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Murine Respiratory Mycoplasmosis in F344 and LEW Rats: Evolution of Lesions and Lung Lymphoid Cell Populations

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By comparison of two rat strains, LEW and F344, which are known to differ in susceptibility to *Mycoplasma pulmonis* respiratory disease, it was shown that differences in lesion severity and progression were associated with changes in lung lymphocyte populations. Lung lesions in LEW rats developed earlier after infection, became more severe, and were characterized by continued proliferation of all classes of lymphoid cells, T lymphocytes, B lymphocytes, and plasma cells, throughout the 120-day observation period. In contrast, lymphoid proliferation in F344 rats reached a plateau at 28 days and was restricted to an increase in T lymphocytes, immunoglobulin A (IgA)-bearing B lymphocytes, and IgA and IgG plasma cells. Although approximately 10 times as many IgG B cells and 4 times as many IgG plasma cells were found in infected LEW rats as compared with F344 rats, the specific anti-*M. pulmonis* IgG response in the two strains was roughly parallel. The same relationships held true, although to a lesser extent, for specific IgA antibody responses and cellular responses. Whereas lung lesions showed a tendency to resolve in F344 rats by 120 days, severe lesions persisted in LEW rats. The disparity between the cellular response and specific antibody response, the seemingly uncontrolled lymphocyte proliferation in LEW rats, and the mitogenic potential of *M. pulmonis* suggest that differences between LEW and F344 rats in lung lesion severity and progression are related to differences in the degree of nonspecific lymphocyte activation in the two strains, an imbalance in regulation of lymphocyte proliferation in LEW rats, or both.

Murine respiratory mycoplasmosis, due to *Mycoplasma pulmonis*, is a naturally occurring, slowly progressing, chronic disease in laboratory rats (28, 29). By 28 days postinfection, LEW rats develop quantitatively and qualitatively more severe lung lesions than do F344 rats (J. K. Davis, and G. H. Cassell, Vet. Pathol., in press). The increased severity of lung lesions in LEW rats appears to correlate with a greater amount of peribronchial and perivascular lymphoid tissue in these animals, although lymphoid hyperplasia is a prominent lesion in infected rats of both strains. This observation suggests that lymphocytes have a central function in the development of lung lesions.

The present studies were designed to compare the kinetics of lymphoid hyperplasia and parenchymal lung lesions in LEW and F344 rats, define the lymphocyte populations present at various times after infection, identify the classes of antibody produced, and correlate each of these parameters with severity and progression of disease. Antibody production to *M. pulmonis* was used as an indicator of immune recognition and degree of specific responsiveness. The results show that lung lymphocyte numbers and subpopulation distributions are related to development of lung lesions in both F344 and LEW rats and suggest that differences in lung lesion severity and progression are related to differences in degree of nonspecific lymphocyte activation in the two strains, an imbalance in regulation of lymphocyte proliferation in LEW rats, or both.

MATERIALS AND METHODS

Animals. Pathogen-free F344 rats were reared and maintained in Trexler plastic film isolators as previously described (28). Pathogen-free LEW rats, reared and maintained in a similar fashion, were obtained from the Trudeau Institute, Inc., Saranac Lake, N.Y. Intracage ammonia levels were monitored twice weekly, and the bedding was changed as necessary to maintain levels between 19 and 38 mg/liter (25 and 50 ppm) (4). At 2 months of age, F344 or LEW rats were sedated with a combination of fentanyl and droperidol (Innovar-Vet; Pittman Moore, Inc., Washington Crossing, N.J.) and intranasally inoculated with either 0.05 ml of sterile Hayflick broth or 9 × 10^6 colony-forming units of *M. pulmonis* (UAB 6510) in 0.05 ml of Hayflick broth (7). Control rats (those receiving sterile broth) were killed at 0, 60, or 120 days, and *M.*

pulmonis-inoculated animals were killed at 3, 7, 14, 28, 60, or 120 days. An additional F344 control group was killed at 28 days. Lungs from one-half of each group for each time interval were processed for histology (28), and serum and tracheobronchial lavages were obtained. Lungs from the remaining animals were used to harvest lung lymphocytes.

Mycoplasma culture. Tracheobronchial lavages (control and experimental) were used for *M. pulmonis* isolation as described previously (28). Cultures were incubated for 2 weeks at 37°C before they were considered negative.

Lesion scoring. To quantitate the severity of otitis, laryngotracheitis, and rhinitis, histological sections were identified by code and subjectively scored (0 to 3) according to severity of lesions (7). 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 for an experimental group.

Lung lesion areas were measured morphometrically (7). Five specific lesions were measured: (i) bronchial-associated lymphoid tissue (BALT) hyperplasia; (ii) perivascular and peribronchiolar lymphoid infiltration; (iii) bronchial exudate; (iv) bronchiolar exudate; and (v) alveolar consolidation or atelectasis or both. Based on lesion measurements, the following calculations were made: total lymphoid tissue = BALT + perivascular and peribronchiolar lymphoid infiltration; total parenchymal disease = (perivascular and peribronchial lymphoid infiltration + bronchiolar exudate) + alveolar consolidation; and total lesion area = sum of all five lesions. For purposes of analysis and comparison, each of these parameters was expressed as a ratio of lesion area to total lung area.

Antibody measurement. The serum from each individual animal was analyzed for immunoglobulin M (IgM) and IgG anti-*M. pulmonis* antibodies by an enzyme-linked immunosorbent assay (ELISA) (23) and for anti-*M. pulmonis* IgA by indirect immunofluorescence (IMF) (8). ELISA values (IgG and IgM) are expressed as absorbance (400 nm) per 100 min, and IMF values are expressed as titers. Tracheobronchial lavage samples from each group were pooled and analyzed for IgG at conjugate dilutions of 1:100 and tracheobronchial lavage dilutions of 1:2.

Lymphoid cell recovery. Lung mononuclear cells were collected from each animal by a modification of the techniques of Hunninghake and Fauci (15, 24) with the following isolation medium: RPMI 1640 medium (GIBCO, Grand Island, N.Y.) buffered to pH 7.3 with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; GIBCO) plus 1% L-glutamine (Difco Laboratories, Detroit, Mich.), 10% fetal bovine serum (Difco), 100 U of penicillin G per ml, 5 mg of DNase (GIBCO) per liter, and 50 μg of streptomycin per ml (15, 24).

The lungs were removed and mechanically disaggregated for 1.5 min with an impacter (Stomacher 80; Dynatech Laboratories, Inc., Alexandria, Va.). The resulting cell suspension was passed through a 250-μm mesh nylon screen to remove fragments. Fragments from selected animals in each group were examined histologically to determine the efficiency of lymphocyte recovery. The resulting cell suspensions were centrifuged at 250 × *g* for 20 min at 4°C, washed five times in RPMI 1640 medium, and passed over a Ficoll-Hypaque gradient to isolate mononuclear cells (1). Mononuclear cells were washed twice, and phagocytic cells were removed by allowing them to ingest carbonyl iron (10 mg/ml), followed by magnetic sedimentation. Remaining cells were incubated in plastic tissue culture flasks at 37°C for 45 min with 0.1% latex beads (0.8 μm; Dow Chemical, Indianapolis, Ind.) per 2 × 10^7 cells (40). Less than 0.1% of the adherent cells possessed surface immunoglobulin as determined by IMF examination with anti-rat $F(ab)_2$ antisera; thus, this step did not cause selective loss of B-lymphocytes. The nonadherent cell population from each rat was counted, and the viability (by trypan blue exclusion), phagocytotic ability (37), pinocytotic ability (10), and morphological appearance (Wright-Giemsa stain) were determined. In selected cases, dichlorotriazinylaminofluorescein (DTAF)-labeled (3) $F(ab)_2$ fragments of anti-alveolar macrophage serum (31) were used to confirm morphological and functional evidence of the absence of large numbers of macrophages in the final cell population.

Enumeration of lymphoid cell populations. The final cell populations were washed eight times with 20 to 50 volumes of phosphate-buffered saline (pH 7.4) containing 0.02% sodium azide and 10 mg/ml bovine serum albumin (42). Samples of the purified lymphoid population from the lungs of each rat were then examined by IMF (42) for T and B lymphocytes and plasma cells. After the appropriate labeling steps, the cells were examined by alternate-phase and IMF microscopy (Leitz Orthoplan). A total of 300 mononuclear cells were counted per slide, excluding ciliated and phagocytic (containing latex or iron particles or both) cells. The percentage of unclassifiable mononuclear cells, those not staining with any of the sera, was computed by subtraction. The data per animal were converted to absolute numbers.

B lymphocytes were detected with either DTAF-labeled $F(ab)_2$ fragments (3) or the intact IgG fraction of rabbit anti-rat $F(ab_2)$ serum (ABS) obtained from E. D. Crum, Cleveland Veterans Administration Hospital, Cleveland, Ohio. This serum has been shown not to react with rat T lymphocytes (12). Anti-thymocyte serum was prepared in rabbits by the protocol of Balch and Feldman (2) and was sequentially absorbed with rat kidney cells. (3×), glutaraldehyde-precipitated rat kidney homogenates (3×), rat erythrocytes (3×), rat liver cells (1×), and rat B lymphocytes (2×) bound to a glass bead column. This column was prepared by sequential incubation of glass beads for 2 h with normal rat serum, ABS, and rat spleen cells. RPMI 1640 medium (500 ml) was used to wash the column after each step. An IgG fraction of absorbed anti-thymocyte-serum was prepared by salt fractionation and DEAE chromatography, and either the entire fraction or $F(ab)_2$ fragments (20) prepared from the gamma globulin fraction were labeled with DTAF (3). Neither the intact absorbed anti-thymocyte serum and ABS nor their $F(ab)_2$ fragments cross-reacted in preliminary double-labeling experiments in which DTAF–anti-thymocyte serum and rhodamine-labeled ABS or the converse were used. In addition, neither serum labeled cells that phagocytosed latex beads.

B lymphocyte subclasses were enumerated by either direct or indirect IMF (42), using DTAF-labeled rabbit gamma globulin fractions of anti-rat IgG and

IgM or goat anti-rat IgA or by using unlabeled sera followed by fluorescein-labeled goat-anti-rabbit or rabbit-anti-goat globulin. Anti-IgM, -IgG, and -IgA were purchased from Miles Laboratories, Inc., Elkhart, Ind., and were class specific by immunoelectrophoresis, gel diffusion, and ELISA with purified myeloma proteins (23). Both the direct and indirect methods stained the same percentage of rat spleen cells and did not stain macrophages. Normal rabbit serum was used as a control in the indirect method, whereas preincubation with unlabeled immune serum served as a control for direct IMF.

When indirect IMF was used to enumerate cell classes, care was taken to guard against spurious results due to binding of reagents to Fc receptors or reaction of specific anti-immunoglobulin reagents to rat immunoglobulin bound to Fc receptors. Cells were washed extensively before use of Ficoll-Hypaque gradients to prevent conformational changes in Fc receptors, which make removal of attached immunoglobulin difficult (1). Phagocytes present served as an internal control since they did not stain with any reagent, as they would if the reagents were binding nonspecifically. The percentage of cells staining with direct labeled $F(ab)_2$ was compared with the total percentage of cells staining with anti-IgM, -IgG, and -IgA. If Fc-receptor binding was a problem, then the percentage obtained by addition of the percentages stained by each class would exceed that obtained with anti-$F(ab)_2$.

Cytoplasmic labeling was used to detect intracellular immunoglobulin (18). Samples of the lymphoid cell preparation in phosphate-buffered saline–bovine serum albumin were cytocentrifuged onto glass slides, fixed for 20 min in 95% alcohol–5% acetic acid at −20°C, and stained with anti-$F(ab)_2$ serum or with anti-rat IgG, IgM, or IgA antisera. Cells with cytoplasmic immunoglobulin were designated as plasma cells.

Statistical analysis. Titer values for serum anti-*M. pulmonis* IgA were converted to titer counts to avoid a skewed distribution for statistical analysis, but the means were expressed as average titers (30). All nonparametric data were analyzed by the rank sum test. Other data were analyzed by the analysis of variance technique with the Duncan multiple-range test modified for unequal samples (27) used to detect which means were different. Correlation analysis was used to determine which variables were closely linked. A value of $P < 0.05$ was accepted as significant.

RESULTS

Isolation of *M. pulmonis*. *M. pulmonis* was isolated sporadically from lung lavages of F344 and LEW infected animals through 7 days post-inoculation. Thereafter, the organism was isolated from all experimentally infected animals. Control animals were negative for *M. pulmonis*.

Development of upper respiratory tract lesions. Neither LEW nor F344 control rats showed histological changes in the upper respiratory tract. The earliest lesion seen in infected rats was a mild, acute, mucopurulent rhinitis found in LEW rats 3 days postinfection (lesion index not significantly different from controls). By 7 days, significant ($P < 0.05$) rhinitis and laryngotracheitis were present in all LEW and F344 rats. The peak severity of rhinitis occurred at 14 days postinfection in both strains (lesion indices, 0.93 ± 0.09 and 0.50 ± 0.17 for LEW and F344 rats, respectively) and did not diminish by 120 days postinfection. The peak severity of laryngotracheitis did not occur until 28 days postinfection (lesion indices, 1.0 ± 0.00 and 0.94 ± 0.09 for LEW and F344 rats, respectively) and did not abate.

Significant otitis media did not occur until 14 days postinfection and peaked at 28 days (lesion indices, 0.90 ± 0.15 and 0.61 ± 0.17 for LEW and F344 rats, respectively). Although these lesions did not regress in LEW rats, there was a significant reduction ($P < 0.05$) in the severity of otitis media in F344 rats by 60 days (lesion index, 0.30 ± 0.07).

Development of lung lesions. Lung lesions were not observed at any time in control rats. As noted previously (28), rats normally have lymphoid tissue in the walls of major bronchi, i.e., BALT. The normal ratio of BALT area to lung area averaged 0.002 for both LEW and F344 rats.

BALT hyperplasia was the first major histological change noted in the lungs after *M. pulmonis* infection. Significant BALT hyperplasia occurred 14 days earlier in LEW rats than in F344 rats and was consistently more extensive in this strain (Fig. 1). When BALT hyperplasia was maximal in F344 rats (28 days post-inoculation), the BALT/lung ratio was still two times greater in LEW rats.

Inoculated LEW rats also developed extensive parenchymal disease and thus more severe overall lung disease than F344 rats (Fig. 1). Accumulation of mononuclear cells and small numbers of polymorphonuclear leukocytes in alveoli was the major component of parenchymal disease (80 to 90%) in LEW rats, with perivascular and peribronchiolar lymphoid infiltration contributing significantly (up to 10% of the total parenchymal disease ratio). This peripheral lymphoid tissue comprised from 10 to 20% of the total lymphoid ratio in LEW rats. In contrast, inoculated F344 rats did not develop significant parenchymal disease. A few of these animals developed peribronchiolar and perivascular lymphoid infiltrates, but the extent was minimal. Thus, the total lung lesion ratio was essentially a reflection of BALT hyperplasia in F344 rats. Although extensive lung lesions persisted in LEW rats, those present in F344 rats showed a tendency to resolve by 120 days.

Total lung lymphoid cells. Histological examination of sections of residual lung fragments (those remaining after mechanical disaggregation) from both control and infected rats revealed that most lymphoid cells were removed

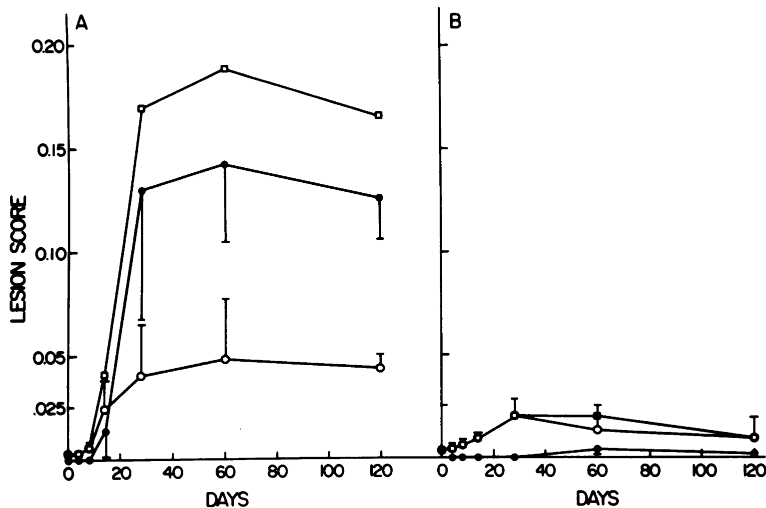

FIG. 1. Lung lesion ratios in LEW (A) and F344 (B) rats. Each point represents the mean for four to six animals. Standard deviation bars have been omitted for the total lesion/lung ratio curve (□) for clarity. BALT/lung ratios for infected rats (○) first became significantly different from that for controls at 14 and 28 days postinfection for LEW ($P < 0.001$) and F344 ($P < 0.06$) rats, respectively. The parenchymal lesion/lung ratio (●) for LEW rats was significantly different ($P < 0.001$) from that for controls at 28 days postinfection. Total lesion/lung ratios for infected rats were significantly different ($P < 0.001$ and 0.0027 for LEW and F344 values, respectively) from that for controls at 28 days postinfection. The 120-day total lesion ratio for F344 rats was not significantly different from that for controls.

from the lungs by the isolation technique used. The small number of remaining lymphoid cells were usually in the submucosa of the bronchi.

Regardless of the source (control or infected F344 or LEW rats), the final cell population was composed of 85 to 95% viable cells, 90 to 95% lymphocytes, and less than 5% macrophages. Epithelial cells accounted for most of the dead cells. Erythrocytes were rarely seen in the starting population, indicating that blood contamination was minimal. Of the final cell population, 1 to 2% contained latex or iron particles or both and were considered functional macrophages. These cells served as a control in later steps to allow detection of IMF staining due to Fc receptors.

The total number of lymphoid cells recovered from the lungs of control rats of both strains remained constant throughout the observation period (Fig. 2). However, the total number recovered from infected animals increased markedly in both strains (Fig. 2). In LEW rats, the increase was apparent by 14 days and continued throughout 120 days. However, the increase in infected F344 rats was not striking until 28 days after inoculation. Even then, more than twice as many lymphoid cells were recovered from infected LEW rats as from infected F344 animals. As time post-inoculation increased, the magnitude of this difference between the strains increased.

Class distribution of lymphoid cells recovered from control rats. There were differences in the subpopulations of lymphoid cells recovered from control animals killed at various times after inoculation with sterile broth. In LEW rats, these differences consisted of a decrease in unidentified mononuclear cells (from $9.4 \times 10^5 \pm 1.0 \times 10^5$ to $4.7 \times 10^5 \pm 2.0 \times 10^5$; $P < 0.001$) accompanied by an increase in both B lymphocytes and plasma cells (Table 1). The increase in total B lymphocytes was due to a significant increase ($P < 0.01$) for all B cell subclasses, i.e., cells carrying IgM, IgG, or IgA on their surfaces. However, only IgM and IgA plasma cells increased significantly; $P < 0.005$ and $P < 0.04$, respectively.

In control F344 rats, the unidentified mononuclear cell numbers did not change, but there was an increase in T lymphocytes ($5.4 \times 10^5 \pm 0.4 \times 10^5$ at day 0 to $6.5 \times 10^5 \pm 0.8 \times 10^5$ at day 60; $P < 0.006$), B lymphocytes, and plasma cells at 60 days post-inoculation (Table 1). This increase was due to an increase in IgG B lymphocytes ($P < 0.004$) and IgM and IgA plasma cells ($P < 0.01$ and $P < 0.0001$, respectively). The absolute numbers of IgG B lymphocytes and IgA plasma cells at 120 days remained significantly higher than the earlier 0- and 28-day values, even though the total B lymphocyte and plasma cell numbers were not statistically higher than earlier values.

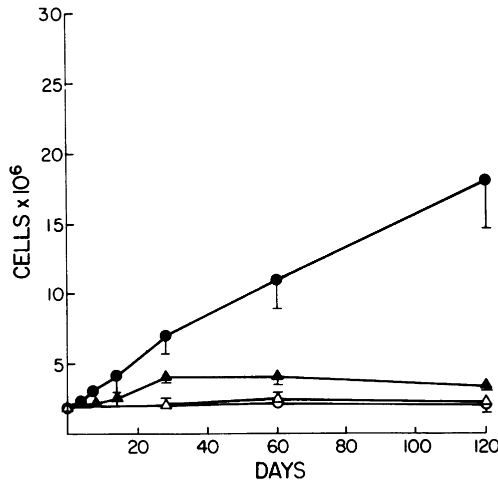

FIG. 2. Total number of lymphoid cells isolated from lungs of LEW (○) and F344 (△) control and *M. pulmonis*-infected LEW (●) and F344 (▲) rats. Points represent the mean ± standard deviation for four to six animals. LEW means differed from each other at $P < 0.0001$; the 14-day mean for infected rats was different from earlier time points as well as from that for controls. The 28-, 60-, and 120-day means for infected F344 rats were significantly different from that for controls ($P < 0.0001$) but not from each other.

Class distribution of lymphoid cells recovered from infected rats. The absolute numbers of T lymphocytes, B lymphocytes, and plasma cells recovered from the lungs of *M. pulmonis*-infected rats of both strains are shown in Fig. 3. The increase in cells of all three classes in both strains after infection with *M. pulmonis* greatly exceeded changes described earlier for control rats. T and B lymphocyte populations reached levels significantly different from that of controls 1 to 2 weeks earlier in LEW rats. All three classes increased constantly with time in LEW rats, whereas in F344 rats, a maximum was reached by 28 or 60 days post-inoculation.

The B lymphocyte increase in *M. pulmonis*-inoculated F344 rats was due almost entirely to a significant increase in cells carrying IgA on their surfaces ($P < 0.0001$). There was an approximate doubling of the IgA B cells by 28 days ($3.2 \times 10^5 \pm 0.05 \times 10^5$ at time 0 and $6.2 \times 10^5 \pm 0.7 \times 10^5$ at 28 days). In contrast, all B cell classes increased significantly in LEW rats.

The number of IgM B cells from infected LEW rats reached levels statistically different ($P < 0.01$) from control values ($5.0 \times 10^5 \pm 2.3 \times 10^5$ for infected rats; $1.8 \times 10^5 \pm 0.006 \times 10^5$ for control rats) by 14 days postinfection. The numbers of IgG and IgA B lymphocytes in infected LEW rats first became significantly different ($P < 0.01$) from control values at 28 days (IgG, $0.4 \times 10^5 \pm 0.2 \times 10^5$ at day 0 to $3.6 \times 10^5 \pm 0.9 \times 10^5$ at day 28; IgA, $1.8 \times 10^5 \pm 0.4 \times 10^5$ at day 0 to $13.1 \times 10^5 \pm 2.1 \times 10^5$ at day 28). Both subclasses continued to increase in infected LEW rats, reaching $15.5 \times 10^5 \pm 2.9 \times 10^5$ IgG and $29.7 \times 10^5 \pm 6.2 \times 10^5$ IgA B lymphocytes at 120 days postinfection.

In both strains, the total number of B lymphocytes as determined by IMF staining with ABS did not differ significantly from the total determined by addition of the number of cells in each subclass.

Plasma cell classes in infected LEW and F344 rats are shown in Table 2. Cells with intracellular immunoglobulin of each class, IgM, IgG, and IgA, increased after *M. pulmonis* inoculation in each strain. In LEW rats, only IgG plasma cells reached a plateau. In F344 rats, plasma cells showed no significant increases after 28 days (IgG) or 60 days (IgM, IgA) postinfection.

In infected LEW rats, unidentified mononuclear cells significantly decreased ($P < 0.001$) after infection from $8.1 \times 10^5 \pm 2.1 \times 10^5$ cells at 3 days to $3.1 \times 10^5 \pm 2.4 \times 10^5$ cells at 28 days. There was no change until 120 days, when these cells had increased to $6.1 \times 10^5 \pm 3.9 \times 10^5$; however, the number was still significantly lower than that seen in control animals. In infected F344 rats, unidentified mononuclear cells averaged $9.8 \times 10^5 \pm 0.4 \times 10^5$ cells at 3 days postinfection (approximately equal to controls), increased to maximum ($12.7 \times 10^5 \pm 1.5 \times 10^5$) at 28 days ($P < 0.0001$), and decreased to control levels by 120 days.

Antibody response. In LEW rats, anti-*M. pulmonis* antibody of the IgM class peaked 7 days

TABLE 1. B lymphocytes and plasma cells isolated from the lungs of control LEW and F344 rats

Rat strain	Days post-inoculation with sterile broth	No. of B lymphocytes isolated ($\times 10^5$)[a]	No. of plasma cells isolated ($\times 10^4$)[a]
LEW	0	2.8 ± 0.1[b]	7.6 ± 1.4[b]
	60	4.0 ± 0.4[b]	15.1 ± 2.0[c]
	120	8.0 ± 1.2[c]	10.2 ± 2.4[b]
F344	0	4.4 ± 0.5[b]	9.7 ± 1.3[b]
	28	5.1 ± 0.3[b]	12.2 ± 1.4[b]
	60	6.2 ± 1.0[c]	21.0 ± 5.2[c]
	120	5.3 ± 0.05[b]	11.5 ± 1.4[b]

[a] Numbers represent mean absolute numbers of specific cell type ± standard deviation for four to six animals.

[b,c] A significant difference in mean absolute numbers existed at $P < 0.0001$ and $P < 0.004$ for LEW and F344 B lymphocytes, respectively, and $P < 0.0001$ for both LEW and F344 plasma cells. Letter superscripts are used to identify which means are different within a strain. Means within the same strain with the same superscript are not statistically different.

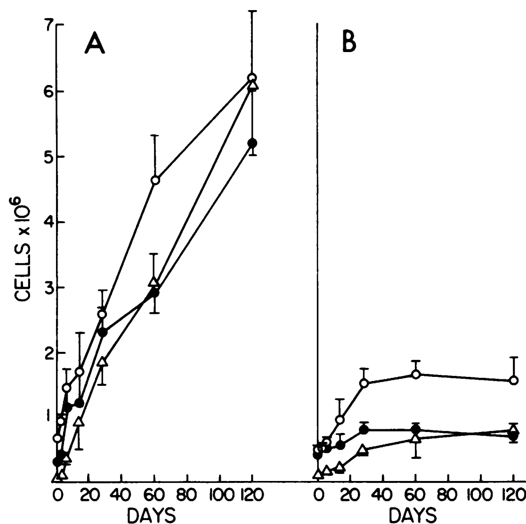

FIG. 3. Classes of lymphoid cells isolated from lungs of *M. pulmonis*-inoculated LEW (A) and F344 (B) rats. Points represent mean ± standard deviation for four to six animals. Significant differences occurred in each group of means from both strains ($P <$ 0.0001). LEW T and B lymphocytes were significantly higher than those of controls at 7 days post-inoculation, whereas plasma cell numbers were significantly higher at 28 days. F344 means were significantly higher than those for controls at 14 days for T lymphocytes and 28 days for both B lymphocytes and plasma cells and showed no significant increases after 28 days for lymphocytes and 60 days for plasma cells. Symbols: ○, T cells; ●, B cells; △, plasma cells.

postinfection (mean ELISA value, 0.39 ± 0.08) and slowly declined thereafter but never returned to that of control animals (mean IgM ELISA value for LEW control rats, <0.05). In contrast, IgM levels rose slowly in infected F344 rats and did not become significantly different from controls until 120 days postinfection (mean IgM ELISA value, 0.12 ± 0.01 for F344 infected rats and <0.05 for F344 control rats).

LEW rats showed an earlier rise in anti-*M. pulmonis* IgG than did F344 rats (significantly different [$P < 0.01$] from controls at 7 and 14 days for LEW and F344 rats, respectively). However, the shape of the antibody production curve was similar for both strains, and by 60 days postinfection, F344 rats had approximately as much circulating IgG antibody as did LEW rats (mean IgG ELISA values, 0.79 ± 0.05 and 0.81 ± 0.02, respectively). The ELISA IgG value for control LEW and F344 rats was <0.02.

Only in anti-*M. pulmonis* antibody of the IgA class at 28 days post-inoculation did F344 rats show a higher antibody response (1:32 mean IMF titer) than did LEW rats (1:4 mean IMF titer). At all other time points, infected LEW rats had an equivalent or higher serum IgA titer than did infected F344 rats. In general, LEW and F344 IgA titers were within one dilution of each other. Control rats of both strains consistently had IMF titers of ≦1:2.

Anti-*M. pulmonis* IgG was detected in pooled tracheobronchial lavages from 14 days through 120 days in *M. pulmonis*-inoculated LEW rats and from 28 days onward in infected F344 animals.

Correlations. Those variables that showed a high degree of correlation (r = 0.7 to 0.9) with lung lesions are shown in Table 3. In addition to those relationships shown in the table, the absolute number of T lymphocytes correlated highly with absolute numbers of B lymphocytes, plasma cells, IgG- and IgA-producing plasma cells, and IgA B lymphocytes in both LEW and F344 rats. In infected LEW rats, but not in infected F344 rats, T lymphocyte numbers also correlated highly with IgM and IgG B cells.

DISCUSSION

The results of the present studies clearly show that F344 rats do not develop as severe pulmonary lesions after *M. pulmonis* inoculation as do LEW rats and that the difference is not simply a delay in lesion development in F344 rats. Neither does the difference appear to be related to mere organism persistence in one strain versus another. Numbers of *M. pulmonis* in the lower respiratory tract sufficient to allow recovery from tracheobronchial lavages were present in both F344 and LEW rats at 7 days post-inoculation and persisted in the lungs of both strains for up to 120 days. This observation strongly suggests that the difference in lung lesion severity is related to differences in the type or degree of host response. This hypothesis is verified by the striking differences found in the lymphoid cell responses of the two strains of rats.

The present studies are the first to define the changes in total lung lymphoid cells during development and progression of a specific disease. Although they do not determine the origin of the cells involved (i.e., local versus those from the circulating pool), the results strongly suggest that the collection method used provides a representative sample of the lymphoid cells present. The number of lymphoid cells recovered always correlated highly with the amount of lymphoid tissue measured by histological evaluation. In both LEW and F344 *M. pulmonis*-infected rats, the total number of lung lymphocytes became statistically different from controls at the same time that BALT hyperplasia, total lymphoid tissue in infected lungs, or both became significantly different from control lungs. In addition, histological analysis of lung fragments consistently showed only a limited num-

TABLE 2. Classes of plasma cells isolated from the lungs of infected LEW and F344 rats

Rat strain	Days post-inoculation	No. of cells ($\times 10^5$) isolated with the following class of cytoplasmic immunoglobulin[a]:		
		IgM	IgG	IgA
LEW	3[b]	0.4 ± 0.08^{c}	0.1 ± 0.01^{c}	0.7 ± 0.2^{c}
	7	$2.0 \pm 0.06^{c,d,e}$	0.3 ± 0.1^{c}	1.9 ± 0.9^{c}
	14	$3.2 \pm 1.8^{d,e}$	0.5 ± 0.4^{c}	4.2 ± 1.8^{c}
	28	$2.8 \pm 0.07^{c,d,e}$	3.6 ± 1.0^{d}	12.1 ± 1.9^{d}
	60	4.7 ± 0.05^{e}	8.2 ± 2.0^{e}	19.5 ± 3.0^{e}
	120	8.7 ± 3.5^{f}	7.5 ± 1.7^{e}	45.4 ± 2.9^{f}
F344	3[b]	0.6 ± 0.04^{c}	0.1 ± 0.06^{c}	0.7 ± 0.02^{c}
	7	0.8 ± 0.1^{c}	0.2 ± 0.08^{c}	0.7 ± 0.3^{c}
	14	0.2 ± 0.8^{c}	1.0 ± 0.3^{d}	0.8 ± 0.3^{c}
	28	0.4 ± 0.2^{c}	1.9 ± 0.6^{e}	2.3 ± 0.5^{d}
	60	1.0 ± 0.3^{d}	1.8 ± 0.8^{e}	4.7 ± 1.0^{e}
	120	0.9 ± 0.4^{d}	2.2 ± 0.6^{e}	4.3 ± 1.1^{e}

[a] Numbers represent mean ± standard deviation of the absolute number of cells with the appropriate intracellular immunoglobulin.
[b] No 3-day value was statistically different from control values.
[c-f] A significant difference existed for all plasma cell classes in both strains ($P < 0.001$). Letter superscripts are used to indicate differences for each cell class within each strain. Means within the same strain with the same letter superscript are not different.

ber of lymphocytes remaining after subjection to the isolation procedure.

As described in other studies (13, 21, 22, 26, 36), including our own previous studies in rats (15), a significant number of cells morphologically indistinguishable from lymphocytes failed to stain with either T or B cell reagents. Furthermore, they failed to stain with anti-alveolar macrophage serum and were not adherent, phagocytic, or pinocytic. Thus, by definition, they were not classical macrophages. They may represent a subpopulation of lymphocytes possessing surface markers other than those examined in these studies or perhaps activated T lymphocytes bearing low concentrations of identifiable surface markers (6).

With the exception of unidentified mononuclear cells, all lung lymphocyte populations in LEW rats increased linearly after infection. This seemingly uncontrolled lymphocyte prolifera-

TABLE 3. Correlation coefficients between lung lesion variables and lymphoid cell or immune response variables

Rat strain	Lymphoid or immune response variable	Correlation coefficient for:				
		Alveolar consolidation	BALT hyperplasia	Total lymphoid tissue	Total parenchymal disease	Total lung disease
LEW	IgG antibody	—[a]	0.84	0.96	0.70	0.76
	IgA antibody	—	0.70	0.85	—	—
	Total lymphoid cells	0.85	0.82	0.79	0.82	0.79
	T cells	0.86	0.86	0.84	0.86	0.82
	IgG lymphocytes	0.79	0.75	0.72	0.77	0.73
	IgG plasma cells	0.90	0.89	0.87	0.91	0.87
	IgM lymphocytes	0.76	0.75	0.74	0.70	0.73
	IgM plasma cells	0.80	0.82	0.80	0.79	0.76
	IgA lymphocytes	0.87	0.83	0.80	0.84	0.81
	IgA plasma cells	0.80	0.76	0.74	0.77	0.74
F344	IgG antibody	—	—	0.72	—	—
	IgA antibody	—	—	0.81	—	—
	Total lymphoid cells	—	0.91	0.94	0.79	0.91
	T cells	—	0.87	0.90	0.80	0.88
	IgG plasma cells	—	0.86	0.86	0.73	0.81
	IgA lymphocytes	—	0.90	0.87	0.70	0.82
	IgA plasma cells	—	—	—	0.78	—

[a] —, Correlation coefficient of less than 0.7.

tion was in contrast to that observed in infected F344 rats. In the latter, increases were noted only in T lymphocytes, plasma cells (predominantly IgG and IgA), and IgA B lymphocytes. More importantly, each responding cell type reached a maximum at either 28 or 60 days after infection in F344 rats, whereas each cell type continued to increase throughout the 120-day observation period in LEW rats.

Serum antibody responses in the IgG and IgA classes did not follow the expected pattern based upon the differences observed in the cellular responses. For example, approximately 10 times as many IgG B cells and 4 times as many IgG plasma cells were found in infected LEW rats as compared with F344 rats, yet the specific anti-*M. pulmonis* IgG response in the two strains was roughly parallel. The same relation held true, although to a lesser extent, for specific IgA antibody responses and IgA cellular responses.

The simplest explanation for the differences in antibody responses as compared with absolute numbers of cells is a difference in the affinity of the antibody produced between the two strains. ELISA values are strongly influenced by antibody affinity (5); thus, if the affinity of the anti-*M. pulmonis* antibodies produced by LEW rats was considerably lower than that produced by F344 rats, there might not be large differences in the ELISA values even if LEW rats actually produced more antibody in terms of milligrams of protein. Alternatively, the two strains may differ in the proportion of antibody present in local versus systemic compartments, thus accounting for the differences.

The use of lung lavages to quantitate local antibody requires the assumption that either the entire lung is available to the lavage fluid or at least equal proportions of the lungs are available in all animals. These assumptions are obviously false when the lungs are affected with a pneumonic disease. The correct method would require homogenization of the lungs and quantitation of local antibody in the homogenate. Quantitation of local antibody was not attempted in this study because homogenization would have precluded histological examination of the lungs and isolation of viable lung lymphocytes. For this reason, compartmentalization differences between the two rat strains cannot be dismissed.

Neither of the above hypotheses, however, totally explains the continued cell proliferation seen in LEW rats as compared with the leveling off in F344 rats. Immune responses and presumably lymphoid cell proliferation are known to be under various control mechanisms which may involve T lymphocytes, macrophages, feedback inhibition by antibody, or all three (25, 38, 39). It is possible that there is a difference in control mechanisms between the two strains. The recent availability of monoclonal antisera that recognize rat T helper and suppressor lymphocytes (6, 41) should allow experimental examination of this hypothesis.

Another possibility that would explain both the differences in cell proliferation and the antibody responses is that only part of the proliferating lymphoid cells may be responding specifically to *M. pulmonis*. This organism is known to be a polyclonal B cell activator and a nonspecific mitogen for both rat B and T lymphocytes in vitro (32–34). Interestingly, much of the work demonstrating nonspecific mitogenicity for *M. pulmonis* has utilized splenic lymphocytes from LEW rats (33). These rats are also known to be high responders to other mitogens (35). Although the mitogenic potential has not been evaluated in F344 rats, these animals have been shown to be low responders to other mitogens and chemically defined antigens (11, 14, 38). It thus seems reasonable to suggest that the differences in lung lesions between LEW and F344 rats may be related to strain variations in the level of mycoplasma mitogen-induced lymphocyte proliferation. Mitogenic preparations of mycoplasmal membranes inoculated intranasally in LEW rats produce pathological effects similar to those induced by viable *M. pulmonis* (32). Hyperresponsiveness to polyclonal activators has been suggested to play a role in other chronic inflammatory diseases, such as lupus and periodontitis (9, 16).

Murine respiratory mycoplasmosis in LEW and F344 rats would seem to be an ideal model system in which to investigate the role(s) of nonspecific mitogenicity and regulatory mechanisms in chronic lung disease. These two rat strains share major histocompatibility antigens and many of the same lymphocyte differentiation antigens (11, 17, 19). In fact, skin graft rejection between the two strains is delayed (19), and kidney grafts are often accepted (17). Thus, cell cooperation between macrophages and lymphocytes or between different lymphocyte populations might occur across the strain barrier. If so, this would provide a means of investigating the role of specific cell types in control of mycoplasmal antigen or mitogen mediated proliferation or both and ultimately their relationship to lesion production or lung defense.

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