mean (standard deviation) of antibody activities from one experiment.
[d] Significant difference ($P \leq 0.05$) between C3 and B6 mice.

DISCUSSION

M. pulmonis infection of mice results in both acute and chronic inflammatory diseases of the respiratory tract (6). Cassell et al. (6) described chronic MRM in mice infected with high doses ($\geq 10^5$ CFU) of the organism. Although our results did not demonstrate the bimodal death curve, the lung lesions we report are identical to those described previously (6). C3H/HeN and C57BL/6N mice differ in the early (acute) stages of MRM (9), but little is known about the development of chronic MRM in these mouse strains. The resistant C57BL/6N mice have more effective nonspecific clearance mechanisms, which probably accounts for the relative lack of acute inflammatory lung disease in them in comparison with that in C3H/HeN mice. However, previous evidence indicates that C3H/HeN mice develop greater antibody responses of the IgM and IgG classes to *M. pulmonis* in acute MRM (10). This difference in humoral immunity may affect the progression of chronic MRM. The purpose of the present study was to evaluate the development of chronic respiratory disease and humoral im-

TABLE 4. Comparison of serum IgG subclass responses in *M. pulmonis*-infected C3H/HeN and C57BL/6N mice[a]

Day p.i.	Mouse strain[b]	Relative antibody titer[c]			
		IgG1	IgG2a	IgG2b	IgG3
7	C3	1.0 (1.0)	1.0 (2.2)	1.6 (2.5)	2.0 (6.3)
	B6	1.0 (1.6)	1.0 (2.5)	4.0 (2.5)	7.9 (3.2)
14	C3	79.4 (2.0)[d]	63.1 (2.5)[d]	251.2 (1.3)	79.4 (1.6)
	B6	12.6 (2.3)	1.3 (2.0)	158.5 (1.6)	50.1 (2.5)
21	C3	398.0 (2.5)[d]	501.2 (1.3)[d]	1,000.0 (1.4)	398.1 (2.0)
	B6	79.4 (1.5)	20.0 (1.9)	1,258.0 (1.3)	200.0 (2.5)
28	C3	1,584.9 (1.6)[d]	1,000.0 (1.2)[d]	1,995.3 (1.3)	316.2 (1.8)
	B6	158.5 (2.2)	63.1 (1.3)	2,511.9 (1.6)	398.1 (1.7)
63	C3	3,162.3 (1.5)[d]	1,258.9 (1.3)[d]	2,512.0 (1.6)	631.0 (2.5)
	B6	501.2 (3.2)	251.2 (1.2)	5,012.0 (1.6)	316.2 (5.5)

[a] Mice (n = 4 per time point per strain) were inoculated intranasally with 10^4 CFU of *M. pulmonis*. Antibody activity of sera was determined at the indicated time by bioassay analysis of ELISA data and expressed as the relative titers within each class of antibody.
[b] C3, C3H/HeN; B6, C57BL/6N.
[c] Geometric mean (standard deviation) of antibody activities from one experiment.
[d] Significant difference ($P \leq 0.05$) between two strains.

mune responses in both C57BL/6N and C3H/HeN mice after infection with *M. pulmonis.*

The lesions were qualitatively the same in both mouse strains throughout the respiratory tract. Disease in nasal passages and tracheas was apparent at 7 days p.i., while lung disease was present at all subsequent time points. The lung lesions were neutrophil-rich exudate in airways, hyperplasia and dysplasia of airway epithelium, submucosal lymphoid hyperplasia, and lung parenchymal consolidation. C3H/HeN mice had dramatically more severe lung lesions than C57BL/6N mice at time points to 63 days p.i. The severity of tracheal disease was also greater in C3H/HeN mice. In contrast, C57BL/6N mice had equivalent or more severe epithelial and lymphoid hyperplasia in nasal passages. This is similar to results described for acute disease in mice where the difference in disease was associated with nonspecific clearance of organisms (25). Thus, C3H/HeN and C57BL/6N mice differed in the severity of chronic MRM in the lower, but not the upper, respiratory tract after infection with *M. pulmonis.*

The number of recoverable *M. pulmonis* organisms from each tissue corresponded to the severity of chronic disease. As with the lesion scores, there was little or no difference between the mouse strains in the number of organisms recovered from the nasal passages or trachea. In contrast, the number of organisms recovered from the lungs of C3H/HeN was 2 to 5 logs higher than that from C57BL/6N mice, which corresponds with the difference in lung disease between the two strains. It is also important to note that the number of organisms recovered from each strain did not change significantly in any of the tissues during the course of study. These results are consistent with the view that early (<7 days), nonspecific pulmonary clearance accounts for the initial differences in numbers of organisms which, in turn, result in the differences in severity of chronic, as well as acute, disease in these mouse strains. In fact, we have shown that C57BL/6N mice more efficiently clear *M. pulmonis* from their lungs than C3H/HeN mice within 3 days p.i. (24). In addition, we have shown that C57BL/6-*scid/scid* mice are no more susceptible to disease or infection than immunocompetent mice, supporting the importance of nonspecific or innate host defenses in controlling mycoplasma disease (3). It is not known what innate host mechanisms are responsible for limiting the continued growth of organisms and thereby maintaining differences in disease severity in C3H/HeN and C57BL/6N mice.

The appearance of *M. pulmonis*-specific antibody in serum was similar in both strains of mice after infection and consistent with previous studies (5, 27, 28). IgM was the first antibody to be detected, at 7 days; this was followed by IgG and IgA, which were detected at 14 and 21 days, respectively. However, C3H/HeN mice had significantly greater IgM titers at 7 days than C57BL/6N mice, with no difference observed at the other time points. These data are in agreement with those reported previously for the acute disease in these strains of mice (10, 24). We did not detect a significant difference in serum IgA levels between the mouse strains, which contrasts with a previous report that C3H/HeN mice produce greater amounts of IgA in bronchial secretions than C57BL/6N mice (20). However, the major difference in antibody responses was in the levels of *M. pulmonis*-specific IgG, since susceptible C3H/HeN mice developed much greater levels of this antibody class than resistant C57BL/6N mice.

The strain difference in serum concentrations of *M. pulmonis*-specific IgG was reflected in the contribution of the IgG subclasses to the total IgG response. C3H/HeN mice produced 5 to 10 times higher IgG1 titers and up to 45 times higher levels of IgG2a than C57BL/6N mice at time points after 7 days p.i. In addition, stepwise regression analysis demonstrated that IgG1 and IgG2a levels in both strains of mice were the primary predictors of the serum IgG responses. These results show that the higher IgG responses in C3H/HeN mice were due to the preferential increase of specific antibody of the IgG1 and IgG2a subclasses but not to a difference between the two mouse strains in the IgG subclasses which contribute to the total IgG.

Our results are similar to findings in our previous studies in which susceptible LEW rats produced more total IgG following *M. pulmonis* infection than resistant F344 rats did (30). However, LEW and F344 rats differ in the IgG subclasses contributing to the total IgG response (30), and disease severity is probably due to differences in regulatory T-cell function. Our results do not support the view that C3H/HeN and C57BL/6N mice qualitatively differ in T-cell or cytokine responses. However, it is interesting to note that IgG2a antibody responses are promoted by Th1 cells, through gamma interferon production (1, 22, 23), and we have shown that gamma interferon mRNA is induced in both C3H/HeN and C57BL/6N mice within days (14) and can be detected in total lung RNA from mice with chronic MRM (unpublished results).

Antibody can influence the progression of mycoplasma disease since passive transfer of antibody has been shown to prevent disease in experimentally infected mice (5, 32). In addition, *M. pulmonis*-specific antibody of the IgG1, IgG2a, and IgG2b subclasses have mycoplasmacidal effects when incubated with the organisms prior to inoculation (31). However, in both resistant and susceptible strains of mice, there was no apparent change in the numbers of organisms present in any tissue despite the development of large IgG subclass responses. These results correspond to *M. pulmonis* disease in rats, where serum antibody does not account for differences in the severity of chronic respiratory disease in different strains of rats (30). Also, once infection in the lung is established, adaptive immune responses are ineffective in clearing the organisms. However, C3H/HeSnJ *scid/scid* mice developed arthritis and peritonitis and had increased mortality 17 to 20 days p.i. (3). Thus, lymphocyte responses appear to prevent dissemination of infection and disease even though they are unable to allow recovery from respiratory tract disease.

Although lymphocyte responses can protect against infection and disseminated disease, they also may play a role in the development of lung lesions after *M. pulmonis* infection. The higher serum IgG responses developed in the strain of mice (C3H/HeN) with the more severe lung disease and the higher number of organisms, similar to *M. pulmonis* disease in rats (30). These results suggest that specific immune responses contribute to disease, but if the difference in chronic disease in C3H/HeN and C57BL/6N mice is due solely to exacerbation by antibody, one would expect that the difference in disease severity would be present throughout the respiratory tract, not just the lung. However, C3H/HeSnJ mice developed severe lung disease and exhibited characteristic clinical signs of *M. pulmonis* disease, in contrast to C3H/HeSnJ-*scid/scid* mice, which do not have functional lymphocytes (3). Thus, these results are consistent with the view that lymphocyte responses can have a role in exacerbation of lesions, but antibody responses alone are probably not responsible. Additional work is needed to fully characterize the role of adaptive immunity in disease pathogenesis.

In summary, this study demonstrates that C3H/HeN mice develop more severe lung lesions, harbor more organisms in the lungs, and mount a stronger serum IgG antibody response than C57BL/6N mice do during chronic MRM. Effective innate host defenses in C57BL/6N mice are probably the critical de-

terminant in the differences between these mouse strains in acute and chronic diseases due to *M. pulmonis*. In addition, the development of serum antibody does not result in recovery from chronic lung disease and infection in mice. Ongoing studies are evaluating whether lymphocyte responses play a protective role in preventing dissemination of *M. pulmonis* to extrapulmonary tissues and death and whether lymphocyte responses contribute to disease severity and clinical symptoms. A similar association of lymphocyte responses and disease is found in *M. pneumoniae* disease in humans (19). Future studies will evaluate the role of lymphocyte activation in the development of lesions and the genetic susceptibility of mice to respiratory mycoplasmosis.

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