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Strain Differences in Susceptibility to Murine Respiratory Mycoplasmosis in C57BL/6 and C3H/HeN Mice

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Not only is murine respiratory mycoplasmosis, due to *Mycoplasma pulmonis*, a complication of biomedical research, it provides excellent animal models to study the development of a naturally occurring respiratory disease induced by an infectious agent. The understanding of pathogenic mechanisms of disease can be greatly facilitated by studying genetic differences in susceptibility. Five strains of mice with various *H-2K* haplotypes were examined for their susceptibility to murine respiratory mycoplasmosis; of these, C57BL/6 and C3H/HeN mice were chosen for additional study. There were no significant differences in the incidence of infection in either the upper or lower respiratory tract or in the severity of upper respiratory tract lesions in the two strains as determined at 14 days postinfection. In striking contrast, the C57BL/6 mice were significantly more resistant to the development of gross and microscopic lung lesions and to death due to pneumonia as shown by an almost 100-fold difference in the 50% lethal dose, 50% gross pneumonia dose, and 50% microscopic lesion dose. The most apparent differences in lung lesions between the two strains were in the severity of acute lesions of the bronchial epithelium, the amount of mixed inflammatory response in the alveoli, and the amount of lymphoid infiltrates. All were significantly more severe in C3H/HeN mice. In addition, more C3H/HeN mice developed antibody responses to *M. pulmonis*. The amount of antibody correlated with lesion severity in both strains.

Approximately 60% of all barrier-maintained and nearly all conventionally maintained commercially available rodents are known to be infected with mycoplasmas (5). The most important of these is *Mycoplasma pulmonis*, the etiological agent of murine respiratory mycoplasmosis (MRM). Not only can this disease be a significant cause of mortality, but *M. pulmonis* alters lung cell populations (12, 25), respiratory physiology (16), and the response to other pulmonary insults, including both infectious agents (15) and carcinogens (21). Experimentally induced MRM in mice provides excellent animal models in which to study respiratory disease induced by an infectious agent. *M. pulmonis* alone can produce all of the lesions of the naturally occurring disease, and quantitative dose relationships have been demonstrated (2, 6, 17).

As previously demonstrated for MRM in rats (10, 12), differences between different strains of animals in the development of disease provide ideal tools for the dissection of mechanisms of disease production. MRM differs considerably between the rat and the mouse (2, 6). When given high doses of *M. pulmonis*, mice develop acute disease (2, 6, 17); rats do not develop acute disease even when given comparable numbers of organisms per gram of body weight (2, 6). In general, the disease in mice progresses more rapidly, with development of lung lesions by 7 days postinfection. These early lesions are characterized primarily by neutrophils and edema in the alveolar spaces. Mice that survive this acute phase develop chronic suppurative bronchitis and bronchiolitis characterized by marked peribronchial lymphoid cuffing and a mixed inflammatory response consisting of both neutrophils and macrophages in the alveoli. The latter phase morphologically resembles MRM in rats; however, even at this stage, there is less hyperplasia and squamoid change of respiratory epithelium and more extensive accumulation of neutrophils in airways of mice than in rats. Therefore, it can be expected that clear delineation of strain differences in susceptibility in mice will highlight mechanisms unique from those responsible for lesion differences between rat strains. In addition, genetic analysis would be facilitated in mice due to the larger number of congenic and recombinant inbred strains.

The purpose of the present study was to determine if there were differences between commonly available mouse strains in susceptibility to MRM and to define the parameters by which these differences could be investigated. Differences in susceptibility to *M. pulmonis* were initially evaluated in five mouse strains. C3H/HeN and C57BL/6 strains were chosen to quantify the degree of susceptibility. The C57BL/6 strain was more resistant to development of pulmonary lesions. Morphometric analysis of lung lesions showed that lung lesion differences were most pronounced in the extent of lymphoid accumulation and mixed inflammatory responses in the alveoli.

MATERIALS AND METHODS

Animals. For the initial study, five strains of mice (C57BL/6, C3H/HeN, C3H × C57BL/6 F1, CBA, and B10.D2) were chosen to represent different *H-2K* haplotypes. Male mice of each strain were obtained from Jackson Laboratory, Bar Harbor, Maine, and shipped to the University of Alabama at Birmingham behind bacteriological filter media. Ten percent of the animals were sacrificed upon arrival and examined by culture and histology for

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mycoplasmas or other murine pathogens. No mycoplasmas or other pathogens were isolated.

C57BL/6 and C3H/HeN mice were chosen for additional study based on the results of the initial experiments and because of their availability as germfree animals. Germfree animals were used to avoid possible differences in MRM susceptibility related to normal microbial flora. To further ensure that differences were genetic instead of environmental, breeding colonies of these strains were established in plastic film isolators (Germ-Free Supply Division, Standard Safety Equipment Co., Palatine, Ill.) at the University of Alabama at Birmingham from known germfree stock obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. Isolators were monitored for contamination by quarterly fecal cultures, monthly enzyme-linked immunosorbent assay (ELISA) for immunoglobulin G (IgG) and IgM antibodies to *M. pulmonis* and *M. arthritidis* (3, 5, 9), quarterly necropsy and histological examination of retired breeders, and serologic tests for rodent viruses on sera from retired breeders and mice used in experiments. Mice were tested by either hemagglutination inhibition, complement fixation, or ELISA by Microbiological Associates (Bethesda, Md.) for pneumonia virus of mice, reovirus-3, Theiler's GDVII, polyomavirus, Sendai virus, minute virus of mice, ectromelia, mouse adenovirus, mouse hepatitis virus, and lymphocytic choriomeningitis virus. No murine pathogens were detected by these procedures. Weanling (4 to 6 weeks of age) mice were matched for age and sex and used for all further experiments. The level of intracage ammonia was measured (1) during experiments and was consistently less than 25 ppm.

Mycoplasmas. All experiments were performed with the CT strain of *M. pulmonis*. CT was isolated from a mouse with MRM and identified as a pure culture of *M. pulmonis* by immunofluorescence (13). Stock cultures of CT were grown in mycoplasma broth containing (per liter): 22.5 g of Frey mycoplasma broth base (GIBCO Laboratories, Grand Island, N.Y.), 0.20 g of DNA (degraded free-acid type IV; Sigma Chemical Co., St. Louis, Mo.), 730 ml of distilled water, and 2.0 ml of 1% phenol red (Fisher Chemical Co., Springfield, N.J.). These components were autoclaved for 15 min and allowed to cool, and then 10 ml of reconstituted CVA enrichment (GIBCO), 200 ml of filter-sterilized, gamma globulin-free, heat-inactivated horse serum (GIBCO), 10 ml of 50% glucose (J. T. Baker Chemical Co., Phillipsburg, N.J.), and 32 mg of Cefobid (Roerig Pharmaceutical Co., New York, N.Y.) were added. Solid medium was made by adding 10.0 Noble agar (Difco Laboratories, Detroit, Mich.) before autoclaving. Organisms were frozen in broth in 1-ml aliquots at −70°C. Thawed ampoules contained an average of 2×10^9 CFU/ml. For inoculation of mice, thawed ampoules were mixed and diluted in 10-fold increments. Each dilution was quantitated by culture at the time of inoculation and contained the expected number of organisms plus or minus 5%.

Quantitative mycoplasma cultures of tissues from experimental animals were performed as follows. Aseptically collected lungs, tracheas, and larynxes were homogenized in motor-driven, sterile, ground-glass homogenizers (Kontes Biomedical Products, Vineland, N.J.). The heads were transected just rostral to the eyes, the soft tissues and teeth were removed, and the remaining bony structures were crushed. Homogenates and nasal tissues were sonicated (model W220 F; Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) at full power for 30 s in ice water and quantitatively cultured by serial dilution and plating. Although homogenates of tissues are reported to inhibit mycoplasmal growth, this effect is reduced or eliminated by dilution. In these experiments, the organisms grew well except in the first dilution tubes and in plated undiluted homogenate. Cultures were incubated for 2 weeks at 37°C before they were considered negative.

Biological endpoints. The biological parameters of the 50% lethal dose (LD_{50}) and the 50% gross pneumonia dose (PD_{50}) as well as the 50% infectious dose (ID_{50}) and the 50% microscopic lesion dose (MLD_{50}) for various levels of the respiratory tract were calculated by the method of Reed and Muench (19). These parameters were all based on a 14-day period; i.e., the ID_{50} represents the dose necessary to establish an infection detectable by culture at 14 days postinoculation. Mice of each strain were lightly anesthetized with pentobarbital and inoculated intranasally with 10-fold dilutions of the stock culture. All surviving mice were killed at 14 days postinfection.

Lesion scoring. Lung lesions were measured morphometrically with the Bioquant II Image Analysis System (R & M Biometrics, Nashville, Tenn.), using an adaptation of the method we have previously described for rat lung lesions (10, 11). A standard reference section was cut from each lung lobe so that the major bronchus was cut in longitudinal section. Based on the reported descriptions of MRM in mice (17), the following areas and lengths were measured on each lobe: (i) the total length of bronchial epithelium, (ii) the total length of bronchiolar epithelium, (iii) the total lung area, (iv) the length of acute (thinned or missing epithelial cells) bronchial epithelial lesions, (v) the length of acute bronchiolar lesions, (vi) the length of hyperplastic bronchial lesions, (vii) the length of hyperplastic bronchiolar lesions, (viii) the area of acute inflammation (neutrophils and edema), (ix) the area of chronic inflammation (macrophages), (x) the area of mixed inflammation (areas with both neutrophils and macrophages), (xi) lymphoid areas associated with bronchi, (xii) lymphoid areas associated with bronchioles, (xiii) lymphoid areas associated with blood vessels, (xiv) area of bronchial exudates, and (xv) area of bronchiolar exudates. The lungs were also examined for areas of hemorrhage, abscesses, and "acinar gland formation" (17) due to hyperplastic type II epithelial cells, but these lesions were not found in the experimental animals at 14 days and were not included. From the above parameters, the following calculations were made: total bronchial lesions = acute + hyperplastic bronchial lesions; total bronchiolar lesions = acute + hyperplastic bronchiolar lesions; total epithelial lesions = total bronchial + total bronchiolar lesions; total lymphoid area = bronchial lymphoid areas + bronchiolar lymphoid areas + vascular lymphoid areas; total area of exudation = bronchial + bronchiolar exudation; and total lung lesion area = lymphoid area + area of exudation + acute inflammation + chronic inflammation + mixed inflammation. For purposes of analysis and comparison, each of these parameters was expressed as a ratio to either the total (bronchial or bronchiolar) lung epithelial length or the total lung area.

This method for quantifying lung lesions is based on the assumption that most if not all lung lesions develop centrifugally in mice as is true for rats (10). To test the validity of this assumption, 15 lung lobes with lesions of MRM ranging from severe to mild were cut in serial section, and these parameters were measured on the standard reference section and on four additional sections cut at different equally spaced planes. In addition, the standard reference sections from these 15 lobes were recoded and examined at five

different times by the same pathologist to determine the operator variability.

Nasal cavity lesions were measured in the same manner as the lung lesions. Reference sections were selected at the levels of: (i) the first incisors, (ii) the third palatine ridge, and (iii) the midpoint of the eyes. The following lesions were measured on each section: (i) length of acute epithelial lesions (missing or squamoid epithelium), (ii) length of the chronic epithelial lesions (hyperplasia), (iii) area of lymphoid nodules (primarily the pharyngeal tonsils), (iv) area of submucosal lymphoid infiltrates, (v) area of submucosal glands, (vi) area of neutrophils in submucosa, (vii) area of chronic inflammation in submucosa (macrophages), and (viii) area of exudates in the lumen. These lesion measurements were expressed as a ratio to the total length of respiratory plus olfactory epithelium, the total cross-sectional area of the nasal passages, or the total submucosal area.

Because the difficulty of obtaining standard histological sections precluded the use of morphometry, the severity of otitis was subjectively scored (as 0 to 3) (18). In addition, subjective scoring was performed for nasal passage lesions. For comparison of group scores, the sum of scores for each organ from individual animals divided by the sum of maximal scores possible gave the lesion index for that organ. A lesion index of 1.0 was the most severe change possible.

Antibody measurement. The serum from each animal was analyzed for IgM and IgG anti-*M. pulmonis* antibodies by an ELISA (14). Previous studies have shown that IgA antibodies are not detected until 14 days after intranasal inoculation and that serum antibody reflects antibody produced locally (4). Serum was collected at the time of spontaneous death of animals or at the termination of the experiment at 14 days postinfection. ELISA values (expressed as A_{400} per 100 min) from experimental animals were compared with those of the eight control sera from known pathogen-free mice as described previously (3). A value greater than the mean of the control sera plus 2 standard deviations was considered positive.

Statistical analysis. Incidence figures were analyzed by the chi-square test, and nonparametric data were analyzed by the Kruskal-Wallis test (8). All other data were analyzed by the analysis of variance technique (22). Correlation analysis was used to determine the association between measurement of lung lesions on the standard reference section as compared with use of all lung sections and the association between ELISA values and lesion ratios. A value of $P \leq 0.05$ was accepted as significant.

RESULTS

Strain comparisons. Six mice per dose (10-fold dilutions ranging from 5×10^1 to 5×10^7 CFU per animal) for five strains of mice were used to determine the LD_{50} and PD_{50} for each strain (Table 1). The dose required to produce these endpoints was highest in *H-2K*^{b} mice (C57BL/6 strain), intermediate in *H-2K*^{d} mice (B10.D2 strain), and lowest in *H-2K*^{k} mice (C3H/HeN and CBA strains). The LD_{50} for *H-2K*$^{b/k}$ mice (C3B6 F1 hybrid strain) was intermediate between the parental haplotypes.

TABLE 1. Resistance and susceptibility of inbred mouse strains to *M. pulmonis*

Strain	*H-2K* haplotype	LD_{50} (CFU/animal)	PD_{50} (CFU/animal)
C57BL/6	*b*	$>5 \times 10^7$	2.9×10^5
C3H/HeN	*k*	9.3×10^5	4.7×10^4
C3B6 F1	*b/k*	7.5×10^6	ND[a]
CBA	*k*	3.8×10^5	1.7×10^4
B10.D2	*d*	7.5×10^6	ND

[a] ND, Not done.

TABLE 2. ID_{50} and MLD_{50} for C3H/HeN and C57BL/6 mice 14 days after intranasal inoculation of *M. pulmonis*

Strain and site in respiratory tract	ID_{50} (CFU/animal)	MLD_{50} (CFU/animal)
C3H/HeN		
Nasal passages	1.5×10^3	5.0×10^4
Larynx	2.1×10^3	ND[a]
Trachea	1.5×10^4	ND
Lung	8.5×10^3	2.0×10^4
Middle ear	ND	7.9×10^3
C57BL/6		
Nasal passages	1.4×10^3	2.0×10^5
Larynx	1.2×10^4	ND
Trachea	1.5×10^4	ND
Lung	2.0×10^4	2.5×10^5
Middle ear	ND	3.2×10^4

[a] ND, Not done.

To verify the LD_{50} and PD_{50} using germfree animals and to determine the ID_{50} for the nasal passages, larynx, trachea, and lungs, 72 C3H/HeN mice and an equivalent number of C57BL/6 mice were inoculated intranasally with 10-fold dilutions of *M. pulmonis* (12 mice of each strain per dilution) ranging from 5×10^6 to 5×10^1 CFU per mouse in C3H/HeN and from 5×10^7 to 5×10^2 CFU per mouse in C57BL/6 mice. All surviving mice were killed at 14 days postinoculation, and their lungs were examined for gross lesions and serum was collected. The respiratory tracts from half of the mice in each group were processed for histology and morphometric analysis of lesions, and the remainder were used for quantitative culture of the nasal passages, larynxes, tracheas, and lungs.

Biological 50% endpoints. All spontaneous deaths occurred between 3 and 10 days postinfection. All respiratory tissues were examined grossly and microscopically. Surviving mice were killed and examined similarly at 14 days. As expected from the initial experiment, the incidences of death and gross pneumonia for C3H/HeN mice were significantly higher than for C57BL/6 mice given corresponding doses ($P < 0.007$ for incidence of death and $P < 0.001$ for gross pneumonia). The LD_{50} and PD_{50} were $>5 \times 10^7$ and 1.2×10^6 CFU per animal, respectively, for C57BL/6 mice and 1.4×10^6 and 2.2×10^4 CFU per animal, respectively, for C3H/HeN mice. In fact, the LD_{50} for C57BL/6 mice could not be calculated as it was higher than the highest dose given. The MLD_{50} and ID_{50} for both strains are given in Table 2. While there was no significant difference between the two strains in the incidence of infection at different regions of the respiratory tract, there was a trend for higher doses to be required in the C57BL/6 mice, especially for the larynx and lungs. The incidence of microscopic lung lesions in C3H/HeN mice was significantly higher than in C57BL/6 mice given corresponding doses ($P < 0.05$), but this was not true for lesions of the nasal passages or middle ears. The MLD_{50} was 2.5×10^5 CFU per animal for C57BL/6 mice and 2.0×10^4 CFU per animal for C3H/HeN mice.

Culture data. Although there were no significant differ-

TABLE 3. Numbers of *M. pulmonis* recovered from the respiratory tract in C57BL/6 and C3H/HeN mice

Inoculated dose (CFU/animal)	Culture site	CFU ± SD recovered	
		C57BL/6	C3H/HeN
5×10^5	Nasal passages	$(1.1 \pm 0.2) \times 10^7$	$(2.2 \pm 3.4) \times 10^7$
	Larynx	$(1.2 \pm 2.0) \times 10^6$	$(3.5 \pm 3.9) \times 10^6$
	Trachea	$(1.5 \pm 2.0) \times 10^6$	$(7.6 \pm 0.003) \times 10^8$ [a]
	Lung	$(1.2 \pm 2.1) \times 10^5$	$(8.0 \pm 14) \times 10^8$ [a]
5×10^4	Nasal passages	$(5.7 \pm 10.0) \times 10^5$	$(1.6 \pm 2.5) \times 10^6$
	Larynx	$(3.3 \pm 2.6) \times 10^5$	$(3.0 \pm 2.3) \times 10^3$
	Trachea	$(3.4 \pm 4.5) \times 10^3$	$(5.7 \pm 6.5) \times 10^5$
	Lung	$(3.2 \pm 3.4) \times 10^3$	$(4.9 \pm 9.6) \times 10^5$
5×10^3	Nasal passages	0 ± 0	$(1.7 \pm 3.1) \times 10^6$ [a]
	Larynx	$(1.2 \pm 2.4) \times 10^4$	$(9.3 \pm 14.0) \times 10^5$ [a]
	Trachea	0 ± 0	$(5.6 \pm 6.9) \times 10^5$ [a]
	Lung	$(6.0 \pm 12.0) \times 10^2$	$(3.5 \pm 6.7) \times 10^6$

[a] C3H/HeN value is significantly higher than C57BL/6 value at $P \leq 0.05$ based on the Kruskal-Wallis test.

ences in the incidence of infection at various levels of the respiratory tract, there were significant differences in the numbers of organisms (Table 3). Note that only the doses from 5×10^3 to 5×10^5 CFU are shown. All C3H/HeN mice that received more than this dose died before 14 days postinoculation, and there were no organisms isolated from the majority of animals of either strain which were inoculated with smaller doses. Within the C3H/HeN strain, there was a significant difference ($P = 0.02$) in the CFU recovered from the lungs between animals from which organisms were isolated which did not have gross lesions ([0.87 ± 1.4] $\times 10^6$ CFU per lung) and animals from which organisms were isolated which did have gross lesions ([40.4 ± 97.7] $\times 10^6$ CFU per lung).

Upper respiratory tract lesions. Nasal lesions and otitis media were qualitatively and quantitatively similar in both strains. The primary lesion in the nasal passages (Fig. 1) was suppurative rhinitis with squamoid changes of the respiratory and olfactory epithelium. Variable amounts of mononuclear infiltrates were in the submucosa, and the pharyngeal tonsils were enlarged. Lymphoid nodules were significantly larger ($P < 0.001$) in C3H/HeN mice (lesion ratio, 0.03 ± 0.01) than in C57BL/6 mice (0.01 ± 0.01). Otherwise, there were no significant differences in severity of nasal passage lesions between the two strains based on either subjective scores or lesion ratios.

The primary lesion in the middle ears was purulent otitis media accompanied by infiltrates of mononuclear cells and neutrophils in the lamina propria. There was no significant difference between lesion scores for the two strains of mice.

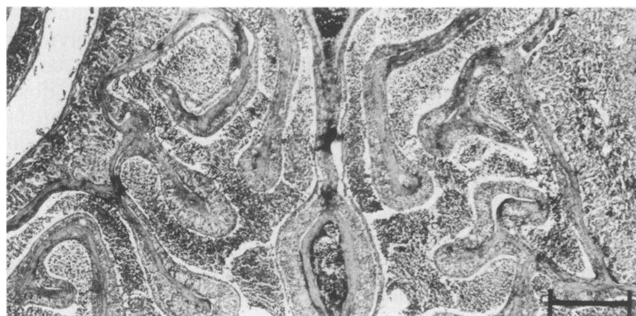

FIG. 1. Nasal passages from a C3H/HeN mouse that received 5×10^5 CFU of viable *M. pulmonis* 14 days previously. The lumen of the nasal cavity is filled with neutrophils, and epithelial cells have been lost from the turbinates. Bar = 280 μm.

Lung lesions. Lung lesions were qualitatively similar in both mouse strains, although different doses were required to produce a similar level of lesion severity in the two strains, i.e., C57BL/6 mice required more than a log higher dose of *M. pulmonis* than C3H/HeN mice required to produce a similar level of lesion severity (Fig. 2). The primary lesion was suppurative bronchopneumonia with squamoid changes of the respiratory epithelium. Prominent cuffing of bronchi, bronchioles, and blood vessels by lymphoid cells was common at all doses and was the principal lesion at lower doses in both strains. Atelectasis and consolidation of the parenchyma were most severe adjacent to airways and occurred more frequently at higher doses in both strains.

When the sections from 15 different lobes were analyzed on five different days, there was no significant daily variation in measurement of lung lesions. The correlation between lesion ratios measured on a single reference slide per lobe and ratios measured using five slides per lobe was between 0.4 and 0.6 (moderate degree of correlation) for bronchial epithelial lesions and above 0.75 (very good to excellent correlation) for all area lesions with the exception of bronchiolar exudation which was usually underestimated. Bronchiolar epithelial lesions also were underestimated when only a single reference slide was used. However, bronchiolar exudates and bronchiolar epithelial lesions are relatively minor components of the total lesion area or total epithelial lesions, respectively, and generally represent less than 1% of the total. Therefore, the assumption that mouse lung lesions developed centrifugally seemed valid, and the reference slide method was used to compare lung lesions between the two mouse strains.

Lung lesion ratios were compared between the two strains only for groups in which both strains had received equal doses of *M. pulmonis* (5×10^5 to 5×10^2 CFU per animal) and at least four animals survived for 14 days. The mean lung lesion ratios for each lesion type are shown in Table 4. Mixed inflammatory lesions ($P < 0.03$), bronchial lymphoid areas ($P < 0.004$), area of bronchial exudates ($P < 0.021$), area of bronchiolar exudates ($P < 0.002$), total lymphoid areas ($P < 0.002$), total areas of exudation ($P < 0.02$), and total lung lesions ($P < 0.002$) were also significantly affected by the inoculated dose of *M. pulmonis*. In addition, vascular lymphoid areas were significantly affected by the dose of

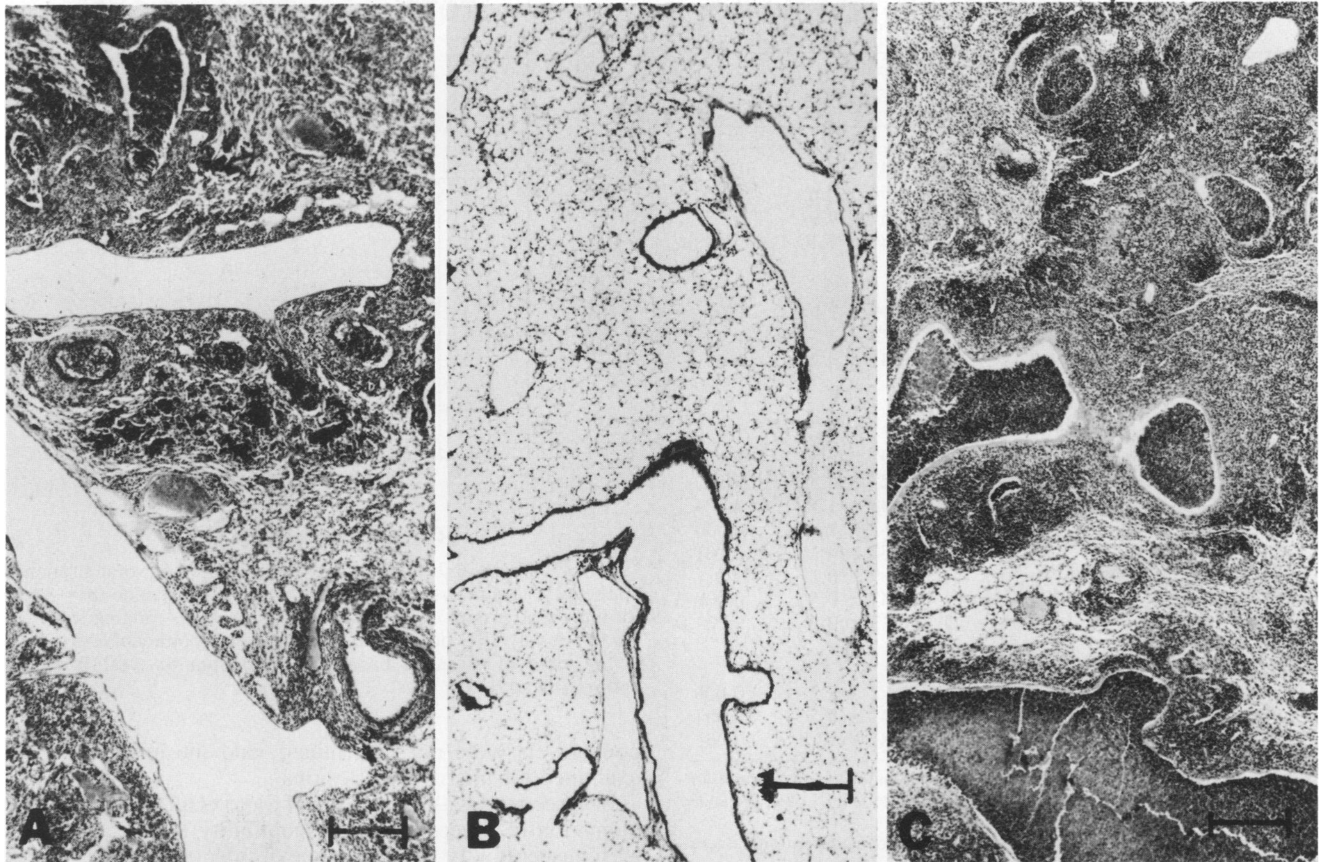

FIG. 2. Sections of left lobe of lung from mice infected with *M. pulmonis* 14 days previously. Bars = 370 μm. (A) C3H/HeN Mouse given 5×10^5 CFU. Note extensive lymphoid infiltrates, neutrophils in lumen of airways, and mixed inflammatory response in alveoli. The clear structures are pulmonary veins. (B) C57BL/6 mouse given the same dose of *M. pulmonis* as the mouse in panel A. Lesions are limited to a few lymphoid cells around the vessels and airways. (C) C57BL/6 mouse given 5×10^7 CFU, 100 times as many organisms as in panels A and B. Note that the lesions are similar to those in panel A.

organism ($P < 0.004$) although there were no significant strain differences.

In most parameters, there was a strain-dose interaction which represented a direct correlation within each strain between the dose of organisms given and lesion severity. The strain differences and dose effects are shown graphically for bronchial epithelial lesions, area of mixed inflammatory response, total lymphoid areas, and total lung lesions in Fig. 3 to 6, respectively.

Immune responses. The incidence of antibody production was higher in C3H/HeN than in C57BL/6 mice for both IgG and IgM classes at most doses used in these experiments (Table 5). All control mice were serologically and culturally negative. The relationship between amount of *M. pulmonis* antibody and selected lesion ratios for the lung and the number of CFU recovered from the nasal passages and lungs is shown in Table 6.

DISCUSSION

These studies provide convincing quantitative evidence for strain differences in susceptibility to MRM in laboratory mice. The data in Table 1 suggest that these genetic differences are related to the major histocompatibility complex as is susceptibility to *Mycoplasma arthritidis* (another murine mycoplasma)-induced toxicity and death (7). It appears that haplotype $H\text{-}2K^b$ mice may be resistant to *M. pulmonis*-induced respiratory disease while haplotype $H\text{-}2K^k$ mice are susceptible. Furthermore, it appears that resistance and susceptibility may be codominant traits. However, more mouse strains, especially those with the $H\text{-}2K^b$ haplotype, need to be examined, and additional studies with the F_2 or backcross generations or both need to be performed to confirm both the linkage to the major histocompatibility complex and the mode of inheritance.

Both biological parameters and quantitative measurements of lesion severity can be used to identify strain differences in susceptibility. As might be expected within each strain, the LD_{50} requires the greatest number of organisms, followed by the PD_{50}, the MLD_{50}, and the ID_{50}. Within each strain, the similarity of the ID_{50} for various sites suggests that by 14 days postinfection the organisms have spread throughout the respiratory tract in all animals that become infected. Even though there was no significant difference between C57BL/6 and C3H/HeN mice in incidence of infection or in incidence of lesions in the upper respiratory tract, the difference in incidence and severity of lung lesions between the two strains coupled with the differences in numbers of organisms suggests that the mechanism for these differences may be more closely related to disease production or establishment of the organism in the lung than to susceptibility to infection per se and probably involves a basic mechanism of lung defense.

Morphometric analysis identified mixed inflammatory responses in the alveoli, lymphoid infiltration (especially that

TABLE 4. Comparison of lung lesion ratios between C57BL/6 and C3H/HeN mice[a]

Lesion type	Lung lesion ratio[a]		P value[b]
	C57BL/6	C3H/HeN	
Epithelial lesions			
Acute bronchial	0.04 ± 0.4	1.42 ± 1.5	0.01
Acute bronchiolar	0.10 ± 0.5	0.54 ± 0.6	0.11
Hyperplastic bronchial	0 ± 0	0.57 ± 0.9	ND[c]
Hyperplastic bronchiolar	0 ± 0	0.84 ± 1.1	0.18
Calculated lesions			
Total bronchial lesions	0.04 ± 0.4	1.99 ± 2.0	0.05
Total bronchiolar lesions	0.10 ± 0.5	1.38 ± 1.5	0.05
Total epithelial lesions	0.14 ± 0.6	3.37 ± 4.2	0.05
Area lesions			
Acute inflammation	0 ± 0	5.92 ± 7.8	0.10
Chronic inflammation	0.09 ± 0.2	0.28 ± 0.8	ND
Mixed inflammation	0.01 ± 0.1	1.48 ± 1.5	0.004
Bronchial lymphoid	0.12 ± 0.2	1.32 ± 1.8	0.006
Bronchiolar lymphoid	0 ± 0.1	0.50 ± 0.3	<0.001
Vascular lymphoid	0.10 ± 0.1	0.09 ± 0.1	ND
Bronchial exudates	0.02 ± 0.1	0.33 ± 0.5	0.008
Bronchiolar exudates	0.01 ± 0.1	0.13 ± 0.2	0.004
Calculated lesions			
Total lymphoid	0.25 ± 0.5	1.72 ± 2.2	0.006
Total exudation	0.03 ± 0.1	0.43 ± 0.5	0.007
Total lung lesions	0.38 ± 0.3	9.81 ± 12.8	0.002

[a] Numbers represent the mean ± standard deviation for four groups of four to six mice of each strain which received from 5×10^6 to 5×10^2 CFU of viable *M. pulmonis* per animal.
[b] *P* value indicates the probability that there are no significant differences between the corresponding lesion ratio for the two strains. $P \leq 0.05$ was accepted as indicating that a significant difference did in fact exist.
[c] ND, No *P* value could be calculated by the analysis of variance technique. The lesion may have been extensive in those lungs in which it did occur.

around the airways), and exudation in the airways as being the primary differences in lung lesions between C57BL/6 and C3H/HeN mice. Surprisingly, there was no significant difference in the amount of lymphoid tissue around blood vessels between the two strains, suggesting that there were no differences in the number of lymphoid cells recruited from the circulating pool. As bronchial and bronchiolar tissue is not prominent in normal mouse lungs, it was expected that the majority of lymphocytes present would be recruited. The differences in lymphoid infiltration between the two strains of mice may have been due to differences in proliferation of lymphocytes around the airways or differences in the migration of recruited lymphocytes to the airways by 14 days, or both.

Saito et al. (20) compared the incidence of lung lesions and *M. pulmonis* isolation in randomly bred ICR, NIH, C3H/HeN, CF#1, and ddY mice and found that gross lesions occurred in 40 to 70% of ICR mice and in less than 5% of ddY mice after caging with mice experimentally infected with *M. pulmonis*. The other mouse strains had intermediate lesion incidence. There were no differences in the isolation frequencies of *M. pulmonis* among the strains and no difference in the growth rates of the organism in the trachea. Differences in the number of organisms in the lung were related to the presence or absence of pneumonia rather than to the strain of mice. Furthermore, there was little difference in the concentration of complement-fixing antibodies between the ICR and ddY mice. Class-specific antibody levels were not determined, and the lungs were not examined for microscopic lesions.

In agreement with the results of Saito et al., we found little indication of differences in susceptibility to infection or at least susceptibility to persistence of infection. However, we did find differences in the numbers of organisms at 14 days between C57BL/6 and C3H/HeN mice. Also, in contrast to Saito et al., we found considerable differences in the antibody responses between the two strains when given the same initial antigenic load, with a much lower incidence of antibody-positive animals in the C57BL/6 strain. This may

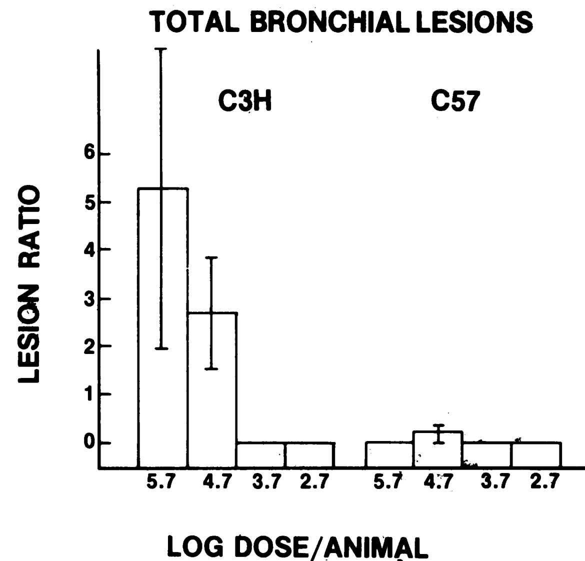

FIG. 3. Mean and standard deviation (bars) of total bronchial epithelial lesion ratios for C3H/HeN and C57BL/6 mice ($n = 4$ to 6 for individual groups) given doses of *M. pulmonis* ranging from 5×10^2 to 5×10^5 CFU per animal. The bronchial epithelial lesion ratio is the ratio of diseased bronchial epithelium to total bronchial epithelial length.

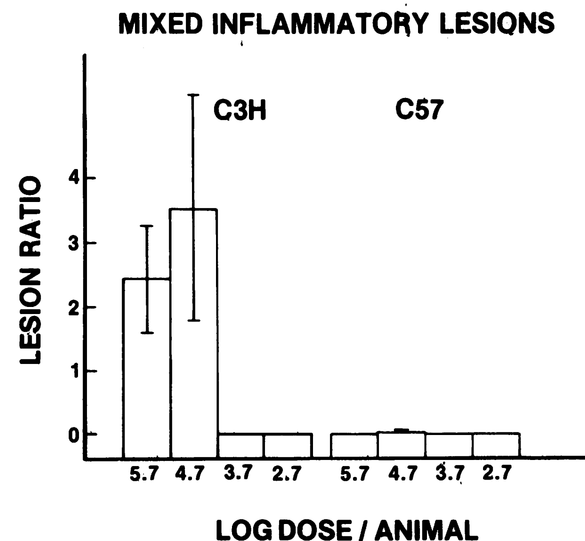

FIG. 4. Mean and standard deviation (bars) of mixed inflammatory lesion ratios for C3H/HeN and C57BL/6 mice ($n = 4$ to 6 for individual groups) given doses of *M. pulmonis* ranging from 5×10^2 to 5×10^5 CFU per animal. The mixed inflammatory lesion ratio is the ratio of the area of mixed inflammation to the total lung area.

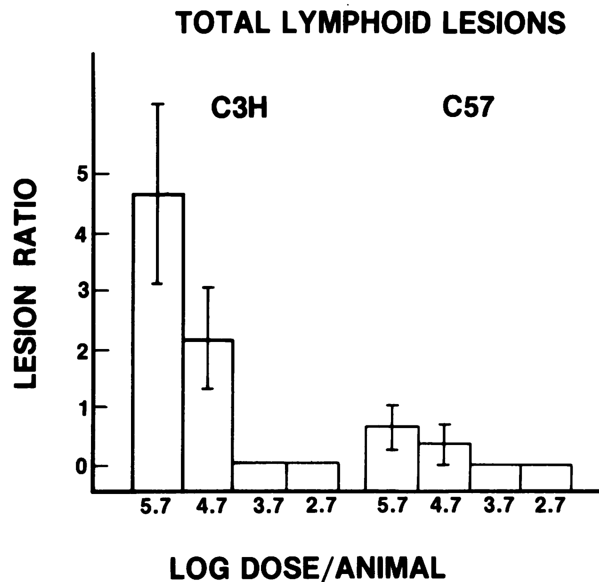

FIG. 5. Mean and standard deviation (bars) of total lymphoid lesion ratios for C3H/HeN and C57BL/6 (n = 4 to 6 for individual groups) given doses of *M. pulmonis* ranging from 5×10^2 to 5×10^5 CFU per animal. The total lymphoid lesion ratio is the ratio of the area of total lymphoid tissue to the total lung area.

be related to the difference in the methods chosen to measure antibody. The ELISA will measure all antibodies of a given class regardless of function, while complement fixation only measures antibody with complement-fixing ability. In the first 14 days of infection, serum antibody responses did not seem to provide any protection against ongoing MRM in either strain even though there was clear histological evidence of inflammatory responses sufficient to allow serum antibody access to the lung parenchyma. This is in contrast to the protective effects of prexisting antibody against development of *M. pulmonis*-induced lung lesions (C3H and outbred CBA mice [2, 23, 24]). Based on the good-to-excellent correlation between antibody responses and lung lesions in both strains and the higher incidence of antibody responses in C3H/HeN mice, it appears likely that antibody responses are closely related to the presence or absence of lesions. However, a role for local antibody in resistance remains a possibility.

Comparison of the results of the present studies with those previously obtained with LEW and F344 rats (2, 10–12) suggests that the mechanisms of resistance or susceptibility are likely to be different in the two species. In rats, strain differences were striking for lesions of both the upper and lower respiratory tract, while in mice the differences were primarily seen only in the lung. The principal differences in lung lesions in rats were in the degree of lymphoid hyperplasia and lymphoid infiltration, which was more severe in LEW rats, and in alveolar consolidation due primarily to mononuclear cells (from 14 through 120 days postin-

TABLE 5. Antibody responses in C3H/HeN and C57BL/6 mice after intranasal inoculation of *M. pulmonis*

Dose (CFU)	% ELISA positive			
	C3H/HeN		C57BL/6	
	IgM	IgG	IgM	IgG
5×10^7 [a]	ND[b]	ND	87	87
5×10^6	ND[c]	ND[c]	100	100
5×10^5	100	100	90	60[d]
5×10^4	100	80	54[d]	18[d]
5×10^3	60	20	0[d]	0
5×10^2	40	10	0[d]	0
5×10^1 [e]	40	10	ND[e]	ND[e]
Controls[f]	0	0	0	0

[a] 5×10^7 CFU were given to C57BL/6 mice only.
[b] ND, Not done.
[c] All C3H/HeN animals given 5×10^6 CFU died before the end of the 14-day observation period.
[d] Incidence of ELISA-positive animals was significantly less ($P < 0.05$) in C57BL/6 mice than in the corresponding C3H/HeN mice.
[e] 5×10^1 CFU were given to C3H/HeN mice only.
[f] Inoculated intranasally with 50 μl of sterile broth.

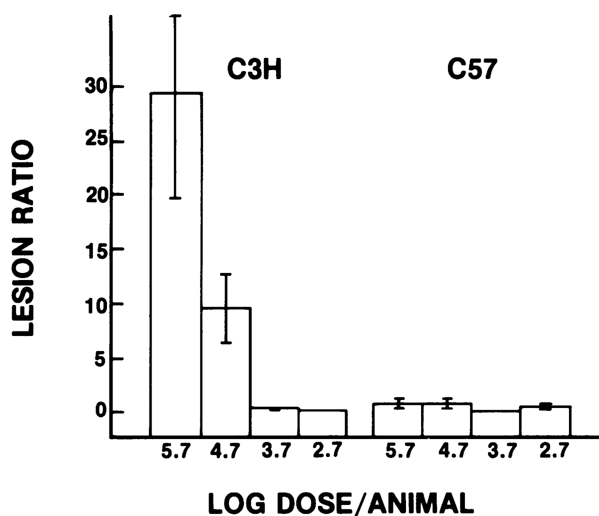

FIG. 6. Mean and standard deviation (bars) of total lung lesion ratios for C3H/HeN and C57BL/6 mice (n = 4 to 6 for individual groups) given doses of *M. pulmonis* ranging from 5×10^2 to 5×10^5 CFU per animal. The total lung lesion ratio is the ratio of the total area of all parenchymal lung lesions to the total lung area.

TABLE 6. Correlation between antibody responses to *M. pulmonis* lesion ratios and culture results in C57BL/6 and C3H/HeN mice

Antibody response	Independent variable	Correlation coefficient[a]	
		C57BL/6	C3H/HeN
IgG	Mixed inflammatory response	0.91	0.69
	Total lymphoid tissue	0.84	0.73
	Total lung lesions	0.79	0.48
	Nasal passage CFU	0.63	0.44
	Lung CFU	0.57	0.52
IgM	Mixed inflammatory response	0.52	0.73
	Total lymphoid tissue	0.53	0.67
	Total lung lesions	0.41	0.53
	Nasal passage CFU	0.88	0.76
	Lung CFU	0.56	0.51

[a] Numbers represent correlation coefficient between antibody response and lesion ratio. Correlations from 0 to 0.25 indicate little or no relationship, those from 0.5 to 0.75 indicate a moderate to good relationship, and those above 0.75 indicate a very good to excellent relationship.

fection), which was seen only in the LEW strain (10). The histological differences in lesions of the mouse lung between C3H/HeN and C57BL/6 mice are comparable with the exception that neutrophils (mixed inflammatory response) are a more predominant component of the alveolar lesions in mice.

The strain differences in susceptibility to MRM in mice should provide additional model systems with which to dissect the mechanisms of lesion production. Not only are the differences striking, they are easily quantified both by biological endpoints and by morphometric analysis. The availability of numerous inbred mouse strains, congenic lines, and recombinant inbred strains should allow identification of the genetic mechanism of resistance with the caveat that most strains will have to be rederived by cesarean section to exclude indigenous mycoplasmal infection (5).

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