INFECTION AND IMMUNITY, Nov. 1989, p. 3570–3575
0019-9567/89/113570-06$02.00/0

Vol. 57, No. 11

Serum Antibody Does Not Account for Differences in the Severity of Chronic Respiratory Disease Caused by *Mycoplasma pulmonis* in LEW and F344 Rats

JERRY W. SIMECKA,[1]* JERRY K. DAVIS,[1,2] AND GAIL H. CASSELL[1]

Department of Microbiology,[1] and Department of Comparative Medicine,[2] University of Alabama at Birmingham, University Station VH 507A, Birmingham, Alabama 35294

Received 15 May 1989/Accepted 25 July 1989

Chronic respiratory disease in rats, resulting from *Mycoplasma pulmonis* infection, is useful in the study of the immunological mechanisms in similar inflammatory diseases and provides a unique opportunity to study the interactions between systemic and mucosal immune systems in a naturally occurring infection. The present study examined the serum antibody responses to *M. pulmonis* in strains of rats which differ in disease progression and severity; LEW rats developed more severe disease than did F344 rats. Serum antibody responses were evaluated as to their levels, isotypes, and antigens recognized. Infected LEW rats produced greater or equal levels of the major classes of serum antibody to *M. pulmonis* than did infected F344 rats, suggesting that development of serum antibody responses alone does not resolve lesions and is not responsible for the difference in disease severity found in LEW and F344 rats. Although LEW rats produced higher responses in all subclasses of immunoglobulin G (IgG), the specific IgG response of LEW rats was composed predominately of IgG1 and IgG2a subclasses, while IgG2b was the major component of the IgG response in F344 rats. Finally, LEW rats responded more quickly to *M. pulmonis* antigens than did F344 rats, and there was no difference in the antigens eventually recognized by each strain, confirming previous work which suggested that LEW rats do not exhibit an unresponsiveness to a specific antigen(s) of *M. pulmonis*.

Murine respiratory mycoplasmosis (MRM), resulting from *Mycoplasma pulmonis* infection, is a chronic, naturally occurring respiratory disease of laboratory rats and is a good model of mycoplasmal respiratory diseases found in many animal species (3). The disease is characterized by a large influx of mononuclear cells into the submucosa of the respiratory tract, which first appears in the nasal passages and spreads downward into the lungs (5, 20). Eventually, the disease can progress to severe lesions of the lung parenchyma.

We have shown that differences in disease progression and severity exist between two strains of rats. After infection, LEW rats develop more severe upper and lower respiratory tract lesions than do F344 rats (6). In particular, LEW rats can develop severe lesions in the lung parenchyma caused solely by *M. pulmonis* infection, whereas the disease in F344 rats is predominately associated with the mucosal surfaces and larger airways. Also, lung lesions begin to resolve in F344 rats after 28 days postinfection, while the lesions in LEW rats continue to increase (8). Increased susceptibility to lung lesions in LEW rats is associated with increased infiltration of all classes of lymphocytes throughout the course of disease. However, in F344 rats, the numbers of lung lymphocytes do not increase after 28 days postinfection and only restricted lymphocyte classes increase in the first month of infection (8).

F344 and LEW rats differ in their abilities to respond nonspecifically (7) and specifically (21) to immunologic stimuli. Lymphocytes from LEW rats, compared with those from F344 rats, proliferate to a greater degree in response to mitogens, including *M. pulmonis*-associated mitogen (7). In contrast, F344 rats produce higher levels of *M. pulmonis*-specific serum immunoglobulin G (IgG) antibody, after intraperitoneal immunization with nonreplicating *M. pulmonis* antigens, than do LEW rats, but IgM serum antibody responses do not differ. The high IgG responses to *M. pulmonis* in F344 rats was almost exclusively due to the development of much higher specific responses in the IgG2b subclasses. Further analysis by Western blots (immunoblots) revealed that F344 rats consistently produced antibody to more protein antigens than did LEW rats after immunization with nonreplicating antigens. However, it did not appear that LEW rats were unresponsive to any particular antigen; rather, LEW rats varied as to which antigens that antibody responses were developed.

To further examine the role that host immune responses have in determining progression of MRM, serum antibody responses specific for *M. pulmonis* were compared in infected LEW and F344 rats. In contrast to rats immunized with nonreplicating *M. pulmonis* antigens (21), infected LEW rats produced greater or equal levels of the major classes of serum antibody to *M. pulmonis* than did infected F344 rats, regardless of the dose of infecting organisms and the time postinfection. Although LEW rats produced higher levels of IgG to the organism than F344 rats after infection, the total IgG response in LEW rats consisted primarily of the IgG1 and IgG2a subclasses, while IgG2b was the major component in the total IgG response of F344 rats. Also, LEW rats responded more quickly to *M. pulmonis* antigens than did F344 rats. Furthermore, there was no difference in the antigens eventually recognized by each strain, confirming that LEW rats do not exhibit an unresponsiveness to a specific antigen(s) of *M. pulmonis*. These results suggest that serum antibody responses alone do not resolve lesions; also, differences in antibody levels are probably not responsible for the difference in disease severity found in LEW and F344 rats.

* Corresponding author.

MATERIALS AND METHODS

Animals. Pathogen-free LEW and F344 rats (8 to 12 weeks old), reared and maintained in Trexler-type plastic film isolators, were used (19). Pathogen-free status of animal colonies was monitored by serological (including enzyme-linked immunosorbent assay [ELISA] for serum anti-mycoplasmal antibodies) and cultural techniques as described earlier for mycoplasmal, viral (Sendai virus, pneumonia virus, sialodacryoadenitis virus, Kilhyam rat virus, H-1 virus, reovirus-3, GD-VII virus, lymphocytic choriomeningitis virus, mouse adenovirus [Charles River Biotechnical Services, Wilmington, Mass.]), fungal, and other bacterial pathogens. The colony was consistently negative for all pathogens, and the animals were negative for IgG and IgM anti-mycoplasmal antibodies. LEW and F344 rats were age (±2 weeks) and sex matched and also cocaged to minimize variability caused by environmental factors. Animals were intranasally inoculated with 50 or 100 μl of *M. pulmonis* containing 0.9×10^7 to 2×10^7 CFU, unless otherwise indicated.

To obtain serum samples, rats were anesthetized with a combination of ketamine hydrochloride (Bristol Laboratories, Syracuse, N.Y.) and xylazine (5 mg/ml; Rhompun; Haver-Lockhart, Shawnee, Kans.). Blood was collected by tail bleed or cardiac puncture for the determination of the levels of specific antibody. Serum samples were stored at −20°C until ready for use.

Mycoplasma. *M. pulmonis* UAB 6510 was originally isolated from the lungs of a rat with natural MRM. The isolate was cloned three times by using the medium described by Hayflick (15) and was identified as a pure culture of *M. pulmonis* by immunofluorescence (9). *M. pulmonis* was grown in mycoplasma medium (7, 15) and stored in 1-ml portions at −70°C. The number of *M. pulmonis* CFU was determined by inoculation of agar plates with serial dilutions of the stock in broth media. In selected experiments, mycoplasma were concentrated by centrifugation before the inoculation of animals.

Antibodies. Alkaline phosphatase (AP) goat anti-rat IgG (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.), sheep anti-rat IgA (BSI/Serotec, Indianapolis, Ind.), and goat anti-rat IgM (Organon Teknika, Malvern, Pa.) were used to detect antigen-specific rat IgG, IgA, and IgM, respectively, by ELISA. The subclass distribution of IgG was determined by using sheep antisera to rat IgG2a and rat IgG2b (Pel-Freez Biologicals, Rodgers, Ark.) conjugated with AP type III (Sigma Chemical Co., St. Louis, Mo.) (11) and by using AP-conjugated sheep anti-rat IgG1 and anti-rat IgG2c (BSI/Serotec).

Antibody levels. The level of antibodies in the sera were compared with ELISAs as described earlier (21). Serial dilutions of each serum sample were added in triplicate to microdilution wells coated with a *M. pulmonis* cell lysate. After overnight incubation, AP-labeled secondary antibodies were added to each of the wells at a dilution giving minimal nonspecific binding to antigen-coated wells. After a 5-h incubation, AP substrate was added to each of the wells. After incubation at 37°C, the optical densities at 405 nm were found by using a Titertek Multiskan ELISA reader (Flow Laboratories, Inc., McLean, Va.). The relative antibody activities were determined by using a parallel line bioassay (1). A serum sample, in a particular assay, was given an arbitrary activity of 3,000 and used as the standard to determine the activity of the other serum samples. Comparisons between sera activities were made only with values obtained within a single ELISA run to eliminate daily variation within the assay. This type of analysis produced antibody titers highly correlated ($r > 0.85$) with results found with endpoint titration of antisera (unpublished data).

Western blots. To examine the antigens recognized by serum antibodies, Western blots were done as previously described (21). *M. pulmonis* antigens were separated by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose. Rat sera were diluted 1:100 and allowed to react with antigens on the nitrocellulose strips. Biotinylated goat anti-rat IgG (whole molecule [Sigma]), which reacts with all antibody classes (21; unpublished observations), and avidin-horseradish peroxidase (Sigma) were used to reveal the antibody reactions. Preimmune rat sera had no reactivity in the immunoblot procedures.

Statistics. The data were analyzed by using the Mann-Whitney U test, analysis of variance, and the Duncan means ratio test after logarithmic transformation of the data (1). Also, multiple linear regressions after logarithmic transformation of antibody titers were done, followed by analysis by partial regression coefficients (1, 10). A probability (P) of less than 0.05 was accepted as significant.

RESULTS

Antibody responses in rats infected with different doses of organisms. LEW and F344 rats were infected with 10-fold dilutions of *M. pulmonis* that ranged from 10^4 to 10^9 CFU. Twenty-eight days after infection, serum was collected from each of these animals and the amounts of specific IgM and IgG, including IgG2a and IgG2b subclasses, were determined. As previously described (6), all animals had microscopic lesions characteristic of MRM in the upper respiratory tract, and lung parenchymal disease in LEW rats dramatically increased at an inoculating dose of about 10^6 CFU and higher of *M. pulmonis*. For both LEW and F344 rats, there was no significant effect caused by the inoculating dose of organisms on the serum antibody responses of any isotype. For all doses of organisms, LEW antibody responses were either equal to or greater than F344 antibody responses. Significant differences between the LEW and F344 rats were found in IgG ($P = 0.016$), IgG2a ($P = 0.0024$), and IgM ($P = 0.009$); however, LEW IgG2b responses were slightly higher but insignificant ($P = 0.0778$).

Development of serum antibody responses after infection. In contrast to immunization with nonviable *M. pulmonis* (21), the above experiments showed that during infection LEW rats produced higher or equal levels of IgG and IgG2b than did infected F344 rats. One possible explanation for these conflicting observations is that F344 rats initially produce a greater response than LEW rats and that the response at 28 days postinfection by F344 rats decreased to a level below that of LEW rats because of its effectiveness in arresting the progression of disease. To investigate this possibility and to fully evaluate the development of the other antibody class responses, LEW and F344 rats were intranasally infected with 9×10^6 CFU of *M. pulmonis*. Three rats of each strain were killed, and sera were collected at the following times after infection: 3 days, 7 days, 14 days, 28 days, 2 months, and 4 months. The levels of specific IgM, IgA, and IgG, including all IgG subclasses, to *M. pulmonis* were determined in serum. LEW rats produced higher responses than did F344 rats in all classes of antibody ($P < 0.05$) (Fig. 1). IgM and IgA responses were significantly higher in LEW rats by 7 days postinfection, while their IgG responses were

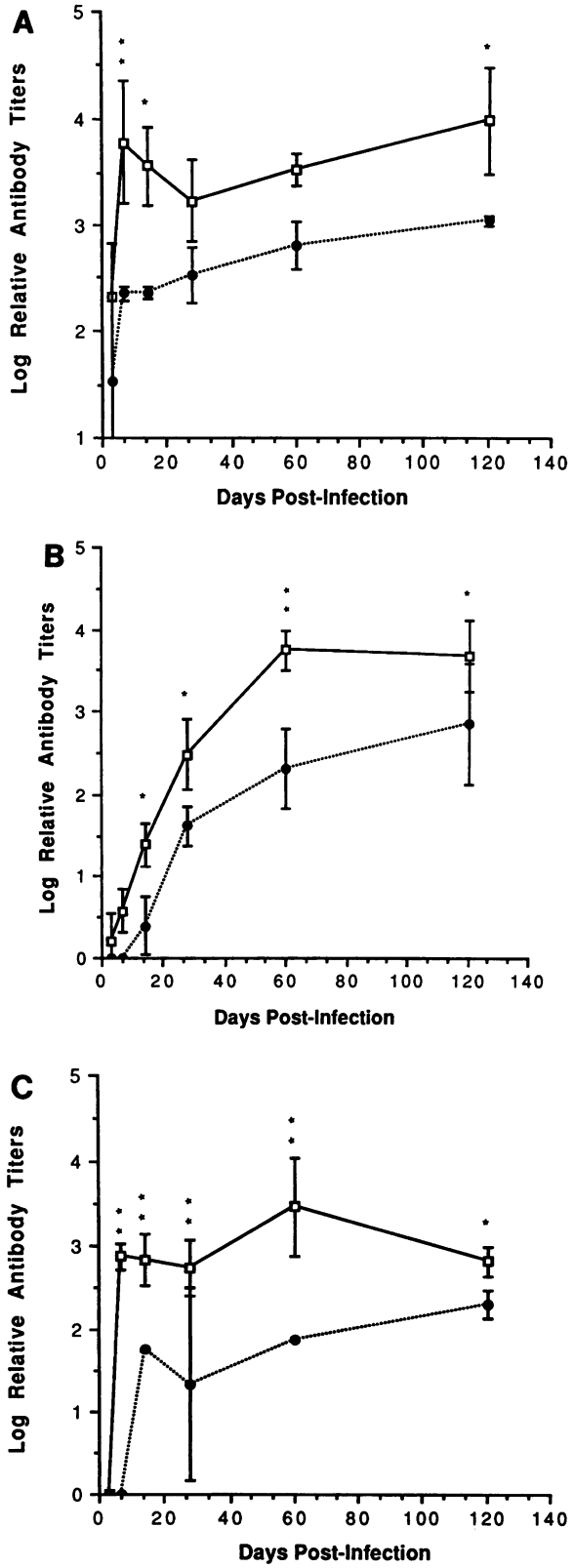

FIG. 1. Development of serum antibody responses after infection. The levels of anti-*M. pulmonis* IgM (A), IgG (B), and IgA (C) from LEW (□) and F344 (●) rats produced at various times postinfection were determined. LEW rats produced antibody specific for *M. pulmonis* at higher levels than did F344 rats. * and **, Significant difference between responses in LEW and F344 rats at $P < 0.05$ and $P < 0.01$ levels, respectively. Error bars represent standard deviation of the antibody titers at that time point.

significantly higher by 14 days. IgM and IgA responses leveled off at 7 to 14 days after infection, by 2 months postinfection LEW and F344 IgG responses leveled off, and little or no increase in antibody levels was observed thereafter.

As differences in IgG subclass responses had previously been shown after immunization with nonviable *M. pulmonis* between LEW and F344 rats (21), *M. pulmonis*-specific IgG subclass responses were also determined. LEW rats produced consistently higher responses in all but the IgG2c subclass (Fig. 2). At 7 and 14 days postinfection, LEW IgG2c responses were higher than those found in F344 rats. In 28 days postinfection and beyond, the differences between IgG2c responses in F344 and LEW rats were not significant, with P values of 0.068, 0.241, and 0.269 at 28, 60, and 120 days postinfection.

The relative contributions of each of the IgG subclasses to the total IgG response were analyzed for each rat strain. Secondary antibodies may differ in the numbers of epitopes recognized on the serum antibodies, the ratios of enzyme to antibody molecule, or avidities; this does not allow direct comparisons to be made between the antibody subclasses. Thus, the data were analyzed by multiple linear regression. This analysis indicated that LEW and F344 rats differed in the primary IgG subclasses which contribute to the total IgG response. Significant contributions to the total IgG response in LEW rats were made by IgG1 ($P < 0.05$) and IgG2a ($P < 0.05$) subclasses, whereas the only significant contribution to the F344 IgG response to *M. pulmonis* was the IgG2b subclass ($P < 0.02$). The IgG2c responses in both F344 and LEW rats appeared to be minor components of the total IgG responses, although another possibility is that the AP-conjugated anti-IgG did not strongly recognize this subclass. However, as the IgG2c responses in LEW and F344 rats at later time points did not contribute significantly to the difference in LEW and F344 IgG responses, it is unlikely that IgG2c antibody is a dominant component of the total IgG response.

Antigens recognized by antibody. Antisera from LEW and F344 rats (7, 14, and 28 days postinfection) were reacted with immunoblots of *M. pulmonis* antigens. LEW rats produced antibody to more *M. pulmonis* antigens than did F344 rats at 7 days after infection (Fig. 3). However, there was no difference in the antigens recognized by antibody from LEW and F344 rats at 28 days postinfection, although a difference in titer of the antibody is apparent. These results do confirm the suggestion from our previous study (21) that LEW rats do not exhibit an unresponsiveness to a specific antigen of *M. pulmonis*.

DISCUSSION

Specific immune responses are able to influence the progression of MRM in rats. Systemic immunization of F344 rats with live or killed *M. pulmonis* decreases the severity and incidence of subsequent disease (4). The most dramatic effect is on the incidence of lung disease, which drops from greater than 70% in control animals to 0 after immunization. In mice, passive transfer of immune sera protects against the development of acute pneumonia after infection with *M. pulmonis* (2, 5). In contrast, passive transfer of immune sera

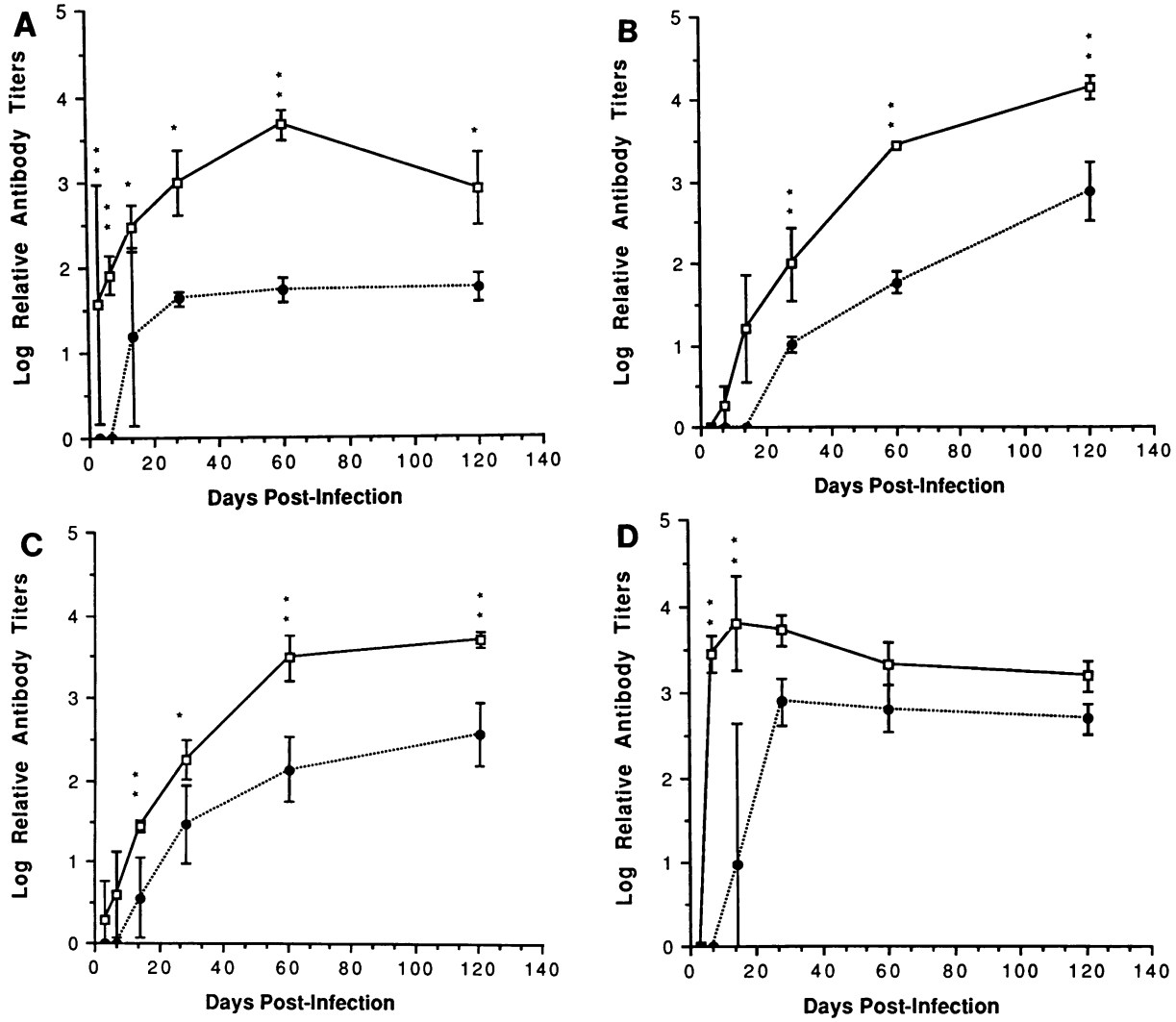

FIG. 2. Development of IgG subclass responses after infection. The levels of anti-*M. pulmonis* IgG1 (A), IgG2a (B), IgG2b (C), and IgG2c (D) from LEW (□) and F344 (●) rats produced at various times postinfection were determined. LEW rats produced IgG subclass antibody specific for *M. pulmonis* at higher levels than did F344 rats, except for the IgG2c responses, which were the same at later time points. * and **, Significant difference between responses in LEW and F344 rats at $P < 0.05$ and $P < 0.01$ levels, respectively. Error bars represent standard deviation of the antibody titers at that time point.

in rats does not prevent *M. pulmonis* infection or disease (2). However, the studies in rats were performed with a single injection of antisera at the time of infection, which may have little influence on the progression of a chronic disease caused by the clearance of the transferred antibody. Thus, there was no clear evidence that serum antibody does or does not affect the progression of chronic disease in rats.

Immunization studies with nonviable mycoplasma suggest the possibility that the difference in the severity and progression of disease in LEW and F344 rats was due to differences in their ability to produce antibody to *M. pulmonis* (21); however, the present studies indicate that serum antibody responses alone are not able to protect rats from the development of lung lesions. LEW rats develop severe parenchymal lung lesions after infection with *M. pulmonis*, while F344 rats have minimal parenchymal disease after infection; in addition, the severity of submucosal inflammation is also greater in LEW rats (6). As shown in these studies, infected LEW rats produced either similar or higher levels of serum anti-*M. pulmonis* activity in all antibody classes than were found in infected F344 rats, irrespective of the time after infection. In fact, LEW rats responded more quickly to *M. pulmonis* antigens than did F344 rats, and there was no difference in the protein antigens eventually recognized by each strain, confirming the suggestion that LEW rats do not exhibit an unresponsiveness to a specific antigen(s) of *M. pulmonis*. Almost all the antibody activity to *M. pulmonis* is removed after protease treatment of the organism (18); thus, it is unlikely that differences in antibody responses to a major antigen(s) have been missed in the present studies with immunoblotting which detects protein-containing antigens. Thus, the lack of lesions, including those of the lung parenchyma, cannot be explained by the presence of protective antibody found only in F344 rats.

In LEW rats at 28 days postinfection, the severity of middle ear and lung parenchymal lesions increased (6) while antibody responses were the same. Thus, the increased severity of lesions in LEW rats, compared with that in F344

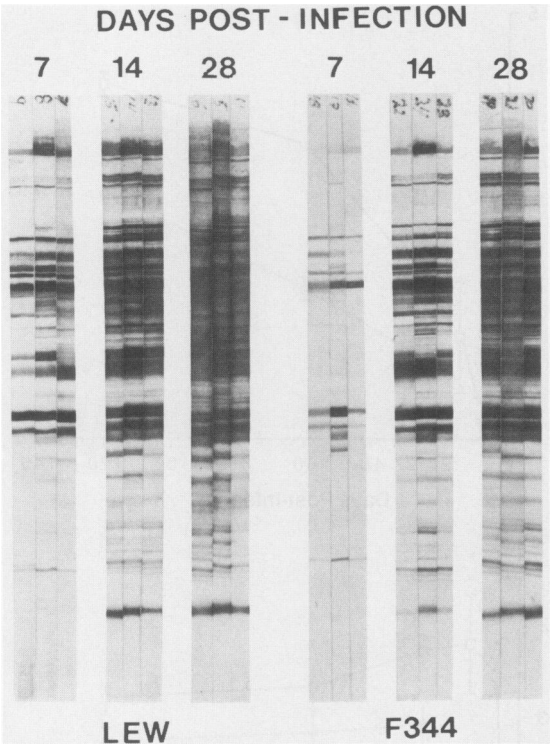

FIG. 3. Electrophoresis of antigens recognized by serum antibody. Antisera from F344 and LEW rats were reacted with immunoblots of *M. pulmonis* antigens which were revealed by biotinylated anti-rat IgG (heavy and light chain) and avidin peroxidase. LEW rats produced antibody to antigens slightly earlier than did F344 rats, but there was no difference in the antigens recognized by 28 days postinfection.

rats, is not likely to be antibody mediated. This does not preclude a role of antibody in the pathogenesis of the disease. Antibody, in conjunction with Fc receptor-bearing cells, may determine the course of the disease. Antibody alone may be responsible for some of the immunopathology in LEW rats, especially in the earlier stages of the disease where LEW rats respond rapidly to infection, but it is probably not a major component, as MRM lesions in rats are predominately a mononuclear cell infiltration (6, 8) which is not typical for antibody complex-plus-complement-mediated lesions.

There is an apparent paradox between the previous report demonstrating that F344 rats develop higher anti-*M. pulmonis* IgG2b responses after immunization with killed organisms (21) and the present studies, which show that LEW rats developed higher responses after infection with live organisms. However, infected LEW rats had higher responses in all IgG subclasses, and although comparisons cannot be made directly between relative antibody titers of different classes, the subclasses that appeared to contribute the most to the total specific IgG response of LEW rats are IgG1 and IgG2a when the results were analyzed by multiple linear regression. In F344 rats, the total specific IgG response was primarily IgG2b. The subclass distribution is consistent with the previous immunization studies (21). Thus, the present studies suggest that the total IgG response to *M. pulmonis* in LEW rats is much higher than expected because of infection, but the IgG subclass distribution of the responses is the same as previously indicated (21).

The factors contributing to the higher-than-expected IgG response in LEW rats are unknown. As mentioned above, this may be related to the actual numbers of organisms present in the respiratory tract at various times after infection. Also, the increased migration of cells to the site of infection in LEW rats may result in an increase in the number of activated B cells either by direct stimulation of lymphoid cells or the increased transport of antigen to local lymph nodes via active or passive transport mechanisms (12–14). However, another possible explanation for the higher-than-expected IgG responses in LEW rats is that chronic stimulation of lymphocytes by the B-cell mitogen of *M. pulmonis* may influence the antibody responses in infected rats. This is plausible, considering that LEW lymphocytes respond more to this mitogen than do F344 lymphocytes in vitro (7). Also, recent studies from our laboratory (S. E. Ross, J. K. Davis and G. H. Cassell, submitted for publication) demonstrate that the *M. pulmonis* mitogen can directly stimulate B cells to proliferate and differentiate without any preference for IgG subclass. Thus, *M. pulmonis* mitogen could account for both the enhanced IgG responses in LEW rats and the retention of IgG subclass distribution as suggested above.

Although serum antibody does not appear to be responsible for the differences in the disease in LEW and F344 rats, antibody production at the sites of infection may still play an essential role in protection from disease. Serum antibody levels are not truly representative of the antibody activity found at the tissue site. For example antibody responses in secretions have an increased proportion of IgA relative to serum (16); in addition, serum IgA also does not contribute significantly to rat bronchial secretions (17). However, infected LEW rats have higher numbers of *M. pulmonis*-specific antibody-forming cells in the respiratory tract than do F344 rats (J. W. Simecka, P. Patel, J. K. Davis, and G. H. Cassell, submitted for publication), and these numbers are roughly proportional to the antibody responses in serum. Further studies on the distribution of antibody-producing cells are needed not only to understand the role of antibody in disease progression but to further understand the effect of chronic inflammatory responses on immune function.

In summary, the results from these studies demonstrate that, unlike acute pneumonia in mice (5), the development of serum antibody responses alone do not protect against parenchymal lung disease in rats infected with *M. pulmonis*. Thus, there appear to be fundamental differences between the protective immune mechanisms involved in the chronic respiratory disease in rats, compared with acute disease which develops in mice after infection with *M. pulmonis*, although the chronic disease in mice may have similar mechanisms. In addition, cell-mediated immunity may also play an important role in MRM. Passive transfer of immune cells can prevent the production of disease in F344 rats but does not prevent it in mice (2). Also, immune LEW lymphocytes fail to proliferate in response to *M. pulmonis* antigen in vitro, while F344 lymphocytes respond (21). Thus, future studies will examine the mechanisms of cell-mediated immunity present in infected LEW and F344 rats. Further analysis of the differences in immune mechanisms operative in MRM in rats and mice may be important in the future development of vaccination protocols.

ACKNOWLEDGMENTS

We thank Ginger Gambill for her excellent technical help during the course of these studies. We also thank Suzanne Ross and Harold

Watson for their helpful suggestions and criticisms of the manuscript.

This work was supported by Public Health Service grant HL19741 to G.H.C. from the National Heart, Lung, and Blood Institute. Jerry W. Simecka is a Parker B. Francis Fellow in Pulmonary Research.

LITERATURE CITED

1. **Armatradge, P.** 1977. Statistical methods in medical research. Blackwell Scientific Publications, Oxford, England.
2. **Cassell, G. H.** 1982. The pathogenic potential of mycoplasmas: Mycoplasma pulmonis as a model. Rev. Infect. Dis. **4**:S18–S34.
3. **Cassell, G. H., W. Clyde, Jr., and J. K. Davis.** 1985. Mycoplasmal respiratory infections, p. 65–106. *In* S. Razin and M. F. Barile (ed.), The mycoplasmas, vol. 4. Academic Press, Inc., Orlando, Fla.
4. **Cassell, G. H., and J. K. Davis.** 1978. Protective effect of vaccination against *Mycoplasma pulmonis* respiratory disease in rats. Infect. Immun. **21**:69–75.
5. **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.
6. **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.
7. **Davis, J. K., J. W. Simecka, J. S. P. Williamson, S. E. Ross, M. M. Juliana, R. B. Thorp, and G. H. Cassell.** 1985. Nonspecific lymphocyte responses in F344 and LEW rats: susceptibility to murine respiratory mycoplasmosis and examination of cellular basis for strain differences. Infect. Immun. **49**:152–158.
8. **Davis, J. K., R. B. Thorp, R. B. Maddox, M. B. Brown, and G. H. Cassell.** 1982. Murine respiratory mycoplasmosis in F344 and LEW rats: evolution of lesions and lung lymphoid cell populations. Infect. Immun. **36**:720–729.
9. **Del Guidice, R. A., and M. Barile.** 1974. Immunofluorescent procedures for mycoplasma identification. Dev. Biol. Stand. **23**:134–137.
10. **Dowdey, S., and S. Wearden.** 1989. Statistics for research. John Wiley & Sons, Inc., N.Y.
11. **Engvall, E., and P. Permann.** 1972. Enzyme-linked immunosorbent assay ELISA. III. Quantitation of specific antibodies by enzyme labeled anti-immunoglobulin in antigen coated tubes. J. Immunol. **109**:129–135.
12. **Green, G. M.** 1973. Alveolobronchiolar transport mechanisms. Arch. Intern. Med. **131**:109–114.
13. **Harmsen, A. G., M. J. Mason, B. A. Muggenburg, N. A. Gillett, M. A. Jarpe, and D. E. Bice.** 1987. Migration of neutrophils from lung to trachobronchial lymph node. J. Leukocyte Biol. **41**:95–103.
14. **Harmsen, A. G., B. A. Muggenburg, M. B. Snipes, and D. E. Bice.** 1985. The role of macrophages in particle translocation from lungs to lymph nodes. Science **230**:1277–1280.
15. **Hayflick, L.** 1965. Tissue cultures and mycoplasmas. Tex. Rep. Biol. Med. **23**(Suppl. 1):285–303.
16. **Kaltreider, H. B., and M. K. Chan.** 1976. The class-specific immunoglobulin composition of fluids obtained from various levels of the canine respiratory tract. J. Immunol. **116**:423–429.
17. **Lemaitre-Coelho, I., M. Yamakido, P. C. Montgomery, A. E. Langendries, and J. P. Vaerman.** 1982. Selective excretion of IgA in rat bronchial secretions: lack of significant contribution from plasma IgA. Immunol. Commun. **11**:441–453.
18. **Minion, F. C., M. B. Brown, and G. H. Cassell.** 1984. Identification of cross-reactive antigens between *Mycoplasma pulmonis* and *Mycoplasma arthritidis*. Infect. Immun. **43**:115–121.
19. **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.
20. **Schoeb, T. R., M. K. Davidson, and J. R. Lindsey.** 1982. Intracage ammonia promotes growth of *Mycoplasma pulmonis* in the respiratory tract of rats. Infect. Immun. **38**:212–217.
21. **Simecka, J. W., and G. H. Cassell.** 1987. Serum antibody and cellular responses in LEW and F344 rats following immunization with *Mycoplasma pulmonis* antigens. Infect. Immun. **55**: 731–735.