An Essential Role for Non–Bone Marrow–Derived Cells in Control of *Pseudomonas aeruginosa* Pneumonia

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MyD88 is an adapter protein required for the induction of proinflammatory cytokines by most Toll-like receptors (TLR), and *Pseudomonas aeruginosa* expresses ligands for multiple TLRs. MyD88^{−/−} (KO) mice are highly susceptible to aerosolized *P. aeruginosa*, failing to elicit an early inflammatory response and permitting a 3-log increase in bacterial CFU in the lungs by 24 h after infection. We hypothesized that alveolar macrophages are the first cells to recognize and kill aerosolized *P. aeruginosa* in an MyD88-dependent fashion due to their location within the airways. To determine which cells in the lungs mediate MyD88-dependent defenses against *P. aeruginosa*, we generated radiation bone marrow (BM) chimeras between MyD88KO and wild-type (WT) mice. MyD88KO mice transplanted with MyD88KO BM (MyD88KO→MyD88KO mice) displayed uncontrolled bacterial replication, whereas all other chimeras controlled the infection by 24 h. However, at 4 h, both MyD88KO→MyD88KO and WT→MyD88KO mice permitted intrapulmonary bacterial replication, whereas MyD88KO→WT and WT→WT mice did not, indicating that the source of BM had little impact on the early control of infection. Similarly, the genotype of the recipient rather than that of the BM donor determined early neutrophil recruitment to the lungs. Whereas intrapulmonary TNF- α and IL-1 β production were associated with WT BM, levels of the CXC chemokines MIP-2 and KC as well as GM-CSF were associated with recipient genotype. We conclude that lung parenchymal and BM-derived cells collaborate in the MyD88-dependent response to *P. aeruginosa* infection in the lungs in mice.

Keywords: alveolar macrophages; lung parenchyma; MyD88; neutrophils; *Pseudomonas aeruginosa*; Toll-like receptors

Pseudomonas aeruginosa (PA) is a ubiquitous Gram-negative bacterium that is found in the soil and water and is also an important opportunistic pathogen. PA is a leading cause of hospital acquired pneumonia and of chronic airway infection in individuals with cystic fibrosis (CF) (1, 2). MyD88 is an adapter protein required for the induction of proinflammatory cytokines by all Toll-like receptors (TLR) with the possible exception of TLR3 (3). PA expresses ligands for multiple TLRs including lipopolysaccharide (LPS) for TLR4, flagellin for TLR5, and lipoproteins for TLR2, all of which likely contribute to innate recognition and clearance of PA. Innate immunity has an essential role in host defense against acute PA pneumonia, as MyD88^{−/−} (MyD88KO) mice exposed to aerosolized PA fail to elicit early neutrophilic inflammation and succumb to uncontrolled bacterial replication within 24 h of infection (4, 5). Surprisingly,

MyD88KO mice clear aerosolized *Staphylococcus aureus* from the lungs, demonstrating the pathogen-specific role of MyD88-dependent signaling for pulmonary host defense (4).

The cellular components of host defense against PA in the lower respiratory tract are incompletely understood. Resistance to PA pneumonia is associated with a rapid influx of neutrophils to the lungs that is essential to control the infection (4). Depleting circulating PMN or blocking the CXCR2 chemokine receptor and, hence, neutrophil recruitment, greatly increased mortality from PA pneumonia relative to control mice (6). Alveolar macrophages (AM) ingest PA after respiratory infection (4, 7, 8) and contribute to the protective inflammatory response. Depletion of > 95% of AM before infection was associated with blunted cytokine responses, reduced neutrophilic inflammation, impaired bacterial clearance, and reduced survival in PA pneumonia (9, 10), whereas depletion of 80% of AM did not significantly affect neutrophil recruitment or bacterial clearance after PA infection (7). Airway epithelial cells also express proinflammatory mediators in response to PA infection (11, 12) but their role in host defense against PA pneumonia is unknown.

The severe phenotype of MyD88KO mice provides an opportunity to explore the roles of specific components of innate immunity to PA pneumonia through selective reconstitution. We hypothesized that alveolar macrophages, by virtue of their location within the airways, would mediate the earliest control of bacterial replication as well as proinflammatory responses to aerosolized PA. MyD88 expression in macrophages has been previously demonstrated (13). We therefore constructed radiation bone marrow (BM) chimeras between MyD88KO and wild-type (WT) mice to examine the relative contribution of BM and non-BM-derived MyD88-dependent signals in the control of PA replication in the lungs.

MATERIALS AND METHODS

Mice

MyD88KO mice (from S. Akira, Osaka University, Japan) were backcrossed to C57Bl/6 mice for six generations. C57Bl/6 congenic CD45.1 WT mice were either purchased from the Jackson Laboratory (Bar Harbor, ME) or bred in-house at the University of Washington. All mice were maintained in ventilated cages (Thoren Caging System, Hazelton, PA) and fed Picolab Rodent Diet 20 (Labdiet, St. Louis, MO). Water was supplied through an automatic watering system, and cages and bedding were autoclaved. Animals were only handled in a laminar flow hood. The facilities at the University of Washington are specific pathogen-free for mouse hepatitis virus, mouse parvovirus, minute virus of mice, reovirus-3, pneumonia virus of mice, epizootic diarrhea of infant mice, Theiler's murine encephalomyelitis virus, lymphocytic choriomeningitis virus, ectromelia, sendai virus, sialodacryoadenitis virus, rat parvovirus, *Mycoplasma pulmonis*, pinworms, and fur mites. All methods were approved by the Institutional Animal Care and Use Committee.

BM Chimeras

Male and female mice 8–16 wk of age were lethally irradiated with 950 rads using a ¹³⁷Cs source and injected intravenously 3–6 h later with 2–4 $\times 10^6$ T cell-depleted BM cells from female donors. T cell depletion

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was performed using anti-CD4 (RL-172), anti-CD8 (3.168), and anti-Thy1.2 (30-H12) followed by Low-Tox-M Rabbit Complement (Cedarlane, Hornby, BC, Canada) as previously described (14). We found that MyD88KO mice were susceptible to bacterial infection 1 wk after irradiation; therefore, all mice were placed on Baytril (either oral or subcutaneous; enrofloxacin, Bayer Corporation, Shawnee Mission, KS) for 10 d to 2 wk following irradiation. Three separate experiments over a 1-yr period (with two extending to 24 h) were performed.

The degree of chimerism was assessed by measuring CD45.1 and CD45.2 expression by blood leukocytes and bronchoalveolar lavage (BAL) cells. Eight weeks after reconstitution, whole blood was obtained from the retroorbital sinus. Red blood cells were lysed in 1× Immuno-Lyse (Coulter, Miami, FL). Washed cells were blocked with 5% goat serum in FACS buffer (1% BSA in PBS), then stained with R-phycoerythrin-conjugated anti-CD45.1 (PharMingen, San Diego, CA) and fluorescein-isothiocyanate-conjugated anti-CD45.2 (PharMingen) antibodies. Cells were analyzed on a FACScan (BD Biosciences, San Jose, CA) using CellQuest Pro software. Cells obtained by BAL from a subset of mice at 12 wk after irradiation were stained and analyzed using the same procedure, in the absence of red blood cell lysis.

PA Infection and Tissue Harvesting

Mice were exposed to aerosolized PA (PAK strain, a gift from Steve Lory, Harvard University) in a whole body chamber 12–14 wk after irradiation, and tissues were processed as previously described (4). For each experiment, an aliquot of bacteria was thawed, inoculated into 10 ml LB broth, incubated for 6 h at 37°C in a shaking incubator, then diluted 1:100 into 100 ml LB broth and incubated for an additional 16 h. The plateau phase bacteria were washed twice and resuspended in 20 ml PBS containing 10 mM magnesium chloride. This suspension contained $\sim 3 \times 10^9$ CFU/ml (estimated by optical density and confirmed by quantitative culture) and was transferred to two jet nebulizers (8 ml each) for aerosolization. Immediately, 4 h, and 24 h after infection, mice were killed with pentobarbital (10 mg IP) in accordance with the AVMA Panel on Euthanasia, then exsanguinated by closed cardiac puncture. Bacterial deposition was determined in each experiment by harvesting the left lungs immediately after infection from four mice representing one or more of the experimental groups. The tissue was homogenized in 1 ml PBS and quantitatively cultured on LB agar using the pour plate method. At 4 h and 24 h time points, left lungs and spleens were harvested from three to six mice in each group for quantitative culture. The lung homogenates then were diluted 1:1 in lysis buffer containing 2× protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany), incubated on ice for 30 min, and centrifuged at $1,200 \times g$ before storage of supernatants at -80°C . The right lungs were lavaged with four 0.5-ml aliquots of 0.9% NaCl supplemented with 0.6 mM EDTA for determination of cell counts by hemacytometer and differentials from cytocentrifuge specimens stained with Diff Quik (Dade Behring, Duding, Switzerland). TNF- α , IL-1 β , MIP-2, and KC were measured in lung homogenates by sandwich ELISA using DuoSet reagents (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. GM-CSF was measured with antibody-coated microbeads using a kit purchased from R&D Systems and the BioPlex version of Luminex 100 analyzer (BioRad, Hercules, CA), both according to the manufacturer's instructions.

Histopathology

Right lungs were fixed after lavaging by inflation to 15 cm pressure with 4% paraformaldehyde then removed and stored at 4°C in the same fixative. The entire right lung was embedded in paraffin and the block faced to the approximate mid-point so that all lobes were present. Approximately 5–6- μm sections were then collected and stained with hematoxylin and eosin. A veterinary pathologist examined four to six sections from individual mice in a manner blinded to genotype and time after infection. Peribronchial and perivascular inflammation were scored on a scale of 0–4, with a score of 0 representing the absence of leukocytes and a score of 4 indicating maximal accumulation relative to other sections. Parenchymal inflammation also was scored on a scale of 0–4, with a score of 0 representing normal tissue and a score of 4 indicating widespread interstitial and/or alveolar infiltration. The severity of necrosis was also scored on a 0–4 scale with 4 representing the maximum observed relative to other sections. Scores for inflammation

and necrosis within treatment groups were averaged. The severity score represents a sum of these averages.

Statistical Analysis

Data from three separate experiments (all showing similar results) were pooled. The data were not normally distributed, and were analyzed for significant differences with the Kruskal-Wallis test followed by Dunn's post test for multiple pairwise comparisons, using Prism software (GraphPad Software, San Diego, CA). A *P* value of < 0.05 was considered significant. Data are expressed as median with range of 25th–75th percentile. Correlation was analyzed by the Spearman coefficient using Prism software.

RESULTS

Blood Cell and Alveolar Macrophage Reconstitution of BM Chimeras

Radiation BM chimeras were generated between MyD88KO (CD45.2) mice and C57Bl/6 congenic CD45.1 WT mice. Previous irradiation of WT mice has been shown to increase susceptibility to PA relative to un-irradiated mice (8). Therefore, four groups of mice were compared: WT→WT (WT BM into a WT mouse), MyD88→MyD88 (MyD88KO bone marrow into a MyD88KO mouse), WT→MyD88 (WT BM into a MyD88KO mouse), and MyD88→WT (MyD88KO BM into a WT mouse). Eight weeks after irradiation and BM transfer, reconstitution of circulating leukocytes was determined by staining leukocytes with anti-CD45.1 and anti-CD45.2. We found that 93% (median, range 84–98%) of blood cells were reconstituted with BM-derived cells in all mixed chimeras. To evaluate reconstitution of AM, we performed BALs on a subset of mice at 12 wk after irradiation. Analysis of Wright-Giemsa stained cell smears confirmed that monocytes/macrophages constituted $> 95\%$ of cells in BALs from uninfected mice. Flow cytometric analysis revealed that 95% (median, range 88–99%) of BAL cells were of BM origin, demonstrating good reconstitution in all of the experiments.

Figure 1. Early PA control requires MyD88 expression by non-BM-derived cells. Total CFU per lung are plotted on a $\log_{10}$ scale at either time 0 (deposition), or 4 h or 24 h after infection. Each symbol represents one mouse. The source of BM is listed first, followed by the recipient genotype. The line denotes the median of values for each group. The total number of mice examined were as follows: WT→WT (squares)—16 at 4 h, 10 at 24 h; MyD88→MyD88 (triangles)—13 at 4 h, 7 at 24 h; WT→MyD88 (inverted triangles)—14 at 4 h, 10 at 24 h; MyD88→WT (diamonds)—16 at 4 h, 10 at 24 h. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 for differences between the two groups spanned by the lines.

MyD88 Expression in Non-BM Cells Is Essential for Early Control of PA Replication

Deposition of PA by aerosol was highly reproducible between experiments, yielding 1.78×10^5 CFU/lung ($1.16\text{--}2.05 \times 10^5$, median and 25th–75th percentile) (Figure 1). All mice survived to the predetermined time points. At 4 h after infection, WT→WT mice already had begun to control bacterial replication, because the bacterial count (0.68×10^5 , $0.61\text{--}0.79 \times 10^5$ CFU/lung) was 2.6-fold lower than in the initial inoculum. Similar results were obtained in MyD88→WT mice. In marked contrast, MyD88→MyD88 mice and in WT→MyD88 mice had not controlled bacterial replication, as the lungs of these mice contained 1.1–1.3-fold more bacteria than initially deposited ($P < 0.01$ versus the other two groups). At 24 h, all MyD88→MyD88 mice showed uncontrolled bacterial replication (Figure 1) and exhibited progressive dissemination with bacterial replication in the spleen (data not shown), whereas WT→MyD88 and MyD88→WT mice had greatly reduced bacterial counts, although the WT→MyD88 group still had significantly higher lung counts than the WT→WT group (0.03×10^5 CFU, $0.016\text{--}0.042 \times 10^5$, versus 0.01×10^5 CFU, $0.005\text{--}0.015 \times 10^5$, $P < 0.05$). Therefore, the genotype of the recipient rather than the BM determined 4 h CFU in the lungs.

Severe Tissue Injury in the Absence of MyD88

Lungs of mice challenged with PA 4 and 24 h earlier were evaluated histologically (Figure 2). The magnitude of histologic changes was consistent with lavage findings (*see below*). A tissue injury score summarizing inflammation and necrosis was gener-

ated as depicted in Table 1. The most severe changes were seen in MyD88→MyD88 mice infected for 24 h. Lungs from these mice had severe necrosis of airways and some surrounding tissues accompanied by dense accumulations of neutrophils and necrotic debris (*) within and around vessels and bronchioles. In contrast, lung sections from WT→MyD88 mice at 24 h had lesions similar to those in WT→WT or MyD88→WT mice in that they were characterized by perivascular, and less so, alveolar neutrophil accumulations (N) with rare necrosis. At 4 h after PA challenge there was a noticeable lack of neutrophils in MyD88 recipient mice, with only few mononuclear cells present in perivascular locations (M). This contrasted with WT recipients, in which perivascular neutrophil accumulations were readily recognized.

MyD88 Expression in Non-BM Cells Is Essential for Early PMN Recruitment to the Lung

Inflammatory cell recruitment into the airways was analyzed by enumeration and identification of BAL cells. Total cell counts at 4 h were greatest in WT→WT mice (2.3×10^6 , $1.7\text{--}2.6 \times 10^6$), followed by MyD88→WT (1.1×10^6 , $0.9\text{--}1.6 \times 10^6$), then WT→MyD88 (0.4×10^6 , $0.3\text{--}0.5 \times 10^6$) and finally MyD88→MyD88 (0.3×10^6 , $0.2\text{--}0.4 \times 10^6$). More than 90% of the cells in this latter group consisted of mononuclear cells (MN) with the morphology of AM, which were within 3-fold of the mononuclear cell counts in other groups (Figure 3A) and were not different from WT→WT mice at 4 h.

Neutrophils, which were completely absent in BALs from unexposed mice, constituted the majority of recruited cells into the airways (Figure 3B). At 4 h, neutrophil recruitment in MyD88→MyD88 mice was more than 100-fold lower than in WT→WT mice ($P < 0.001$). Neutrophil recruitment was also significantly

Figure 2. Absence of MyD88 on both BM- and non-BM-derived cells correlates with severe tissue injury. Photos show representative changes in lungs from chimeric mice at 4 and 24 h after infection. M indicates mononuclear cell accumulation; N indicates neutrophil accumulation, and * identifies foci of necrosis and neutrophil accumulation. Original magnification was either $\times 20$ or $\times 40$ as marked. Sections from 10 mice at 4 h after infection and 6 mice at 24 h after infection from each group were examined. The bar represents $\sim 100 \mu\text{m}$ ($\times 20$) or $50 \mu\text{m}$ ($\times 40$).

TABLE 1. LUNG HISTOLOGIC SCORES

Chimera	Time after Infection	Inflammation PB, PV, A	Necrosis B, V, A	Predominant Inflammatory Cell Type	Combined Injury Score (Necrosis + Inflammation)
M88→M88	4 h	0.625	0	Mononuclear	0.625
	24 h	7.75	5.75	PMN	13.50
WT→M88	4 h	0.5	0	Mononuclear	0.5
	24 h	4.125	0	PMN	4.125
WT→WT	4 h	3.5	0.25	PMN	3.75
	24 h	4.125	0.5	PMN	4.625
M88→WT	4 h	1.625	0	PMN	1.625
	24 h	2.375	0	PMN	2.375

Definition of abbreviations: A, alveolar; B, bronchiolar; PB, peribronchial; PV, perivascular; V, vascular.
n = 4 for each group at each time point, mean value shown.

lower in WT→MyD88 KO mice ($P < 0.001$), whereas MyD88→WT counts did not differ significantly from WT→WT. Thus, as for bacterial clearance, the genotype of the recipient mouse rather than the BM determined PMN recruitment to the

lungs at 4 h. At 24 h, all four groups of mice had large numbers of PMN in the lungs (Figure 3B).

MyD88 Expression in BM Cells Is Essential for Early TNF- α and IL-1 β Production, but Non-BM-Derived Cells Preferentially Produce KC, MIP-2, and GM-CSF

To explore the basis for the intermediate phenotype of the MyD88→WT and WT→MyD88 mice compared with the other two groups, we also analyzed cytokines and chemokines in lung homogenates (Figure 4). By 4 h, TNF- α and IL-1 β in the lungs of WT→WT mice was ~ 8 -fold greater than in MyD88→MyD88 mice. BM-derived cells, and most likely AM, appeared to be the primary source of TNF- α and IL-1 β , because WT→MyD88 mice produced amounts similar to WT→WT mice, whereas MyD88→WT mice produced much less of these cytokines. It is possible that the residual 5% of AM not derived from donor BM could have contributed to production of TNF- α and IL-1 β in MyD88→WT mice. In contrast to the results with TNF- α and IL-1 β , non-BM-derived cells appeared to be the predominant source of GM-CSF and the chemokines KC and MIP-2 at 4 h, although BM-derived cells also contributed to chemokine production. Moreover, the concentrations of KC and MIP-2 in the different chimeras correlated directly and closely with the magnitude of neutrophil recruitment at 4 h ($P < 0.0001$ for KC and $P = 0.0044$ for MIP-2), which in turn corresponded best to the genotype of the recipient rather than the genotype of the BM donor.

By 24 h, cytokine and chemokine concentrations in the lungs had declined in all but the MyD88→MyD88 mice, consistent with the clearance of bacteria in all groups with the exception of these latter mice.

DISCUSSION

Clearance of inhaled PA from the lungs requires MyD88, and the data presented here demonstrate that MyD88-dependent signals from both BM and non-BM cells are required to control PA replication. The complex interplay between BM-derived and non-BM derived cells after PA infection in the lung is evident in this study. Within the first 4 h of infection, expression of MyD88 in non-BM cells is required for initial bacterial containment, as well as for early chemokine production and neutrophil recruitment to the lungs. By contrast, robust early TNF- α and IL-1 β production was dependent on MyD88 expression in BM-derived cells, but was not sufficient for early control of the infection. By 24 h, expression of MyD88 in either BM or non-BM cells was sufficient to eliminate PA, in contrast to the uncontrolled replication seen in mice that completely lacked MyD88.

Inflammatory recruitment of neutrophils to the lungs is coordinately regulated by chemokine, cytokine, and adhesion mole-

Figure 3. Early PMN recruitment requires MyD88 expression by non-BM-derived cells. (A) Total mononuclear cells (MN) in BAL from each mouse is plotted on a log₁₀ scale at 4 and 24 h after infection. Cells from a subset of mice killed at 12 wk after irradiation to determine AM reconstitution are shown as Unexposed. (B) Total PMN in BAL from each mouse is plotted on a log₁₀ scale at 4 and 24 h after infection. Line denotes median of values for each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for differences between the two groups spanned by the lines.

Figure 4. Early proinflammatory cytokine production is BM-dependent, whereas chemokine production is non-BM-dependent. TNF- α , IL-1 β , KC, MIP-2, or GM-CSF production in lung homogenates at 4 h (A) or 24 h (B) as measured by ELISA or luminex in each mouse. Line denotes median of values for each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for differences between the two groups bracketed by the ends of the lines.

cule upregulation (reviewed in Ref. 15). In this study, we found that the number of PMN recruited to the lungs at 4 h is directly proportional to the amount of KC and MIP-2 measured in lung homogenates. Production of these CXC chemokines was dependent on lung parenchymal cells. Furthermore, by 24 h, the large numbers of PMN in MyD88→MyD88 mice paralleled and may have resulted from the delayed production of MIP-2 in this group. This indicates that late neutrophil recruitment can occur even in the absence of MyD88. Delayed inflammation in the MyD88→MyD88 mice may have been a consequence of the extraordinary bacterial load and the marked tissue injury that were unique to these mice. In the other mice, expression of MyD88 either by BM- or non-BM-derived cells was sufficient for both neutrophil recruitment and bacterial clearance, thereby minimizing tissue injury. Production of GM-CSF, a cytokine important for survival and proliferation of neutrophils and macrophages (16), was detected only at 4 h and was dependent on MyD88 expression in non-BM cells, suggesting a potential indirect role for the lung parenchyma in neutrophil and macrophage function in the lungs.

Control of PA replication in the lungs has previously been demonstrated to be critically dependent on BM-derived cells, including PMN and AM (6, 7, 10). Unexpectedly, our results suggest that MyD88 expression by these cells is not required to eliminate PA from the lungs. It is likely, however, that MyD88-deficient cells are being activated indirectly, by TLR-independent pathways. For example, IFN- γ activated MyD88KO macrophages can kill *Listeria monocytogenes* *in vitro* (17), and the orphan receptor TREM-1 is expressed on both PMN and AM and can activate myeloid cells in a MyD88-independent fashion (reviewed in Ref. 18). Whether PA expresses or induces a ligand for TREM-1 is currently unknown.

A previous report demonstrated that neutrophil recruitment in response to aerosolized LPS is dependent on expression of TLR4 in BM-derived cells (19). The authors demonstrated that equivalent numbers of neutrophils were recruited to the lungs of TLR4KO mice reconstituted with WT BM and to the lungs of WT mice after aerosol challenge with LPS. In our experiments with live PA, however, MyD88KO mice reconstituted with WT BM recruited 19-fold fewer neutrophils than WT mice. It is possible

that the inability of MyD88-deficient cells to transduce signals downstream of the IL-1 and IL-18 receptors contributed to the differences in findings in the current study and the study with aerosolized LPS. However, neither IL-1 nor IL-18 deficiency alone appears to be sufficient to compromise lung defense against PA (20, 21), and a more likely reason for this discrepancy is our use of whole PA bacteria, which express multiple TLR ligands in addition to LPS. Consistent with this notion, TLR4 expression in BM cells was not sufficient to restore WT levels of PMN recruitment to the lungs in TLR4-deficient (C3H/HeJ) mice after aerosol challenge with PA (our unpublished observations).

The identity of the non-BM cells that mediate early MyD88-dependent responses to PA is unknown, although epithelial or endothelial cells are the most likely candidates. It is possible that the early MyD88-dependent control of bacterial replication at 4 h is due to direct activation by PA of lung epithelial cells, which have been demonstrated to express TLRs (22, 23). These cells may directly kill PA through the production and release of antimicrobial peptides, as previously demonstrated in *in vitro* experiments with tracheobronchial epithelial cells (24). It is possible that BM-derived cells gave rise to lung epithelial cells as several studies have demonstrated (reviewed in Ref. 25), although recent reports dispute these findings (26, 27). However, such cells would constitute a small minority of the lung epithelium and thus would be likely to play no more than a minor role. Other data support the role of epithelial cells in pulmonary inflammation in response to aerosol challenge. Using transgenic mice expressing dominant-negative I κ B α under the control of promoters specific for lung epithelial cells (28, 29), two different groups showed diminished inflammation after aerosol or intranasal instillation of LPS in transgenic mice relative to transgene-negative controls.

In contrast to aerosol challenge, expression of TLR4 in non-BM cells appears to be essential for neutrophil recruitment to the lungs following systemic LPS administration (30). In that study, TLR4-deficient neutrophils were sequestered in capillaries of mice expressing TLR4 in non-BM cells within 4 h of intraperitoneal injection of LPS, and the authors speculated that TLR4 expression in the endothelium was required for this recruitment.

Our results demonstrate that non-BM-derived cells mediate

the earliest MyD88-dependent responses to aerosolized PA. We hypothesize that it is epithelial cells that mediate this response, as they are the first non-BM-derived cells to directly contact PA. A formal test of which parenchymal cell types are involved in TLR- and MyD88-dependent lung inflammation and host defense after intrapulmonary or systemic challenge will require tissue-specific ablation of MyD88 or TLRs in epithelial but not endothelial cells.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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