The Development of Early Host Response to *Pseudomonas aeruginosa* Lung Infection Is Critically Dependent on Myeloid Differentiation Factor 88 in Mice*

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Toll-like receptors (TLR) induce distinct patterns of host responses through myeloid differentiation factor 88 (MyD88)-dependent and/or -independent pathways, depending on the nature of the pathogen. *Pseudomonas aeruginosa* is an opportunistic Gram-negative bacterium, a major cause of nosocomial pneumonia, and a common cause of pulmonary infections in both compromised and non-compromised individuals and cystic fibrosis patients. The role of the TLR-MyD88 pathway in *P. aeruginosa*-induced lung infection in vivo was examined in this study. MyD88−/− mice demonstrated an impaired clearance of *P. aeruginosa* from the lung. Little or no neutrophil recruitment was observed in the airways of MyD88−/− mice following *P. aeruginosa* lung infection. This observation was associated with a reduced production of inflammatory mediators that affect neutrophil recruitment, including macrophage-inflammatory protein-2, tumor necrosis factor, and interleukin-1β in the airways of MyD88−/− mice. Similarly, MyD88−/− mice showed inhibited NF-κB activation in the lung following *P. aeruginosa* infection. Interestingly, *P. aeruginosa* infection induced a 7.5-fold increase in TLR2 mRNA expression in the lungs of MyD88+/+ mice. Furthermore, host responses to *P. aeruginosa* lung infection in TLR2−/− and TLR4 mutant mice were partially inhibited compared with the responses of respective control mice. Taken together, our results indicate that the MyD88-dependent pathway is essential for the development of early host responses to *P. aeruginosa* infection, leading to the clearance of this bacterium, and that TLR2 and TLR4 are involved in this process.

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1 The abbreviations used are: TLR, Toll-like receptors; MyD, myeloid differentiation; TRIF, Toll-interleukin-1 receptor domain-containing adaptor molecule-1; TRAM, TRIF-related adaptor molecule; TNF, tumor necrosis factor; IL, interleukin; BALF, bronchoalveolar lavage fluid; MPO, myeloperoxidase; CFU, colony-forming units; RT, reverse transcriptase; ELISA, enzyme-linked immunosorbent assay; IFN, interferon; MIP, macrophage-inflammatory protein; IRF, IFN-regulatory factor; RANTES, regulated on activation normal T cell expressed and secreted; LPS, lipopolysaccharide.

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A Critical Role for MyD88 in P. aeruginosa Lung Infection

P. aeruginosa infection (24–27). In vivo, decreased expression of TLR4 appears to correlate with impaired resistance to P. aeruginosa infection (25). In vitro, TLR2 and TLR5 are involved in P. aeruginosa flagella-induced activation of epithelial cells (24), and TLR2 and TLR4 are involved in monocyte and macrophage activation by a component of P. aeruginosa alginate (26). However, the contribution of the TLR-MyD88 pathway to the effective host response to P. aeruginosa lung infection remains a critical area of investigation.

Here we demonstrate that MyD88-deficient (MyD88−/−) mice showed little or no neutrophil recruitment or production of the neutrophil attractants MIP-2, TNF, and IL-1β and had an impaired ability to clear P. aeruginosa from the lung, which vastly contrasted with the findings of robust MIP-2, TNF, and IL-1β production, neutrophil infiltration, and bacterial clearance demonstrated in wild type animals. These findings suggest an essential role for the MyD88-dependent pathway in the host defense against P. aeruginosa lung infection in vivo. The different immune responses seen in MyD88−/−, TLR2−/−, and TLR4 mutant mice suggest that the host defense against P. aeruginosa lung infection may involve multiple members of the MyD88-dependent TLR/IL-1 superfamily.

MATERIALS AND METHODS

Mice—MyD88−/− mice backcrossed eight times to the C57BL/6 background and TLR2-deficient mice (TLR2−/−, C57BL/6 background) were kindly provided by Dr. S. Akira (Osaka University, Osaka, Japan) (28). C57BL/6 mice were purchased from Charles River (Wilmington, MA). MyD88−/− mice were matched with C57BL/6 mice for age and sex. C3H/HeJ mice (TLR4 mutant) and control, C3H/HeOuJ, (TLR4+/+) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The protocols were approved by the University Committee on Laboratory Animals, Dalhousie University, in accordance with the guidelines of the Canadian Council on Animal Care.

Lung Infection with P. aeruginosa and Collection of Lung and Bronchoalveolar Lavage Fluid—P. aeruginosa strain 8821 (a gift from Dr. A. Chakrabarty, University of Illinois, Chicago) is a mucoid strain isolated from a cystic fibrosis patient (29). Mice were infected intranasally with 1 × 104 or 1 × 105 CFU of P. aeruginosa. After 4, 8, or 24 h, mice were sacrificed by cardiac puncture, and phosphate buffer solution containing soybean trypsin inhibitor (100 μg/ml) were mixed with equal volumes of 0.5% cetyltrimethylammonium chloride (250 μM) that does not disperse red blood cells (the supernatant was stored at −80°C for later analysis of cytokines. The pellet was resuspended and homogenized in 0.5% cetyltrimethylammonium chloride (4 μl/mg lung tissue) and centrifuged as above. The clear extract was used for the MPO assay.

BALF (10 μl) was plated on agar dish and incubated for 24–48 h, and then bacterial CFU were counted. For the detection of cytokines and MPO activity, BALF was centrifuged at 1500 rpm for 5 min at 4°C. Supernatants were used for cytokine analysis. The pellets were resuspended in 1 ml of NH4Cl (0.15 M) and spun as before to lyse red blood cells. Supernatants were discarded, and the pellets were resuspended in 0.5% cetyltrimethylammonium chloride (250 μl/mouse) and centrifuged, and the clear extracts were used for MPO assay.

Electrophoretic Mobility Shift Assays—A consensus double-stranded NF-κB oligonucleotide (Promega, Madison, WI) (5′-AGT TGA GGG GAC TCC CCC CCG C-3′) was used for the electrophoretic mobility shift assay. Probe labeling was accomplished by treatment with T4 kinase (Promega) in the presence of 32P-adenosine triphosphate (Amersham Biosciences). Labeled oligonucleotides were purified on a Sephadex G-25M column (Amersham Biosciences). 10 μg of nuclear protein was added to a 10-μl volume of binding reaction with 1 μg of poly(dI-dC) (Amersham Biosciences) and incubated at room temperature for 15 min. Labeled double-stranded NF-κB oligonucleotide was added to each reaction mixture, which was incubated at room temperature for 30 min and separated by electrophoresis on a 6% polyacrylamide gel in 0.5 × Tris-boric acid-EDTA buffer. Gels were vacuum-dried and subjected to autoradiography. Cold competition was carried out by adding 1 μl (1.75 μM) of specific unlabeled double-stranded probe to the reaction mixture. Unlabeled double-stranded oligonucleotide (1 μl, 1.75 μM) that does not bind NF-κB was used for nonspecific competition (data not shown). Polyclonal antibodies to p50 and p65 (1 μg/10 μl) (Santa Cruz Biotechnology) were used for supershift assays for NF-κB proteins (data not shown).

Histology—Mice lungs were fixed in 10% formalin overnight and then in 100% ethanol for paraffin embedding and sectioning. Slides were deparaffinized with Citrisolv (Fisher) and rehydrated through decreasing concentrations of ethanol. Slides were stained with Harris hematoxylin-eosin to illustrate lung histology.

Statistics—Data are presented as mean ± S.E. of the indicated number of experiments. Statistical significance was determined by assessing means with analysis of variance and the Tukey-Kramer multiple comparison test or by using an unpaired t test. Differences were considered significant at p < 0.05.

FIG. 1. Impaired clearance of P. aeruginosa from the lungs of MyD88−/− mice. MyD88−/− and MyD88−/− mice were challenged intranasally with P. aeruginosa (mucoid strain 8821). After 24 h, the BALF (a) and the right lungs (b) were collected for bacterial colony counting. Data are the mean ± S.E. of 6 mice/group.

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RESULTS

Impaired Clearance of P. aeruginosa in MyD88$^{-/-}$ Mice—

We used MyD88-deficient mice to examine the role of MyD88 in the host defense against P. aeruginosa lung infection in vivo. MyD88$^{+/+}$ and MyD88$^{-/-}$ mice were inoculated intranasally with 1 x 10$^7$ CFU/mouse. Mice that were not treated with bacteria or those that received saline were used as controls (saline). Total RNA isolated from the lungs was subjected to real-time RT-PCR analysis for MIP-2 expression. Data are expressed as fold increase relative to saline-treated MyD88$^{+/+}$ mice. Data are the mean ± S.E. of 3–5 mice/group (*, p < 0.05, compared with the saline group).

**FIG. 3. Diminished MIP-2 expression in MyD88-deficient mice following P. aeruginosa lung infection.**

*a*, lung tissue was collected from MyD88$^{+/+}$ and MyD88$^{-/-}$ mice 4 h after intranasal administration of P. aeruginosa (Psa) strain 8821 (1 x 10$^7$ CFU/mouse). Mice that were not treated with bacteria or those that received saline were used as controls (saline). Total RNA isolated from the lungs was subjected to real-time RT-PCR analysis for MIP-2 expression. Data are expressed as fold increase relative to saline-treated MyD88$^{+/+}$ mice. Data are the mean ± S.E. of 3–5 mice/group (*, p < 0.05, compared with the saline group).

**FIG. 2.** Defective neutrophil recruitment into the airways of MyD88$^{-/-}$ mice following P. aeruginosa lung infection. MyD88$^{+/+}$ and MyD88$^{-/-}$ mice were inoculated intranasally with P. aeruginosa (Psa) (mucoid strain 8821, 1 x 10$^7$ CFU/mouse). Mice that were not treated with bacteria or those that received saline were used as controls (saline). a and b, after 4 or 8 h, BALF and lung tissue were collected for the determination of MPO activities. Data are the mean ± S.E. of 5–10 mice/group (*, p < 0.05, compared with the saline group). c–f, 8 h after infection, mice were sacrificed, and the upper lobe of the left lung was collected for hematoxylin-eosin staining. c, MyD88$^{+/+}$ mouse lung at x40; d, MyD88$^{-/-}$ mouse lung at x10; e, MyD88$^{+/+}$ mouse lung at x40; f, MyD88$^{-/-}$ mouse lung at x100.

**RESULTS**

Impaired Clearance of P. aeruginosa in MyD88$^{-/-}$ Mice—

We used MyD88-deficient mice to examine the role of MyD88 in the host defense against P. aeruginosa lung infection in vivo. MyD88$^{+/+}$ and MyD88$^{-/-}$ mice were inoculated intranasally with 1 x 10$^7$ CFU of P. aeruginosa strain 8821. BALF and lung tissue were collected 24 h later for the detection of viable bacteria by counting CFU. MyD88$^{+/+}$ mice are able to clear P. aeruginosa (Fig. 1). Significantly higher CFU counts can be seen in both the BALF and lung tissue from MyD88$^{-/-}$ mice (Fig. 1), suggesting that MyD88$^{-/-}$ mice demonstrate impaired bacterial clearance in comparison with MyD88$^{+/+}$ mice.

**Impaired Neutrophil Recruitment to the Lung in MyD88$^{-/-}$ Mice**—Given that neutrophils are essential for the clearance of P. aeruginosa during acute lung infection (15), we tested whether the impaired bacterial clearance in MyD88$^{-/-}$ mice...
was caused by defective neutrophil infiltration. MyD88\(^{+/+}\) and MyD88\(^{-/-}\) mice were inoculated intranasally with \(P.\ aeruginosa\) (Psac) (mucoid strain 8821, \(1 \times 10^7\) CFU/mouse). Mice that were not treated with bacteria or those that received saline were used as controls (saline). After 4 or 8 h, BALF and lung tissue were collected for the determination of IL-1\(\beta\) and TNF by ELISA. In contrast, there was little MIP-2 production in MyD88\(^{-/-}\) mice. In contrast, there was little response of IL-1\(\beta\) or TNF in lung tissue or BALF from MyD88\(^{-/-}\) mice. It is also noteworthy that in wild type mice, \(P.\ aeruginosa\)-induced IL-1\(\beta\) remained largely in the lung tissue (Fig. 4b), whereas the majority of TNF was secreted in the BALF (Fig. 4c).

Given that IFN-\(\gamma\) and IL-12 have been implicated in \(P.\ aeruginosa\) lung infection (35, 36), these two cytokines were examined in both MyD88\(^{+/+}\) and MyD88\(^{-/-}\) mice. Mice were challenged for 4 or 8 h with an intranasal inoculation of \(P.\ aeruginosa\) (strain 8821, \(1 \times 10^7\) CFU/mouse). The BALF and lung tissue homogenates were used to determine the levels of production of IFN-\(\gamma\) and IL-12 by ELISA. In contrast to the robust response of neutrophil attractants MIP-2, IL-1\(\beta\), and TNF, there was little IFN-\(\gamma\) and IL-12 response in both MyD88\(^{+/+}\) and MyD88\(^{-/-}\) mice after 4 or 8 h of \(P.\ aeruginosa\) lung infection (data not shown).

To examine the role of MyD88 in \(P.\ aeruginosa\)-induced NF-\(\kappa\)B activation, lung tissue homogenates from \(P.\ aeruginosa\)-infected (4 h) or untreated mice were used to isolate nuclear extracts for the determination of NF-\(\kappa\)B activation by electrophoretic mobility assay. \(P.\ aeruginosa\)-induced NF-\(\kappa\)B activation was seen in wild type mice (Fig. 5). In contrast, \(P.\ aeruginosa\)-induced NF-\(\kappa\)B activation was markedly reduced in MyD88\(^{-/-}\) mice. These data are consistent with the defect of \(P.\ aeruginosa\)-induced mediator production in MyD88\(^{-/-}\) mice (Fig. 3c).

Increased TLR2, but Not TLR4, mRNA Expression in the Lung after \(P.\ aeruginosa\) Stimulation.—Because MyD88 is important in TLR signaling, the lack of a \(P.\ aeruginosa\)-induced response in MyD88\(^{-/-}\) mice suggests that TLR may have a
critical role in \( P. \) aeruginosa-induced host responses. To examine whether \( P. \) aeruginosa infection induces changes in TLR2 and TLR4 expression, lung tissue from \( P. \) aeruginosa-infected (4 h) MyD88\(^{+/+}\) and MyD88\(^{-/-}\) mice was used to determine mRNA levels by real-time RT-PCR. Results were expressed as the -fold increase relative to the level in wild type untreated mice. As shown in Fig. 6a, the basal levels of TLR2 expression in MyD88\(^{+/+}\) and MyD88\(^{-/-}\) mice were similar. Interestingly, \( P. \) aeruginosa infection induced a greater increase in TLR2 expression in MyD88\(^{+/+}\) mice (7.5-fold) than in MyD88\(^{-/-}\) mice (1.7-fold) (Fig. 6a), supporting the concept that TLR2 is involved in \( P. \) aeruginosa lung infection. However, little change in TLR4 level in the lung was found in MyD88\(^{+/+}\) and MyD88\(^{-/-}\) mice (Fig. 6b).

To examine whether TLR4 plays a role in \( P. \) aeruginosa-induced up-regulation of TLR2 expression, TLR4 mutant mice were used. Lung tissue from saline-treated or \( P. \) aeruginosa-infected (4 h) mice was subjected to real-time RT-PCR analysis for TLR2 mRNA. Data are expressed as the -fold increase relative to the level in saline-treated mice. As shown in Fig. 6a, \( P. \) aeruginosa infection had little effect on TLR2 expression, suggesting an important role for TLR4 in \( P. \) aeruginosa-induced TLR2 expression.

\( P. \) aeruginosa-induced Differential Responses in TLR2\(^{+/+}\) and TLR4 Mutant Mice—To determine the specific contributions of TLR2 and TLR4 in the development of early immune responses in the lung following \( P. \) aeruginosa infection, TLR2\(^{-/-}\) and TLR4 mutant mice as well as their corresponding control mice were infected intranasally with \( P. \) aeruginosa strain 8821 for 4 h. Lung homogenates were used to determine neutrophil infiltration (MPO) and cytokine production. TLR2\(^{-/-}\) mice showed a partial decrease in neutrophil recruitment compared with wild type mice (Fig. 7a). However, a significant \( P. \) aeruginosa-induced MIP-2 and IL-1\( \beta \) production was observed in TLR2\(^{-/-}\) mice (Fig. 7, b–d), suggesting that TLR2 alone may not be a major component in mediating \( P. \) aeruginosa-induced responses. Similarly, TLR4 mutant mice showed \( P. \) aeruginosa-induced production of MIP-2, IL-1\( \beta \), and TNF when compared with saline-treated mice (Fig. 7, f–h).

Compared with TLR4\(^{+/+}\) mice, TLR4 mutant mice demonstrated a significant decrease of neutrophil recruitment and MIP-2, IL-1\( \beta \), and TNF production (Fig. 7, e–h).

**DISCUSSION**

Depending on the nature of the microbial infection, the mechanism of the host defense varies, including variations in the receptor usage, signaling pathways, cellular participants, and the pattern of gene expression (10, 15). The mechanisms of the host defense against \( P. \) aeruginosa lung infection in vivo remain incompletely defined. The lung has a unique relationship with the environment and has developed distinct strategies to defend itself from microbial invasion with innate immune mechanisms that are primarily responsible for the elimination of bacterial organisms (37, 38). \( P. \) aeruginosa, which generates a mucoid phenotype after colonizing lung tissue, appears to have a specialized relationship with the lung (39). \( P. \) aeruginosa-induced lung damage is the major cause of death in cystic fibrosis patients (11) and accounts for 40% of the cause of deaths in people with ventilator-associated pneumonia (13). We attempted to determine the role of the TLR-MyD88 pathway in the host defense against \( P. \) aeruginosa lung infec-
tion. Consistent with a recent finding (40), our results suggest that the MyD88-dependent pathway is a central component of the initiation of *P. aeruginosa*-induced early immune responses in the lung, leading to the clearance of this bacterium. Because neutrophils play a major role in the clearance of *P. aeruginosa* from the lung, the defective clearance of *P. aeruginosa* seen in MyD88<sup>−/−</sup> mice is likely caused by a deficient recruitment of neutrophils into the airways. Given that MyD88<sup>−/−</sup> neutrophils appear to have a normal migratory ability (8), the defective influx of neutrophils into the airways is most likely the result of insufficient production of neutrophil attractants in the lung of MyD88<sup>−/−</sup> mice as observed in this study. Accordingly, our results support the model in which, during acute *P. aeruginosa* lung infection, MyD88 is absolutely required for the early

**FIG. 7.** *P. aeruginosa*-induced differential immune responses in the lung in TLR2<sup>−/−</sup> and TLR4 mutant mice. Lung tissue and BALF were collected from TLR2<sup>−/−</sup> and TLR4 mutant mice and their respective control mice after intranasal infection with *P. aeruginosa* (Psa) (mucoid strain 8821, 1 × 10⁷ CFU/mice) for 4 h. Mice that were not treated with bacteria or those that did not receive saline served as controls (saline). Lung homogenates were used to determine MPO activity (a and e) and MIP-2 (b and f) and IL-1β (c and g) production. TNF (d and h) was measured using BALF samples. Data are the mean ± S.E. of 12–16 mice/group.
production of the cytokines and chemokines MIP-2, IL-1β, and TNF, which are responsible for neutrophil recruitment and subsequent bacterial clearance. However, this does not exclude the possibility of the involvement of MyD88-independent pathways such as the TRIF/TRAM pathway in the P. aeruginosa-induced host response, especially in the later phase of the infection. Several IFN-regulatory factor (IRF) 3-regulated cytokines and chemokines such as RANTES and IFN-α inducible protein 10 are induced by the TRIF/TRAM pathway in response to bacterial LPS stimulation (41, 42). RANTES and IFN-α inducible protein 10 are up-regulated during P. aeruginosa infection (43). Thus, the role for the TRIF/TRAM pathway in P. aeruginosa-induced lung infection requires further study.

Given the importance of MyD88 in TLR signaling (1) and the circumstantial evidence of the association between P. aeruginosa and TLR2 or TLR4 (24–26), immune responses to P. aeruginosa infection were examined in MyD88−/−, TLR2−/−, and TLR4 mutant mice, with the goal of assessing the relative contribution of these molecules as well as additional TLRs in P. aeruginosa infection. Apparently, the pattern of P. aeruginosa-induced immune responses in TLR2−/− or TLR4 mutant mice is different from that seen in MyD88−/− mice. These results suggest that neither TLR2 nor TLR4 functions individually as the only component responsible for P. aeruginosa-induced immune response in the lung in vivo. This difference suggests that additional TLRs, such as TLR5 (24, 44), or synergistic effects between different TLRs (45) may be involved. Nevertheless, the increase of TLR2 expression in the lung after infection and the partial inhibition of immune responses in TLR2−/− mice support a role for TLR2 in P. aeruginosa-induced lung inflammation. This is consistent with recent in vitro studies (46) demonstrating that TLR2 is involved in the activation of macrophages and monocytes by mannuronic acid polymers, a component of P. aeruginosa alginate that is specifically related to the mucoid phenotype (26).

Interestingly, TLR2 is mobilized into an apical lipid raft receptor complex in epithelial cells after P. aeruginosa stimulation. In addition, TLR2 together with TLR5 and gangliotetraosylceramide have been shown to be involved in P. aeruginosa flagellum-induced epithelial cell activation in vitro (24).

Although the level of TLR4 mRNA was not changed, the inhibition of P. aeruginosa-induced neutrophil infiltration and production of MIP-2, IL-1β, and TNF in TLR4 mutant mice supports a role for TLR4 in P. aeruginosa lung infection. However, even the nearly complete inhibition in MyD88−/− mice, a significant P. aeruginosa-induced production of MIP-2, IL-1β, and TNF was observed in TLR4 mutant mice. Other studies (47, 48) have demonstrated P. aeruginosa-induced immune responses in C3H/HeJ mice. Thus, other TLRs in addition to TLR4 or synergistic effects between TLRs may be involved in host responses to P. aeruginosa lung infection.

The increase in TLR2 mRNA expression and the unchanged TLR4 mRNA level in P. aeruginosa-infected lungs suggest that TLR2 and TLR4 may have different roles during P. aeruginosa infection. It is likely that the contribution of TLR2 may increase when the infection progresses, considering the 7.5-fold increase of TLR2 mRNA in the lungs of P. aeruginosa-infected wild type mice. The P. aeruginosa-induced increase of TLR2 mRNA was not observed in TLR4 mutant mice. This suggests that P. aeruginosa up-regulates TLR2 through activation of TLR4. It is widely recognized that TLR4 is associated with LPS-induced tolerance before exposure to bacterial LPS, leading to a state of hyporesponsiveness to subsequent LPS stimulation (49). Thus, it is possible that TLR4 plays a major role during the early phase of P. aeruginosa infection, whereas TLR2 may play a greater role as the infection progresses.

It has been well established that MyD88 transduces cell surface signals to transcription factor NF-κB, which regulates NF-κB-dependent gene expression, including TNF and IL-1β (1). P. aeruginosa-induced NF-κB activation has been reported previously (50) and was confirmed in our study in the infected lung tissue using an electrophoretic mobility shift assay. The defective production of TNF and IL-1β in MyD88−/− mice is likely caused by the blockade of signaling from TLRs to NF-κB in these animals, because P. aeruginosa-induced NF-κB activation in the lung was markedly reduced in MyD88−/− mice.

Interestingly, unlike RANTES and IFN-α inducible protein 10, which can be regulated in a MyD88-NF-κB-independent manner (42), MyD88−/− mice showed an almost complete deficiency in MIP-2 expression of both mRNA and protein levels. RANTES and IFN-α inducible protein 10 contain IRF binding sites in their promoters and are regulated through the TRIF/TRIF/37 pathway during TLR activation (42, 51). In contrast, there is no report regarding the existence of an IRF-binding site in the MIP-2 promoter. Although the MIP-2 promoter contains a conserved NF-κB consensus motif (52), TLR-mediated MIP-2 production appears to be NF-κB-independent because the NF-κB inhibitor pyrrolidinedithiocarbamate blocks LPS-induced TNF and IL-1β production but has no effect on MIP-2 expression (53). Thus, MyD88 may regulate MIP-2 production through additional transcription factors rather than through NF-κB. Alternatively, MyD88-dependent MIP-2 production may be secondary to the production of TNF and IL-1β because IL-1β and TNF are able to regulate MIP-2 expression (54, 55). Thus, the role for MyD88 in the host defense against P. aeruginosa lung infection is likely to rely on the interplay of multiple P. aeruginosa-induced mediators in the lung, such as MIP-2, TNF, and IL-1β.

In summary, our results suggest that the MyD88-dependent pathway plays an essential role in P. aeruginosa-induced early immune responses, including MIP-2, IL-1β, and TNF production and subsequent neutrophil recruitment and bacterial clearance. Multiple MyD88-dependent TLRs, including TLR2 and TLR4, may be involved in the host defense against P. aeruginosa lung infection.

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The Development of Early Host Response to *Pseudomonas aeruginosa* Lung Infection Is Critically Dependent on Myeloid Differentiation Factor 88 in Mice

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The development of early host response to *Pseudomonas aeruginosa* lung infection is critically dependent on myeloid differentiation factor 88 in mice.

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**Page 49320:** In Fig. 7, *a–d*, the labels for the *left two bars* in the graphs should read *TLR2*/+*/+* (not *TLR2*/−/*−*), while only the labels for the *right two bars* represent data from *TLR2*/−/*−* mice. In Figure 7, *e–h*, the label for the *left two bars* in the graphs should read *TLR4*/+*/+* (not *TLR4*/−/*−*), and the labels for the *right two bars* represent data from *TLR4* mutant mice. Therefore, in Fig. 7 the labels on the *bottom left-hand side* of all the graphs should read */+*/+, not */−*/−. The correct figure is shown following.

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