

Luminex
complexity simplified.

Guava® SARS-CoV-2 Multi-Antigen Antibody Assay

New assay for SARS-CoV-2 antibody detection on your flow cytometer
For Research Use Only. Not for use in diagnostic procedures.



Learn More >



Airway Epithelial MyD88 Restores Control of *Pseudomonas aeruginosa* Murine Infection via an IL-1–Dependent Pathway

This information is current as of September 23, 2021.

Lilia A. Mijares, Taming Wangdi, Caroline Sokol, Robert Homer, Ruslan Medzhitov and Barbara I. Kazmierczak

J Immunol 2011; 186:7080-7088; Prepublished online 13 May 2011;

doi: 10.4049/jimmunol.1003687

<http://www.jimmunol.org/content/186/12/7080>

Supplementary Material <http://www.jimmunol.org/content/suppl/2011/05/13/jimmunol.1003687.DC1>

References This article **cites 38 articles**, 19 of which you can access for free at: <http://www.jimmunol.org/content/186/12/7080.full#ref-list-1>

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

**average*

Subscription Information about subscribing to *The Journal of Immunology* is online at: <http://jimmunol.org/subscription>

Permissions Submit copyright permission requests at: <http://www.aai.org/About/Publications/JI/copyright.html>

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at: <http://jimmunol.org/alerts>

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2011 by The American Association of
Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.



Airway Epithelial MyD88 Restores Control of *Pseudomonas aeruginosa* Murine Infection via an IL-1–Dependent Pathway

Lilia A. Mijares,^{*,1,2} Tamding Wangdi,^{†,1} Caroline Sokol,^{‡,3} Robert Homer,^{§,¶}
Ruslan Medzhitov,^{||,#} and Barbara I. Kazmierczak^{†,*,**}

The opportunistic human pathogen *Pseudomonas aeruginosa* causes rapidly progressive and tissue-destructive infections, such as hospital-acquired and ventilator-associated pneumonias. Innate immune responses are critical in controlling *P. aeruginosa* in the mammalian lung, as demonstrated by the increased susceptibility of MyD88^{-/-} mice to this pathogen. Experiments conducted using bone marrow chimeric mice demonstrated that radio-resistant cells participated in initiating MyD88-dependent innate immune responses to *P. aeruginosa*. In this study we used a novel transgenic mouse model to demonstrate that MyD88 expression by epithelial cells is sufficient to generate a rapid and protective innate immune response following intranasal infection with *P. aeruginosa*. MyD88 functions as an adaptor for many TLRs. However, mice in which multiple TLR pathways (e.g., TLR2/TLR4/TLR5) are blocked are not as compromised in their response to *P. aeruginosa* as mice lacking MyD88. We demonstrate that IL-1R signaling is an essential element of MyD88-dependent epithelial cell responses to *P. aeruginosa* infection. *The Journal of Immunology*, 2011, 186: 7080–7088.

Pseudomonas aeruginosa is an opportunistic human pathogen that causes both acute respiratory infections, such as ventilator-associated pneumonia, and chronic infections, such as those seen in individuals with cystic fibrosis (1). Bacterial production and translocation of type 3 secretion system effector proteins are associated with increased morbidity and mortality in acutely infected human patients and in murine pneumonia models (2, 3). These bacterial effectors are injected into host cells and are capable of causing cell necrosis, apoptosis, cytoskeletal re-

arrangements, and disruption of cell–cell contacts (4). Immune compromise—both local, as seen in epithelial wounds or burns, or systemic, as seen following ablative chemotherapy—increases the risk of *P. aeruginosa* infection, and current medical practice often involves prophylactic use of anti-pseudomonal antibiotics in high-risk patient populations (1).

P. aeruginosa elicits rapid innate immune responses that are necessary for bacterial clearance in acute pneumonia models and that require MyD88-dependent signals. MyD88^{-/-} animals cannot control *P. aeruginosa* replication following inoculation of this organism into the murine respiratory tract (5, 6). Humans with mutations resulting in loss of MyD88 function have also been described and often develop pyogenic bacterial infections owing to *P. aeruginosa* early in life (7). MyD88 is an adaptor for multiple TLRs and for the IL-1 and IL-18 receptors. The activation of these receptors ultimately leads to NF-κB nuclear translocation and proinflammatory cytokine and chemokine production (8). Rapid pulmonary neutrophil recruitment is a prominent consequence of the innate response to *P. aeruginosa* and is required for bacterial control and clearance (9).

Because TLRs, IL-1R, and IL-18R are expressed by a variety of tissues and cell types, it is not immediately obvious which cell types orchestrate a MyD88-dependent response to *P. aeruginosa* in the lung. Hajjar et al. (10) addressed this question by constructing bone-marrow chimeras between wild-type and MyD88^{-/-} knockout mice. They observed that MyD88^{-/-} animals reconstituted with a wild-type bone marrow nonetheless showed delayed neutrophil recruitment and bacterial control following pulmonary infection with *P. aeruginosa*, arguing that non-bone marrow-derived cells played a nonredundant role in the recognition of and response to bacteria in the lung. Several recent studies have suggested that these non-bone marrow-derived responses might be due to TLR5-dependent recognition of flagellated bacteria by lung epithelial cells (11, 12). However, this recognition mechanism would be inoperative in the case of infections by nonflagellated bacteria, and it might be circumvented by the ability of *P. aeruginosa* to rapidly downregulate flagellin synthesis upon exposure to secreted neutrophil elastase in the lung (13, 14).

*Graduate Program in Microbiology, Yale University School of Medicine, New Haven, CT 06520; †Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06520; ‡Graduate Program in Immunobiology, Yale University School of Medicine, New Haven, CT 06520; §Department of Pathology, Yale University School of Medicine, New Haven, CT 06520; ¶Pathology and Laboratory Medicine Service, Veterans Affairs Connecticut Healthcare System, West Haven, CT 06516; ||Department of Immunobiology, Yale University School of Medicine, New Haven, CT 06520; #Howard Hughes Medical Institute, Chevy Chase, MD 20815; and **Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06520

¹L.A.M. and T.W. contributed equally to this work.

²Current address: National Institutes of Health/National Human Genome Research Institute, Bethesda, MD.

³Current address: Department of Medicine, Massachusetts General Hospital, Boston, MA.

Received for publication November 5, 2010. Accepted for publication April 15, 2011.

This work was supported by Grants F31 AI061882 (to L.A.M.) and R01 AI081825 (to B.I.K.) from the National Institute of Allergy and Infectious Diseases (National Institutes of Health), the Howard Hughes Medical Institute (to R.M.), an Investigator Award from the Patrick and Catherine Weldon Donaghue Medical Research Foundation (to B.I.K.), and an Investigator in the Pathogenesis of Infectious Diseases Award from the Burroughs Wellcome Fund (to B.I.K.).

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health.

Address correspondence and reprint requests to Dr. Barbara Kazmierczak, Yale University School of Medicine, 333 Cedar Street, Box 208022, New Haven, CT 06520. E-mail address: barbara.kazmierczak@yale.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: BAL, bronchoalveolar lavage; CCSP, Clara cell secretory protein; hpi, hours postinfection; LTA, lipoteichoic acid; MTEC, mouse tracheal epithelial cell; poly(I:C), polyinosinic:polycytidylic acid.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/\$16.00

We have used a novel transgenic mouse model in which MyD88 expression is limited to epithelial cells of the airways to determine whether these cells play a nonredundant role in initiating protective immune responses during acute *P. aeruginosa* infection. Our findings indicate that *P. aeruginosa* is recognized by a combination of MyD88-independent and -dependent signaling events in the lung, and that IL-1R signaling by epithelial cells significantly contributes to the MyD88 dependence of this response.

Materials and Methods

Mice

Animal studies were performed in compliance with U.S. Department of Health and Human Services *Guide for the Care and Use of Laboratory Animals*. All animal protocols were approved by the Yale University Institutional Animal Care and Use Committee. Mice were housed in isolator cages under pathogen-free conditions. MyD88^{-/-} (N9) mice in a C57BL/6 background have been described (15). C57BL/6 control animals were purchased from the National Cancer Institute.

Transgenic mice with a functional MyD88 construct under the control of the CC10 promoter were constructed as follows. cDNA encoding murine MyD88 was amplified and ligated downstream of the rat CC10 promoter in a modified pBluescript II SK(+) plasmid. The resulting construct was linearized and purified, then injected into (C57BL/6J × SJL/J)F₂ embryos to generate CC10-MyD88 transgenic mice. Resulting founder mice were backcrossed onto the C57BL/6J background three times and then crossed with MyD88-deficient mice (N9) to generate CC10-MyD88 transgenic mice deficient in endogenous MyD88. These mice were crossed to MyD88^{-/-} N9 animals to produce MyD88^{-/-} transgene-positive and MyD88^{-/-} transgene-negative mice used to generate Figs. 2 and 3. The presence of the transgene was confirmed by PCR amplification of genomic DNA. We continued to backcross founder mice onto a C57BL/6J background; these N6 animals were subsequently crossed with MyD88^{-/-} mice (N9) to again generate CC10-MyD88 transgenic mice deficient in endogenous MyD88. Crosses of these mice with MyD88^{-/-} (N9) animals were used to produce the transgene-positive and transgene-negative animals used to generate Figs. 4 and 5. Littermate transgene-positive and transgene-negative mice were compared in all experiments.

IL-1R blockade with IL-1Ra

IL-1Ra (anakinra; Genentech) was administered to mice i.p. (10 mg/kg) daily for 4 d; control mice were injected with an equal volume of PBS. Mice were infected on day 4 of IL-1Ra treatment.

Bacteria

P. aeruginosa strains PA103 and PAK were maintained at -80°C as a 15% glycerol stock. Freshly plated bacteria were used to inoculate overnight cultures of Luria-Bertani medium. Bacteria were subcultured 1:50 into fresh Luria-Bertani medium and grown for 4 h, then diluted to an OD₆₀₀ of 0.06 into fresh Luria-Bertani medium and grown for 2 h, ensuring that bacteria used for infection were reproducibly in log phase. The titer of the bacterial inoculum was adjusted to a final concentration of $\sim 1.25 \times 10^6$ CFU/ml (PA103) or 1.25×10^7 CFU/ml (PAK) based on OD₆₀₀. Inoculum titer was determined for each infection by plating serial dilutions of the inoculum on Vogel-Bonner medium agar plates.

Mouse infections

Groups of 8- to 10-wk-old sex-matched mice were lightly anesthetized with methoxyflurane (2–4%) or isoflurane (20% in propylene glycol), then intranasally infected with PA103. Mice were euthanized 4, 12, and 24 h postinfection. Bronchoalveolar lavage (BAL) was performed using 3×1 ml cold PBS. BAL fluid supernatants were stored at -20°C for subsequent cytokine measurements. Cell pellets were treated with RBC lysis buffer prior to counting and preparation of cytopins, which were stained with a modified Wright-Giemsa technique (Diff-Quik) to perform manual differential counts.

To determine bacterial recovery from organs, lungs, liver, and spleen were removed, weighed, homogenized in PBS, and passed through a sterile screen to obtain a single-cell suspension. Samples were serially diluted and plated on Vogel-Bonner medium agar. Colonies were counted after 24 h incubation at 37°C.

Histopathology, immunohistochemistry, and immunofluorescence

Following BAL, perfused mouse lungs were fixed with Streck tissue fixative at 4°C overnight. Paraffin embedding of fixed lungs, sectioning, and H&E staining were carried out by Yale University Histopathology staff. Slides were reviewed in a blinded fashion by a pathologist (R.H).

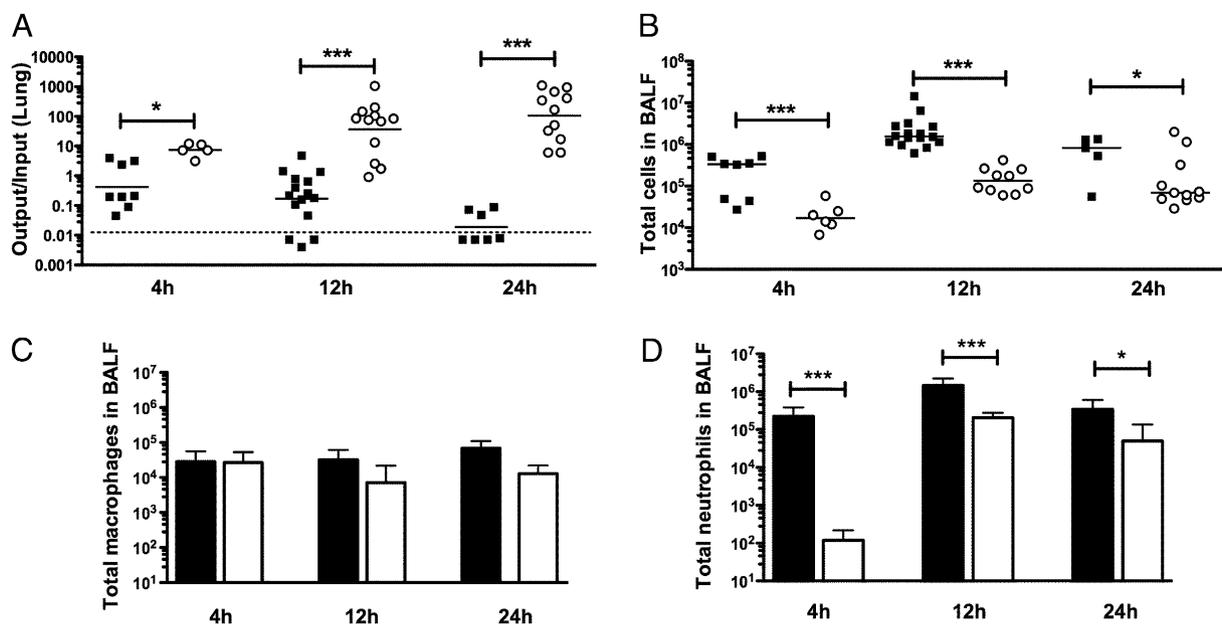


FIGURE 1. MyD88 is required for control of the nonflagellated *P. aeruginosa* strain PA103 in the lung. Wild-type (■) and MyD88^{-/-} (○) mice were intranasally infected with $\sim 5 \times 10^4$ CFU *P. aeruginosa* strain PA103, then sacrificed 4, 12, or 24 hpi. **A**, Lung bacterial burden expressed as number of bacteria recovered per lung (output) divided by measured inoculum administered (input). Each point represents an individual animal; the line indicates the geometric mean for each group. The dotted line shows the limit of detection. **B**, Total cells in BAL specimens from wild-type and MyD88^{-/-} mice at indicated times postinfection; lines indicate medians. Cytopins of BALs were stained with Diff-Quik to allow enumeration of macrophages (**C**) and neutrophils (**D**). Bars show medians \pm interquartile range. Log-transformed data were analyzed using ANOVA followed by Bonferroni posttests to compare outcomes at each time point. * $p < 0.05$, *** $p < 0.001$.

Prior to Ab staining, sections were deparaffinized, rehydrated, and steamed in Ag retrieval solution. Slides were blocked with 2% fish gelatin in PBST (PBS with 0.2% Triton X-100) before overnight exposure to primary Ab. Slides were washed with PBST, probed with a secondary Ab for an hour, washed again, and mounted with coverslips.

Clara cells were stained with goat anti-Clara cell secretory protein (1:3000) (gift from B. Stripp, University of Pittsburgh) and HRP-conjugated donkey anti-goat antiserum (1:5000), then developed with diaminobenzidine+ substrate-chromogen. Slides were imaged using a Nikon microscope with a SPOT color CCD camera. Images were assembled and labeled using Photoshop 7.0 (Adobe).

MyD88 was localized with rabbit anti-MyD88 (1:150) (Novus Biologicals, Littleton, CO) and Alexa Fluor 647 goat anti-rabbit IgG (Invitrogen). Slides were examined using a Bio-Rad MRC-1024 confocal microscope under identical laser and camera settings. Figures were assembled and labeled using Photoshop 7.0 (Adobe).

Mouse tracheal epithelial cell culture

Mouse tracheal epithelial cells (MTECs) were isolated and cultured as described by You et al. (16). MTECs (2×10^5) were seeded into collagen-coated 12-mm Transwells and cultured in MTEC/Plus medium in 5% CO₂ at 37°C. When transepithelial resistance was ≥ 1000 ohms/cm², apical chamber medium was removed to establish an air-liquid interface. Every other day MTEC/NS medium was replaced in the basal chamber and the apical chamber was washed with warm PBS (Ca/Mg-free) to remove dead cells. Cultures were used for experiments 15–20 d after air-liquid interface conditions were established. Cells were apically stimulated with 10 ng LPS in 100 μ l PBS (InvivoGen); an equal volume of PBS was added to control wells.

Measurement of MyD88 gene expression in MTECs

RNA was harvested from membranes using the RNeasy kit (Qiagen) according to the manufacturer's protocols. An on-column DNA digestion step was included. cDNA was synthesized using 1 μ g purified RNA, OligodT_{12–18} primer (Invitrogen), and SuperScript III reverse transcriptase (Invitrogen). For quantitative real-time PCR, 20 ng cDNA was added to iQ SYBR Green Supermix (Bio-Rad) and amplified using primers QMyD883F (5'-ATGATCCGGCAACTAGAACAG-3') and QMyD883R (5'-TCGTCAGAAACAACACCAC-3'). To control for the amount of total RNA, the same reaction was carried out using primers QGapdhF (5'-TGTCATGGGTGTGAACCACGAGAA-3') and QGapdhR (5'-GAGCCCTTCCTCAATGCCAAAGTT-3'), which amplify the GAPDH transcript. Reactions were run in a DNA Engine Opticon 2 (Bio-Rad) and analyzed using Opticon Monitor 3.1 software (Bio-Rad). Relative amounts of MyD88 transcript were calculated according to Pfaffl (17).

Peritoneal macrophage isolation and stimulation

Thioglycolate-elicited peritoneal macrophages were harvested and seeded to 24-well tissue culture dishes at a density of 5×10^5 cells/well in DMEM plus 10% FBS. Cells were stimulated in triplicate with lipoteichoic acid (LTA; 10 μ g/ml), LPS (50 ng/ml), or polyinosinic:polycytidylic acid (poly(I:C); 25 μ g/ml) for 18 h. All ligands were purchased from InvivoGen.

Measurement of cytokines

TNF α , MIP-2, and KC in tissue culture supernatants were measured by sandwich ELISA (R&D Systems) according to the manufacturer's protocol. Cytokine and chemokine levels in BAL fluid were measured using Beadlyte multiplex cytokine detection systems from Millipore or Bio-Rad according to the manufacturer's protocol.

Statistical analysis

Statistical analysis was performed using Prism 5.0 software (GraphPad Software). Raw (cytokine amounts) or log-transformed data (bacterial burden, recruited cell count numbers) were normally distributed and were analyzed by ANOVA followed by a Bonferroni multiple comparison test for all experiments involving three or more experimental groups. The pairwise comparisons between wild-type and MyD88^{-/-} cytokine levels were analyzed using *t* tests. A two-tailed *p* value <0.05 was considered significant.

Results

MyD88^{-/-} is required for control of nonflagellate *P. aeruginosa* PA103

MyD88 is required for control replication of several *P. aeruginosa* strains in the mouse lung, including flagellated laboratory strains

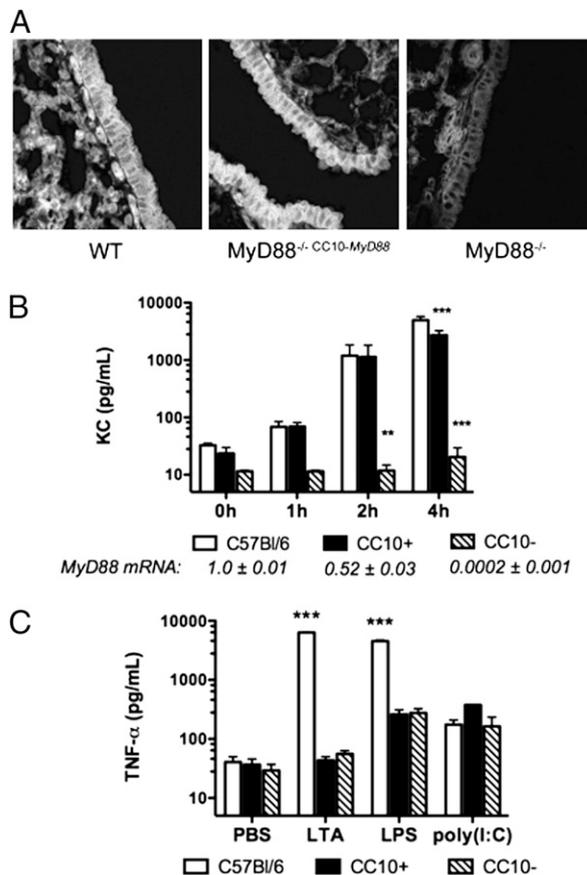


FIGURE 2. MyD88^{-/-CC10-MyD88} mice express MyD88 in airway epithelial cells but not in macrophages. *A*, Lung sections prepared from C57Bl/6 (wild-type [WT]), MyD88^{-/-CC10-MyD88}, and MyD88^{-/-} were stained with anti-MyD88 Ab as described in *Materials and Methods*. Original magnification $\times 400$. *B*, Murine tracheal epithelial cells from C57Bl/6 (WT; white columns), *CC10-MyD88* transgene-positive (black columns), and *CC10-MyD88* transgene-negative (striped columns) mice cultured on Transwells at an air-liquid interface were treated with LPS (100 ng/ml) in triplicate. KC concentrations were measured in supernatants by ELISA. Means \pm SD are plotted for a representative experiment (repeated twice). The relative amounts of MyD88 transcript (normalized to GAPDH) expressed by each cell culture were determined by quantitative RT-PCR at the end of the experiment and are reported as means \pm SD. ANOVA followed by Bonferroni posttest was used to determine whether responses of transgene-positive or -negative cultures differ significantly from those of wild-type cells at each time point. *C*, Peritoneal macrophages harvested from wild-type (white columns), *CC10-MyD88*⁺ (black columns), and *CC10-MyD88*⁻ (striped columns) mice were stimulated in triplicate with LTA (10 μ g/ml), LPS (50 ng/ml), or poly(I:C) (25 μ g/ml) for 18 h. TNF- α levels in culture supernatants were measured by ELISA; bars represent means \pm SD of a representative experiment (repeated twice). ANOVA followed by Bonferroni posttest showed that wild-type macrophage responses to LTA and LPS, but not to poly(I:C), differ significantly from those of transgene-positive or -negative macrophages. $^{**}p < 0.01$, $^{***}p < 0.001$.

and a cystic fibrosis patient isolate (5, 6). We confirmed that this was also the case for the well-characterized human pneumonia isolate, PA103, which does not express flagellin and secretes a distinct complement of type 3 secretion system effectors, namely ExoU (a phospholipase A₂) and ExoT (a bifunctional GTPase-activating protein/ADP ribosyl transferase). Wild-type and MyD88^{-/-} mice were intranasally infected with ~ 0.05 the LD₅₀ inoculum of PA103 ($\sim 5 \times 10^4$ CFU), then euthanized at 4, 12, or 24 h postinfection (hpi) to measure bacterial replication

and host immune response (Fig. 1). Rapid neutrophil recruitment to airways was observed at 4 hpi in wild-type animals, but absent in *MyD88*^{-/-} mice at this early time point. Delayed neutrophil recruitment was observed in *MyD88*^{-/-} animals, but was insufficient to control bacterial replication in the lung. These results are consistent with those reported by Skerrett et al. (6) using *MyD88*^{-/-} mice infected with larger inocula of the flagellated *P. aeruginosa* strain PAK. Of note, recruitment of neutrophils to lungs of *MyD88*^{-/-} mice (via non-*MyD88*-dependent stimuli) prior to *P. aeruginosa* infection conferred a significant survival advantage in the study of Koh et al. (9), suggesting that *MyD88*-deficient neutrophils are capable of clearing *P. aeruginosa*. The inocula used in this particular study were extremely low (38–256 CFU/mouse) and no survival benefit was observed in mice infected with 775 CFU/animal, suggesting that clearance of *P. aeruginosa* occurs most effectively at high neutrophil-to-bacteria ratios. This may be especially important for PA103, as the ExoU effector that it translocates into host cells is capable of lysing recruited neutrophils via its phospholipase A₂ activity (18).

Construction of a transgenic mouse in which *MyD88* expression is restored to cells of the airway epithelium

To address the role of airway epithelial cells in *MyD88*-dependent responses to bacterial infection, we constructed a transgenic mouse in which *MyD88* is expressed from a CC10 promoter. The CC10 promoter element is active in cells of the bronchiolar epithelium and normally drives the expression of CCSP (19). We confirmed that the distribution of CCSP was similar between wild-type, *MyD88*^{-/-}, and transgene-positive *MyD88*^{-/-}*CC10-MyD88* animals by immunohistochemistry (data not shown). We next examined expression of *MyD88* in the lungs of these mice by immunofluorescence. A far-red secondary Ab was used to visualize positive cells to minimize tissue autofluorescence, which interfered with signal acquisition in the FITC and rhodamine channels. Wild-type animals showed robust staining for *MyD88* in multiple cell types, including airway epithelial cells (Fig. 2A). Transgene-positive *MyD88*^{-/-}*CC10-MyD88* animals showed positive staining with anti-*MyD88* Ab that was restricted to cells within the bronchiolar epithelium. Neither *MyD88*^{-/-} nor transgene-negative littermate controls showed staining with anti-*MyD88* Ab (Fig. 2A and data not shown).

Cultured primary MTECs respond to LPS in a *MyD88*-dependent fashion by secreting chemokines such as KC (20). We demonstrated that the *CC10-MyD88* transgene restored the ability of epithelial cells purified from mouse trachea and differentiated at an air-liquid interface to respond to LPS (Fig. 2B).

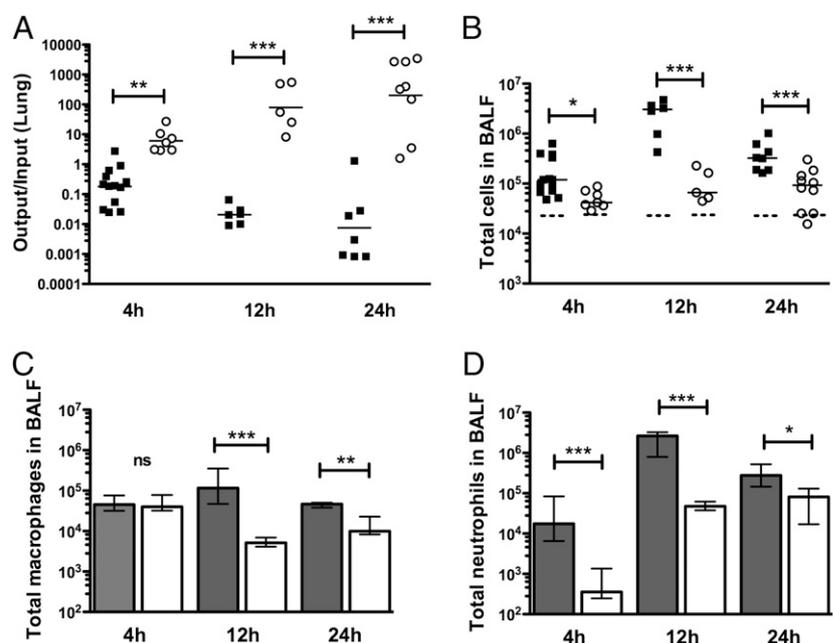
We did not expect the CC10 promoter to be active in bone marrow-derived cells. We confirmed this by measuring the response of macrophages isolated from C57BL/6, transgene-positive, and transgene-negative mice to TLR ligands. As shown in Fig. 2C, there was no response to the TLR2 ligand LTA by macrophages harvested from transgene-positive or transgene-negative mice; this result is expected, as TLR2 signaling is mediated solely by *MyD88*. Macrophages from wild-type, transgene-positive, and transgene-negative mice all showed equivalent responses to the *MyD88*-independent ligand poly(I:C), whereas responses of transgene-positive and -negative macrophages to LPS were equally blunted as compared with wild-type macrophages. This reflects the presence of both *MyD88*-dependent and -independent pathways downstream of TLR4. Thus, the *CC10-MyD88* transgene did not restore *MyD88*-dependent macrophage signaling.

Expression of *MyD88* in airway epithelial cells is sufficient for *P. aeruginosa* clearance in the lung

We tested whether transgenic expression of *MyD88* in airway epithelial cells was sufficient to restore a protective innate immune response to *P. aeruginosa* in mice lacking endogenous *MyD88*. Transgene-positive (*CC10-MyD88*⁺) animals and sex- and age-matched transgene-negative (*CC10-MyD88*⁻) littermate controls were infected with PA103 and examined 4, 12, and 24 hpi. *CC10-MyD88*⁺ animals showed lower bacterial burdens in the lung than did *CC10-MyD88*⁻ littermate controls at all time points (Fig. 3A). At 24 hpi, *CC10-MyD88*⁺ mice had cleared >99% of the infecting inoculum (geometric mean), similar to C57BL/6 mice (>98% inoculum cleared), and no bacteria were recovered from liver or spleen. In contrast, *CC10-MyD88*⁻ mice, similar to *MyD88*^{-/-} animals, were unable to control PA103 replication in the lung and had detectable bacteria in liver and spleen (Fig. 3A and data not shown).

The total number of cells recruited to the airways also differed significantly between transgene-positive and -negative animals at all time points examined (Fig. 3B). Although transgene-negative

FIGURE 3. Expression of *MyD88* in the airway epithelium is sufficient to control *P. aeruginosa* infection. *MyD88*^{-/-}*CC10-MyD88* (*CC10-MyD88*⁺; ■/gray columns) and transgene-negative littermate controls (*CC10-MyD88*⁻; ○/white columns) were infected with $\sim 5 \times 10^4$ CFU PA103, then sacrificed 4, 12, and 24 hpi. Lung bacterial burden (A) was determined as described previously; each symbol represents an individual animal, and the line indicates the geometric mean. Total cell counts (B) and numbers of macrophages (C) and neutrophils (D) in BAL samples were determined as described previously. Medians are indicated for each group; error bars show interquartile range. Log-transformed data were analyzed using ANOVA followed by Bonferroni posttests to compare outcomes at each time point. The dashed lines in B show the median number of cells harvested from uninfected mice of each genotype ($n = 4$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ns, not significant.



mice had higher bacterial burdens in lung, the total number of cells present in BAL fluid was lower and comparable to that observed in uninfected transgene-positive or -negative mice. At 4 hpi, this difference in total cells was primarily attributable to decreased numbers of neutrophils in the airways of transgene-negative mice (Fig. 3D). At later time points, fewer macrophages and neutrophils were present in *CC10-MyD88^{-/-}* BAL fluid. In addition to diminished recruitment of neutrophils to the airways, increased phagocyte killing by ExoU-secreting bacteria is likely to contribute to this reduction in both macrophages and neutrophils at later time points during infection (18). The recruitment of neutrophils to the airways was not observed in BAL fluid harvested from uninfected transgene-positive or -negative mice, with manual differentials yielding <0.5% polymorphonuclear cells ($n = 4$ mice). Thus, airway inflammation is a response to infection and not the consequence of transgene expression.

Lung histology of infected wild-type, *CC10-MyD88⁺*, and *MyD88^{-/-}* animals was examined at 4, 12, and 24 hpi and was consistent with BAL findings (Fig. 4 and data not shown). By 24 hpi, *MyD88^{-/-}* lungs showed pronounced airspace obliteration, airway epithelial ulceration, and tissue hemorrhage. In marked contrast, *CC10-MyD88⁺* mice showed minimal findings of alveolar consolidation and, similar to wild-type mice, no evidence of epithelial ulceration at this time point.

Production of IL-1 β is independent of MyD88 in PA103 infected mice

Rapid recruitment of neutrophils to the airways was strongly correlated with bacterial clearance in both wild-type and transgenic mice. To understand which signaling events contributed to efficient recruitment, we measured BAL fluid concentrations of a panel of chemokines and cytokines in wild-type and mutant mice at 4 hpi. Most cytokines and chemokines were undetectable in BAL fluid collected from control uninfected wild-type or *MyD88^{-/-}* mice (Table I). Many remained undetectable or at low levels (<20 pg/ml) in wild-type mouse BAL fluid at 4 hpi (including IL-12/IL-23 (p40), IL-17, and IFN- γ) or showed modest increases (20–75 pg/ml) (IL-1 α , IL-5, RANTES). Several, however, were strongly induced in wild-type mice but not in *MyD88^{-/-}* counterparts (Fig. 5). IL-1 β did not follow this pattern, as both wild-type and *MyD88^{-/-}* mice showed similar elevations of this cytokine early postinfection.

Cytokines and chemokines were also measured in BAL fluid samples collected at 4 hpi from *CC10-MyD88* transgene-positive and -negative littermate mice (Fig. 5). Direct comparisons to *MyD88* (N9) mice were not made, as the transgenic animals are not fully backcrossed onto the C57BL/6 background. Expression of the *MyD88* transgene in epithelial cells resulted in significantly higher levels of IL-6, MIP-1 β , KC, MIP-2, and GM-CSF in BAL fluid as compared with littermate controls, indicating that epithelial cell *MyD88* signaling contributed significantly to the expression of neutrophil chemoattractants and activators in this setting. We again observed comparable levels of IL-1 β in BAL fluid from transgene-positive and -negative mice. These cytokine responses were not attributable to expression of the transgene itself, as BAL fluid samples collected from the lungs of uninfected transgene-positive mice did not show increased expression of any of these cytokines, and indeed they were comparable to those observed in BAL fluid collected from uninfected transgene-negative animals.

IL-1R blockade with anakinra prevents neutrophil recruitment to the transgenic mouse lung and impairs bacterial clearance

The *MyD88* dependence of innate immune responses to *P. aeruginosa* is frequently interpreted as a requirement for TLR-

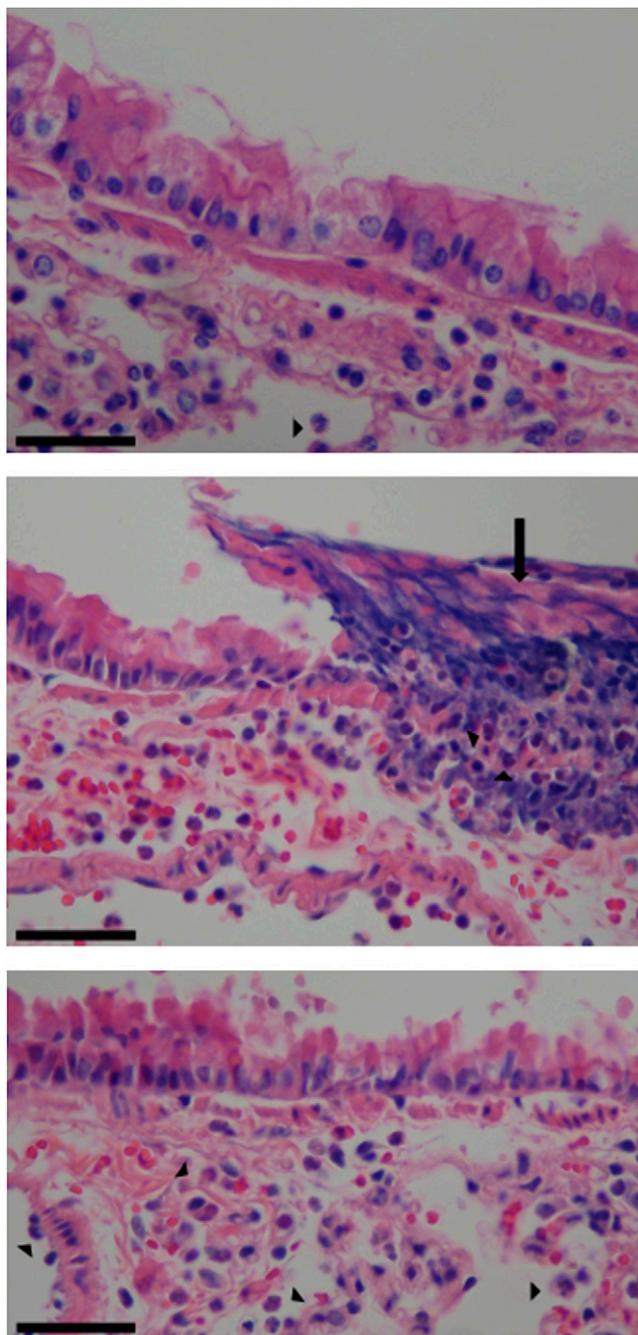


FIGURE 4. Lung histopathology following *P. aeruginosa* PA103 infection shows a distinct inflammatory response in *MyD88^{-/-}CC10-MyD88* mice. H&E-stained paraffin-embedded sections were prepared from *CC10-MyD88* transgene-positive (top panel), *MyD88^{-/-}* (middle panel), and wild-type (bottom panel) mouse lungs harvested 24 hpi. *MyD88^{-/-}* lungs showed epithelial ulceration (arrow), which was absent from transgene-positive and wild-type lungs. Arrowheads indicate neutrophils. Scale bars, 50 μ m.

mediated recognition of this pathogen. Nonetheless, infection of mice in the absence of TLR2, TLR4, and TLR5 signaling does not phenocopy infection of *MyD88* knockout mice (21), suggesting that either additional TLRs are involved in this process, or that other *MyD88*-adapted receptors (e.g., IL-1R, IL-18R) play important roles in recognition. As increased IL-1 β is one of the initial responses seen after PA103 infection, we tested the importance of IL-1R signaling in the *CC10-MyD88⁺* mice by blocking this pathway with IL-1Ra (anakinra). PBS-pretreated

Table I. Cytokine and chemokine levels in BAL fluid

	Uninfected MyD88 ^{-/-}	Uninfected C57BL/6	C57BL/6 4 h after PA103 Infection
IL-1 α	nd	0.80 \pm 0.04	31.6 \pm 10.1
IL-1β	nd	nd	102.6 \pm 14.2
IL-2	nd	nd	nd
IL-3	nd	nd	nd
IL-4	nd	nd	1.5 \pm 0.5
IL-5	nd	nd	75.6 \pm 11.8
IL-6	nd	nd	1794 \pm 262
KC	nd	nd	1129 \pm 139
IL-9	nd	nd	15.5 \pm 3.9
IL-10	18.2 \pm 0.7	nd	15.7 \pm 3.1
IL-12/IL-23 (p40)	14.2 \pm 1.9	nd	10.7 \pm 4.7
IL-12	nd	nd	5.6 \pm 3.2
IL-13	nd	nd	nd
IL-17	nd	nd	18.1 \pm 4.1
TNF-α	nd	nd	674.8 \pm 108.9
MIP-1β	9.4 \pm 1.5	nd	495.4 \pm 64.2
GM-CSF	nd	nd	97.4 \pm 26.0
IFN- γ	nd	nd	5.1 \pm 2.0
RANTES	nd	nd	45.7 \pm 3.0
MCP-1	nd	nd	284.4 \pm 30.4
VEGF	10.8 \pm 2.9	nd	13.7 \pm 1.6

Cytokine concentrations are expressed in pg/ml (mean \pm SD, $n = 3-5$). Cytokines and chemokines showing >100-fold induction postinfection are in bold. nd, not detected.

CC10-MyD88⁺ and *CC10-MyD88⁻* animals were used as controls. Both transgene-negative animals and transgene-positive animals pretreated with IL-1Ra showed a modest increase in bacterial recovery from lungs as compared with transgene-positive controls at 4 hpi (Fig. 6A). Transgene-positive mice treated with IL-1Ra showed a defect in neutrophil recruitment comparable to that of transgene-negative littermates (Fig. 6D). By 24 hpi, transgene-positive mice had largely cleared PA103 from the lung, whereas both transgene-negative and IL-1Ra-treated transgenic mice had significantly higher lung bacterial burdens (Fig. 6B). Thus, IL-1R signaling by epithelial cells was required for early neutrophil recruitment in *CC10-MyD88⁺* mice, and the absence of this specific innate response was associated with a significant defect in bacterial clearance.

We assayed cytokines and chemokines in BAL fluid collected from IL-1Ra-treated animals and controls (Fig. 5). IL-1R blockade had no significant effect on IL-6 or MIP-1 β production, suggesting that other MyD88-dependent receptors played primary roles in the elaboration of these cytokines following PA103 infection of transgene-positive mice. In contrast, median levels of GM-CSF and of the CXC chemokines MIP-2 and KC were lower in IL-1Ra-treated mice as compared with untreated *CC10-MyD88⁺* controls. Diminished levels of these chemoattractants likely contribute to the observed defect in neutrophil recruitment that we observed (22).

Discussion

In summary, our findings demonstrate that MyD88 signaling restricted to airway epithelial cells is sufficient to generate a protective innate immune response in mice infected with *P. aeruginosa*. Previous studies have shown that epithelial cells play a nonredundant role in innate immune responses. Transgenic mice in which NF- κ B was specifically repressed in airway epithelial cells had attenuated proinflammatory responses to endotoxin instillation compared with transgene-negative littermate controls (23, 24). Infections of radiation chimeras constructed between wild-type and MyD88^{-/-} mice likewise showed MyD88 signaling in non-bone marrow-derived cells was required for proinflammatory responses, as wild-type recipients of either wild-type

or MyD88^{-/-} bone marrow showed more robust rapid neutrophil recruitment than did MyD88^{-/-} recipients reconstituted with either wild-type or MyD88-deficient bone marrow (10). Wild-type recipients of MyD88^{-/-} bone marrow had diminished numbers of lung bacteria at both 4 and 24 hpi as compared with controls lacking MyD88 in both donor- and recipient-derived cells. Thus, MyD88 expression by bone marrow cells was not required for bacterial control in this pneumonia model. Our work extends these findings by demonstrating that MyD88 signaling restricted to airway epithelial cells is sufficient to generate protective responses following *P. aeruginosa* infection.

A requirement for MyD88 signaling is often interpreted as evidence of a requirement for TLR signaling. However, the immune defect of *P. aeruginosa* infected MyD88-deficient mice is more severe than that of mice in which signaling via multiple TLR pathways is absent (21). Our study demonstrates that a key role of epithelial cell MyD88 is to serve as an adaptor for IL-1R, as treatment of transgenic mice with the receptor antagonist IL-1Ra inhibited the secretion of neutrophil chemoattractants and the recruitment of neutrophils to the airways to levels comparable to those observed in transgene-negative animals. We were interested to observe that IL-1Ra-treated transgenic animals had an intermediate defect in bacterial clearance at a later time point (24 hpi) as compared with transgene-positive and -negative animals (Fig. 6B). This argues that both IL-1R-dependent and -independent MyD88-mediated epithelial signals measurably contribute to clearance. The mechanistic basis for this observed difference in clearance remains under investigation.

We have recently demonstrated that the rapid neutrophilic response to *P. aeruginosa* pulmonary infection in mice requires caspase-1 activity in bone marrow-derived cells and intact IL-1R signaling (25). The present study shows that IL-1R signaling limited to airway epithelial cells is sufficient for such a response, and it is consistent with a model in which efficient pathogen recognition requires participation of both bone marrow-derived cells (e.g., alveolar macrophage inflammasome activation) and epithelia. Of note, we observed that IL-1 β production was largely independent of MyD88 in *P. aeruginosa* infected mice (Fig. 5), an observation that has been made in other in vivo infection models

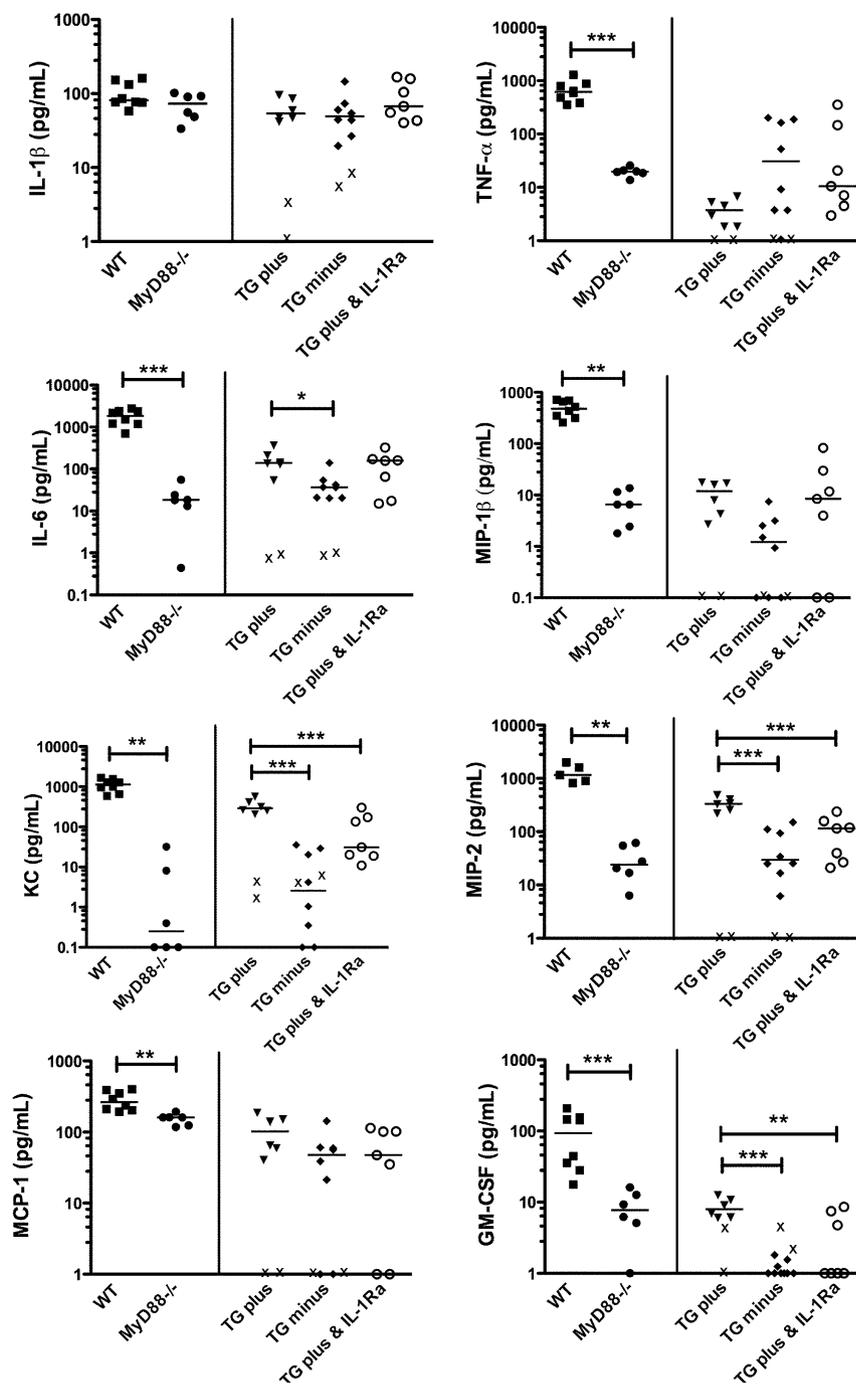


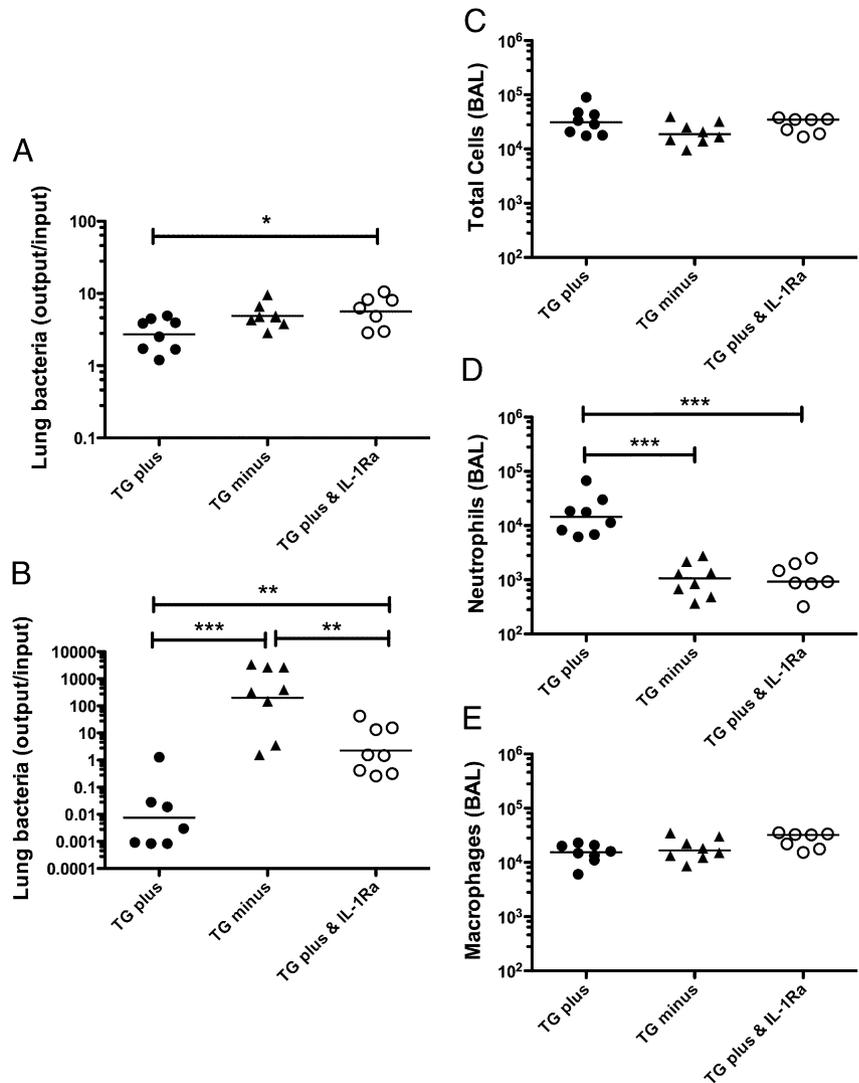
FIGURE 5. Cytokine and chemokine levels in infected wild-type, mutant, and transgenic (TG) mice BAL fluid at 4 hpi. BAL fluid was collected from mice described in Fig. 1 (wild-type [WT] [■] and MyD88^{-/-} [●]) and Fig. 5 (*CC10-MyD88*⁺, TG plus [▼]; *CC10-MyD88*⁻, TG minus [◆]; and *CC10-MyD88*⁺ mice pretreated with IL-1Ra, TG plus & IL-1Ra [○]). Cytokine and chemokine levels were measured using the Beadlyte assay (Bio-Rad), and medians for each group are indicated. Data points on the x-axis were below the level of detection for the assay. The *p* values for WT versus MyD88^{-/-} mice were calculated using *t* tests. The *p* values for pairwise comparisons between TG plus, TG minus, and TG plus & IL-1Ra mice were calculated using ANOVA followed by Bonferroni posttests. ×, BAL cytokine and chemokine levels for uninfected TG plus and TG minus mice. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

(26). *P. aeruginosa* activates caspase-1 via the NLRC4 (IPAF) inflammasome in bone marrow-derived and alveolar macrophages, resulting in pro-IL-1 β processing and secretion (27–30). In this in vitro system, secretion requires two signals: the first, induction of pro-IL-1 β expression triggered by a TLR ligand, IL-1, or TNF- α (31, 32) followed by a second, incompletely understood signal that leads to activation of the inflammasome (33). If the first signal is also required for IL-1 β secretion in vivo, it could be provided to macrophages in MyD88^{-/-} mice by LPS interactions with TLR4/TRIF or by TNF- α , which is detected (albeit at very low levels) in BAL fluid from these mice. Wild-type mice do secrete more IL-1 β than do MyD88^{-/-} animals when infected with flagellin-expressing *P. aeruginosa* strains, such as PAK (Supplemental Fig. 1 and Ref. 10), which we attribute to TLR5-mediated induction of pro-IL-1 β . Importantly, however, induction of IL-1 β

secretion is also observed in PAK infected MyD88^{-/-} mice. Pro-IL-1 β can be cleaved and activated not only by caspase-1 but also by other proteases, such as serine proteases expressed by neutrophils and mast cells (34, 35). These cells are absent in the MyD88^{-/-} airway at 4 hpi, however, and they are unlikely to contribute substantially to measured IL-1 β at this time.

TNF- α secretion is largely dependent on MyD88 signaling and was not restored by the *CC10-MyD88* transgene: median TNF- α levels in BAL fluid from infected MyD88^{-/-} and *CC10-MyD88*⁺ mice were 19.7 and <10 pg/ml, respectively. Significantly higher TNF- α levels were measured in PA103 infected C57BL/6 mice BAL fluid (median, 675 pg/ml); the likely source of TNF- α is bone marrow-derived cells, based on the radiation chimera experiments of Hajjar et al. (10). TNF- α makes a significant contribution to host defense in *Pseudomonas*-infected wild-type

FIGURE 6. Inhibition of IL-1R signaling attenuates early neutrophil recruitment and decreases bacterial clearance in infected MyD88^{-/-}CC10-MyD88 mice. CC10-MyD88⁺ (transgene [TG] plus) (●), CC10-MyD88⁻ (TG minus [▲]), and CC10-MyD88⁺ mice pretreated with IL-1Ra (TG plus & IL-1Ra [○]) were infected with PA103 and euthanized at 4 or 24 hpi. Lung bacterial burden was determined at 4 (A) and 24 hpi (B) and is expressed as output/input (line indicates geometric mean). Each point represents an individual animal. Total cell (C), neutrophil (D), and macrophage (E) counts in BAL fluid were determined at 4 hpi; lines indicate medians for each group. Log-transformed data were analyzed using ANOVA followed by Bonferroni posttests to compare outcomes at each time point. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.



mice (36). Indeed, IL-1 and TNF- α both elicit rapid NF- κ B activation, cytokine production, and neutrophil recruitment following bacterial infection, and these innate responses are profoundly attenuated only when both pathways are interrupted, as has been described in IL-1R/TNFR-deficient animals infected with pneumococcus or *Escherichia coli* (37, 38). Our data demonstrate, however, that IL-1R signaling is necessary and sufficient to elicit a rapid and protective response in the absence of TNF signals, as in these transgenic mice that fail to produce TNF.

Acknowledgments

We thank Carla Weibel for excellent assistance with animal experiments, B. Stripp (University of Pittsburgh) for CCSP-specific antiserum, and Peter Peduzzi (Yale University) for helpful discussion regarding statistical analysis.

Disclosures

The authors have no financial conflicts of interest.

References

1. Sadikot, R. T., T. S. Blackwell, J. W. Christman, and A. S. Prince. 2005. Pathogen-host interactions in *Pseudomonas aeruginosa* pneumonia. *Am. J. Respir. Crit. Care Med.* 171: 1209–1223.
2. Hauser, A. R., E. Cobb, M. Bodi, D. Mariscal, J. Vallés, J. N. Engel, and J. Rello. 2002. Type III protein secretion is associated with poor clinical outcomes in

- patients with ventilator-associated pneumonia caused by *Pseudomonas aeruginosa*. *Crit. Care Med.* 30: 521–528.
3. Roy-Burman, A., R. H. Savel, S. Racine, B. L. Swanson, N. S. Revadigar, J. Fujimoto, T. Sawa, D. W. Frank, and J. P. Wiener-Kronish. 2001. Type III protein secretion is associated with death in lower respiratory and systemic *Pseudomonas aeruginosa* infections. *J. Infect. Dis.* 183: 1767–1774.
4. Hauser, A. R. 2009. The type III secretion system of *Pseudomonas aeruginosa*: infection by injection. *Nat. Rev. Microbiol.* 7: 654–665.
5. Power, M. R., Y. Peng, E. Maydanski, J. S. Marshall, and T.-J. Lin. 2004. The development of early host response to *Pseudomonas aeruginosa* lung infection is critically dependent on myeloid differentiation factor 88 in mice. *J. Biol. Chem.* 279: 49315–49322.
6. Skerrett, S. J., H. D. Liggitt, A. M. Hajjar, and C. B. Wilson. 2004. Cutting edge: myeloid differentiation factor 88 is essential for pulmonary host defense against *Pseudomonas aeruginosa* but not *Staphylococcus aureus*. *J. Immunol.* 172: 3377–3381.
7. von Bernuth, H., C. Picard, Z. Jin, R. Rankla, H. Xiao, C.-L. Ku, M. Chrabieh, I. B. Mustapha, P. Ghandil, Y. Camcioglu, et al. 2008. Pyogenic bacterial infections in humans with MyD88 deficiency. *Science* 321: 691–696.
8. Medzhitov, R. M. 2001. Toll-like receptors and innate immunity. *Nat. Rev. Immunol.* 1: 135–145.
9. Koh, A. Y., G. P. Priebe, C. Ray, N. Van Rooijen, and G. B. Pier. 2009. Inescapable need for neutrophils as mediators of cellular innate immunity to acute *Pseudomonas aeruginosa* pneumonia. *Infect. Immun.* 77: 5300–5310.
10. Hajjar, A. M., H. Harowicz, H. D. Liggitt, P. J. Fink, C. B. Wilson, and S. J. Skerrett. 2005. An essential role for non-bone-marrow-derived cells in control of *Pseudomonas aeruginosa* pneumonia. *Am. J. Respir. Cell Mol. Biol.* 33: 470–475.
11. Feuillet, V., S. Medjane, I. Mondor, O. Demaria, P. P. Pagni, J. E. Galán, R. A. Flavell, and L. Alexopoulou. 2006. Involvement of Toll-like receptor 5 in the recognition of flagellated bacteria. *Proc. Natl. Acad. Sci. USA* 103: 12487–12492.

12. Morris, A. E., H. D. Liggitt, T. R. Hawn, and S. J. Skerrett. 2009. Role of Toll-like receptor 5 in the innate immune response to acute *P. aeruginosa* pneumonia. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 297: L1112–L1119.
13. Jyot, J., A. Sonawane, W. Wu, and R. Ramphal. 2007. Genetic mechanisms involved in the repression of flagellar assembly by *Pseudomonas aeruginosa* in human mucus. *Mol. Microbiol.* 63: 1026–1038.
14. Mahenthalingam, E., M. E. Campbell, and D. P. Speert. 1994. Nonmotility and phagocytic resistance of *Pseudomonas aeruginosa* isolates from chronically colonized patients with cystic fibrosis. *Infect. Immun.* 62: 596–605.
15. Adachi, O., T. Kawai, K. Takeda, M. Matsumoto, H. Tsutsui, M. Sakagami, K. Nakanishi, and S. Akira. 1998. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity* 9: 143–150.
16. You, Y., E. J. Richer, T. Huang, and S. L. Brody. 2002. Growth and differentiation of mouse tracheal epithelial cells: selection of a proliferative population. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 283: L1315–L1321.
17. Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 29: 2003–2011.
18. Diaz, M. H., C. M. Shaver, J. D. King, S. Musunuri, J. A. Kazzaz, and A. R. Hauser. 2008. *Pseudomonas aeruginosa* induces localized immunosuppression during pneumonia. *Infect. Immun.* 76: 4414–4421.
19. Stripp, B. R., P. L. Sawaya, D. S. Luse, K. A. Wikenheiser, S. E. Wert, J. A. Huffman, D. L. Lattier, G. Singh, S. L. Katyal, and J. A. Whitsett. 1992. *cis*-Acting elements that confer lung epithelial cell expression of the CC10 gene. *J. Biol. Chem.* 267: 14703–14712.
20. Raoust, E., V. Balloy, I. Garcia-Verdugo, L. Touqui, R. Ramphal, and M. Chignard. 2009. *Pseudomonas aeruginosa* LPS or flagellin are sufficient to activate TLR-dependent signaling in murine alveolar macrophages and airway epithelial cells. *PLoS ONE* 4: e7259.
21. Skerrett, S. J., C. B. Wilson, H. D. Liggitt, and A. M. Hajjar. 2007. Redundant Toll-like receptor signaling in the pulmonary host response to *Pseudomonas aeruginosa*. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 292: L312–L322.
22. Tsai, W. C., R. M. Strieter, B. Mehrad, M. W. Newstead, X. Zeng, and T. J. Standiford. 2000. CXC chemokine receptor CXCR2 is essential for protective innate host response in murine *Pseudomonas aeruginosa* pneumonia. *Infect. Immun.* 68: 4289–4296.
23. Poynter, M. E., C. G. Irvin, and Y. M. W. Janssen-Heininger. 2003. A prominent role for airway epithelial NF- κ B activation in lipopolysaccharide-induced airway inflammation. *J. Immunol.* 170: 6257–6265.
24. Skerrett, S. J., H. D. Liggitt, A. M. Hajjar, R. K. Ernst, S. I. Miller, and C. B. Wilson. 2004. Respiratory epithelial cells regulate lung inflammation in response to inhaled endotoxin. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 287: L143–L152.
25. Wangdi, T., L. A. Mijares, and B. I. Kazmierczak. 2010. In vivo discrimination of type 3 secretion system-positive and -negative *Pseudomonas aeruginosa* via a caspase-1-dependent pathway. *Infect. Immun.* 78: 4744–4753.
26. Mayer-Barber, K. D., D. L. Barber, K. Shenderov, S. D. White, M. S. Wilson, A. Cheever, D. Kugler, S. Hieny, P. Caspar, G. Núñez, et al. 2010. Caspase-1 independent IL-1 β production is critical for host resistance to *Mycobacterium tuberculosis* and does not require TLR signaling in vivo. *J. Immunol.* 184: 3326–3330.
27. Franchi, L., J. Stoolman, T. D. Kanneganti, A. Verma, R. Ramphal, and G. Núñez. 2007. Critical role for Ipaf in *Pseudomonas aeruginosa*-induced caspase-1 activation. *Eur. J. Immunol.* 37: 3030–3039.
28. Miao, E. A., R. K. Ernst, M. Dors, D. P. Mao, and A. Aderem. 2008. *Pseudomonas aeruginosa* activates caspase 1 through Ipaf. *Proc. Natl. Acad. Sci. USA* 105: 2562–2567.
29. Sutterwala, F. S., L. A. Mijares, L. Li, Y. Ogura, B. I. Kazmierczak, and R. A. Flavell. 2007. Immune recognition of *Pseudomonas aeruginosa* mediated by the IPAF/NLRC4 inflammasome. *J. Exp. Med.* 204: 3235–3245.
30. Galle, M., P. Schotte, M. Haegman, A. Wullaert, H. J. Yang, S. Jin, and R. Beyaert. 2008. The *Pseudomonas aeruginosa* type III secretion system plays a dual role in the regulation of caspase-1 mediated IL-1 β maturation. *J. Cell. Mol. Med.* 12(5A): 1767–1776.
31. Dinarello, C. A. 2009. Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* 27: 519–550.
32. Franchi, L., T. Eigenbrod, and G. Núñez. 2009. Cutting edge: TNF- α mediates sensitization to ATP and silica via the NLRP3 inflammasome in the absence of microbial stimulation. *J. Immunol.* 183: 792–796.
33. Brodsky, I. E., and D. Monack. 2009. NLR-mediated control of inflammasome assembly in the host response against bacterial pathogens. *Semin. Immunol.* 21: 199–207.
34. Guma, M., L. Ronacher, R. Liu-Bryan, S. Takai, M. Karin, and M. Corr. 2009. Caspase 1-independent activation of interleukin-1 β in neutrophil-predominant inflammation. *Arthritis Rheum.* 60: 3642–3650.
35. Lin, T.-J., R. Garduno, R. T. M. Boudreau, and A. C. Issekutz. 2002. *Pseudomonas aeruginosa* activates human mast cells to induce neutrophil trans-endothelial migration via mast cell-derived IL-1 α and β . *J. Immunol.* 169: 4522–4530.
36. Sadikot, R. T., H. Zeng, M. Joo, M. B. Everhart, T. P. Sherrill, B. Li, D. S. Cheng, F. E. Yull, J. W. Christman, and T. S. Blackwell. 2006. Targeted immunomodulation of the NF- κ B pathway in airway epithelium impacts host defense against *Pseudomonas aeruginosa*. *J. Immunol.* 176: 4923–4930.
37. Jones, M. R., B. T. Simms, M. M. Lupa, M. S. Kogan, and J. P. Mizgerd. 2005. Lung NF- κ B activation and neutrophil recruitment require IL-1 and TNF receptor signaling during pneumococcal pneumonia. *J. Immunol.* 175: 7530–7535.
38. Mizgerd, J. P., M. R. Spieker, and C. M. Doerschuk. 2001. Early response cytokines and innate immunity: essential roles for TNF receptor 1 and type I IL-1 receptor during *Escherichia coli* pneumonia in mice. *J. Immunol.* 166: 4042–4048.