Strain Differences in Alveolar Neutrophil Infiltration and Macrophage Phenotypes in an Acute Lung Inflammation Model

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Pulmonary infection is a major cause of mortality and morbidity, and the magnitude of the lung inflammatory response correlates with patient survival. Previously, we have shown that neutrophil migration into joints is regulated by arthritis severity quantitative trait loci (QTLs). However, it is unclear whether these QTLs contribute to the regulation of lung inflammation in pneumonia. Therefore, to more clearly define the factors regulating acute inflammatory responses in the lung, we examined two inbred rat strains, DA and F344, that differ in these QTLs and their susceptibility to joint inflammation. Staphylococcal cell wall components lipoteichoic acid (LTA) and peptidoglycan (PGN), administered intratracheally, significantly increased the numbers of neutrophils retrieved in the bronchoalveolar lavage fluid (BALF). F344 had approximately 10-fold more neutrophils in the BALF compared with DA (P < 0.001) and higher BALF concentrations of total protein, tumor necrosis factor-α and macrophage inflammatory protein 2. LTA/PGN administration in DA×F344 congenic strains (Cia3d, Cia4, Cia5a, and Cia6) resulted in inflammation similar to that in DA, demonstrating that the genes responsible for the differences in pulmonary inflammation are not contained within the chromosomal intervals carried by these congenic strains. Alveolar macrophages (AMs) isolated from naïve F344 stimulated in vitro with LTA/PGN produced significantly higher levels of keratinocyte-derived chemokine and macrophage inflammatory protein 2 than alveolar macrophages from DA rats. The differences were related to differential mitogen-activated protein kinase phosphorylation. We conclude that the factors contributing to inflammation can be site and challenge dependent. A better understanding of site-specific inflammation may lead to more effective treatment of acute lung inflammation and injury.

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Online address: http://www.molmed.org
doi: 10.2119/molmed.2010.00064

INTRODUCTION

Pulmonary infection is a major cause of mortality and morbidity (1), particularly in patients who are critically ill (2,3). The innate immune response plays a critical role in host defense. This concerted host reaction to bacteria is influenced by the complex interplay between genetics, epigenetics and the environment and can be organ specific (4–7). Staphylococcus aureus is a leading cause of hospital-acquired and community-associated pneumonia and is typically associated with severe disease (8–10). Although many studies have led to a better understanding of the epidemiology, resistance mechanisms and potential virulence, there is still a paucity of knowledge about the mechanisms involved in the in vivo host immune response to S. aureus infections (11).

Lipoteichoic acid (LTA) and peptidoglycan (PGN) are components of the cell wall of Gram-positive bacteria, such as S. aureus, and can stimulate the generation of proinflammatory cytokines and activate leukocytes in vitro and in vivo (12–16). LTA and PGN can also induce lung inflammation in mice (16) and F344 rats (17). F344 rats have previously been shown to be arthritis resistant, whereas DA rats are susceptible to this inflammatory disease (18). Results of previous studies have demonstrated that neutrophil and macrophage migration into an inflammatory site, such as the synovial-like air pouch injected with carrageenan, is genetically regulated (19). Specifically, arthritis-susceptible DA rats have greater neutrophil and macrophage migration into the synovial inflammatory site than arthritis-resistant F344 rats (19). The difference in neutrophil migration in that
model is regulated by the arthritis-severity quantitative trait loci (QTLs) Cia4 and Cia6 (19), located on rat chromosomes 7 and 8, respectively. Moreover, other arthritis QTLs, such as Cia3d and Cia5a, have also been shown to regulate joint inflammatory responses (18,20).

Neutrophils and macrophages are critical to the pathogenesis of acute lung injury (21,22), rheumatoid arthritis (23,24) and other inflammatory diseases. Cytokines, produced by neutrophils and macrophages such as tumor necrosis factor-α (TNF-α), interleukin-8 (IL-8), macrophage migration inhibitory factor (MIF) and high mobility group box 1 (HMGB1) mediate arthritis damage and acute lung injury (25–30). These data suggest that arthritis and acute lung injury share some inflammatory mechanisms. However, it remains unclear whether the genetic differences between DA and F344 rats, in particular in arthritis QTLs, are associated with differences in the regulation of lung inflammation.

In the present study we used a pneumonia model in which lung inflammation was induced by Gram-positive bacterial components. We examined the differences in pulmonary inflammation between DA and F344 rats and investigated whether these differences are regulated by arthritis QTLs and what other factors may influence differences in lung inflammation.

MATERIALS AND METHODS

Reagents

LTA and PGN from S. aureus (Sigma-Aldrich, St Louis, MO, USA) were dissolved in 0.9% saline to the target concentration, stored at −20°C, and used within 60 d.

Animals

All experiments involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Feinstein Institute for Medical Research.

Arthritis-resistant F344 and arthritis-susceptible DA rats were purchased from Harlan Sprague Dawley (Indianapolis, IN, USA). DA.F344(Cia3d), DA.F344(Cia4), DA.F344(Cia5a) and DA.F344(Cia6) congenics were generated as previously described (18,20). Briefly, arthritis QTLs were introgressed from F344 into DA background rats through 8 to 10 genotype-guided backcrosses followed by at least 5 intercrosses. All the animals were housed in a pathogen-free environment, under standard light and dark cycles, with free access to food and water.

In Vivo Experimental Protocol

Rats were males 8- to 14-wks old and were F344 (n = 7), DA (n = 7), or the congenic strains DA.F344(Cia3d), DA.F344(Cia4), DA.F344(Cia5a) and DA.F344(Cia6) (n = 5/congenic strain) with body weights of 201–305 g. Rats were anesthetized with isoflurane. The tracheas were surgically exposed and LTA 10 μg/kg and PGN 33 μg/kg (a dose that we have shown previously to induce lung inflammation [31]) were instilled intratracheally. The animals were allowed to recover from anesthesia and have access to food and water ad libitum. After 18 h, the rats were euthanized and the lungs were lavaged twice with saline (0.9% w/v, 35 mL/kg/lavage). The BALF was centrifuged, and cells were mixed with 0.5 mL 0.2% saline for 10 s to lyse any erythrocytes. The cells were then resuspended in 10 mL Hanks’ Balanced Salt Solution (HBSS) and recentrifuged, and the macrophage pellet was collected. The resulting macrophages were 99% pure, as determined by HEMA 3 Stain Set stain (Fisher Scientific, Kalamazoo, MI, USA) (Figure 1A, B), and were 98% viable, based on trypan blue exclusion. The cells were resuspended at 2.5 × 10^5 cells/mL in RPMI 1640 with 1% fetal bovine serum and 1% penicillin/streptomycin.

In Vitro Alveolar Macrophage Chemokine Response to LTA and PGN

For the in vitro studies, F344 and DA rats (n = 5/group) were euthanized without prior instillation. The lungs were lavaged 5× with saline (0.9% w/v, 35 mL/kg/lavage). The BALF was centrifuged, and cells were mixed with 0.5 mL 0.2% saline for 10 s to lyse any erythrocytes. The cells were then resuspended in 10 mL Hanks’ Balanced Salt Solution (HBSS) and recentrifuged, and the macrophage pellet was collected. The resulting macrophages were 99% pure, as determined by HEMA 3 Stain Set stain (Fisher Scientific, Kalamazoo, MI, USA) (Figure 1A, B), and were 98% viable, based on trypan blue exclusion. The cells were resuspended at 2.5 × 10^5 cells/mL in RPMI 1640 with 1% fetal bovine serum and 1% penicillin/streptomycin.
Macrophages from each animal were distributed to three different wells at 1 mL/well in 24-well low-attachment cell culture plates (Corning Costar, Cambridge, MA, USA) and cultured in medium, as described above, to which LTA 0.5 μg/mL + PGN 1.65 μg/mL, LTA 1.0 μg/mL + PGN 3.3 μg/mL or an equal volume of 0.9% saline (control) was added, for 6 h at 37°C in 5% CO₂. The cell culture medium was then centrifuged and the supernatant stored at –80°C for the chemokine assay.

**In Vitro Alveolar Macrophage Mitogen-Activated Protein Kinase Activation Study**

To explore the differences in mitogen-activated protein kinase (MAPK) activation between these two strains, alveolar macrophages (AMs) lavaged from F344 and DA strains were stimulated by LTA and PGN in vitro (LTA 1.0 μg/mL + PGN 3.3 μg/mL). The whole cell lysates were collected at different time points (0, 1 and 6 h). The MAPK pathway markers (phosphorylated extracellular signal-regulated kinases 1 and 2 [ERK1/2] and P38) were determined in the cell lysates by Western blot. In addition, total ERK1/2 and total P38 protein and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control were determined by stripping the same membrane after the phosphorylated forms had been assessed. Toll-like receptor 2 (TLR2) levels in AMs from F344 and DA were determined by Western blot. Antibodies used for Western blots (phosphor-P38, total-P38, phosphor-ERK1/2 and GAPDH) as an internal control were determined by stripping the same membrane after the phosphorylated forms had been assessed. Toll-like receptor 2 (TLR2) levels in AMs from F344 and DA were determined by Western blot. Antibodies used for Western blots (phosphor-P38, total-P38, phosphor-ERK1/2 and GAPDH) were obtained from Cell Signaling Technology (Danvers, MA, USA). Total ERK1/2 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). TLR2 antibody was purchased from Abcam (Cambridge, MA, USA).

**In Vitro MAPK Inhibition Study**

To further detect the role of MAPK ERK1/2 and P38 pathways in the regulation of chemokine production, the effects of MAPK inhibition on KC and MIP-2 production were examined. AMs were prepared by using the method described above. The specific inhibitors for p38 (SB-203580; EMD Chemicals, Gibbstown, NJ, USA) and ERK1/2 (PD-98059; Calbiochem, San Diego, CA, USA) were added to the AMs of F344 and DA rats 30 min prior to LTA/PGN stimulation (LTA 1.0 μg/mL + PGN 3.3 μg/mL). After 6 h, cell culture medium was collected. KC and MIP-2 in the medium were measured by using an ELISA method as described below. To exclude the possibility that the inhibitors may have caused cell damage, a cytotoxicity study was performed at the same inhibitor doses and contact time, and the culture medium was assessed for lactate dehydrogenase (LDH) activity (Takara Bio, Shiga, Japan).

**BALF Total Cell and Differential Cell Counts**

BALF cells were mixed with 0.5 mL of 0.2% saline for 10 s to lyse any residual erythrocytes, and the cells were then resuspended in 10 mL HBSS. The total cell number retrieved in the BALF was determined with a hemocytometer. Slides were prepared using a Shandon cytocentrifuge (Shandon Scientific, London, UK), and cells were stained with an HEMA 3 Stain Set (Fisher Scientific) for differential cell counting. Counts were made on at least 200 cells per slide by an observer blinded to the grouping.

**Cytokine and Chemokine Assays**

TNF-α, KC and MIP-2 were measured by enzyme-linked immunosorbent assay (ELISA) using commercial assay kits.

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Figure 2. Differential cellular inflammatory response in the lung between F344 and DA rats. Following intratracheal instillation of LTA/PGN, both strains (saline control: n = 5/strain; LTA/PGN: n = 7/strain) show increased total numbers of cells in the BALF (A). This increased cell number is predominately due to a neutrophil influx to the lung (B). Although F344 rats had more macrophages in the BALF than DA rats following either saline or LTA/PGN instillation, there was no significant change in the number of macrophages in either strain owing to LTA/PGN (C). MPO was used as an indicator of accumulation of neutrophils within the lung tissue. F344 rats had higher MPO activity in lung tissue than DA rats, following LTA/PGN instillation (D). Bars indicate mean ± SD. **P < 0.01 versus F344 treated by LTA/PGN; *P < 0.05 versus F344 treated with LTA/PGN; ##P < 0.01 versus DA treated with LTA/PGN; #P < 0.05 versus DA treated with LTA/PGN.
(TNF-α: R&D Systems, Minneapolis, MN, USA; KC and MIP-2: Antigenix America, Huntington Station, NY, USA) according to manufacturers’ instructions.

MPO Activity Measurement
MPO was measured by using a commercially available assay kit (CytoStore, Calgary, Alberta, Canada) according to the manufacturer’s instructions. Briefly, lung tissues were weighed and sample lysis buffer was added at 12 mL per gram of tissue, followed by tissue homogenization. Supernatant from each sample homogenate was collected. Tissue sample supernatant (20 μL) was combined with 200 μL of the mixture of chromogen and H₂O₂. Absorbance at 450 nm was measured immediately after addition of chromogen, and again after a 60-sec interval. The absorbance changes during the 60-sec interval were used as a measure of the MPO activity in the sample.

Protein Content Assay
BALF protein concentration was assayed by using the Coomassie (Bradford) Protein Assay Kit (Pierce Company, Rockford, IL, USA). The total protein content was calculated as the product of concentration and BALF volume.

Statistics
Data are presented as mean ± SD. Student t test or one-way ANOVA was used to evaluate the statistical significance of the results. Differences with a P value <0.05 were considered significant.

RESULTS
F344 Rats Have Higher BALF and Lung Tissue Neutrophil Infiltration following LTA and PGN Instillation than DA Rats
Both F344 and DA rats instilled with LTA and PGN showed significant increases in total BALF cell number compared with controls instilled with saline (Figure 2A). However, the cell recruitment into the alveolar space was eight-fold greater in F344 rats than in DA rats (mean ± SD: F344 7.9 ± 2.6 × 10⁶; DA 1.0 ± 0.4 × 10⁴; P < 0.001) (Figure 2A). The increase was predominantly due to neutrophil influx (BALF neutrophil counts: F344 7.0 ± 2.6 × 10⁶ versus DA 6.6 ± 4.5 × 10⁵; P < 0.001) (Figure 2B and Figure 1E, F). Nevertheless, macrophage numbers in the BALF did not change significantly following LTA/PGN instillation in either strain. To determine whether DA neutrophils had been recruited to the lung, but had not crossed into the alveolar space, lung tissue was assessed for neutrophil content based on postlavage MPO assay. However, the lung tissue MPO activity was significantly higher in F344 rats, indicating a more robust response and increased damage. Bars indicate mean ± SD. *P < 0.01 versus F344 treated with LTA/PGN; #P < 0.05 versus F344 treated with LTA/PGN; ##P < 0.01 versus DA treated with LTA/PGN; H P < 0.05 versus DA treated with LTA/PGN; ND, not detectable.

Figure 3. Lung responses to LTA/PGN instillation. F344 rats showed greater inflammatory responses and lung injury to LTA/PGN than DA rats. Following LTA/PGN instillation, there was increased accumulation of cytokines in lung alveolar (A, B, C) and tissue (E, F) samples from both strains. Accumulation of MIP2 (B), TNF-α (C) and protein (D) in the BALF, and MIP-2 in lung tissue (F) were significantly higher in F344 rats, indicating a more robust response and increased damage. Bars indicate mean ± SD. *P < 0.01 versus F344 treated with LTA/PGN; #P < 0.05 versus F344 treated with LTA/PGN; ##P < 0.01 versus DA treated with LTA/PGN; #P < 0.05 versus DA treated with LTA/PGN; ND, not detectable.
LTA/PGN instillation was significantly different in these two strains.

**Inflammatory Mediators in BALF**

**Higher in F344 than DA Rats Following LTA/PGN Stimulation**

Concentrations of TNF-α in BALF and MIP-2 in both BALF and lung tissue following LTA/PGN instillation were significantly higher in samples from F344 rats than in the equivalent samples from DA rats under the same conditions (Figure 3), suggesting increased inflammatory responses in F344 compared with DA. In addition, the total protein in the BALF from F344 rats was higher than from DA rats following challenge, indicating increased tissue damage.

**Higher Baseline Numbers of AMs in BALF, and Enhanced In Vitro AM Response in F344 Compared with DA**

We next examined the number of AMs in naïve (untreated) rats. The baseline number of AMs in the BALF was significantly higher in F344 than DA (mean ± SD cell counts: 15.4 ± 5.2 × 10⁵ versus 8.2 ± 2.3 × 10⁵; P = 0.022, Figure 4C). AMs from each strain were adjusted to the same concentration (2.5 × 10⁵ cells/mL) and stimulated with LTA and PGN in vitro. After 6 h, F344 AMs produced significantly higher concentrations of KC and MIP-2 than DA AMs (Figure 4A, B). These results demonstrate that F344 not only has increased baseline numbers of AMs, but also its AMs produce increased amounts of neutrophil chemoattractants.

**The DA.F344(QTL) Congenic Strains Have Similar Inflammatory Response to LTA and PGN as Their Background DA Strain**

In previous studies we have shown that the specific arthritis QTLs Cia4 and Cia6, located on rat chromosomes 7 and 8, respectively, mediate the difference between DA and F344 in neutrophil migration into a synovial-like cavity in response to carrageenan (19). Therefore, we assessed whether either of these QTLs, as well as two additional arthritis QTLs, Cia3d and Cia5a, might account for a genetically regulated difference in lung inflammation induced with LTA and PGN. Following instillation of LTA/PGN, all four congeneric strains (DA.F344[Cia3d], DA.F344[Cia4], DA.F344[Cia5a] and DA.F344[Cia6]) developed lung inflammatory responses similar to their DA background, including BALF total cell number, BALF neutrophil counts, BALF macrophage counts, BALF protein content and lung tissue MPO activity (Figure 5). These results suggest that these arthritis and inflammation QTLs do not explain the differences between lung inflammation detected in F344 and DA rats.

**Differential MAPK Pathway Responses to TLR2 Stimulation in AMs from F344 and DA Strains**

To further explore the mechanism responsible for the strain differences in KC and MIP-2 release by AMs after LTA + PGN stimulation, we focused on the TLR2-MAPK pathway, which is considered to be a major pathway in the regulation of chemokine production during TLR2 stimulation (32,33).

There were similar levels of TLR2 in the AMs from these two strains (P = 0.69). Furthermore, there were no significant differences between the strains, in baseline total ERK1/2 (P = 0.30) and total p38 (P = 0.36). However, the activated forms,
that is, the phosphorylated p38 and phospho-
ylated ERK1/2 levels were signifi-
cantly higher in the AMs from F344 rats,
after LTA + PGN stimulation (Figure 6).
Similar results were achieved from three
independent repeats. For F344 versus DA
at 1-h and 6-h time points, the p-/t-p38
ratios were 1.47 ± 0.20–fold and 1.83 ±
0.52–fold higher (∗∗∗P = 0.015 and 0.049,
respectively) and the p-/t-ERK1/2 ratios
were 3.09 ± 1.24–fold and 2.59 ± 0.33–fold
higher (∗∗P = 0.044 and 0.001). These results
indicate that different activation (phos-
phorylation) of the MAPK pathway, but
not the total TLR2 expression or total
MAPK member expression, is associated
with the different chemokine production
between these two strains in this AM in
vitro LTA + PGN stimulation model.

Effects of MAPK Inhibition on AM
Chemokine Production
To further examine the role of MAPK path-
way in the regulation of chemokine
production, effects of MAPK inhibition
on KC and MIP-2 production were as-
sessed. In our pilot study, both p38 in-
hibitor SB-203580 and ERK1/2 inhibitor
PD-98059 showed a dose-dependent in-
hibition of LTA/PGN stimulated KC
and MIP-2 production. The minimum
doses that can produce maximum in-
hibitory effects (SB-203850 20 μmol/L
and PD-98059 50 μmol/L) were then
chosen and used for subsequent experi-
ments. Results showed that both SB-
203850 and PD-98059 decreased
LTA/PGN-induced KC and MIP-2 pro-
duction (Figure 7A, B). In addition, if
the two inhibitors were used in combi-
nation, this led to further inhibition of
KC and MIP-2 production, compared
with the LTA/PGN-stimulated group
(Figure 7A, B). The cytotoxicity study
showed that both the single and com-
bined administrations did not increase
cytotoxicity (LDH release) compared
with the control group (Figure 7C).

DISCUSSION
Lung inflammation and pneumonias
are major and common causes of mor-
bidity and mortality, particularly in
cases associated with S. aureus (10).
Neutrophils have a central role in acute
lung inflammation (34,35), but the local
factors regulating neutrophil influx into
the lung remain incompletely under-
stood. In this study, we used a model of
acute lung inflammation induced by
LTA and PGN, components of the
Gram-positive bacteria cell membrane
that activate macrophages via TLR2
(16,36,37), to explore the factors regulat-
ing neutrophil influx into the lung. We
demonstrated that the autoimmunity-
and arthritis-susceptible DA rats and
the resistant F344 rats developed acute
pulmonary inflammation induced by
LTA and PGN, as indicated by increased
numbers of neutrophils and high levels
of inflammatory mediators in the BALF.
Unexpectedly, F344 rats had signifi-
cantly higher numbers of BALF neu-
trophils and macrophages, and their
macrophages produced increased levels
of neutrophil chemotactic factors such
as KC and MIP-2. These observations differ from those previously described in studies of acute inflammation in the synovial-like air pouch using carrageenan challenge (19,20), raising the possibility of tissue-specific regulation of inflammatory responses, with different cell types (for example, AMs versus synovial macrophages and synovial fibroblasts) implicated in the tissue responses to injury. Specifically, these two studies used different stimuli. LTA/PGN stimulate TLR2 to induce the inflammatory responses. Our results indicate that LTA/PGN stimulate the TLR2-MAPK-chemokine pathway leading to increased chemokine production and finally increased neutrophil infiltration. However, carrageenan is a high molecular weight sulfated polygalactan, stimulating inflammatory responses through different pathways than LTA/PGN. It has been reported that carrageenan induces IL-8 through a distinct Bcl10 pathway (38). Reactive oxygen species (39), lipoxygenase (40,41), and TLR4 (42) may also be involved in carrageenan-induced inflammation. In addition, these two studies explored inflammation at the different tissue sites. The lung has its own specific inflammatory cell population distinct from the joints. In the current study we found that lung residential cells, AMs, are particularly associated with the different inflammation in these two strains. It has been reported that macrophages from different sites have different inflammatory responses (43). We believe that the combination of different inflammatory stimuli activating different cell-signaling pathways at different stimulatory sites may account for the strain differences observed between this study and previous synovial-like air pouch studies (19,20).

Our observations demonstrate that neutrophil migration into the lung, and AM activation in response to bacterial products, are different between these two strains. In an attempt to determine the location of the trait-regulatory genes, we tested four congenic strains generated between DA and F344 (DA.F344[QTL] congenics) and known to regulate arthritis (Cia3, Cia4, Cia5a and Cia6) and acute inflammatory responses in the synovial-like air pouch model injected with carrageenan (Cia4 and Cia6) (19). However, all four congenics had BALF findings consistent with their background genome (DA) and excluding a contribution by the introgressed F344 intervals, which suggests that the differences in lung inflammation were not due to genes contained within these chromosomal intervals carried by these congenic strains.

Our previous studies with F344 and DA rats demonstrated that the two strains have similar peripheral blood neutrophil numbers and similar neutrophil chemotaxis in response to chemoattractants (19). These findings suggest that neutrophil responses themselves do not cause the differences in the BALF cell numbers. To explore the reason why more neutrophils migrate to lung in F344 rats, we focused on differences within AMs. The AM is a resident lung-specific cell population and has been reported to play a key role in acute lung injury caused by sepsis, hemorrhage, mechanical ventilation and aspiration (44–48). AMs are one of the first cell types to encounter lung pathogens, or in this study, the instilled bacterial products LTA and PGN. Our results demonstrate that there are differences in both the number of cells present, and in the quality of the AM response between the DA and F344 strains. BALF from F344 rats contained approximately 50% more AMs than from the DA strain. In addition to the higher numbers of AMs in the F344 strain, these cells also showed higher KC and MIP-2 production per cell following LTA and PGN.
during in vitro stimulation. KC and MIP-2, (also known as CXCL1 and CXCL2), belong to the CXC chemokine family and are major neutrophil chemoattractants acting via the neutrophil surface receptor CXCR2 (49,50). Neutralization of CXC chemokines (49) or their receptor (45,51–55) results in reduction of neutrophil recruitment into the lung following a variety of stimuli. AMs are a major source of chemokines in the alveolar space and respond directly to bacterial products such as bacterial lipopolysaccharide and gram-positive cell wall products such as LTA (50) and toxins such as enterotoxin A (56). In our study, LTA and PGN stimulation resulted in a dose-dependent elevation of KC and MIP-2 production, with F344 AMs producing approximately 50% more MIP-2 and 100% more KC than DA AMs. The significantly increased production of chemokines by F344 AMs may explain, at least partially, the different lung neutrophil influx detected between the two strains.

To further explore the mechanism involved in increased chemokine production by F344 AMs, we focused on the major chemokine production pathway during LTA + PGN stimulation, that is, the TLR2-MAPK pathway (32,33). Our results show that there are no differences in the basal expression of TLR2 and total MAPK members (p38 and ERK1/2) between the two strains. However, the phosphorylation of p38 and ERK1/2 was significantly higher in the F344 group after LTA + PGN stimulation. Furthermore, our results also showed that both p38 and ERK1/2 inhibitors can inhibit LTA/PGN-induced KC and MIP-2 production. If both of these two MAPK pathways are inhibited at the same time, a substantial inhibition of chemokines is achieved, which includes almost 100% of stimulated MIP-2 production and 75% of stimulated KC production. This observation indicates that the p38 and ERK1/2 pathways are two major cell-signaling pathways in LTA/PGN-induced KC and MIP-2 accumulation. The differences in MAPK pathway activation in these two strains can, at least partially, explain the differences in KC and MIP-2 production by AMs following LTA/PGN stimulation.

In conclusion, we have shown that there are significant differences in the regulation of pulmonary inflammation induced by bacterial products in the two different strains of rats. In particular, the differential regulation can be seen in neutrophil infiltration to the alveolar spaces. These differences are associated with different AM phenotypes and, specifically, differences in MAPK signaling pathways. The data presented should lead to a better understanding of the compartmentalization of the lung inflammatory response and tissue-specific regulation of the immune responses. Further study of these particular influences on the inflammatory processes may lead to more effective treatment of acute lung inflammation and injury.

ACKNOWLEDGMENTS
This work was supported by NIH R01-HL 081655 (EJ Miller) and NIH R01-AR46213, R01-AR052439 and R01-AI54348 (PS Gulko).

DISCLOSURE
The authors declare that they have no competing interests as defined by Molecular Medicine, or other interests that might be perceived to influence the results and discussion reported in this paper.
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