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MyD88 Mediates Neutrophil Recruitment Initiated by IL-1R but Not TLR2 Activation in Immunity against *Staphylococcus aureus*

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Summary

MyD88 is an important signaling adaptor for both TLR and IL-1R family members. Here, we evaluated the role of TLR2/MyD88 and IL-1R/MyD88 signaling in host defense against *S. aureus* by using a cutaneous infection model in conjunction with bioluminescent bacteria. We found that lesions of *S. aureus*-infected MyD88- and IL-1R-deficient mice were substantially larger with higher bacterial counts compared with wild-type mice. In contrast, TLR2-deficient mice had lesions that were only moderately larger with minimally higher bacterial counts. In addition, MyD88- and IL-1R- but not TLR2-deficient mice had severely decreased recruitment of neutrophils to the site of infection. This neutrophil recruitment was not dependent upon IL-1R/MyD88 signaling by recruited bone marrow-derived cells, suggesting that resident skin cells utilize IL-1R/MyD88 signaling to promote neutrophil recruitment.

Introduction

MyD88 (myeloid differentiation factor 88) is a signaling adaptor molecule that is critical for an effective immune response against a wide range of microbial pathogens (Akira and Takeda, 2004; Martin and Wesche, 2002). Much of the recent scientific literature has focused on the role of MyD88 in relationship to Toll-like receptor (TLR) signaling, since TLRs have a cytoplasmic TIR (Toll-IL-1 receptor) domain that interacts with another TIR domain found on MyD88 and its related family members TIRAP/Mal, TRIF, and TRAM (Akira and Takeda, 2004; Martin and Wesche, 2002). This interaction leads to activation of several signaling cascades including the NF-κB pathway, resulting in production of proinflammatory cytokines, chemokines, and upregulation of costimulatory and adhesion molecules involved in innate and acquired immune responses (Akira and Takeda, 2004; Martin and Wesche, 2002). In addition, MyD88 is essential for transducing signals from IL-1R family members, including IL-1R, IL-18R, and several orphan receptors (Adachi et al., 1998; Fitzgerald and O'Neill, 2000).

We chose to investigate the role of MyD88-mediated immune responses in host defense by using *Staphylococcus aureus* cutaneous infection as a model. This gram-positive extracellular bacterium is an important human pathogen, becoming increasingly resistant to antibiotic therapy (Lowy, 1998; Melles et al., 2004; Fridkin et al., 2005). Although *S. aureus* infections usually originate locally in the skin, invasive and life-threatening infections are common sequelae, including abscesses of various organs, cellulitis, lymphangitis, osteomyelitis, arthritis, pneumonia, meningitis, endocarditis, and sepsis (Lowy, 1998). Nevertheless, many of the studies of the innate immune response to *S. aureus* have been carried out in vitro or in systemic infection models. For example, in vitro experiments have demonstrated that TLR2 can recognize lipopeptides and lipoteichoic acid of *S. aureus* (Takeuchi et al., 2001, 2002). In addition, TLR2- and MyD88-deficient mice have been shown to be more susceptible than wild-type (wt) mice to a systemic *S. aureus* infection (Hoebe et al., 2005; Takeuchi et al., 2000). However, the severity of infection was far greater in MyD88-deficient mice compared with TLR2-deficient mice (Takeuchi et al., 2000). These data suggest that receptors other than TLR2 that signal via MyD88 may contribute to the immune response against this bacterium. Therefore, we chose to compare the role of TLR2 versus IL-1R in host defense against *S. aureus*.

Results

MyD88-Deficient Mice Have Markedly Larger Skin Lesions with Higher Bacterial Counts Compared with Wild-Type Mice after Skin Inoculation with *S. aureus*

Mice deficient in MyD88, an important signaling molecule for members of the TLR/IL-1R family, have been shown to be highly susceptible to a systemic *S. aureus* infection (Takeuchi et al., 2000). To evaluate the role of MyD88 signaling in cutaneous host defense against a localized *S. aureus* infection, we utilized an in vivo bioluminescence cutaneous infection model to track bacterial growth. Wild-type and MyD88-deficient mice were inoculated with a bioluminescent strain of *S. aureus* (2.5×10^6 CFUs/100 μl) (SH1000 strain), and lesion size and in vivo bioluminescence of live, actively metabolizing bacteria within the lesions over time were evaluated (Figures 1A–1E). Wild-type mice developed visible skin lesions on day 2, which expanded in size until day 5 (0.52 ± 0.1 cm^2) and healed by day 14 (Figures 1A and 1B). In contrast, MyD88-deficient mice developed 3-fold larger lesions by day 3, failed to show any signs of healing by day 10, and were consequently euthanized. In separate experiments, we used the common laboratory *S. aureus* strain 8325-4. Unlike the bioluminescent strain SH1000 in Figure 1, strain 8325-4 has a known defect in the *rsbU* gene, which codes for a positive regulator of the σ^B (*sigB*) virulence pathway (Horsburgh et al., 2002). This

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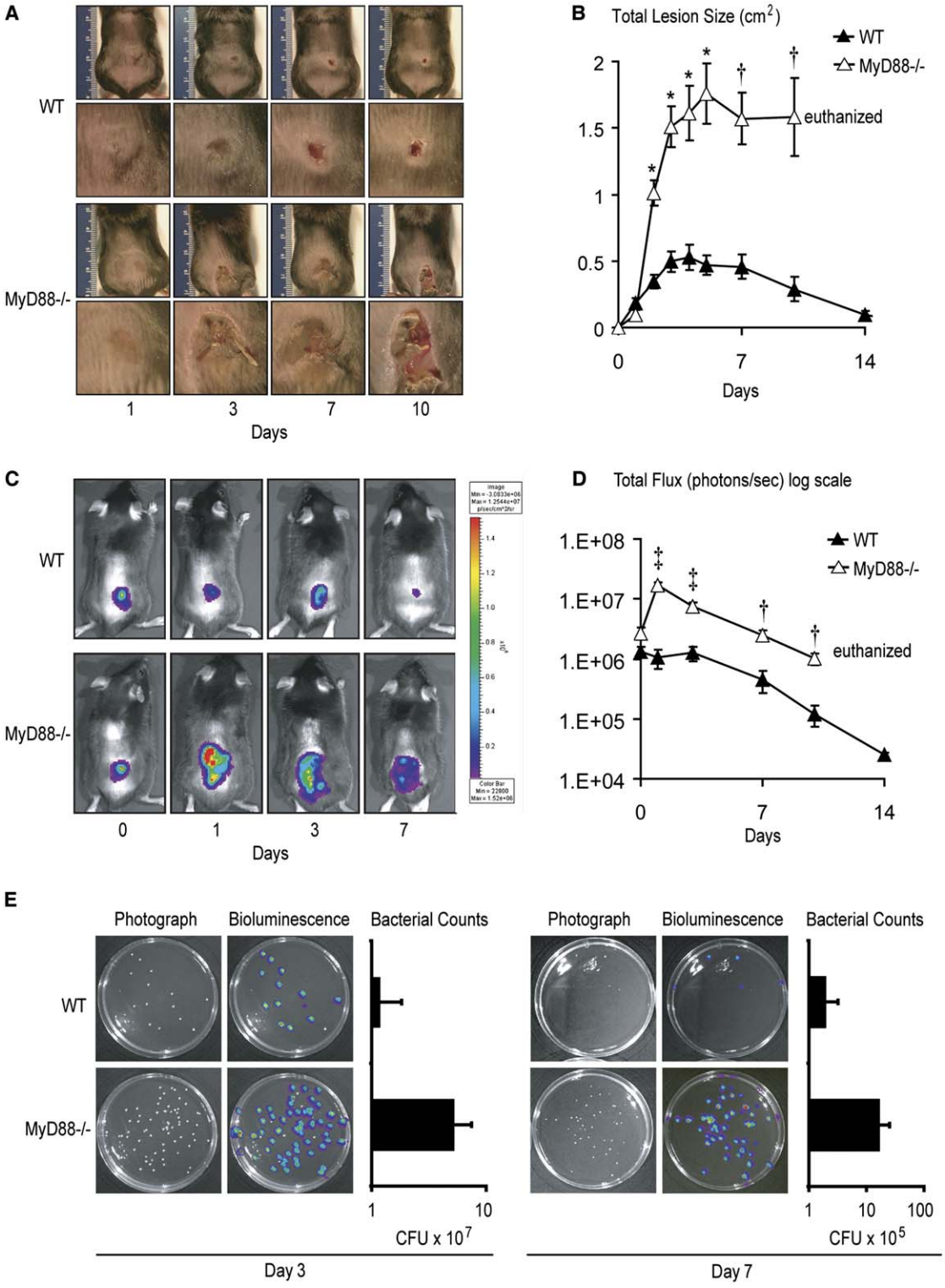

Figure 1. MyD88-Deficient Mice Have Markedly Larger Skin Lesions and Higher Bacterial Counts Compared with Wild-Type Mice

MyD88$^{-/-}$ and wt mice were inoculated subcutaneously with *S. aureus* (2.5 × 10^6 CFUs in 100 μl PBS) (bioluminescent strain SH1000).

(A) Representative photographs.

(B) Mean total lesion size (cm^2) ± SEM.

(C) Representative photographs of in vivo bioluminescence (Xenogen IVIS).

(D) Mean total flux (photons/s) ± SEM.

(E) Representative photographs of bacterial culture plates after overnight culture ± bioluminescence and colony-forming units (CFUs) of *S. aureus* recovered from 8 mm lesional punch biopsies on days 3 and 7.

*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ MyD88$^{-/-}$ versus wt mice (Student's t test).

less virulent *S. aureus* strain required a 20-fold higher inoculum (5×10^7) to induce a similar pattern of skin lesions in wt mice as seen in Figure 1, namely a peak size of $\sim 0.47 \pm 0.1$ cm^2 with complete healing by day 14 (see Figure S1 in the Supplemental Data available with this article online). In MyD88-deficient mice, the 8325-4 strain produced larger lesions than wt mice, but to a lesser degree than the bioluminescent SH1000 strain. This allowed us to follow these lesions in MyD88-deficient mice over a longer period of time. We found that the lesions in MyD88-deficient mice persisted and produced purulent drainage for up to the termination of the experiment at 5 weeks.

To determine whether the larger lesions of MyD88-deficient mice were due to a defect in bacterial clearance, we used in vivo bioluminescence (Xenogen IVIS) to estimate bacterial counts within the lesions of live anesthetized mice (Figures 1C and 1D). After infection with *S. aureus*, wt mice had bioluminescent signals that decreased over 14 days. In contrast, at all time points after the initial inoculation, MyD88-deficient mice had increased bioluminescent signals (color scale) that were 10-fold higher (logarithmic scale) compared with wt mice. Similar to in vivo bioluminescence, numbers of *S. aureus* colony-forming units (CFUs) recovered from 8 mm lesional punch biopsies were almost 10-fold higher in MyD88-deficient mice compared with wt mice on days 3 and 7 (Figure 1E). Thus, MyD88-deficient mice have a defect in bacterial clearance, which may help explain the persistence of skin lesions. Furthermore, to test whether MyD88-deficient mice also have an inherent defect in wound healing, a 5 mm punch biopsy was performed on MyD88-deficient and wt mice in the absence of any bacterial inoculation (Figure S2). We found no difference between MyD88-deficient and wt mice in their ability to heal these lesions. Therefore, the defect in MyD88-deficient mice is likely related to bacterial clearance and not wound healing.

MyD88-Deficient Mice Have a Defect in Neutrophil Recruitment Compared with Wild-Type Mice after Skin Inoculation with *S. aureus*

Since the increased lesion size and bioluminescent signals in MyD88-deficient mice were observed shortly after the infection, histology of lesional skin was evaluated at days 1 and 2 after inoculation with *S. aureus* (Figure 2A). We found that lesions of wt mice had large neutrophilic abscesses in both H&E and anti-Gr-1 (neutrophil marker) mAb-labeled sections. In fact, almost all of the cells of the infiltrate were Gr-1 positive with only a few scattered Mac-3 (macrophage marker)-positive cells (Figure S3). In addition, in wt mice, *S. aureus* bacteria were barely detectable by gram stain, perhaps due to phagocytosis of the bacteria by the neutrophils within the abscess (Figure 2A). In contrast, lesions of MyD88-deficient mice had severely decreased numbers of neutrophils, with no observable abscess formation, and contained a blue-staining band of gram-positive bacteria spanning the entire horizontal length of the section. Thus, MyD88-deficient mice have a severe defect in the recruitment of neutrophils to the site of infection with *S. aureus*. Furthermore, the readily detectable gram-positive bacteria in lesions of MyD88-deficient mice corroborate the results obtained with in vivo bioluminescence and bacterial counts, demonstrating that MyD88-deficient mice have a severe defect in bacterial clearance. There was also significantly less myeloperoxidase (MPO) activity, a marker of neutrophil function, in lesions of MyD88-deficient mice compared with wt mice at 1 day after inoculation with *S. aureus* (Figure 2B).

A previous study with a similar *S. aureus* cutaneous infection model demonstrated that neutrophil-depleted mice (with mAb RB6-8C5) developed large nonhealing skin lesions and failed to clear *S. aureus* from these lesions (Molne et al., 2000). Thus, both MyD88-deficient mice and neutrophil-depleted mice share a similar phenotype and provide evidence that neutrophils play a key role in immunity against cutaneous *S. aureus* infections. Moreover, our results suggest that MyD88 signaling is essential for this neutrophil recruitment to take place.

MyD88-Deficient Mice Have a Defect in Production of Cytokines and Chemokines Involved in Neutrophil Recruitment In Vivo after Skin Inoculation with *S. aureus*

The induction of cytokines and chemokines involved in neutrophil recruitment was evaluated in lesions of MyD88-deficient mice and wt mice. mRNA was harvested from lesions at 6 hr after inoculation and levels of mRNA transcripts were compared, including IL-1β, KC/IL-8, and TNFα (Figure 2C). We found that MyD88-deficient mice have decreased *IL-1β* and *KC* mRNA compared with wt mice ($p < 0.05$). The levels of *TNFα* mRNA were not significantly decreased in lesions of MyD88-deficient mice compared with wt mice.

Protein levels of IL-1β, KC, and MIP2 from lesions were determined by performing ELISAs on homogenized 8 mm punch biopsies of lesional skin at 0, 6, and 24 hr after inoculation with *S. aureus* (Figure 2D). MyD88-deficient mice had significantly decreased levels of IL-1β, KC, and MIP2 compared with wt mice at 6 hr but not at 24 hr after inoculation. Thus, the decreased neutrophil recruitment in MyD88-deficient mice may in part be due to an early decreased production of cytokines and chemokines involved in neutrophil recruitment.

TLR2-Deficient Mice Developed Larger Lesions Compared with Wild-Type Mice but Had No Defect in Neutrophil Recruitment after Skin Inoculation with *S. aureus*

Previous studies have demonstrated that TLR2 can recognize lipopeptides and lipoteichoic acid of *S. aureus* (Takeuchi et al., 2001, 2002). In addition, TLR2-deficient mice are more susceptible to a *S. aureus* systemic infection than wt mice (Hoebe et al., 2005; Takeuchi et al., 2000). To investigate whether TLR2/MyD88 signaling is involved in our localized *S. aureus* infection model, we subcutaneously inoculated wt and TLR2-deficient mice with *S. aureus* as in Figures 1 and 2 (Figures 3A–3F). We found that TLR2-deficient mice developed larger lesions ($\sim$2-fold) with higher bioluminescent signals than wt mice (4- to 5-fold) (Figures 3A and 3B), which was consistent with previous reports (Hoebe et al., 2005; Takeuchi et al., 2000). However, TLR2-deficient mice healed their skin lesions at approximately the same time as wt mice (day 14). Furthermore, lesions of TLR2-deficient mice had slightly smaller neutrophilic abscesses than those of wt mice on day 1 but were virtually identical to those

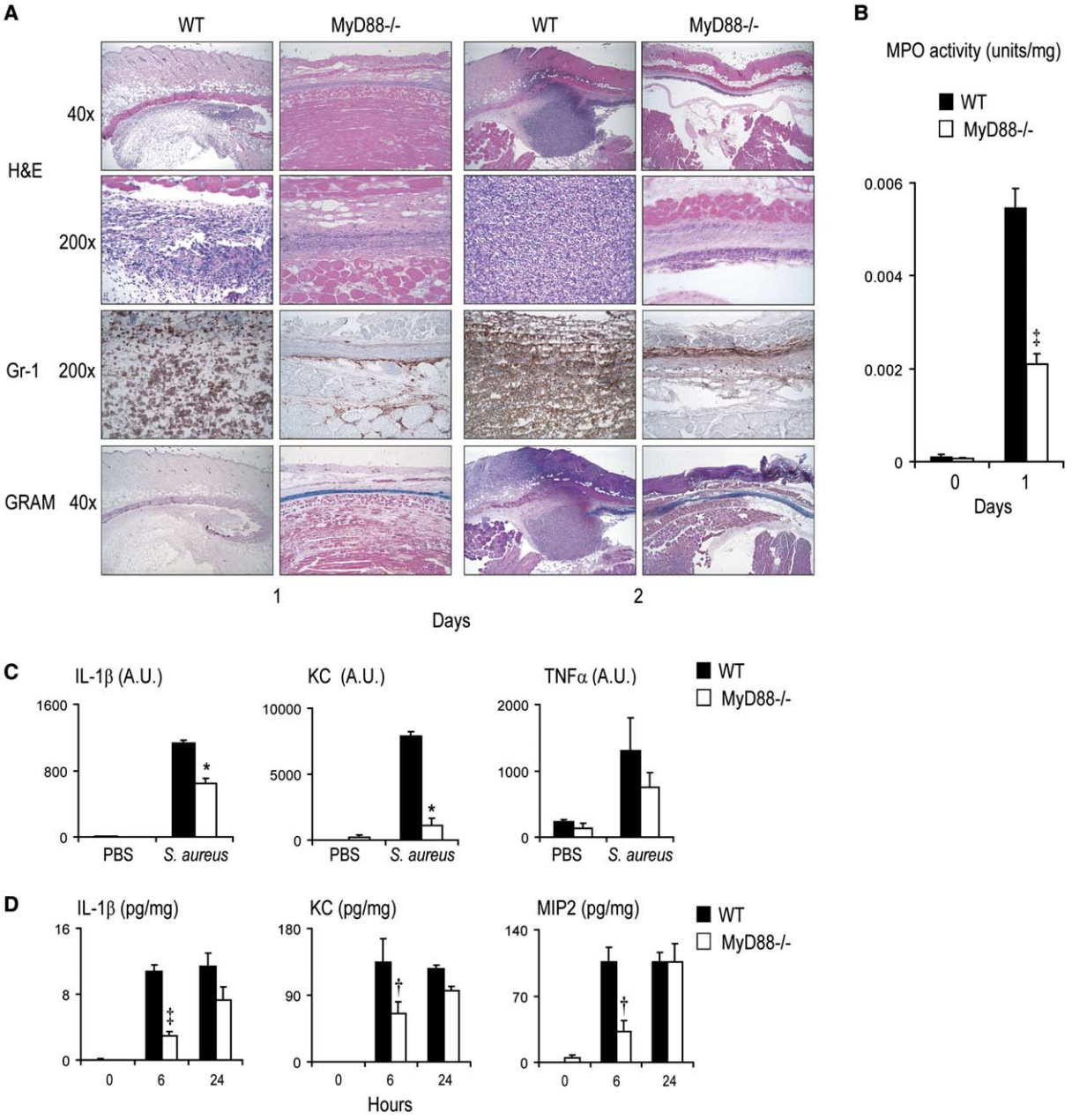

Figure 2. MyD88-Deficient Mice Have a Marked Decrease in Neutrophil Recruitment and in Production of Cytokines and Chemokines after Infection with *S. aureus*

MyD88$^{-/-}$ and wt mice were inoculated subcutaneously with *S. aureus* (2.5 × 10^6 CFUs in 100 μl PBS).

(A) Representative photomicrographs of sections labeled with H&E stain, anti-Gr-1 mAb (immunoperoxidase method), and gram stain of lesional skin at 1 and 2 days after inoculation.

(B) Mean myeloperoxidase (MPO) activity (units/mg tissue) ± SEM.

(C) Mean level of mRNA in arbitrary units (A.U.) ± SEM of *IL-1β, KC,* and *TNFα* at 6 hr after inoculation with *S. aureus* or PBS determined by Q-PCR and normalized to the housekeeping gene *L32*.

(D) Mean protein levels (pg/mg tissue) ± SEM of IL-1β, KC, and MIP2 from tissue homogenates at 0, 6, and 24 hr after infection with *S. aureus*. *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ MyD88$^{-/-}$ versus wt mice (Student's t test).

of wt mice on day 2 (Figure 3C). TLR2-deficient mice also had similar MPO activity and had similar levels of *IL-1β*, *KC*, and *TNFα* transcripts and KC and MIP2 protein levels compared with wt mice (Figures 3D–3F). Interestingly, TLR2-deficient mice had a significant decrease in the level of IL-1β protein compared with wt mice at 6 hr but not at 24 hr after inoculation with *S. aureus* (Figure 3F).

Although the TLR2-deficient mice had larger lesions with increased bioluminescence compared with wt mice (albeit to a much lesser degree than MyD88-deficient mice), TLR2-deficient mice did not have a severe defect in neutrophil recruitment or in production of chemokines such as KC and MIP2 as seen in MyD88-deficient mice. Thus, the defect in neutrophil recruitment observed in

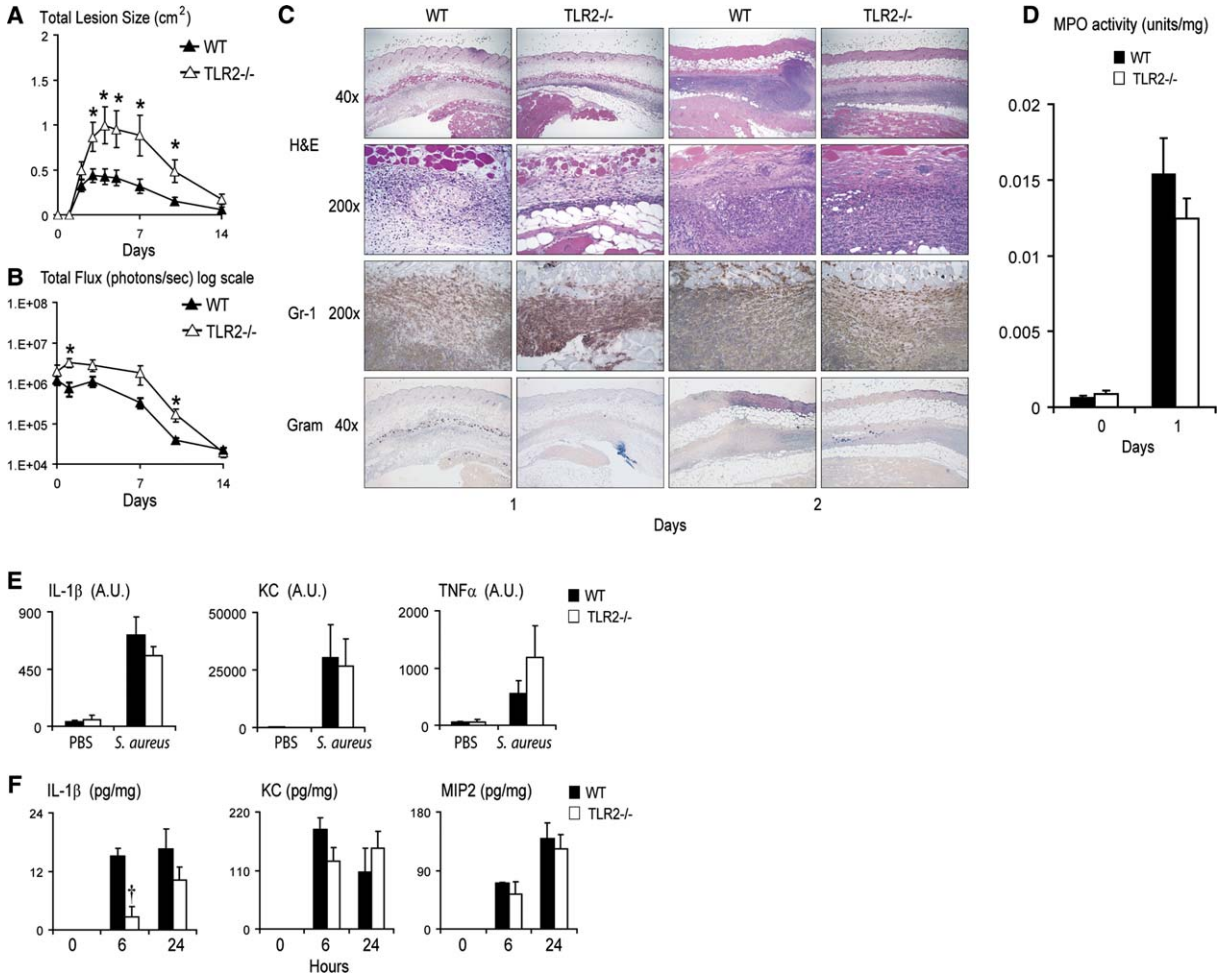

Figure 3. TLR2-Deficient Mice Have Larger Skin Lesions with Higher In Vivo Bioluminescence but Have No Defect in Neutrophil Recruitment Compared with Wild-Type Mice after Infection with *S. aureus*

$TLR2^{-/-}$ and wt mice were inoculated subcutaneously with *S. aureus* (2.5×10^6 CFUs in 100 μl PBS).

(A) Mean total lesion size (cm^2) ± SEM.

(B) Mean total flux (photons/s) ± SEM.

(C) Representative photomicrographs of sections labeled with H&E stain, anti-Gr-1 mAb, and gram stain of lesional skin at 1 and 2 days after inoculation.

(D) Mean myeloperoxidase (MPO) activity (units/mg tissue) ± SEM.

(E) Mean level of mRNA in arbitrary units (A.U.) ± SEM of *IL-1β, KC,* and *TNFα* at 6 hr after inoculation with *S. aureus* or PBS determined by Q-PCR and normalized to the housekeeping gene *L32*.

(F) Mean protein levels (pg/mg tissue) ± SEM of IL-1β, KC, and MIP2 from tissue homogenates at 0, 6, and 24 hr after infection with *S. aureus*.

*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ $TLR2^{-/-}$ versus wt mice (Student's t test).

MyD88-deficient mice could not be explained by the absence of TLR2 signaling alone, indicating that other receptors that signal via MyD88 must be involved.

IL-1R- but Not TNFαR-Deficient Mice Developed Larger Lesions with Increased Bacterial Counts and Had Defective Neutrophil Recruitment Compared with Wild-Type Mice after Skin Inoculation with *S. aureus*

Since IL-1, which signals via the IL-1RI/MyD88 pathway, has been known to be a central player in innate and acquired immune responses in epithelial sites, we hypothesized that IL-1RI/MyD88 signaling may be involved in the immune response to *S. aureus* infection in our model (Murphy et al., 2000; Dinarello, 1996). Wild-type and IL-1RI-deficient mice (designated IL-1R-deficient) were subcutaneously inoculated with *S. aureus* as in Figures 1 and 2 (Figures 4A–4F). Since IL-1 and TNFα are thought to share many of the same proinflammatory responses, we compared these results with those obtained with mice deficient in TNFαRI (designated TNFαR-deficient), which does not signal via MyD88 (Figures 4G–4K; Murphy et al., 2000; Dinarello, 1996; Kielian et al., 2004; Kupper and Fuhlbrigge, 2004).

IL-1R-deficient mice had lesions that were significantly larger and took longer to heal than wt mice (Figure 4A). Furthermore, IL-1R-deficient mice had ~6-fold higher bioluminescence than wt mice, but only at the later time points (day 7 and 10) (Figure 4B). Furthermore, lesions of IL-1R-deficient mice had decreased

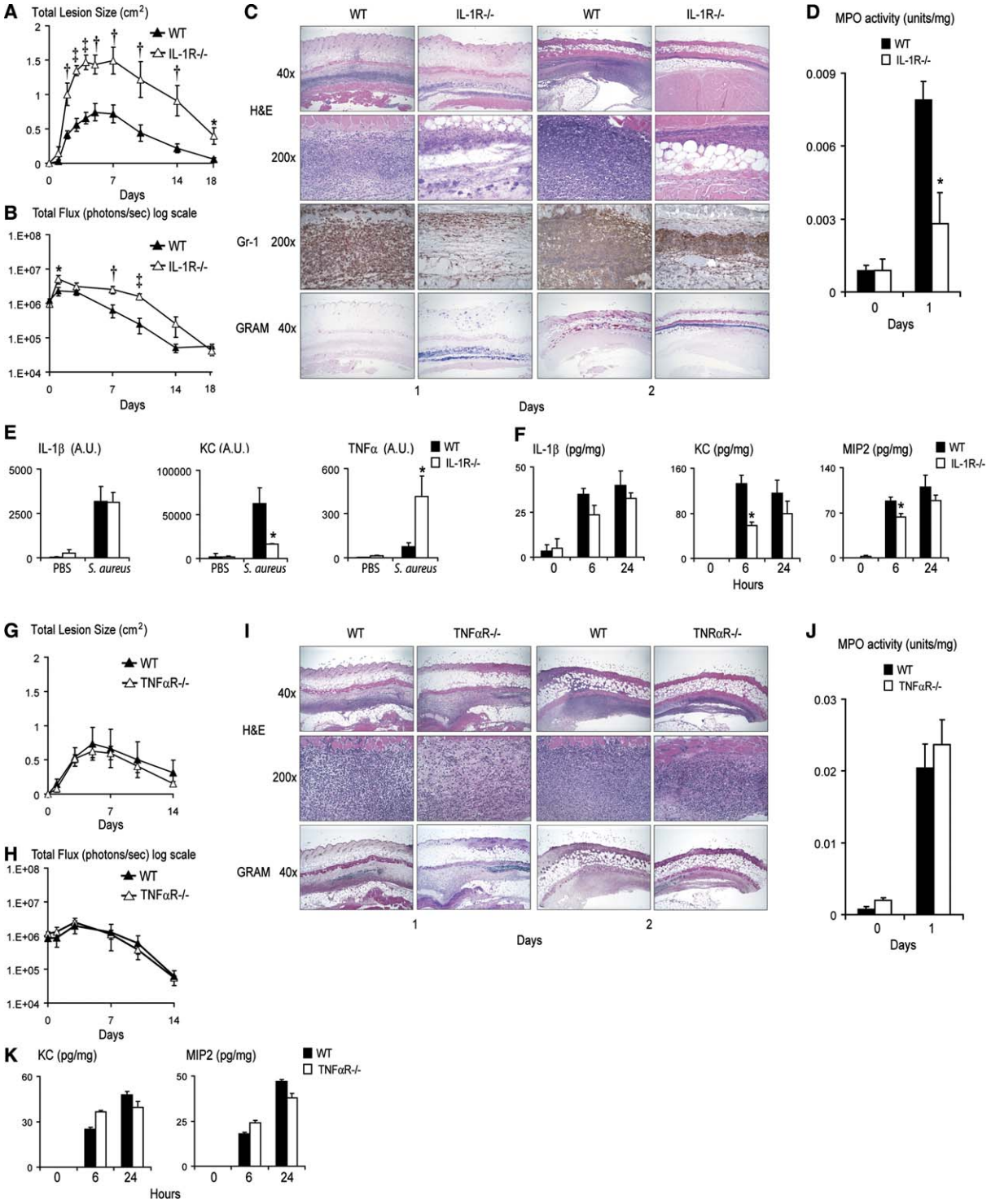

Figure 4. IL-1R- but Not TNFαR-Deficient Mice Have Markedly Larger Skin Lesions with Higher Bacterial Counts and Decreased Neutrophil Recruitment Compared with Wild-Type Mice after Subcutaneous Inoculation with *S. aureus*

IL-1R$^{-/-}$, TNFαR$^{-/-}$, and wt control mice were inoculated subcutaneously with *S. aureus* (2.5 × 10^6 CFUs in 100 μl PBS).

(A and G) Mean total lesion size (cm^2) ± SEM.

(B and H) Mean total flux (photons/s) ± SEM.

(C and I) Representative photomicrographs of sections labeled with H&E stain, anti-Gr-1 mAb, and gram stain of lesional skin at 1 and 2 days after inoculation.

(D and J) Mean myeloperoxidase (MPO) activity (units/mg tissue) ± SEM.

(E) Mean level of mRNA in arbitrary units (A.U.) ± SEM of *IL-1β, KC,* and *TNFα* at 6 hr after inoculation with *S. aureus* or PBS determined by Q-PCR and normalized to the housekeeping gene *L32*.

(F and K) Mean protein levels (pg/mg tissue) ± SEM of IL-1β, KC, and MIP2 from tissue homogenates at 0, 6, and 24 hr after inoculation.

*$p < 0.05$, $^{\dagger}p < 0.01$, $^{\ddagger}p < 0.001$ IL-1R$^{-/-}$ mice or TNFαR$^{-/-}$ mice versus wt mice (Student's t test).

neutrophilic abscess formation, decreased MPO activity, and decreased levels of *KC* mRNA and KC and MIP2 protein levels at 6 hr after inoculation with *S. aureus* compared with wt mice (Figures 4C–4F). Although lesions of both IL-1R-deficient mice and wt mice had similar levels of IL-1β mRNA and protein levels (Figures 4E and 4F), IL-1R-deficient mice cannot respond to IL-1β. Taken together, IL-1R-deficient mice resembled MyD88-deficient mice in that both had substantially larger lesions with higher bacterial counts than wt mice after skin inoculation with *S. aureus*. In addition, both IL-1R- and MyD88-deficient mice had a severe defect in the ability to recruit neutrophils to the site of infection, suggesting that IL-1R/MyD88 signaling is essential for adequate neutrophil recruitment.

Interestingly, lesions of IL-1R-deficient mice had significantly increased levels of *TNFα* mRNA, which may have been a compensatory increase since IL-1R-deficient mice cannot respond to IL-1 (Figure 4E). However, the higher levels of TNFα are less likely to be important in immunity against *S. aureus* in our model, since we found no significant differences in lesion size, in vivo bioluminescence, neutrophilic abscess formation, MPO activity, or induction of KC and MIP2 in lesions of TNFαR-deficient mice compared with wt mice (Figures 4G–4K).

Neutrophil Recruitment in Response to *S. aureus* Infection Is Not Dependent upon MyD88 Expression by Recruited Bone Marrow-Derived Cells

Since the lesions of *S. aureus*-infected mice are comprised of both resident skin cells and recruited bone marrow (BM)-derived cells, we wanted to determine which population of cells utilized IL-1R/MyD88 signaling to promote neutrophil recruitment. Lethally irradiated recipient wt mice were reconstituted with BM from donor wt mice or MyD88-deficient mice to generate two groups: (1) wt mice reconstituted with wt BM (wt BM→wt mice) and (2) wt mice reconstituted with MyD88-deficient BM (MyD88$^{-/-}$ BM→wt mice) (Figure 5). At 8 weeks postreconstitution, these BM-reconstituted mice and normal nonirradiated/nonreconstituted wt and MyD88-deficient mice were subcutaneously inoculated with *S. aureus* as in Figures 1 and 2. We found that MyD88$^{-/-}$ BM→wt mice had lesions that were only moderately larger than those of wt BM→wt mice (Figure 5A). Furthermore, lesions in both groups healed at approximately the same time (day 18). These results were in contrast to lesions of nonirradiated/nonreconstituted MyD88-deficient mice, which had 3-fold larger lesion sizes with no signs of healing and were thus euthanized on day 10. Similarly, in vivo bioluminescence signals of MyD88$^{-/-}$ BM→wt mice were moderately higher than that of wt BM→wt mice, but to levels much less than that of nonirradiated/nonreconstituted MyD88-deficient mice (Figure 5B).

MyD88$^{-/-}$ BM→wt mice, wt BM→wt mice, and nonirradiated/nonreconstituted wt mice all had large neutrophilic abscesses at day 1, whereas nonirradiated/nonreconstituted MyD88-deficient mice had markedly decreased neutrophil recruitment (Figure 5C). Furthermore, there was no significant difference between MPO activity in lesions of nonirradiated/nonreconstituted wt mice compared with MyD88$^{-/-}$ BM→wt mice or wt BM→wt (Figure 5D). To control for BM reconstitution, BM-derived macrophages (BMMs) from all mice were evaluated for the presence of MyD88 mRNA and protein expression (Figure 5E). Functional activity of MyD88-dependent signaling was assayed by measuring *IL-1β* and *IL-6* mRNA in response to the TLR1/2 ligand, Pam3Cys, and the TLR9 ligand, CpG DNA (Figure 5E). Taken together, these results demonstrate that MyD88 signaling by the recruited BM-derived cells is dispensable for neutrophil recruitment after skin inoculation with *S. aureus*.

Neutrophil Recruitment in Response to *S. aureus* Infection Is Dependent upon IL-1R/MyD88 Signaling by Resident Skin Cells and Not by BM-Derived Recruited Cells

We next set out to determine whether IL-1R signaling by BM-derived recruited cells was also dispensable for neutrophil recruitment after skin inoculation with *S. aureus*. As in Figure 5, lethally irradiated recipient wt mice were reconstituted with BM from donor wt mice or IL-1R-deficient mice to generate two groups: (1) wt BM→wt mice and (2) IL-1R$^{-/-}$ BM→wt mice (Figures 6A–6D). IL-1R$^{-/-}$ BM→wt mice had lesion sizes and bioluminescence signals roughly equivalent to those of wt BM→wt mice or wt control mice and significantly less than those of nonirradiated/nonreconstituted IL-1R-deficient mice (Figures 6A and 6B). In addition, lesions of IL-1R$^{-/-}$ BM→wt mice, wt BM→wt mice, and nonirradiated/nonreconstituted wt control mice all developed large neutrophilic abscesses at day 1 (Figure 6C). In contrast, lesions of nonirradiated/nonreconstituted IL-1R-deficient mice had markedly decreased neutrophil recruitment. Furthermore, MPO activity of IL-1R$^{-/-}$ BM→wt mice and of wt BM→wt mice was not significantly different than that of wt control mice (Figure 6D). Similar to results obtained with the MyD88 BM reconstitution experiments, these findings demonstrate that IL-1R signaling by the recruited BM-derived cells is dispensable for neutrophil recruitment and immunity against *S. aureus*.

To evaluate the role of resident skin cells in utilizing IL-1R/MyD88 signaling to promote neutrophil recruitment more directly, we reconstituted IL-1R-deficient recipient mice with either wt or IL-1R-deficient donor BM to generate two groups: (1) wt BM→IL-1R$^{-/-}$ mice and (2) IL-1R$^{-/-}$ BM→IL-1R$^{-/-}$ mice (Figures 6E–6H). Wild-type BM→IL-1R$^{-/-}$ mice and IL-1R$^{-/-}$ BM→IL-1R$^{-/-}$ mice had lesion sizes and bioluminescence signals that were virtually equivalent to those of nonirradiated/nonreconstituted IL-1R-deficient mice and significantly greater (3- to 4-fold) than those of wt control mice (Figures 6E and 6F). In addition, lesions of wt BM→IL-1R$^{-/-}$ mice, IL-1R$^{-/-}$ BM→IL-1R$^{-/-}$ mice, and nonirradiated/nonreconstituted IL-1R$^{-/-}$ mice all had markedly decreased neutrophilic abscess formation, abundant gram-positive bacteria, and decreased MPO activity compared with wt control mice (Figures 6G and 6H). To control for BM reconstitution, BMMs from all mice were assayed for *IL-6* mRNA levels after stimulation with recombinant murine IL-1β (Figure 6I). Taken together, these data suggest that IL-1R signaling by resident skin cells is essential for adequate neutrophil recruitment to a site of a localized *S. aureus* infection in the skin.

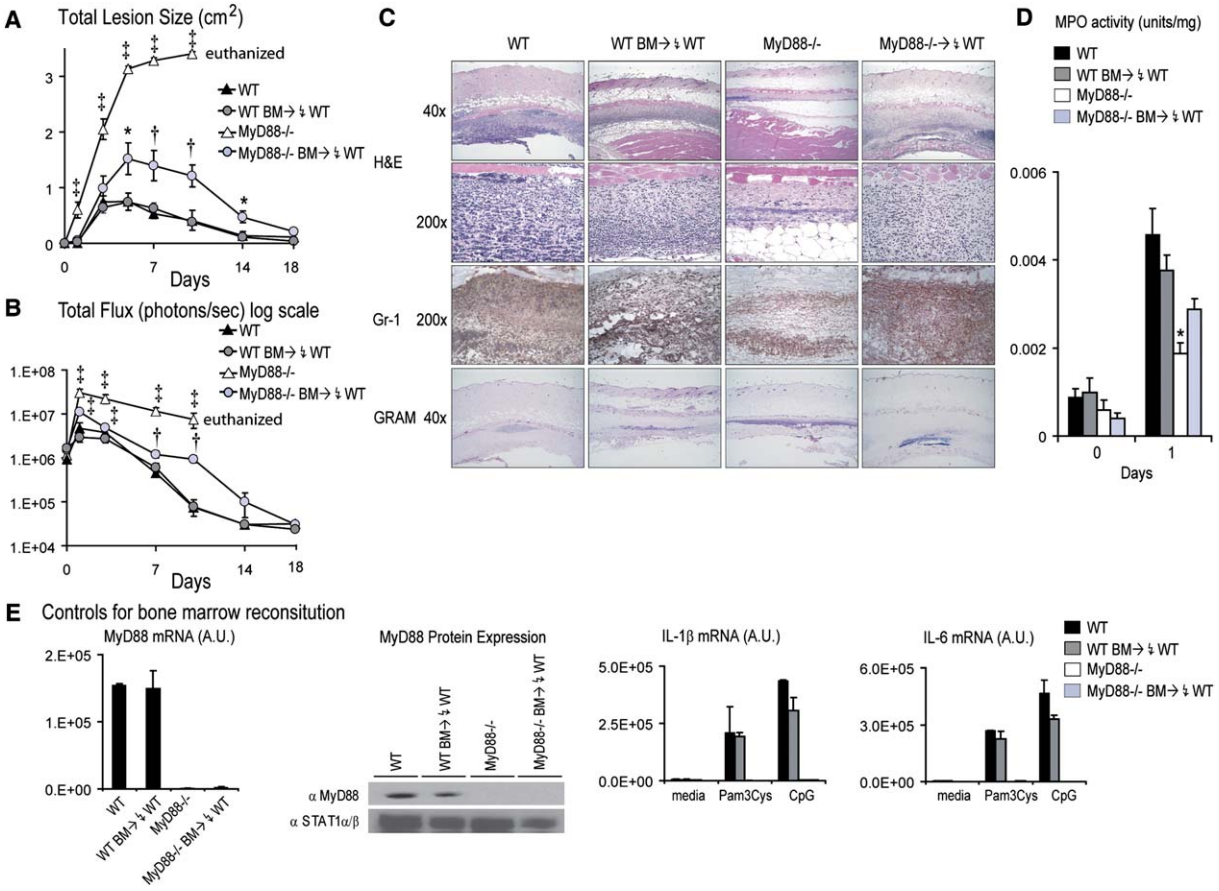

Figure 5. Neutrophil Recruitment in Response to *S. aureus* Infection Is Not Dependent upon MyD88 Expression by Recruited Bone Marrow-Derived Cells

Wild-type recipient mice were lethally irradiated (1000 Rads) and reconstituted with bone marrow (BM) from wt or MyD88$^{-/-}$ donor mice (wt BM→wt and MyD88$^{-/-}$ BM→wt, respectively). After 8 weeks, BM reconstituted mice and nonirradiated/nonreconstituted MyD88$^{-/-}$ and wt control mice were inoculated subcutaneously with *S. aureus* (5 × 10^6 CFUs in 100 μl PBS).

(A) Mean total lesion size (cm^2) ± SEM.

(B) Mean total flux (photons/s) ± SEM.

(C) Representative photomicrographs of sections labeled by H&E stain, anti-Gr-1 mAb, and gram stain of lesional skin at 1 day after inoculation.

(D) Mean myeloperoxidase (MPO) activity (units/mg tissue) ± SEM.

(E) BM-derived macrophages (BMMs) from all mice were assessed for: MyD88 mRNA expression by Q-PCR, MyD88 protein expression by immunoblotting, and MyD88 function by measuring mRNA levels of *IL-1β* and *IL-6* by Q-PCR after stimulation of BMMs with Pam3Cys (1 μg/ml) and CpG (100 nM).

*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ MyD88$^{-/-}$ mice or BM-reconstituted mice versus wt mice (Student's t test).

Discussion

The MyD88 adaptor protein is essential for immunity to microbial pathogens (Akira and Takeda, 2004; Martin and Wesche, 2002; Fitzgerald and O'Neill, 2000). Given that MyD88 is utilized by TLR family members, many studies of microbial infection have focused on the contribution of TLR activation to the MyD88 phenotype (Akira and Takeda, 2004). However, IL-1R family members also utilize MyD88 to induce immune responses (Martin and Wesche, 2002; Adachi et al., 1998; Fitzgerald and O'Neill, 2000). By using a model of *S. aureus* infection in the skin, we found that although MyD88, TLR2, and IL-1R contributed to host defense, MyD88 and IL-1R but not TLR2 were crucial for neutrophil recruitment to the site of infection, an event required for bacterial clearance (Tsuda et al., 2004; Molne et al., 2000). Thus, TLR2 and IL-1R have distinct immunologic roles in host defense against a localized bacterial infection.

Although both TLR2 and IL-1R utilize MyD88 to initiate signaling, there are key differences between these receptors, including: (1) the mechanism of MyD88 activation; (2) the ligands for these receptors; (3) the bioavailability of these ligands; and (4) the cellular distribution of receptors. First, TLR2 signaling via MyD88 is dependent upon interaction with another MyD88 adaptor family member, TIRAP/Mal, whereas IL-1R signaling requires only MyD88 (Horng et al., 2002). Thus, differences between TLR2/MyD88- and IL-1R/MyD88-signaling complexes could influence downstream signaling and result in differential gene activation, such as the production of neutrophil chemoattractants.

Second, the ligands for TLR2 consist of bacterial cell wall components, including lipopeptides and lipoteichoic acid of *S. aureus* (Takeuchi et al., 2001, 2002; Hoebe et al., 2005; Brightbill et al., 1999). In contrast, the ligands for IL-1R consist of IL-1α and IL-1β, which are produced through transcription and translation by

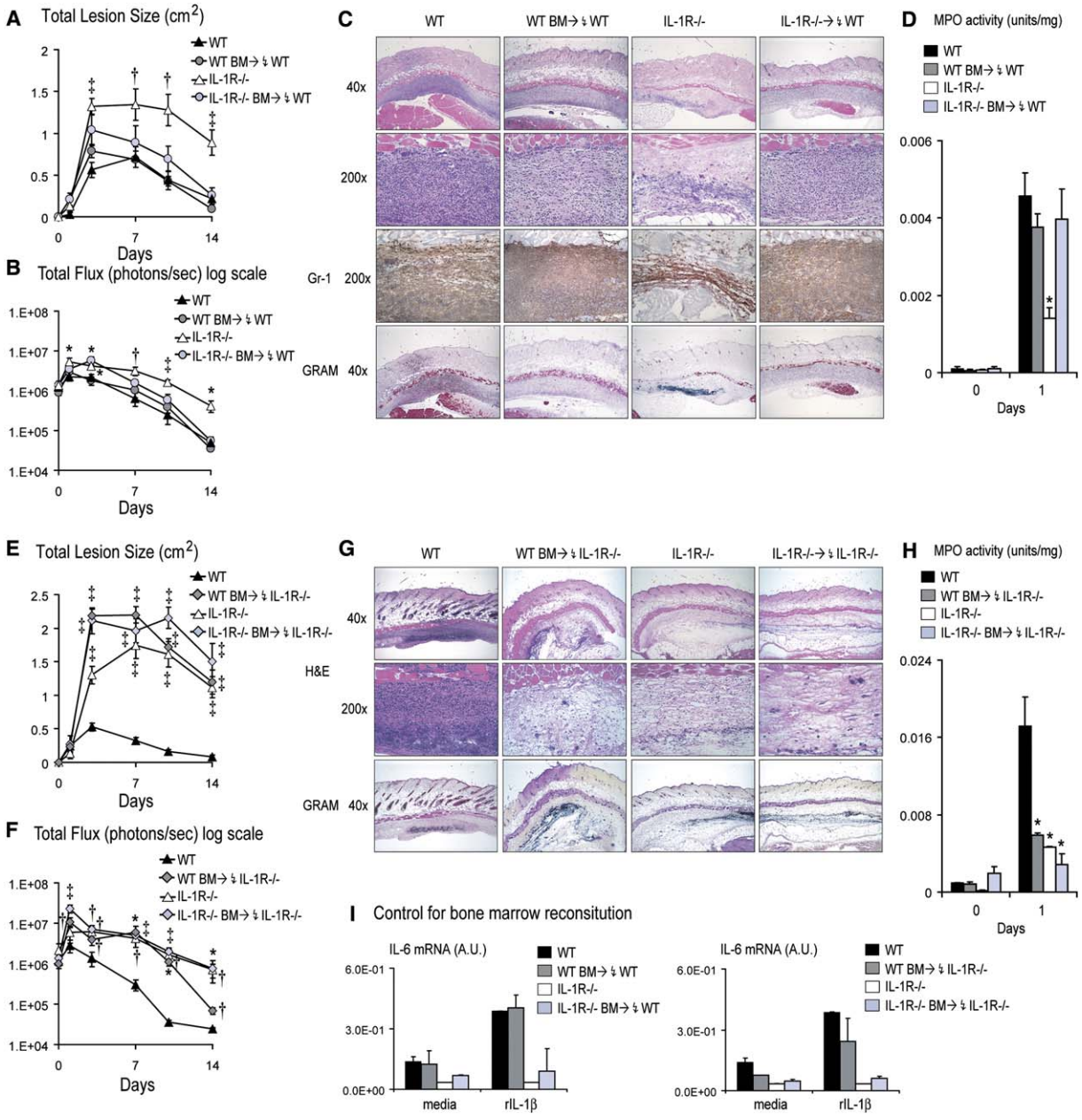

Figure 6. Neutrophil Recruitment in Response to *S. aureus* Infection Is Dependent upon IL-1R/MyD88 Signaling by Resident Skin Cells and Not by Bone Marrow-Derived Recruited Cells

Wild-type and IL-1R$^{-/-}$ recipient mice were lethally irradiated (1000 Rads) and reconstituted with bone marrow (BM) cells from wt or IL-1R$^{-/-}$ donor mice: (A–D) wt BM→wt and IL-1R$^{-/-}$ BM→wt and (E and F) wt BM→IL-1R$^{-/-}$ and IL-1R$^{-/-}$ BM→IL-1R$^{-/-}$. After 8 weeks, BM-reconstituted mice and nonirradiated/nonreconstituted IL-1R$^{-/-}$ and wt control mice were inoculated subcutaneously with *S. aureus* (2.5×10^6 CFUs in 100 μl PBS).

(A and E) Mean total lesion size (cm^2) ± SEM.

(B and F) Mean total flux (photons/s) ± SEM.

(C and G) Representative photomicrographs of sections labeled with H&E, anti-Gr-1 mAb, and gram stain of lesional skin at 1 day after inoculation.

(D and H) Mean myeloperoxidase (MPO) activity (units/mg tissue) ± SEM.

(I) BM-derived macrophages from BM-reconstituted mice and nonirradiated/nonreconstituted IL-1R$^{-/-}$ and wt mice were assessed for IL-1R activity by measuring mRNA levels of *IL-6* by Q-PCR after stimulating BMMs with rIL-1β (50 ng/ml).

*p < 0.05, †p < 0.01, ‡p < 0.001 IL-1R$^{-/-}$ mice or BM-reconstituted mice versus wt mice (Student's t test).

the host (Murphy et al., 2000; Dinarello, 1996). Experiments in vitro have indicated that production of IL-1β is MyD88 dependent (data not shown) (Takeuchi et al., 2000; Kawai et al., 1999). However, we found that IL-1β was produced in vivo in both TLR2- and MyD88-deficient mice, albeit somewhat delayed, reaching levels observed in wt mice by 24 hr after infection with *S. aureus*. Since TLR2-deficient mice can respond to any IL-1α or IL-1β present whereas MyD88- and IL-1R-deficient mice cannot, the bacterial clearance in TLR2-deficient

mice might be primarily or in part mediated by IL-1R activation, which in turn could deliver signals via IL-1R/MyD88 to promote neutrophil recruitment.

Third, since the ligands for TLR2 and IL-1R come from different sources, the bioavailability of these ligands to their corresponding receptors may play a key role in which cell types are activated. TLR2-mediated recognition of bacterial components occurs predominantly in the context of breakdown of the pathogen by inflammatory cells (Akira and Takeda, 2004; Martin and Wesche, 2002). In contrast, IL-1α is produced primarily by keratinocytes and endothelial cells (Murphy et al., 2000; Dinarello, 1996; Steen et al., 1999). In uninfected skin of MyD88-deficient, TLR2-deficient, and wt mice (at time zero), IL-1α was detected within the cells of the epidermis (Figure S4). However, at 24 hr after *S. aureus* inoculation, IL-1α was not only detected in the cells of the epidermis, but also in the dermis and focally within the neutrophilic abscesses. Therefore, IL-1α is localized to distinct compartments within the skin, and its bioavailability to different cell types appears to be time dependent. IL-1β is produced primarily by activated immune system cells such as monocytes/macrophages, dendritic cells, and Langerhans cells (Murphy et al., 2000; Dinarello, 1996; Kupper and Fuhlbrigge, 2004). Activation of these different cell types occurs in an autocrine or paracrine manner during the course of infection. Thus, IL-1α or IL-1β could activate distinct gene programs among the different cell types, resulting in distinct inflammatory responses such as the recruitment of neutrophils. Interestingly, we did observe an early decreased production of IL-1β in TLR2-deficient mice but did not observe a major defect in neutrophil recruitment is these mice. It could be that IL-1α compensated for this early decreased production of IL-1β in TLR2-deficient mice to promote IL-1R/MyD88-dependent neutrophil recruitment.

Fourth, the cellular expression and distribution of IL-1R and TLR2 are different. IL-1R is found ubiquitously, on both resident cells and BM-derived cells (Murphy et al., 2000; Dinarello, 1996). In contrast, TLR2 is primarily expressed on BM-derived cells including neutrophils, monocytes/macrophages, antigen-presenting cells, T and B cells, and with conflicting reports about expression on endothelial and some epithelial cells (Takeuchi et al., 1999; Fan et al., 2003; Kurt-Jones et al., 2002). To delineate which population of cells is important in promoting IL-1R/MyD88-mediated neutrophil recruitment, we utilized techniques of BM reconstitution. We found that neutrophil recruitment and bacterial clearance occurred in wt mice reconstituted with IL-1R-deficient, MyD88-deficient, or wt BM, indicating that IL-1R or MyD88 expression by recruited BM-derived cells had no impact on neutrophil recruitment or in the eradication of the infection. In addition, we found a severe defect in neutrophil recruitment in IL-1R-deficient mice reconstituted with either IL-1R-deficient or wt BM. These data suggest that adequate neutrophil recruitment and bacterial clearance is dependent upon IL-1R/MyD88 signaling by resident skin cells and not by BM-derived recruited cells. Thus, resident skin cells may act as first responders and send appropriate signals via IL-1R/MyD88 activation, such as production of KC and MIP2, to promote neutrophil recruitment. Future studies will utilize additional BM reconstitution experiments to further characterize the functional contribution of IL-1R and TLR2 to the phenotype of MyD88-deficient mice.

It should be mentioned that the results of the present study are consistent with previous studies that have demonstrated the importance of IL-1R/MyD88 signaling for host defense against *S. aureus* in brain abscesses, arthritis, and systemic infections (Hultgren et al., 2002; Kielian et al., 2004; Verdrengh et al., 2004). In addition, the chemokines KC and MIP2 have been shown to be important for neutrophil recruitment in *S. aureus* brain abscesses (Kielian et al., 2001). However, the present study has demonstrated that IL-1R/MyD88 signaling by resident cells at the site of infection is critical for neutrophil recruitment and immunity against *S. aureus* in the skin, where most of these infections originate. We have also demonstrated that production of KC and MIP2 in vivo is dependent upon IL-1R/MyD88 signaling. Our data do not exclude that in addition to TLR2 and IL-1R, other MyD88-dependent receptors, such as TLR9 or IL-18R, may also contribute to host defense against *S. aureus* (Martin and Wesche, 2002; Adachi et al., 1998).

Lastly, it could be that the mechanism(s) leading to neutrophil recruitment is controlled by IL-1R/MyD88 signaling or perhaps another mechanism, such as via TNFαR activation, depending on the site of infection. This may in fact be the case, since a previous study has demonstrated that MyD88 is dispensable for host defense against *S. aureus* pulmonary infections, and other studies have demonstrated that TNFα is essential for neutrophil recruitment in models of acute peritonitis (Skerrett et al., 2004; Zhang et al., 1992; Malaviya et al., 1996).

In summary, we have identified that IL-1R/MyD88 but not TLR2/MyD88 signaling by resident skin cells is critical for neutrophil recruitment to a site of a localized *S. aureus* infection in the skin. From the clinical point of view, the identification of the critical role of IL-1R/MyD88 signaling for promoting neutrophil recruitment to a site of infection raises the possibility that this pathway could be locally targeted to engage the host's own immune responses in the treatment of a microbial infection.

Experimental Procedures

Staphylococcus aureus Strains

All *S. aureus* strains used were derived from the common laboratory strain RN6390, which has a known 11 bp deletion in the *rsbU* gene within the *sigB* operon (Novick, 1990). Strain SH1000 is a derivative of RN6390 with the *rsbU* gene restored (Horsburgh et al., 2002). Strain RN4220 is a derivative of 8325-4 that accepts foreign DNA (Novick, 1990). Strains used in cutaneous bioluminescent mouse infection experiments include the bioluminescent strain ALC2906, which is strain SH1000 containing the shuttle plasmid pSK236 with the *pbp2* (penicillin binding protein 2) promoter fused to the *luxABCDE* reporter cassette from *Photorhabdus luminescens*. In control experiments, strain ALC2506, a nonbioluminescent control SH1000 strain containing pSK236 with a promoterless *luxABCDE* reporter cassette, was used. Strain 8325-4 (kind gift of Prof. Jeffrey F. Miller, Dept. of Microbiology, Immunology, and Molecular Genetics, UCLA, Los Angeles, CA) was used where indicated.

Plasmids

The following plasmids were used to make bioluminescent *S. aureus* strains. pSK236 is an *E. coli* and *S. aureus* shuttle vector (Novick, 1990). pLux is pSK236 containing *luxABCDE* from pSB2025 at *Sal*I

and *Pst*I sites (Qazi et al., 2001). pLux(pbp2-pro) is pLux containing a 986 bp *pbp2* promoter at the *Eco*RI site immediately upstream of *luxABCDE*. pCR2.1 is an *E. coli* vector for direct cloning of PCR fragments (Invitrogen, Carlsbad, CA).

Construction Details of Bioluminescent *S. aureus* Strain

Promoterless *luxABCDE* cassette of *Photorhabdus luminescens* from pSB2025 from *E. coli* strain ALC2468 (JM109 containing pSB2025) was cloned into the *Sal*I/*Pst*I site of pSK236. The promoter of *pbp2* was amplified from chromosomal DNA of *S. aureus* strain RN6390 by PCR with the following promoter-specific primers 5′-CC ACATACTTGTACTTGCCTC-3′ (forward) and 5′-TTCTTAGGCTGAG AAGATCC-3′ (reverse). The resulting 989 bp PCR fragment was cloned into TOPO-TA cloning vector pCR2.1, digested with *Eco*RI, and cloned into *Eco*RI site upstream of the promoterless Lux reporter vector pLux. The correct orientation of the promoter fragment was confirmed by sequencing. Plasmid DNA was electroporated into *S. aureus* strain RN4220 and then into strain SH1000. This bioluminescent strain containing pLux(pbp2) was designated ALC2906. The pLux(pbp2) plasmid in ALC2906 is active during all *S. aureus* growth phases and emits photons only from live, ATP-producing, bacteria.

Preparation of *S. aureus* for Skin Inoculation

S. aureus was streaked onto Tryptic soy agar (Tryptic soy broth [TSB] + 1.5% Bacto Agar) (Becton Dickinson, Sparks, MD; Sigma-Aldrich, St. Louis, MO). Colonies of *S. aureus* were grown overnight at 37°C in a shaking incubator (240 rpm) in TSB. Midlogarithmic phase bacteria were obtained after a 3 hr subculture of 1:100 dilution of the overnight culture. The *S. aureus* bioluminescent strain ALC2906 and nonbioluminescent strain ALC2506 have a chloramphenicol resistance plasmid selection marker, and cultures were performed in the presence of chloramphenicol (10 μg/ml) (Sigma-Aldrich). In other experiments, *S. aureus* strain 8325-4 was prepared similarly, but in the absence of chloramphenicol. Bacterial cells were pelleted, resuspended, and washed three times in PBS. Bacterial concentrations were estimated with a spectrophotometer (Beckman DU 640B Spectrophotometer, Beckman Coulter, Inc., Fullerton, CA) by determining the absorbance at 600 nm (A_{600}). Colony-forming units (CFUs) were verified by plating dilutions of the inoculum onto TSB agar ± chloramphenicol overnight.

Mice

All mice used have a C57BL/6J genetic background. MyD88-deficient mice (F_8), TLR2-deficient mice (F_4), and littermate wt mice were kind gifts from Dr. Shizuo Akira (Osaka University, Osaka, Japan). IL-1RI-deficient mice with *Il1r*^{tm1Imx} targeted mutation (designated IL-1R-deficient mice) (F_5), TNFαRI-deficient mice with *Tnfrsf1a*^{tm1Mak} targeted mutation (designated TNFαR-deficient mice) (F_{12}), and wt mice were obtained from Jackson Laboratories (Bar Harbor, ME).

Mouse Model of Cutaneous *S. aureus* Infection

All procedures were approved by UCLA Animal Research Committee. The mice were shaved on the back and inoculated subcutaneously with 100 μl of midlogarithmic growth phase *S. aureus* strain ALC2906 (~2.5×10^6 CFUs/100 μl = 1:10 dilution of A_{600} of 0.5/ml) in sterile pharmacy grade saline (0.9%) by a 27 gauge needle and a tuberculin syringe (Abbott Laboratories, Chicago, IL). In experiments with strain 8325-4, the concentration used was ~5×10^7 CFUs/100 μl = A_{600} of 0.8/ml. Groups of 4–5 mice were used in each experiment followed by one to two repeats to confirm results. Measurements of total lesion size (cm^2) were made by analyzing digital photographs (Nikon Coolpix 5400) of mice taken every 1–3 days with the software program "Image J" (NIH Research Services Branch [http://rsbweb.nih.gov/ij/]) and a millimeter ruler as a reference.

Quantification of In Vivo *S. aureus*: In Vivo Bioluminescence

In vivo bioluminescence was performed with the Xenogen IVIS imaging system (Xenogen Corporation, Alameda, CA) at the Crump Institute for Molecular Imaging at UCLA as previously described (Francis et al., 2000). Mice were anesthetized via an intraperitoneal injection of a mix of ketamine and xylazine (100 and 20 mg/kg body weight, respectively). Data are presented on color scale overlaid on a gray-scale photograph of mice and quantified as total flux (photons/s) within a circular region of interest (1×10^3 pixels) with Living Image software (Xenogen) (lower limit of detection: 1×10^4 photons/s).

Tissue Embedding and Staining

For histological analysis, lesional 8 mm punch biopsy (Acuderm) specimens were bisected and one half was fixed in formalin (10%) and embedded in paraffin and the other half was embedded in Tissue-Tek OTC compound (Sakura Finetek USA, Inc., Torrance, CA) and frozen in liquid nitrogen. Hematoxylin and eosin (H&E) and gram stains were performed on paraffin sections (4 μm) by the Tissue Procurement & Histology Core Laboratory and by the Histopathology Laboratory at UCLA, according to guidelines for clinical samples.

Immunoperoxidase Labeling

Detection of Gr-1 (Ly-6G)-positive cells and IL-1α expression on frozen cryostat specimens of lesional skin punch biopsy specimens were performed with a biotinylated rat anti-mouse Gr-1 mAb (clone RB6-8C5) (1 μg/ml) (BD Pharmingen, San Diego, CA) or rat anti-mouse IL-1α mAb (clone 40508) (15 mg/ml) (R&D systems) by the immunoperoxidase method as previously described (Miller et al., 2005).

Immunofluorescence and Confocal Laser Microscopy

Double immunofluorescence of Gr-1 (Ly-6G) (neutrophil marker) and Mac-3 (macrophage marker) was performed with a biotinylated rat anti-mouse Gr-1 mAb (clone RB6-8C5) (1 μg/ml) and a FITC-conjugated rat anti-mouse Mac-3 mAb (1 μg/ml) (clone M3/84) or appropriate isotype control mAb (all BD Pharmingen) followed by streptavidin-conjugated Cy™3 (0.2 μg/ml) (Caltag Laboratories, Burlingame, CA) and imaged on a Leica TCS-SP spectral Confocal Inverted Microscope equipped with argon (488 nm blue excitation) and DPSS diode (561 nm yellow excitation) lasers (Leica Microsystems, Heidelberg, Germany) as previously described (Krutzik et al., 2003).

Myeloperoxidase Assay

Myeloperoxidase (MPO) activity from lesional skin was obtained from tissue homogenates (Tissue-tearer, Biospec Products, Bartlesville, OK) of 8 mm punch biopsy specimens (Acuderm) with a MPO assay kit according to the manufacturer's recommendations (Cytostore, Calgary, Alberta, Canada).

Enzyme-Linked Immunosorbent Assay

Protein levels of IL-1β, KC, and MIP2 (pg/mg of tissue) from lesional skin were obtained from tissue homogenates (Tissue-tearer) in 0.01% Triton of 8 mm punch biopsy specimens taken at various time points after *S. aureus* skin inoculation with commercially available ELISA kits (KC and MIP2 from R&D systems; IL-1β from BD Pharmingen).

Bone Marrow Reconstitution

Bone marrow (BM) reconstitution experiments were performed as previously described (Joseph et al., 2004). BM was flushed from tibias and femurs from donor mice, RBC was depleted by ACK lysis buffer, and BM was washed twice in PBS. 1×10^7 BM cells were injected into the tail vein of lethally irradiated (1000 Rads) recipient mice. Reconstituted mice were maintained in autoclaved cages and were administered Baytril (0.5 mg/ml in their drinking water) for the first 3 weeks postirradiation. All experiments were performed 8 weeks after BM reconstitution. To verify the efficiency of reconstitution, BM was removed from euthanized mice and differentiated into BM-derived macrophages (BMMs) as previously described (Doyle et al., 2002). For the MyD88 BM reconstitution, MyD88 mRNA and protein expression was determined by Q-PCR and immunoblotting, respectively. In addition, MyD88 function was evaluated by stimulating BMMs with Pam3Cys (1 μg/ml) (Alexis Biochemicals) or CpG (100 nM) (Invitrogen) and assessed for mRNA expression for *IL-1β* and *IL-6* by Q-PCR. For the IL-1R BM reconstitution, IL-1RI function was evaluated by stimulating BMMs with recombinant murine IL-1β (rIL-1β) (50 ng/ml) (R&D systems) and assessed for mRNA expression of *IL-6* by Q-PCR (see below).

mRNA Quantification

Total RNA was isolated from lesional (*S. aureus*) and control (PBS) homogenized (Kontes RNase Free Pestles, Vineland, NJ) 8 mm punch biopsy specimens performed at 6 hr after inoculation or from cultured BMMs by the use of TRIzol (Invitrogen) according to the manufacturer's recommendations. Quantitative real-time PCR (Q-PCR) was performed as previously described (Doyle et al., 2002). Primer sequences for *IL-1β, TNFα, IL-6, MyD88,* and the normalizer, *L32,* were published previously (Doyle et al., 2002, 2003, 2004). Primers for *KC* were forward, 5′-CACTGCACCCAAACCGAAGT-3′, and reverse, 5′-GGACAATTTTCTGAACCAAGGG-3′. Relative quantities of mRNA per sample were normalized to L32 (Doyle et al., 2002).

Immunoblotting

MyD88 and STAT1 protein expression in BMMs were assayed by immunoblotting by the use of polyclonal antibodies against MyD88 (Prosci) and STAT1 (Santa Cruz Biotechnologies) as previously described (Doyle et al., 2002, 2004).

Statistical Analyses

Data were compared with a Student's t test. All data are expressed as mean ± SEM (standard error of the mean) where indicated. Values of $^{*}p < 0.05$, $^{\dagger}p < 0.01$, and $^{\ddagger}p < 0.001$ were considered statistically significant.

Supplemental Data

Supplemental Data include four figures and can be found with this article online at http://www.immunity.com/cgi/content/full/24/1/79/DC1/.

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References

Adachi, O., Kawai, T., Takeda, K., Matsumoto, M., Tsutsui, H., Sakagami, M., Nakanishi, K., and Akira, S. (1998). Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. Immunity *9*, 143–150.

Akira, S., and Takeda, K. (2004). Toll-like receptor signalling. Nat. Rev. Immunol. *4*, 499–511.

Brightbill, H.D., Libraty, D.H., Krutzik, S.R., Yang, R.B., Belisle, J.T., Bleharski, J.R., Maitland, M., Norgard, M.V., Plevy, S.E., Smale, S.T., et al. (1999). Host defense mechanisms triggered by microbial lipoproteins through Toll-like receptors. Science *285*, 732–736.

Dinarello, C.A. (1996). Biologic basis for interleukin-1 in disease. Blood *87*, 2095–2147.

Doyle, S., Vaidya, S., O'Connell, R., Dadgostar, H., Dempsey, P., Wu, T., Rao, G., Sun, R., Haberland, M., Modlin, R., and Cheng, G. (2002). IRF3 mediates a TLR3/TLR4-specific antiviral gene program. Immunity *17*, 251–263.

Doyle, S.E., O'Connell, R., Vaidya, S.A., Chow, E.K., Yee, K., and Cheng, G. (2003). Toll-like receptor 3 mediates a more potent antiviral response than Toll-like receptor 4. J. Immunol. *170*, 3565–3571.

Doyle, S.E., O'Connell, R.M., Miranda, G.A., Vaidya, S.A., Chow, E.K., Liu, P.T., Suzuki, S., Suzuki, N., Modlin, R.L., Yeh, W.C., et al. (2004). Toll-like receptors induce a phagocytic gene program through p38. J. Exp. Med. *199*, 81–90.

Fan, J., Frey, R.S., and Malik, A.B. (2003). TLR4 signaling induces TLR2 expression in endothelial cells via neutrophil NADPH oxidase. J. Clin. Invest. *112*, 1234–1243.

Fitzgerald, K.A., and O'Neill, L.A. (2000). The role of the interleukin-1/Toll-like receptor superfamily in inflammation and host defense. Microbes Infect. *2*, 933–943.

Francis, K.P., Joh, D., Bellinger-Kawahara, C., Hawkinson, M.J., Purchio, T.F., and Contag, P.R. (2000). Monitoring bioluminescent *Staphylococcus aureus* infections in living mice using a novel luxABCDE construct. Infect. Immun. *68*, 3594–3600.

Fridkin, S.K., Hageman, J.C., Morrison, M., Sanza, L.T., Como-Sabetti, K., Jernigan, J.A., Harriman, K., Harrison, L.H., Lynfield, R., and Farley, M.M. (2005). Methicillin-resistant *Staphylococcus aureus* disease in three communities. N. Engl. J. Med. *352*, 1436–1444.

Hoebe, K., Georgel, P., Rutschmann, S., Du, X., Mudd, S., Crozat, K., Sovath, S., Shamel, L., Hartung, T., Zahringer, U., and Beutler, B. (2005). CD36 is a sensor of diacylglycerides. Nature *433*, 523–527.

Horng, T., Barton, G.M., Flavell, R.A., and Medzhitov, R. (2002). The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. Nature *420*, 329–333.

Horsburgh, M.J., Aish, J.L., White, I.J., Shaw, L., Lithgow, J.K., and Foster, S.J. (2002). sigmaB modulates virulence determinant expression and stress resistance: characterization of a functional rsbU strain derived from *Staphylococcus aureus* 8325-4. J. Bacteriol. *184*, 5457–5467.

Hultgren, O.H., Svensson, L., and Tarkowski, A. (2002). Critical role of signaling through IL-1 receptor for development of arthritis and sepsis during *Staphylococcus aureus* infection. J. Immunol. *168*, 5207–5212.

Joseph, S.B., Bradley, M.N., Castrillo, A., Bruhn, K.W., Mak, P.A., Pei, L., Hogenesch, J., O'Connell, R.M., Cheng, G., and Saez, E. (2004). LXR-dependent gene expression is important for macrophage survival and the innate immune response. Cell *119*, 299–309.

Kawai, T., Adachi, O., Ogawa, T., Takeda, K., and Akira, S. (1999). Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity *11*, 115–122.

Kielian, T., Barry, B., and Hickey, W.F. (2001). CXC chemokine receptor-2 ligands are required for neutrophil-mediated host defense in experimental brain abscesses. J. Immunol. *166*, 4634–4643.

Kielian, T., Bearden, E.D., Baldwin, A.C., and Esen, N. (2004). IL-1 and TNF-alpha play a pivotal role in the host immune response in a mouse model of *Staphylococcus aureus*-induced experimental brain abscess. J. Neuropathol. Exp. Neurol. *63*, 381–396.

Krutzik, S.R., Ochoa, M.T., Sieling, P.A., Uematsu, S., Ng, Y.W., Legaspi, A., Liu, P.T., Cole, S.T., Godowski, P.J., Maeda, Y., et al. (2003). Activation and regulation of Toll-like receptors 2 and 1 in human leprosy. Nat. Med. *9*, 525–532.

Kupper, T.S., and Fuhlbrigge, R.C. (2004). Immune surveillance in the skin: mechanisms and clinical consequences. Nat. Rev. Immunol. *4*, 211–222.

Kurt-Jones, E.A., Mandell, L., Whitney, C., Padgett, A., Gosselin, K., Newburger, P.E., and Finberg, R.W. (2002). Role of Toll-like receptor 2 (TLR2) in neutrophil activation: GM-CSF enhances TLR2 expression and TLR2-mediated interleukin 8 responses in neutrophils. Blood *100*, 1860–1868.

Lowy, F.D. (1998). *Staphylococcus aureus* infections. N. Engl. J. Med. *339*, 520–532.

Malaviya, R., Ikeda, T., Ross, E., and Abraham, S.N. (1996). Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. Nature *381*, 77–80.

Martin, M.U., and Wesche, H. (2002). Summary and comparison of the signaling mechanisms of the Toll/Interleukin-1 receptor family. Biochim. Biophys. Acta *1592*, 265–280.

Melles, D.C., Gorkink, R.F., Boelens, H.A., Snijders, S.V., Peeters, J.K., Moorhouse, M.J., van der Spek, P.J., van Leeuwen, W.B., Simons, G., Verbrugh, H.A., and van Belkum, A. (2004). Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. J. Clin. Invest. *114*, 1732–1740.

Miller, L.S., Sorensen, O.E., Liu, P.T., Jalian, H.R., Eshtiaghpour, D., Behmanesh, B.E., Chung, W., Starner, T.D., Kim, J., and Sieling, P.A. (2005). TGF-alpha regulates TLR expression and function on epidermal keratinocytes. J. Immunol. *174*, 6137–6143.

Molne, L., Verdrengh, M., and Tarkowski, A. (2000). Role of neutrophil leukocytes in cutaneous infection caused by *Staphylococcus aureus*. Infect. Immun. *68*, 6162–6167.

Murphy, J.E., Robert, C., and Kupper, T.S. (2000). Interleukin-1 and cutaneous inflammation: a crucial link between innate and acquired immunity. J. Invest. Dermatol. *114*, 602–608.

Novick, R.P. (1990). The *Staphylococcus* as a molecular genetic system. In Molecular Biology of the *Staphylococcus*, R.P. Novick, ed. (New York: VCH Publishers), pp. 1–40.

Qazi, S.N., Counil, E., Morrissey, J., Rees, C.E., Cockayne, A., Winzer, K., Chan, W.C., Williams, P., and Hill, P.J. (2001). agr expression precedes escape of internalized *Staphylococcus aureus* from the host endosome. Infect. Immun. *69*, 7074–7082.

Skerrett, S.J., Liggitt, H.D., Hajjar, A.M., and Wilson, C.B. (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.

Steen, M.B., Tuck, F.L., and Selvan, R.S. (1999). Spontaneous activation of endothelial cells: a central role for endogenous IL-1alpha. In Vitro Cell. Dev. Biol. Anim. *35*, 327–332.

Takeuchi, O., Hoshino, K., Kawai, T., Sanjo, H., Takada, H., Ogawa, T., Takeda, K., and Akira, S. (1999). Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity *11*, 443–451.

Takeuchi, O., Hoshino, K., and Akira, S. (2000). Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to *Staphylococcus aureus* infection. J. Immunol. *165*, 5392–5396.

Takeuchi, O., Kawai, T., Muhlradt, P.F., Morr, M., Radolf, J.D., Zychlinsky, A., Takeda, K., and Akira, S. (2001). Discrimination of bacterial lipoproteins by Toll-like receptor 6. Int. Immunol. *13*, 933–940.

Takeuchi, O., Sato, S., Horiuchi, T., Hoshino, K., Takeda, K., Dong, Z., Modlin, R.L., and Akira, S. (2002). Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. J. Immunol. *169*, 10–14.

Tsuda, Y., Takahashi, H., Kobayashi, M., Hanafusa, T., Herndon, D.N., and Suzuki, F. (2004). Three different neutrophil subsets exhibited in mice with different susceptibilities to infection by methicillin-resistant *Staphylococcus aureus*. Immunity *21*, 215–226.

Verdrengh, M., Thomas, J.A., and Hultgren, O.H. (2004). IL-1 receptor-associated kinase 1 mediates protection against *Staphylococcus aureus* infection. Microbes Infect. *6*, 1268–1272.

Zhang, Y., Ramos, B.F., and Jakschik, B.A. (1992). Neutrophil recruitment by tumor necrosis factor from mast cells in immune complex peritonitis. Science *258*, 1957–1959.