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STRAIN-RELATED DIFFERENCES OF NONSPECIFIC RESPIRATORY DEFENSE MECHANISMS IN RATS USING A PULMONARY INFECTIVITY MODEL

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A number of animal studies have assessed pulmonary host defense mechanisms by inoculating the lungs with the bacterial agent, Listeria monocytogenes. *Most studies use only a single strain of the animal to be tested; however, strain-related differences in responsiveness to pulmonary toxicants have been well documented. It was the goal of this current investigation to measure the pulmonary defense responses of two different strains of rats in a lung infectivity model. Fischer 344 (F344) and Sprague-Dawley (SD) rats were instilled intratracheally with* 5×10^3 *or* 5×10^5 L. monocytogenes, *and the effect on mortality, lung injury and inflammation, pulmonary bacterial clearance, and alveolar macrophage (AM) function was determined at 3, 5, and 7 days after bacteria treatment. Pulmonary inoculation with the higher (*5×10^5 L. monocytogenes*) dose proved to be highly pneumotoxic to the F344 rats as evidenced by an increase in mortality and more severe lung injury and inflammation when compared with the SD rats. After intratracheal instillation with the lower (*5×10^3 L. monocytogenes*) dose, pulmonary bacterial clearance was slowed and an increase in pulmonary responsiveness was observed for the F344 rats as compared to the SD rats. Specifically, the total number of neutrophils recovered from the lungs and tumor necrosis factor-α secreted by AMs were elevated for the F344 group throughout the 7 days, while cellular chemiluminescence, an index of reactive oxygen species production, and lung albumin and lactate dehydrogenase, indicators of injury, were increased at 3 and 5 days after bacterial instillation. This study demonstrated that respiratory defense function was compromised in F344 rats as evidenced by elevated mortality, slowed pulmonary bacterial clearance, and altered AM function. F344 rats may then represent a sensitive model for the examination of respiratory defense mechanisms after bacterial challenge.*

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Most scientific studies are carried out using a single strain of the test animals; however, strain-related differences in responsiveness to chemicals have been well documented (Kacew & Festing, 1996). This is especially true when comparing the responses of Fischer 344 (F344) and Sprague-Dawley (SD) rats after lung exposure to a variety of drugs and inhaled toxicants. In one study, F344 rats, an inbred strain, were more susceptible to pulmonary toxicity and accumulation of phospholipid after treatment with the antiarrhythmic drug amiodarone as compared to SD rats, an outbred strain (Reasor et al., 1988). Clarke et al. (2000) reported that F344 rats were more sensitive to the pulmonary inflammatory effects of inhaled concentrated urban air particles (CAPs) when compared with SD rats. On the other hand, SD rats were shown to be more responsive to the pulmonary hypertensive effects of monocrotaline (Pan et al., 1993), have an increased tendency to develop alveolar fibrosis after intratracheal instillation of residual oil fly ash particles (Kodavanti et al., 1997), and have an elevated expression of lavage fluid prostaglandin E_2 after short-term ozone exposure (Dye et al., 1999) when compared with F344 rats.

A number of animal studies by our group and others have used the gram-positive, facultative intracellular bacterial agent *Listeria monocytogenes* to assess pulmonary host defense mechanisms (Van Loveren et al., 1988; Jakab, 1993; Reasor et al., 1996; Antonini et al., 2000a, 2000b). *Listeria monocytogenes* has been shown to be an ideal agent for pulmonary defense studies. The initial immune response of the host after *L. monocytogenes* infection is marked by alveolar macrophage (AM) activation and rapid recruitment of AMs and neutrophils (PMNs) to the site of infection (Shen et al., 1998; Seaman et al., 1999). The primary function of pulmonary defense mechanisms is to clear inhaled substances from the lungs, thus keeping the lungs sterile (Sibille & Reynolds, 1990). AMs serve as the first line of cellular defense through their ability to phagocytize inhaled particles, kill microorganisms, and function as an accessory cell in immune responses (Crystal, 1991).

Due to these observed differences in response between F344 and SD rats to pulmonary toxicants, the examination of strain variability would be important in identifying the most sensitive rat strain to be used in a pulmonary infectivity model. It was the objective of current investigation to evaluate the pulmonary defense responses of two different strains of rats after intratracheal inoculation with a bacterial pathogen. F344 and SD rats were instilled intratracheally with *L. monocytogenes*, and the effect on mortality, lung injury and inflammation, pulmonary bacterial clearance, and AM function was determined. This comparison of two different rat strains may be useful in elucidating potential mechanisms involved with alterations in pulmonary defense responses after exposure to bacterial pathogens or to both gaseous and particle pollutants.

MATERIALS AND METHODS

Animals

Young adult male Sprague-Dawley (SD) rats 10 wk of age with a mean body weight of 268.9 ± 5.17 g were obtained from Hilltop Laboratories (Scottdale, PA). Young adult male Fischer 344 (F344) rats 10 wk of age with a mean body weight of 219.2 ± 3.82 g were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). All rats were given a conventional laboratory diet and tap water ad libitum, and allowed to acclimate for 1 wk before use.

Intratracheal Bacteria Inoculation

Listeria monocytogenes (strain 10403S, serotype 1) was obtained as a gift from Rosana Schafer of the Department of Microbiology and Immunology at West Virginia University. *Listeria monocytogenes* was cultured overnight in brain heart infusion broth (Difco Laboratories, Detroit, MI) at 37°C in a shaking incubator. Following incubation, the bacterial concentration was determined spectrophotometrically at an optical density of 600 nm and diluted with sterile saline to the desired concentrations.

The rats from each strain were lightly anesthetized by an intraperitoneal injection of 0.6 ml of a 1% solution of sodium methohexital (Brevital, Eli Lilly, Indianapolis, IN) and inoculated intratracheally with either 5×10^3 or 5×10^5 *L. monocytogenes* in 500 µl of sterile saline, according to the method of Brain et al. (1976). Animals in the vehicle control group were intratracheally dosed with 500 µl of sterile saline.

Mortality/Histopathology

Animal weights and mortality were monitored over the course of the treatment period. Histopathological analysis was performed on the lungs from each group. Rats were euthanized with sodium pentobarbital and the lungs were preserved with 10% buffered formalin by airway fixation at total lung capacity. The lobes of the lungs were removed, sectioned, embedded in paraffin, and stained with hematoxylin and eosin.

Bronchoalveolar Lavage

At 3, 5, and 7 days after bacterial instillation, the rats were deeply anesthetized with an overdose of sodium pentobarbital and then exsanguinated by severing the abdominal aorta. The left bronchus was clamped off, and bronchoalveolar lavage (BAL) was performed on the right lungs of rats from each group. Their right lungs were first lavaged with a 4-ml aliquot of calcium- and magnesium-free phosphate buffer solution (PBS), pH 7.4. This first BAL fluid sample was centrifuged at 500 × g for 10 min and filtered with 0.22-µm sterile filters, and the resultant cell-free supernatant was analyzed for various biochemical parameters. Then the lungs

were lavaged with 6-ml aliquots of PBS until 50 ml was collected. These samples were also centrifuged for 10 min at 500 × g and the cell-free BAL fluid was discarded. The cell pellets from all washes for each rat were combined, washed, and resuspended in 1 ml PBS buffer and evaluated as described later.

Pulmonary Clearance of *L. monocytogenes*

At 3, 5, and 7 days after bacterial instillation, left lungs were removed from all rats in each treatment group. The excised tissues were suspended in 10 ml sterile water, homogenized using a Polytron 2100 homogenizer (Brinkmann Instruments, Westbury, NY), and cultured quantitatively on brain heart infusion agar plates (Becton Dickinson and Co., Cockeysville, MD). The number of viable colony-forming units (CFU) were counted after an overnight incubation at 37°C.

Cellular Evaluation

Total cell numbers were determined using a Coulter Multisizer II and AccuComp software (Coulter Electronics, Hialeah, FL). Cells were differentiated using a Cytospin 3 centrifuge (Shandon Life Sciences International, Cheshire, England); 1.0×10^5 cells were spun for 5 min at 800 rpm and pelleted onto a slide. Cells (200/rat) were identified on cytocentrifuge-prepared slides after labeling with Leukostat stain (Fisher Scientific, Pittsburgh, PA).

Biochemical Parameters of Injury

Within the acellular BAL fluid from the first 4-ml lavage sample, albumin content, a measure of increased permeability of the bronchoalveolar–capillary barrier, and lactate dehydrogenase (LDH) activity, an indicator of general cytotoxicity, were measured. Albumin content was determined colorimetrically at 628 nm based on albumin binding to bromcresol green using an albumin BCG diagnostic kit (Sigma Chemical Company, St. Louis, MO). Measurements were performed with a Cobas Fara II analyzer (Roche Diagnostic Systems, Montclair, NJ). LDH activity was determined by measuring the reduction of pyruvate coupled with the oxidation of NADH at 340 nm according to the method of Wroblewski and LaDue (1955). LDH enzyme reagents were purchased from Roche Diagnostics Systems (Indianapolis, IN).

Luminol-Dependent Chemiluminescence

Luminol-dependent chemiluminescence (CL), a measure of light generation representing reactive oxidant species (ROS) production, was performed with an automated Berthold Autolumat LB 953 luminometer (Wallace, Inc., Gaithersburg, MD) as described previously (Antonini et al., 1994). CL was measured in a total volume of 0.5 ml HEPES buffer. Resting CL was determined by incubating 0.5×10^6 BAL cells at 37°C for 10 min in 0.008 mg% (w/v) luminol (Sigma Chemical Company, St. Louis, MO) followed by

the measurement of CL for 15 min. Luminol is used as an amplifier to enhance detection of the light. Both nonopsonized, insoluble zymosan (2 mg/ml; Sigma Chemical Co., St. Louis, MO) and soluble phorbol myristate acetate (PMA; 0.5 μg/ml; Sigma Chemical Co., St. Louis, MO) were used as stimulants and were added to the assay immediately prior to measurement of CL. Since neutrophils do not respond to unopsonized zymosan, the zymosan-stimulated CL produced is from AMs (Castranova et al., 1990), while PMA-stimulated CL represents light generation from all recovered phagocytes (AMs + PMNs). Measurement of CL was recorded for 15 min at 37°C, and the integral of counts per minute (cpm) versus time was calculated. CL was calculated as the cpm of stimulated cells minus the cpm of the corresponding resting cells.

Tumor Necrosis Factor-α Secretion

BAL cells were suspended at a concentration of 1.0×10^6 cells/ml in essential minimum Eagle's medium (EMEM, Biowhittaker, Walkersville, MD) supplemented with 2 m*M* glutamine, 100 g/ml streptomycin, 100 units/ml penicillin, and 10% heat-activated fetal calf serum, and seeded onto each well of a 24-well tissue culture plate. BAL cells were allowed to adhere to the plates for 2 h at 37°C at 5% CO_2. After the incubation, nonadherent cells were removed by washing three times with EMEM media. The adherent cells, which were found to be >90% AMs, were then incubated in fresh EMEM for 18 h at 37°C at 5% CO_2. The AM-conditioned media was collected, centrifuged, and stored at –70°C until analysis. The content of TNF-α in AM-conditioned supernatants was quantified by an enzyme-linked immunosorbent assay (ELISA) using a commercial kit (BioSource International, Inc., Camarillo, CA). The production of TNF-α was expressed as ng/10^6 AM.

Statistical Analysis

Results are expressed as means ± standard error of measurement (SE). Statistical analyses were carried out with the JMP IN statistical program (SAS, Inc., Belmont, CA). The two rat strains were dosed on the same day with the same bacterial preparation. The low-dose and high-dose *L. monocytogenes* treatments were performed as separate experiments. The significance of the interaction between the two rat strains for the different parameters at each time point was assessed using an analysis of variance (ANOVA). The significance of difference between individual groups was analyzed using the Tukey–Kramer post hoc test. For all analyses, the criterion of significance was set at $p < .05$.

RESULTS

The rats from each strain survived for the entire 7-day period after intratracheal inoculation with the low (5×10^3 *L. monocytogenes*) dose as

well as all SD rats exposed to the higher (5×10^5 *L. monocytogenes*) dose (Figure 1). The F344 rats exposed to the high bacteria dose began to expire as soon as 3 days after inoculation. At 4 days, only 30% of the F344 were still alive, and by 7 days, all the F344 rats had expired after the high bacteria dose treatment.

Inoculation with 5×10^3 *L. monocytogenes* had little effect on body weight for the SD rats, as they continued to gain weight over the 7-day period (Figure 2). For the F344 rats, treatment with the low bacteria dose led to a slight initial loss in body weight, and by 7 days, their weight had nearly returned back to their pre-bacteria-instillation weights. Intratracheal inoculation with the high *L. monocytogenes* dose led to a dramatic loss in body weight for both rat strains at 3 days. By 7 days, the weights of the SD rats had returned to pre-bacteria-instillation weights, while in the F344 rats, the loss in body weight continued at 5 days, and by 7 days all the rats had expired.

Histopathological analyses were performed on the lungs of F344 and SD rats after intratracheal instillation of *L. monocytogenes* (Figure 3). Lungs appeared normal for control rats from both strains prior to *L. monocytogenes* treatment (data not shown). Significant pneumonitis, characterized by a peribronchiolar accumulation of neutrophils, and the appearance of many granulomatous lesions were observed throughout the lungs of F344

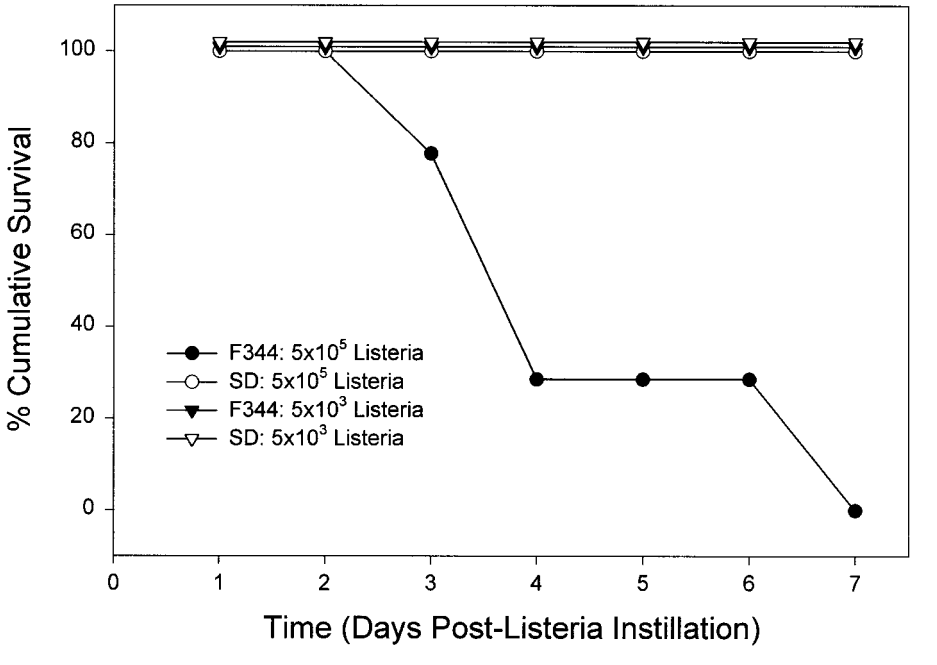

FIGURE 1. Cumulative survival of F344 and SD rats over the 7-day study period. Rats were instilled intratracheally with *L. monocytogenes* (5×10^3 or 5×10^5 bacteria).

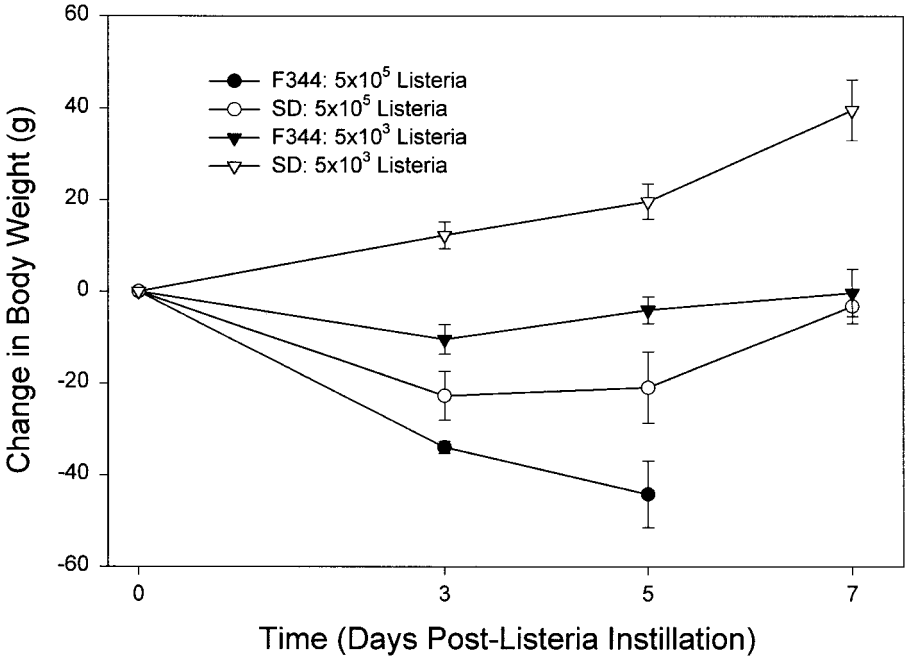

FIGURE 2. Change in body weight of F344 and SD rats over the 7-day study period. Rats were instilled intratracheally with *L. monocytogenes* (5×10^3 or 5×10^5 bacteria). Body weight values were from all the rats available at each time point. Values are means ± SE (n = 8–12/strain at each time point).

rats 5 days after intratracheal inoculation with the low *L. monocytogenes* dose (Figure 3A). Lungs appeared normal for the SD rats 5 days after the same low *L. monocytogenes* dose (Figure 3B). Five days after intratracheal instillation of the high *L. monocytogenes* dose, severe edema, a dramatic infiltration of neutrophils, and multiple granulomatous lesions with amorphous tissue debris were observed in the F344 rats (Figure 3C). Granuloma formation and neutrophil infiltration were observed in the lungs of the SD rats 5 days after intratracheal inoculation with the high *L. monocytogenes* dose (Figure 3D). The lung damage and inflammation observed in the F344 rats at both doses were markedly more extensive and more pronounced than those observed in the SD rats.

In the assessment of lung cell numbers, there was a lower number of AMs recovered from the F344 rats as compared to the SD rats prior to bacteria treatment, and there was no difference in PMNs recovered between the two strains (Figure 4). Intratracheal inoculation with 5×10^3 *L. monocytogenes* had no effect on AM recovery. Significantly fewer AMs were recovered from the lungs of the F344 rats compared to the SD rats at 3 and 5 days postinstillation (Figure 4A), but greater numbers of recovered PMNs for the F344 rats at each time point than for SD rats (Figure 4B). A

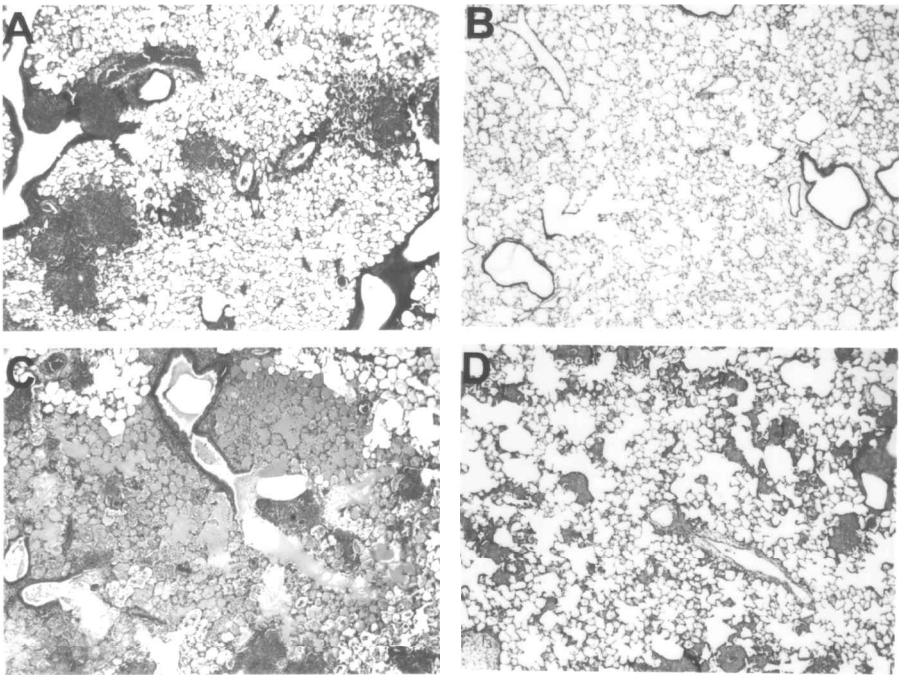

FIGURE 3. Micrographs of rat lungs stained with hematoxylin and eosin 5 days after intratracheal inoculation with *L. monocytogenes*: (A) F344 rats + 5×10^3 *L. monocytogenes*; (B) SD rats + 5×10^3 *L. monocytogenes*; (C) F344 rats + 5×10^5 *L. monocytogenes*; and (D) SD rats + 5×10^5 *L. monocytogenes*. Magnification 4×.

significant increase in the number of recovered PMNs was also observed for the F344 rats at all three time points post bacteria instillation as compared the noninfected F344 control.

In the evaluation of lung injury, there were no differences in BAL LDH and albumin between the two strains prior to bacteria treatment (Figure 5). After intratracheal instillation of 5×10^3 *L. monocytogenes*, there was a significant increase in both BAL LDH and albumin at 3 and 5 days for the F344 rats as compared to the SD rats whose values were not different from pre bacteria treatment levels. By 7 days, BAL LDH and albumin of F344 rats had returned to control levels. A significant increases in BAL LDH and albumin were also observed for the F344 rats at 3 and 5 days postinstillation as compared to the noninfected F344 control.

In the assessment of lung phagocyte function, CL and TNF-α were measured (Figures 6 and 7). Intratracheal instillation of 5×10^3 *L. monocytogenes* caused a significant increase in CL stimulated by both zymosan (a measure of AM CL) and PMA (a measure of both AM and PMN CL) for the F344 as compared to the SD rats at 3 and 5 days post bacteria treatment, with the values returning to control levels by 7 days (Figure 6). PMA-stimulated CL was significantly elevated for the SD groups at 3 days as

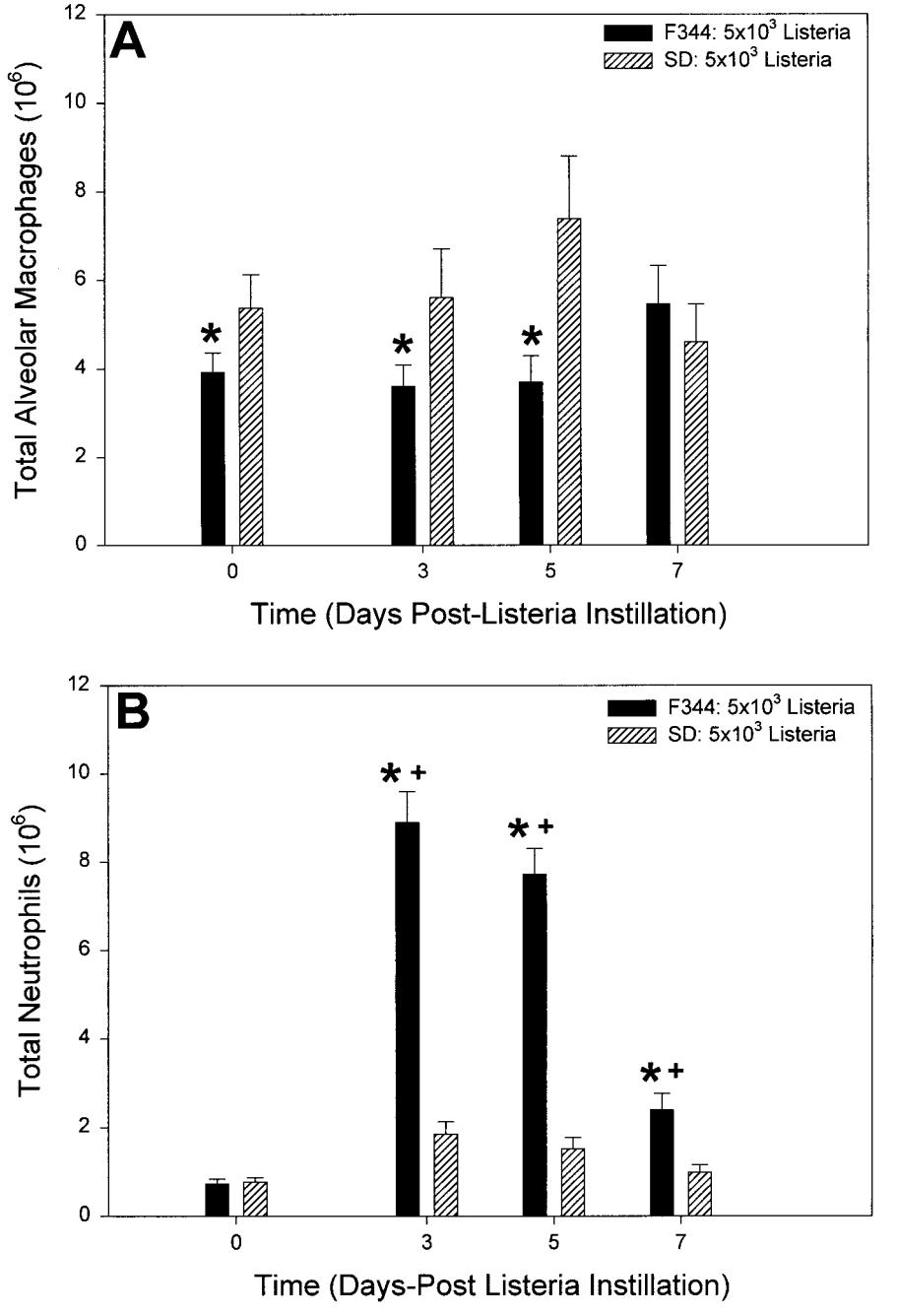

FIGURE 4. Total (A) alveolar macrophages and (B) neutrophils recovered from the lungs of F344 and SD rats after intratracheal instillation with 5×10^3 *L. monocytogenes*. Values are means ± SE (n = 8–12/strain at each time point). Asterisk indicates significantly different from SD group; plus sign, significantly greater than F344 control group ($p < .05$).

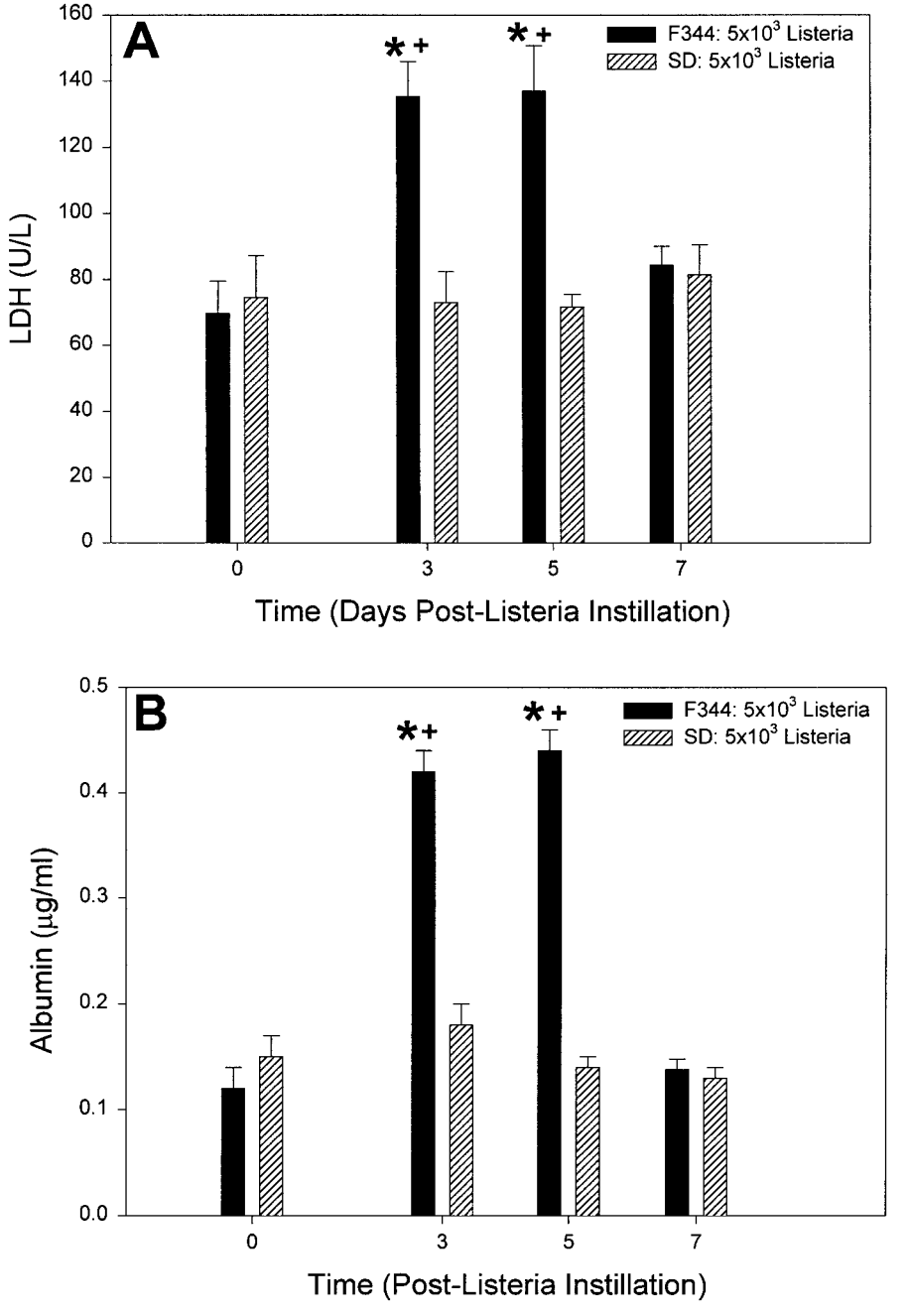

FIGURE 5. (A) LDH activity and (B) albumin of the BAL fluid recovered from the lungs of F344 and SD rats after intratracheal instillation with 5×10^3 *L. monocytogenes*. Values are means ± SE (n = 8–12/strain at each time point). Asterisk indicates significantly greater than SD group; plus sign, significantly greater than F344 control group ($p < .05$).

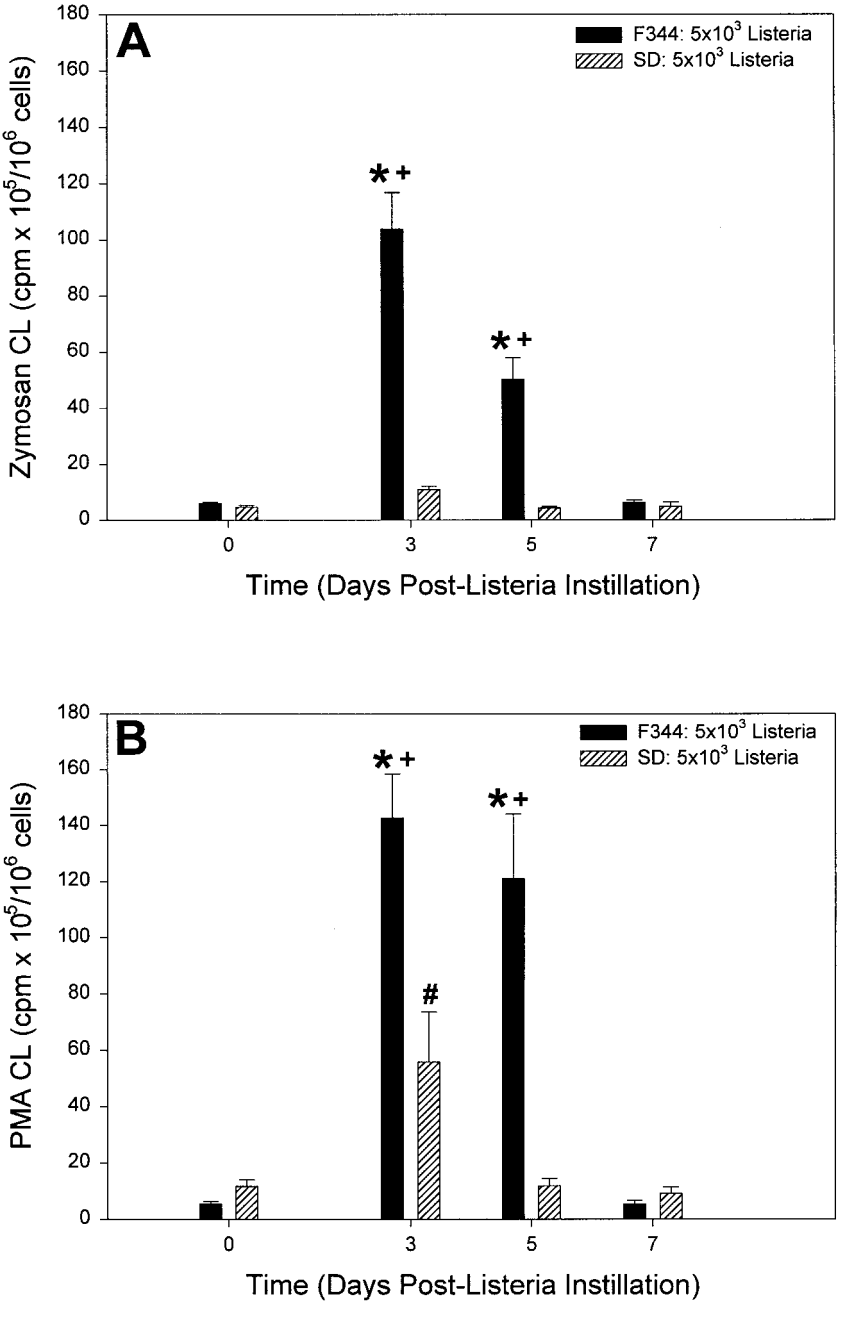

FIGURE 6. (A) Zymosan- and (B) PMA-stimulated chemiluminescence (CL) of cells recovered from the lungs of F344 and SD rats after intratracheal instillation with 5×10^3 *L. monocytogenes*. Values are means ± SE (n = 8–12/strain at each time point). Asterisk indicates significantly greater than SD group; number symbol (#), significantly than SD control group; plus sign, significantly greater than F344 control group ($p < .05$).

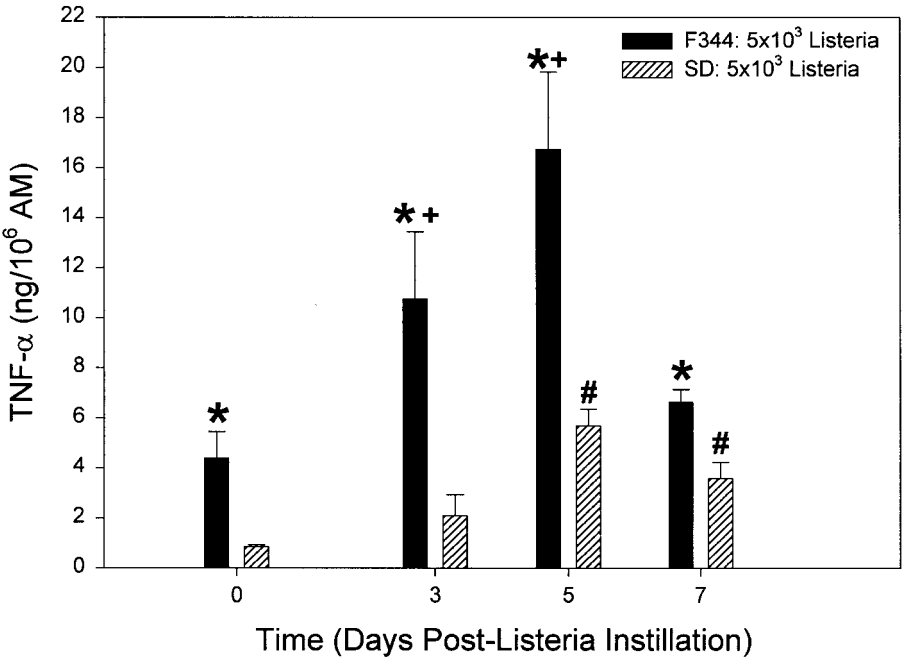

FIGURE 7. TNF-α secreted from AMs recovered from the lungs of F344 and SD rats after intratracheal instillation with 5×10^3 *L. monocytogenes*. Values are means ± SE (n = 8–12/strain at each time point). Asterisk indicates significantly greater than SD group; number symbol (#), significantly than SD control group; plus sign, significantly greater than F344 control group ($p < .05$).

compared to control, while both PMA-stimulated and zymosan-stimulated CL for the F344 rats were significantly enhanced at 3 and 5 days as compared to its noninfected control. For TNF-α produced by AMs, there were significant elevations before and at all time points after bacteria instillation for the F344 rats as compared to the SD rats (Figure 7). Significant increases in AM TNF-α were observed at 5 and 7 days for the SD rats and 3 and 5 days for the F344 rats when compared with their respective controls.

Pulmonary bacterial clearance was determined by counting the CFUs of the left lungs for both rat strains after intratracheal inoculation with 5×10^3 *L. monocytogenes* (Figure 8). The bacteria was cleared much faster from the lungs of SD rats over the 7-day period as compared to the F344 rats. There were substantially more bacteria in the lungs of the F344 rats at all three time points. Dramatic increases in bacterial number in $\log_{10}$ base units of 1.27-, 1.27-, and 1.17-fold were observed for the F344 rats as compared to the SD rats at 3, 5, and 7 days, respectively.

The different lung parameters for both F344 and SD rats were also measured after intratracheal inoculation with the high (5×10^5 *L. monocytogenes*) dose (Table 1). There were significantly more AMs, higher levels of LDH, albumin, and CL, and a greater number of bacteria in the left lung of the F344 rats as compared to the SD rats 3 days after sham challenge.

The other time points of 5 and 7 days post bacteria instillation were also determined for both strains of rats, but due to the elevated mortality for the F344 rats after exposure to the high *L. monocytogenes* dose there were not enough living F344 rats for statistical comparisons with the SD rats. By 7 days, most of bacteria had been cleared from the lungs of the SD rats, and BAL LDH and albumin values and cellular CL were not significantly different when comparing the SD-infected rats and their controls 7 days after bacterial challenge. However, AM and PMN numbers were still significantly elevated in the SD-infected rats 7 days after inoculation as compared to the noninfected SD control values.

DISCUSSION

It has been suggested that detailed studies comparing animal strain differences may help clarify underlying toxic mechanisms of specific chemicals and drugs (Kacew & Festing, 1996). Such studies are mostly conducted using inbred strains in which the genetic characteristics have been fixed, rather than in outbred strains in which individual samples of animals may differ and the phenotype is variable. Also, the observation that strains may differ needs to be addressed in evaluating the potential hazard of a substance, especially when a study involves only a single strain of animal and thus provides no assessment of possible strain variation.

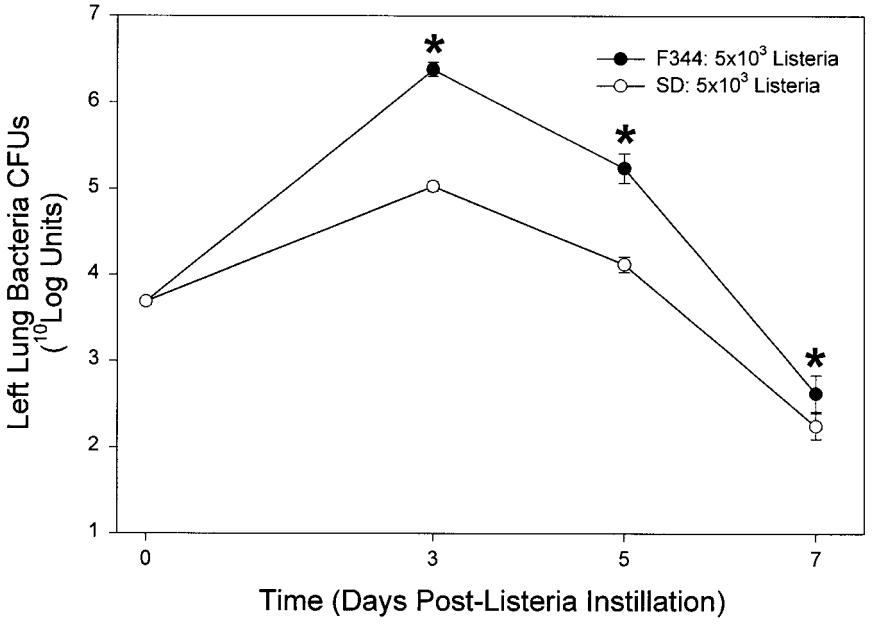

FIGURE 8. Number of bacteria CFUs in the left lung of F344 and SD rats after the intratracheal instillation of 5×10^3 *L. monocytogenes.* Values are means in $\log_{10}$ base units ± SE (n = 5–9). Asterisk indicates significantly greater than SD group ($p < .05$).

TABLE 1. F344 versus SD rats after 5×10^5 *L. monocytogenes* intratracheal inoculation

Lung parameters	F344 rats (± SE)[a]	SD rats (± SE)[a]
AMs (10^6)	3 days: 27.0 ± 5.00[e] 5 days: n.d.[d] 7 days: n.d.	3 days: 10.6 ± 1.43 5 days: 16.5 ± 2.04 7 days: 22.5 ± 3.99
PMNs (10^6)	3 days: 12.0 ± 1.20 5 days: n.d. 7 days: n.d.	3 days: 11.9 ± 2.15 5 days: 19.8 ± 3.09 7 days: 6.22 ± 1.21
LDH (U/L)	3 days: 703 ± 93.4[e] 5 days: n.d. 7 days: n.d.	3 days: 204 ± 23.1 5 days: 259 ± 40.8 7 days: 144 ± 47.8
Albumin (μg/ml)	3 days: 2.20 ± 0.26[e] 5 days: n.d. 7 days: n.d.	3 days: 0.32 ± 0.04 5 days: 0.38 ± 0.08 7 days: 0.12 ± 0.02
Zym CL (cpm x $10^5/10^6$ cell)[b]	3 days: 176 ± 36.7[e] 5 days: n.d. 7 days: n.d.	3 days: 29.4 ± 4.20 5 days: 34.1 ± 5.11 7 days: 2.11 ± 0.22
PMA CL (cpm x $10^5/10^6$ cell)	3 days: 401 ± 104[e] 5 days: n.d. 7 days: n.d.	3 days: 114 ± 9.79 5 days: 58.2 ± 9.11 7 days: 1.72 ± 0.48
Left lung CFU ($\log_{10}$ units)[c]	3 days: 8.36 ± 0.05[e] 5 days: n.d. 7 days: n.d.	3 days: 7.27 ± 0.11 5 days: 4.60 ± 0.16 7 days: 2.54 ± 0.25

[a]Values are means ± SE (n = 5–8).
[b]Zym, zymosan.
[c]CFU, colony-forming units.
[d]n.d., Not determined due to elevated mortality in F344 rats at 5 and 7 days.
[e]Significantly greater than mean value for SD rats.

Differences in responses of inbred and outbred rats after exposure to different pulmonary toxicants have been well documented. In one study, the inbred F344 rat strain has been shown to be more sensitive to oral treatment with the drug amiodarone as compared to the outbred SD rat strain (Reasor et al., 1988). It was observed that a metabolite of amiodarone, desethylamiodarone, accumulated in the lungs and AMs of F344 rats but not significantly in SD rats, suggesting the metabolite may be involved in the drug-induced pulmonary toxicity and phospholipidosis. The differences in amiodarone-induced lung toxicity between strains may also be due to drug bioavailability and distribution. McCloud et al. (1995) demonstrated that amiodarone reached sufficient concentrations to produce an effect only in lungs of the F344 rats. In another study, Clarke et al. (2000) reported that significant pulmonary inflammation was observed in F344 rats after inhalation of 100 μg/m^3 CAPs over a 3-day period, while SD rats did not respond similarly to the same levels of CAPs. SD rats didn't demonstrate significant pulmonary inflammation until exposure to the higher inhaled

CAPs concentration of 500 μg/m^3 over the 3-day exposure period (Clarke et al., 1999).

On the other hand, SD rats have been shown to be more responsive than F344 rats when treated with a variety of other pneumotoxic agents. The intratracheal instillation of residual oil fly ash led to the development of alveolar fibrosis, which was absent in the F344 rats (Kodavanti et al., 1997). This observation was also accompanied with an increased mRNA expression of Fn EIIIA(+), a protein implicated in fibrosis formation. Dye et al. (1999) demonstrated that SD rats responded differently after short-term ozone exposure (0.5 ppm × 8 h) when compared to F344 rats. A significantly greater concentration of prostaglandin E_2 was measured in the BAL fluid of ozone-exposed SD rats. Another chemical, monocrotaline, produces a pulmonary vascular disorder characterized by proliferative pulmonary vasculitis and pulmonary hypertension in SD rats. Pan et al. (1993) showed that F344 rats were more resistant to monocrotaline-induced vascular damage than SD rats, demonstrating no change in right ventricular pressure, which was significantly elevated in the more sensitive SD rats.

Due to the observed variations in responses of F344 and SD rats after exposure to these pneumotoxic substances, it is essential to identify a strain of rat that may be inherently more susceptible to alterations in lung defense processes and the development of pulmonary infection after bacterial challenge. Currently, our laboratory is using a pulmonary infectivity model in rats to investigate the effect of age as well as preexposure to different occupational particles, such as silica, residual oil fly ash, diesel exhaust particles, and welding fumes, on respiratory defense mechanisms. Thus, it was the objective of this study to compare the lung response of an inbred and outbred strain of rat after pulmonary inoculation with *L. monocytogenes* in order to identify a sensitive rat strain to use in our model. It is important to note that the difference in mortality in four different strains of mice has been observed within different strains of mice after exposure to NO_2 and challenged with *Klebsiella pneumoniae* aerosol (Ehrlich, 1980; Gardner, 1982).

Prior to bacterial challenge, only a few differences were observed between the two strains in the baseline values of the parameters that were to be measured. BAL LDH and albumin, the number of PMNs recovered, and cellular CL were not different when comparing noninfected F344 and SD rats. Dye et al. (1999) also saw no difference in most BAL fluid parameters and cell numbers when comparing control F344 and SD rats that had been exposed to air. However, we did observe a significant increase in baseline in the AM inflammatory cytokine TNF-α and a significant decrease in the number of AMs recovered from the F344 rats as compared to the SD rats. One would predict from this observation that the lungs of F344 and SD rats would likely respond differently to bacterial or particle insult.

Pulmonary inoculation with both doses of *L. monocytogenes* proved to be highly pneumotoxic to the F344 rats in comparison to SD rats as evidenced by the presence of severe edema and extensive granuloma formation, elevated inflammation, and a slowed clearance of the bacteria. An increase in mortality was also observed in the F344 rats after intratracheal instillation of the high *L. monocytogenes* dose. In contrast, the SD rats were quite resistant to the low dose of *L. monocytogenes.* Pulmonary inoculation of the SD rats with the low bacteria dose had no effect at all on the number of AMs and PMNs and on BAL levels of LDH and albumin when compared to the SD controls. The SD rats did respond to the high *L. monocytogenes* dose; however, most of the bacteria had been cleared from the lungs, and many of the parameters measured had returned to control levels by 7 days after bacterial challenge. The numbers of AMs and PMNs recovered from the SD-infected rats were still significantly elevated as compared to the noninfected controls throughout the 7 day period.

Significant alterations in AM function were also seen in the F344 rats. The generation of reactive oxygen species, as measured by cellular CL, and the secretion of TNF-α were significantly increased in F344 rats as compared to SD rats. The enhanced pulmonary injury and inflammation observed in the F344 rats could likely be attributed to this overproduction of reactive oxygen species and TNF-α. Activation of AMs and the subsequent release of both reactive oxygen species and TNF-α have been shown to be one mechanism by which pneumotoxic substances injure the lungs (Driscoll et al., 1990; Castranova et al., 1996).

It is interesting to note that the number of AMs recovered from the F344 rats was significantly lower prior to bacterial challenge as well as at 3 and 5 days after instillation of the low *L. monocytogenes* dose as compared to SD rats. At the same time, both CL and TNF-α were significantly elevated, indicating that the AMs recovered from F344 rats were more responsive than the AMs from SD rats. However, this enhancement in reactive oxygen species generation did not appear to have any effect on bacterial killing. Ohya et al. (1998) have indicated that reactive oxygen species play a significant role in bacterial killing only if AMs are activated prior to infection with *L. monocytogenes.* If the AMs are not activated, as was the case in our current investigation, reactive nitrogen intermediates are mainly involved in the macrophage intracellular killing. The number of bacteria present in the lungs of the F344 rats remained elevated throughout the treatment period, suggesting AM number plays a more important role in bacterial clearance as opposed to reactive oxygen species generation. However, if CL per AM and AM per lung are multiplied together for a "net CL/lung" value, there appears to little difference between the F344 and SD rats.

In summary, dramatic strain-related differences were observed when comparing the pulmonary responses of F344 and SD rats after intratracheal inoculation with *L. monocytogenes.* The F344 rats were shown to be more susceptible to pulmonary infection and more sensitive to changes in lung

defense mechanisms, as evidenced by altered AM number and function after bacterial exposure. Thus, F344 rats may represent a sensitive strain of rat to be used in future susceptibility and infectivity studies designed to elucidate the mechanisms that may be responsible for the alterations observed in lung defense responses after exposure to specific environmental particles and gases.

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