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MAJOR ARTICLE

Lack of Receptor for Advanced Glycation End Products Leads to Less Severe Staphylococcal Skin Infection but More Skin Abscesses and Prolonged Wound Healing

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Background. Lack of receptor for advanced glycation end products (RAGE) ameliorates several infections including *Staphylococcus aureus* pneumonia. We sought to investigate the role of RAGE in staphylococcal skin infection in mice.

Methods. Wild-type (WT) and RAGE deficient ($RAGE^{-/-}$) mice were subcutaneously inoculated with *S. aureus* SH1000 strain in abscess-forming dose or necrotic dose. Clinical signs of dermatitis, along with histopathological changes, were compared between the groups.

Results. The skin lesion size was smaller in $RAGE^{-/-}$ mice. Infected $RAGE^{-/-}$ mice expressed lower proinflammatory cytokines in local skins compared to control mice. Low dose of bacteria caused more abscess formation in $RAGE^{-/-}$ mice compared to skin necrosis that was more often observed in WT mice. As a result of more abscess formation, the wound healing was prolonged in $RAGE^{-/-}$ mice. Importantly, $RAGE^{-/-}$ mice had lower bacterial loads in the skin than controls, which is correlated with higher local levels of myeloperoxidase before skin infection. In vitro, enhanced phagocytic capacity of neutrophils and macrophages obtained from $RAGE^{-/-}$ mice compared to control mice was observed.

Conclusions. RAGE deficiency up-regulates phagocytic capacity of phagocytes, resulting in lower bacterial burden in local skin and milder skin lesions in mice with staphylococcal skin infection.

Keywords. receptor for advanced glycation end products; RAGE; *Staphylococcus aureus*; skin infection; mouse.

Staphylococcus aureus, a gram-positive bacterium colonizing humans, often in the anterior nares and the skin, is the leading pathogen responsible for a vast majority of skin and soft tissue infections [1, 2]. Staphylococci can give rise to a diverse spectrum of infectious outcome, ranging from milder pyodermas to severe life-threatening conditions [3]. *Staphylococcus aureus* is one of the leading cause of skin wound infections in chronic wounds [4] and as an infectious postoperative complication [5]. Staphylococcal skin infections are continuously increasing, mainly due to the emergence of antibiotic-resistant, multivirulent bacterial strains and the higher prevalence of immunocompromised individuals [2, 6].

Upon staphylococcal skin infection, the host defense system is activated through recognition of pathogen-associated molecular patterns from bacteria and leads to an acute inflammatory reaction followed by the rapid release of endogenous damage–associated molecular patterns (DAMPs) [7]. This initiates a local immune reaction with production of proinflammatory cytokines and an influx of phagocytes followed by the formation of local skin abscesses or skin necrosis. A receptor for advanced glycation end products (RAGE), a DAMP receptor binding a variety of inflammatory ligands generated during an infectious process, has been implicated to have an important role in inflammation and infection [8]. As RAGE is expressed on a wide variety of immune cells and also in skin tissue [9, 10], it might have an important role in the regulation of local skin infection.

RAGE has been studied in different infections but its definite impact is still controversial, likely depending on the disease model and type of bacteria [11]. Nevertheless, the role of RAGE in different staphylococcal infections is less studied. In the staphylococcal pneumonia model, RAGE-deficient ($RAGE^{-/-}$) mice displayed modestly attenuated lung pathology and lower bacteria in the bronchoalveolar fluid [12]. In the systemic infection model, RAGE deficiency had no impact on the induction of septic arthritis, nor did it influence the survival of mice [13]. Interestingly, when antibiotic-killed *S. aureus* was injected intra-articularly, $RAGE^{-/-}$ mice displayed significantly decreased frequency and severity of synovitis [14].

In terms of cutaneous staphylococcal infections, the only published study, using abscess model with dextran beads, suggests that RAGE does not impact local host defense during *S. aureus* skin infection [15]. This observation prompted us to

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investigate the impact of RAGE in staphylococcal skin infections using a model more closely related to naturally occurring skin disease.

MATERIALS AND METHODS

Mice

C57Bl/6 wild-type (WT) mice were purchased from Charles River Laboratories (Sulzfeld, Germany). RAGE$^{-/-}$ mice were provided by Professor Arnold, Deutsches Krebsforschungszentum, and Professor Nawroth, University Clinical Centre of Heidelberg, Germany [16, 17]. Age-matched 6- to 10-week-old mice of both sexes were used. The mice were kept in the animal facility of the Department of Rheumatology and Inflammation Research, University of Gothenburg. The Ethics Committee of Animal Research of Gothenburg approved the study, and animal experimentation guidelines were strictly followed.

Experimental Protocols for Staphylococcal Skin Infection

Staphylococcus aureus SH1000 strain was prepared and subcutaneously injected to mice as previously described [18–21]. Because injection of different bacterial doses results in distinct clinical manifestations (low dose leads to skin abscesses and high dose causes dermonecrosis) [20], we chose to use both high (2.0×10^7 colony-forming units [CFU]/site) and low (1.0×10^6 CFU/site) doses. The data were pooled from 4 independent experiments for the high-dose setting and 2 experiments for the low-dose setting.

Presence of dermonecrosis was defined as the appearance of brown skin surface, and/or a sunken area of the skin, and/or a red area of de-epithelialization. A healed skin infection was defined as a skin wound fully covered by freshly healed epidermis and lacking clinical signs of tissue necrosis and granulation, inflammatory exudates, or skin abscess.

Bacteriologic Examination of Skin Biopsies

On days 1, 3, and 7 postinfection, the mice were euthanized and their skin was collected for bacteriologic examination as previously described [20, 21]. After the bacteriologic examination, the skin homogenates were spun at 1500*g* for 15 minutes. The supernatants were collected and subsequently used for measurement of cytokines and myeloperoxidase (MPO).

Measurement of Cytokine and MPO Levels in Skin Homogenates

The levels of interleukin 6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor alpha (TNF-α) in supernatants from skin homogenates were quantified using DuoSet enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Abingdon, United Kingdom). The levels of high mobility group box protein 1 (HMGB1) were determined using a 2-step sandwich ELISA kit (IBL International GmbH, Hamburg, Germany). Myeloperoxidase levels were quantified using a mouse MPO ELISA kit (Immunology Consultants Laboratory, Portland, Oregon).

Histopathology

Skin biopsy samples collected on days 3, 7, and 14 in low-dose experiments were fixed and prepared for histopathological evaluation, as previously described [21]. All slides were coded and assessed in a blinded manner by a pathologist.

Phagocytosis Analysis

Mouse whole-blood samples were collected into heparin-containing tubes, and peritoneal leukocytes were collected using peritoneal lavage with 10 mL ice-cold phosphate-buffered saline (PBS). An imaging flow cytometry–based method (ImageStreamX MkII, Amnis) was employed to analyze phagocytic capacity of neutrophils and macrophages, as previously described [22–24]. Unspecific binding was blocked using Fc-block (BioLegend). Blood neutrophils were identified using allophycocyanin (APC)-conjugated rat antimouse Ly6G antibody (BD) and peritoneal macrophages were stained with APC-conjugated rat antimouse F4/80 antibody (BioLegend). To study whether RAGE deficiency impacts the bacterial opsonization, green fluorescent protein (GFP)-expressing *S. aureus* was incubated with 25% mouse sera from both RAGE$^{-/-}$ and WT mice at 37°C for 30 minutes and thereafter mixed with peritoneal leukocytes from Naval Medical Research Institute (NMRI), RAGE$^{-/-}$, and WT mice, respectively. The phagocytosis analysis was performed as mentioned above.

Measurement of Peritoneal Leukocyte Migration Upon Dead S. aureus Stimulation

Staphylococcus aureus strain SH1000 was heat-killed at 95°C for 30 minutes. RAGE$^{-/-}$ and WT mice were then intraperitoneally injected with heat-killed bacteria (1×10^9 dead bacteria) in 150 μL of PBS. Both WT and RAGE$^{-/-}$ mice received equal volume of PBS to determine the baseline level of leukocyte migration responding to PBS injection. The animals were sacrificed 4 hours later and peritoneal leukocytes collected using peritoneal lavage with 5 mL PBS; the cells were quantified by cell counter (Sysmex KX21N) and characterized by fluorescence-activated cell sorting (FACS). The mean cell concentration from the PBS-injected mice was counted as the baseline level of cell migration. The cell migration rate upon heat-killed *S. aureus* stimulation was calculated as the ratio of intraperitoneal leukocyte concentration between heat-killed *S. aureus*–injected mice and baseline levels.

Statistical Analysis

Statistical significance was assessed using the Mann–Whitney *U* test, Fisher exact test, and Mantel–Cox log-rank test as appropriate. Results are reported as the mean ± standard error of the mean unless indicated otherwise. A P value < .05 was considered statistically significant. Calculations were performed using

GraphPad Prism version 7.0b software for Mac (GraphPad, La Jolla, California).

RESULTS

RAGE Deficiency Led to Less Severe Skin Lesions, but More Abscess Formation in Local Infected Skin

To determine the effects of RAGE on the whole course of *S. aureus* skin infection, the skin lesion size, frequency of skin necrosis, and abscess formation postinfection were followed after subcutaneous injection of high-dose and low-dose *S. aureus* (Figure 1).

The skin lesion size was significantly smaller in RAGE$^{-/-}$ mice compared to WT controls in both high- and low-dose experiments (Figure 1A and 1B). No abscess formation was observed during the whole course of infection by using high bacterial inoculum as already from day 2 all skin lesions from both groups turned necrotic (data not shown).

Intriguingly, significantly higher frequency of abscess formation was found in RAGE$^{-/-}$ mice compared to WT mice by using low dose of bacteria. Strikingly, abscess formation was observed in the majority of RAGE$^{-/-}$ mice even at the late stage of skin infection, for example, on day 11 ($P < .05$; Figure 1C). In contrast, WT mice had significantly more skin necrosis than RAGE$^{-/-}$ mice throughout the experiment (Figure 1D). Our clinical observations were also confirmed by histological analysis of infected skin biopsies (Figure 1E–G).

RAGE Deficiency Prolonged Wound Healing in Mice Infected With Low Dose of *S. aureus*

The wound healing process was significantly shorter in WT mice compared with RAGE$^{-/-}$ mice when animals were inoculated with low bacterial dose. All WT animals developed skin necrosis and later open lesions. The first wound healing was found on day 7. All skin lesions from WT mice were fully healed on day 13. In contrast, all RAGE$^{-/-}$ mice developed skin abscesses instead. Some of the abscesses disappeared from day 11. Almost half of the skin abscesses were still visible at the end of experiment on day 15 and were deemed to be unhealed (Figure 2A).

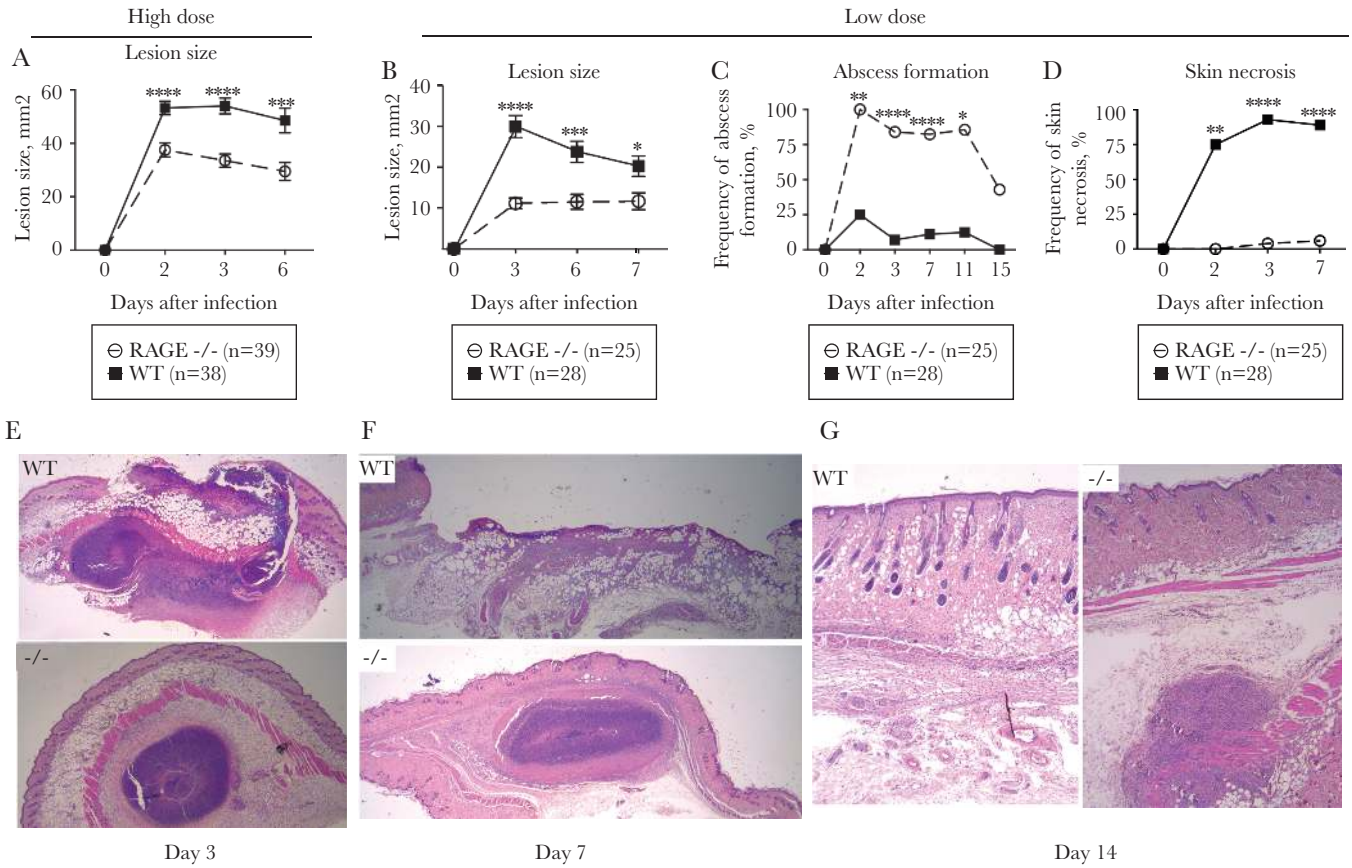

Figure 1. Receptor for advanced glycation end products (RAGE) deficiency led to less severe skin lesions but more abscess formation in *Staphylococcus aureus* skin infection. C57Bl/6 wild-type (WT) mice and RAGE-deficient (RAGE$^{-/-}$) mice were subcutaneously inoculated with *Staphylococcus aureus* SH1000 strains in both high dose (2.0×10^7 colony-forming units [CFU]/site) and low dose (1.0×10^6 CFU/site). The clinical signs of skin infection were followed until the skin wounds were fully healed. The skin lesion size of high dose inoculation (*A*), the skin lesion size of low-dose inoculation (*B*), frequency of abscess formation (*C*), and necrosis frequency (*D*) of skin infection with low-dose bacteria were observed. *E–G*, Pathological changes in the skin of mice infected with low dose of *S. aureus*. Representative images of skin lesions from RAGE$^{-/-}$ mice and C57Bl/6 WT mice are shown on day 3 (*E*), day 7 (*F*), and day 14 (*G*). Statistical evaluations were performed using the Mann–Whitney *U* test (*A* and *B*), with data expressed as mean ± standard error of the mean, and Fisher exact test (*C* and *D*). *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

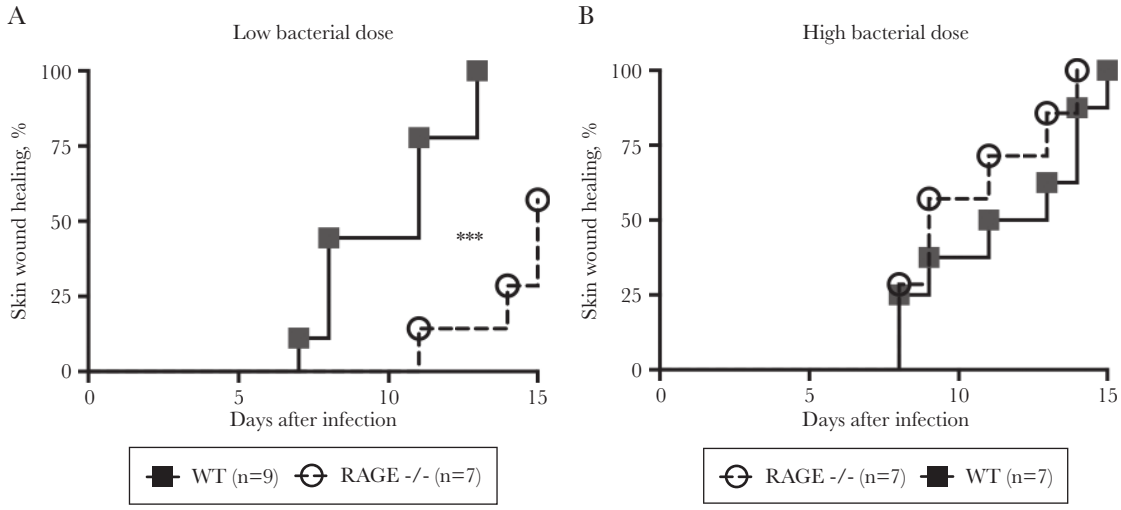

Figure 2. Receptor for advanced glycation end products (RAGE) deficiency prolonged wound healing in mice infected with low-dose *Staphylococcus aureus*. C57Bl/6 wild-type (WT) mice and RAGE-deficient (RAGE $^{-/-}$) mice were subcutaneously inoculated with *S. aureus* SH1000 strain in both low (1.0×10^6 colony-forming units [CFU]/site) and high (2.0×10^7 CFU/site) dose. The skin wound healing time for both low-dose (*A*) and high-dose (*B*) infection was followed for 15 days. Statistical evaluations were performed using the Mantel–Cox log-rank test. ***$P < .001$.

When the high bacterial dose was injected, all animals in both WT and RAGE$^{-/-}$ groups developed skin necrosis and open lesions. There were no significant differences between the groups with regard to wound healing time (Figure 2B).

RAGE Deficiency Led to Better Bacterial Clearance in Local Skin Tissue, Which Correlated to Higher MPO Levels Before Infection

To understand whether the milder skin damage was associated with diminished bacterial growth in skin tissue, the skin biopsies infected with low dose of bacteria were homogenized for CFU counts. There was significantly higher bacterial load in the skin from WT mice from day 1 ($P < .05$) and day 3 ($P < .01$), but the difference disappeared by day 7 when the WT mice started to develop open lesions (Figure 3A). Bacterial counts from the high-dose experiment were also examined on day 3. However, no tangible difference was observed between WT and RAGE$^{-/-}$ mice (Supplementary Figure 1). We speculate that there was an overwhelming effect of bacterial proliferation when there was more bacterial input.

To indirectly identify the level of in situ phagocyte numbers, MPO was measured in the local skin. The MPO levels were significantly higher in healthy RAGE$^{-/-}$ mice compared to WT mice ($P < .05$). Reversely, the levels in RAGE$^{-/-}$ mice became significantly lower on day 3 postinfection ($P < .01$; Figure 3B), suggesting higher degree of neutrophil infiltration in the RAGE$^{-/-}$ skin.

MCP-1 and HMGB1 Levels in RAGE-Deficient Skin Tissues Were Higher Before Infection but Lower After Infection Compared With WT Mouse Skin Tissue

The local levels of cytokines and chemokine were measured in mice infected with a low dose of *S. aureus*. Significantly higher MCP-1 and HMGB1 levels were found in healthy RAGE$^{-/-}$ mice compared with healthy WT mice ($P < .05$; Figure 4). In contrast, both MCP-1 and HMGB1 levels became lower in RAGE$^{-/-}$ mice on day 3 postinfection compared with WT mice ($P < .01$). A similar trend was found with regard to IL-6 and TNF-α (Figure 4). These data suggest that there is a proinflammatory status in RAGE$^{-/-}$ skin compared with skin from WT mice, which might strengthen the first-line host defense against invading microorganisms.

Furthermore, the correlation analysis revealed a positive correlation between the levels of IL-6 and MCP-1 with bacterial loads in the skin on day 1 postinfection in WT mice (Supplementary Table 1).

Migration Rate of Neutrophils Was Enhanced in RAGE$^{-/-}$ Mice

To further understand whether RAGE deficiency affects leukocyte migration to local infection site, heat-killed *S. aureus* was intraperitoneally injected to both WT and RAGE$^{-/-}$ mice. The ratio of intraperitoneal leukocytes between heat-killed bacteria stimulated groups and PBS-injected control groups is shown in Figure 5. The migration rate of total leukocytes from RAGE$^{-/-}$ group tended to increase, but there was no significant difference between the groups (Figure 5A). Interestingly, the migration rate of neutrophils in RAGE$^{-/-}$ mice was significantly higher than WT controls ($P < .01$; Figure 5B). No tangible difference was found regarding the migration rate of macrophages between RAGE$^{-/-}$ and WT mice (Figure 5C). However, rapid influx of neutrophils to the peritoneal cavity was observed already after 4 hours of dead *S. aureus* stimulation in RAGE$^{-/-}$ mice (Figure 5D).

Neutrophils and Macrophages From RAGE$^{-/-}$ Mice Possess Stronger Phagocytic Capacity

To compare the phagocytic capacity, internalization rates of GFP-expressing *S. aureus* by phagocytes were analyzed

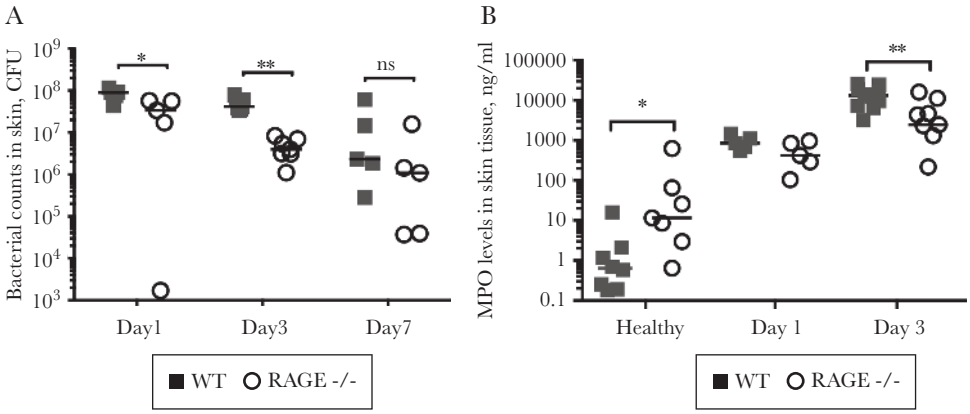

Figure 3. Receptor for advanced glycation end products (RAGE) deficiency led to better bacterial clearance in local skin tissue, which correlated to higher myeloperoxidase levels before infection. C57Bl/6 wild-type (WT) mice and RAGE-deficient ($RAGE^{-/-}$) mice were subcutaneously inoculated with low-dose *Staphylococcus aureus* SH1000 strain (1.0×10^6 colony-forming units [CFU]/site). *A*, Skin biopsies were collected and homogenized for bacterial counts on days 1, 3, and 7 postinfection. *B*, Skin biopsies from both healthy and infected animals were collected and homogenized and the level of myeloperoxidase (MPO) from the supernatants of skin homogenates were detected by enzyme-linked immunosorbent assay. Statistical evaluations were performed using the Mann–Whitney *U* test. Data are presented as scatterplot with line indicating median value. $^*P < .05$; $^{**}P < .01$; ns, not significant.

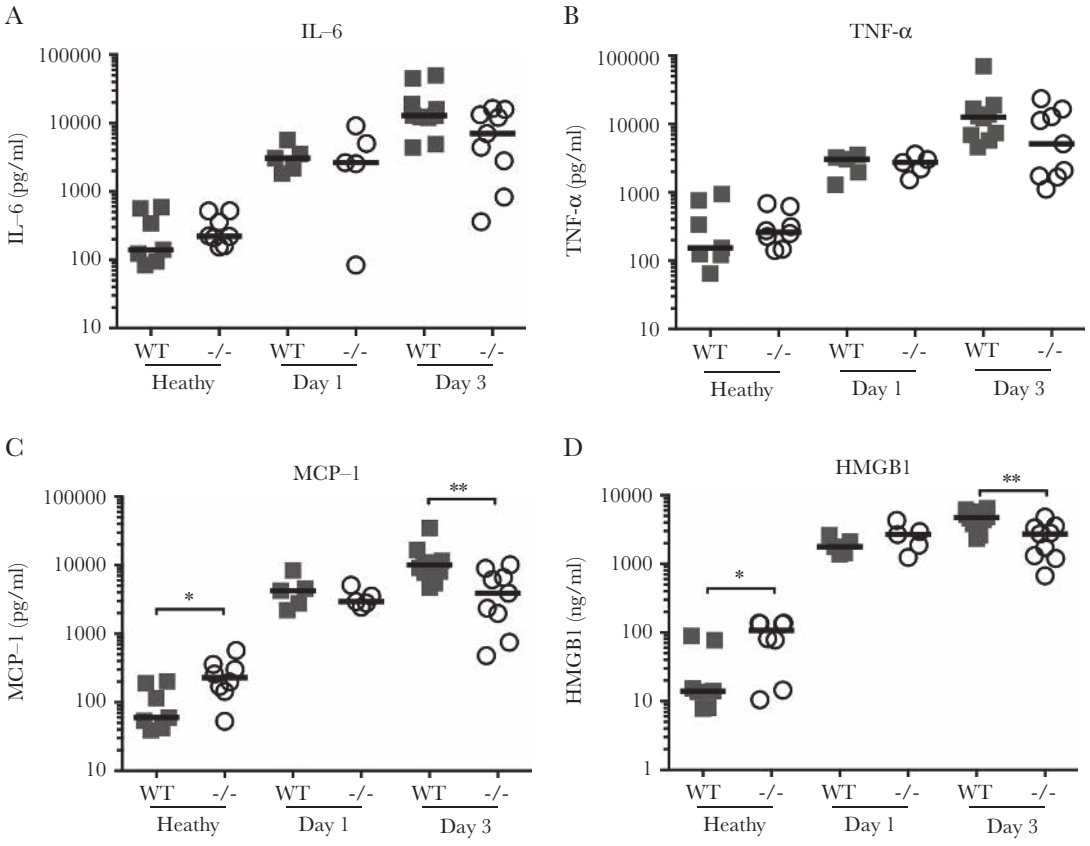

Figure 4. Monocyte chemoattractant protein 1 (MCP-1) and high mobility group box protein 1 (HMGB1) levels in local skin tissues from receptor for advanced glycation end products (RAGE)–deficient ($^{-/-}$) mice were higher before infection but lower postinfection compared with wild-type (WT) controls. C57Bl/6 WT mice and $RAGE^{-/-}$ mice were subcutaneously inoculated with low-dose *Staphylococcus aureus* SH1000 strain (1.0×10^6 colony-forming units/site). The skin biopsies from both healthy and infected animals were collected and homogenized. The level of interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), MCP-1, and HMGB1 from the supernatants of skin biopsy homogenates were detected by enzyme-linked immunosorbent assay. Statistical evaluations were performed using the Mann–Whitney *U* test. Data are presented as scatterplot with line indicating median value. $^*P < .05$; $^{**}P < .01$.

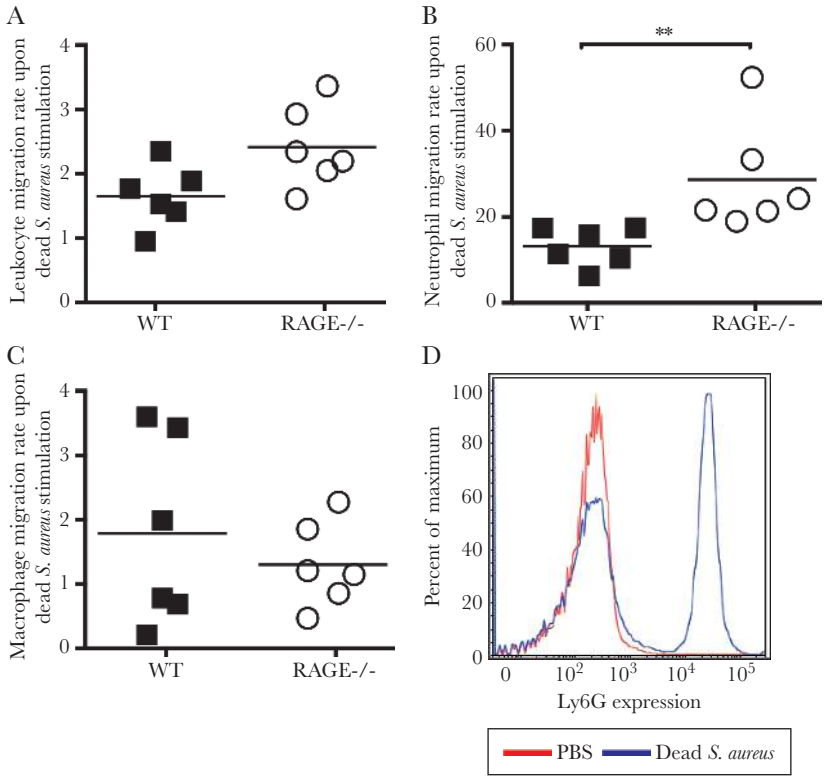

Figure 5. Migration rate of neutrophils upon dead *Staphylococcus aureus* stimulation was enhanced in receptor for advanced glycation end products (RAGE)–deficient mice. C57Bl/6 wild-type (WT) mice and RAGE-deficient (RAGE$^{-/-}$) mice were intraperitoneally injected with heat-killed *Staphylococcus aureus* SH1000 strain (1×10^9 dead bacteria) in 150 µL of phosphate-buffered saline (PBS) and intraperitoneal leukocytes were collected after 4 h. Same volume of PBS was injected to both WT (n = 6) and RAGE$^{-/-}$ mice (n = 6) to determine the baseline level of leukocyte migration responding to PBS injection. The migration rate of total leukocytes (*A*), neutrophils (*B*), and macrophages (*C*) were calculated as the ratio of intraperitoneal leukocyte concentration between heat-killed *S. aureus*–injected mice and baseline levels. *D*, Representative fluorescence-activated cell sorting (FACS) histogram demonstrating the quick migration of neutrophils to peritoneal cavity upon dead *S. aureus* stimulation for 4 hours in RAGE$^{-/-}$ mice. Statistical evaluations were performed using the Mann–Whitney *U* test. Data are presented as scatterplot with line indicating median value. $^{**}P < .01$.

(Figure 6). After incubation for 30 minutes with *S. aureus*, 71%–92% of blood neutrophils from RAGE$^{-/-}$ mice were able to internalize the *S. aureus* compared to 53%–83% of blood neutrophils from WT mice ($P < .01$; Figure 6A). Figure 6B shows the representative images demonstrating how GFP-expressing *S. aureus* is related to neutrophils, for example, whether it is surface-bound, internalized, or not associated.

Intraperitoneal macrophages had weaker capacity to engulf the *S. aureus* compared with blood neutrophils when bacteria were not opsonized: 3%–24% of macrophages devoured GFP-expressing bacteria in RAGE$^{-/-}$ mice and 6%–24% in WT mice (not significant; Figure 6C).

To determine whether the components from different sera affect the phagocytic capacity, peritoneal macrophages from NMRI mice and sera from WT and RAGE$^{-/-}$ mice were used to opsonize the GFP-expressing *S. aureus*. The internalization rates increased from 7% when *S. aureus* was not opsonized to 36% when bacteria were opsonized (Figure 6D), but no significant difference was found between WT and RAGE$^{-/-}$ animals. Interestingly, when both macrophages and sera from RAGE$^{-/-}$ and WT mice were collected and used for opsonization experiment, macrophages from RAGE$^{-/-}$ mice had significantly higher phagocytic capacity compared to macrophages from WT mice (Figure 6E), suggesting that RAGE down-regulates phagocytic capacity of both neutrophils and macrophages.

To further study the physiologic functions of neutrophils, nicotinamide adenine dinucleotide phosphate hydrogen (NADPH)-oxidase was investigated by measuring superoxide release from neutrophils isolated from WT and RAGE$^{-/-}$ mice. Our results indicate that RAGE does not play an important role in neutrophil NADPH-oxidase assembly and activation (Supplementary Figure 2). Furthermore, the bacterial killing capacity of mouse blood was studied by incubating *S. aureus* with whole blood from RAGE$^{-/-}$ mice and WT mice. No significant difference was observed between the groups (Supplementary Figure 3).

DISCUSSION

In the current study, we show that RAGE plays a substantial role in *S. aureus*–induced skin infection. RAGE deficiency leads to stronger immune response, better bacteria elimination, and less severe skin lesions in locally infected skin. Due to better elimination of bacteria, significantly less skin necrosis but more abscesses were formed in RAGE$^{-/-}$ mice compared with WT mice. At the mechanistic level, our data show that (1) higher

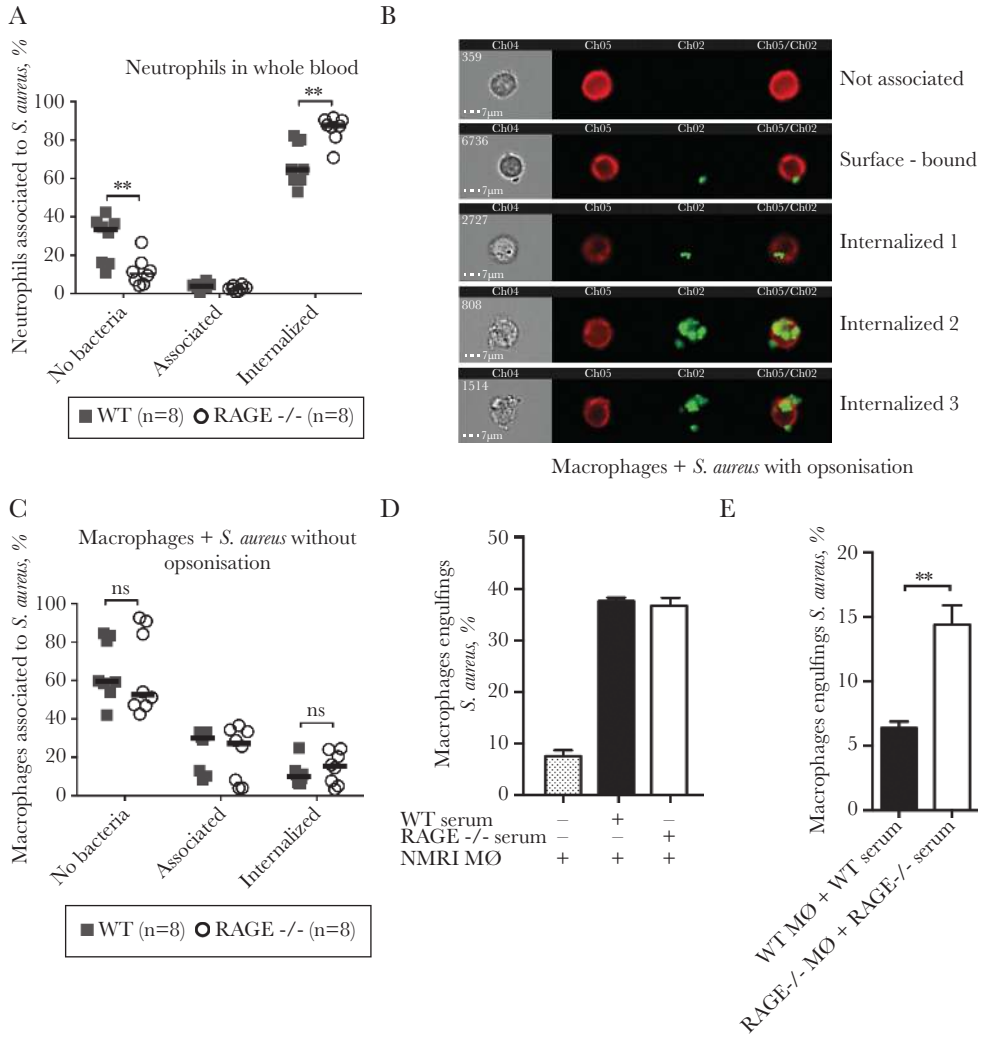

Figure 6. Neutrophils and macrophages from receptor for advanced glycation end product (RAGE)–deficient (–/–) mice possess stronger phagocytic capacity. Peritoneal leukocytes obtained by peritoneal lavage or heparinized blood from both wild-type (WT) and RAGE$^{-/-}$ mice were incubated with green fluorescent protein (GFP)-expressing *Staphylococcus aureus* (multiplicity of infection [MOI] = 5). The internalization wizard in Image Data Exploration and Analysis Software (IDEAS) was used to determine the interaction of the GFP-positive bacteria with phagocytes (not associated, surface bound, or internalized). *A*, Percentages of blood neutrophils interacting with GFP-positive *S. aureus*. *B*, Representative image of neutrophils in association with GFP-expressing *S. aureus* (MOI = 5) analyzed by imaging flow cytometry. *C*, Percentages of peritoneal macrophages (MØ) interacting with GFP-positive *S. aureus* without opsonization. *D*, Percentages of peritoneal macrophages from Naval Medical Research Institute (NMRI) mice internalizing GFP-positive *S. aureus* that was opsonized with either RAGE$^{-/-}$ mouse sera or WT mouse sera. *E*, Percentages of peritoneal macrophages from RAGE$^{-/-}$ mice or WT mice internalizing GFP-positive *S. aureus* that was opsonized with either RAGE$^{-/-}$ mouse sera or WT mouse sera. Statistical evaluations were performed using the Mann–Whitney *U* test. Data are presented as scatterplot with line indicating median value (*A* and *C*) or expressed as mean ± standard error of the mean (*D*). $^{**}P < .01$; ns, not significant.

proinflammatory cytokines and chemokine as well as MPO were found in the local skin tissues from RAGE$^{-/-}$ mice, suggesting increased proinflammatory status in their skin compared to WT mice; (2) migration rate of neutrophils upon intraperitoneal stimulation with dead *S. aureus* was enhanced in RAGE$^{-/-}$ mice compared to WT; and (3) importantly, both neutrophils and macrophages isolated from RAGE$^{-/-}$ mice had better phagocytic capacity than those of control mice.

To our knowledge, there is only one published study investigating the role of RAGE in staphylococcal skin infection. In that study, wild-type and RAGE$^{-/-}$ mice were infected subcutaneously with *S. aureus* mixed with dextran beads. The authors found that abscess size and bacterial burdens were similar in both mouse strains at the primary site of infection, but RAGE$^{-/-}$ mice had lower bacterial counts in their lungs and liver [15]. In contrast, we demonstrate that RAGE$^{-/-}$ mice had lower bacterial counts in infected skin tissue. Certain methodological discrepancies between our study and this study should be mentioned. It is known that appropriate host coagulation function is required for abscess formation [25, 26]. Since dextran has antithrombotic effect, the setting with mixture of bacteria and dextran beads is a good model to study dissemination of a local infection rather than a model to study naturally occurring skin infection. We speculate that addition of beads to *S. aureus* may

cause the overwhelming local inflammation, which masked the differences that we observed in the current study. Also, different staphylococcal strains and bacterial doses could account for discrepancies between this and our current study.

Several studies show similar results whereby RAGE deficiency or inhibition plays a protective role in other models, such as cecal ligation and puncture model [16, 27] and local lung infection models [28–30]. It seems that the role of RAGE in infections might vary in different animal models. We have previously shown that RAGE plays a very limited role in *S. aureus*–induced septic arthritis [13]. In this study, our data compellingly demonstrate that RAGE deficiency leads to improved bacterial clearance, decreased local cytokine levels, and milder skin lesions, suggesting that RAGE is pathogenic in staphylococcal skin infection. We speculate that the importance of RAGE in a specific infection might be dependent on the expression levels of RAGE in the infected organ system. Indeed, it has been shown that RAGE is highly expressed in skin tissue and up-regulated by advanced glycation end products and TNF-α [10].

A paradoxical effect was observed during the course of milder skin infections—the skin necrosis was less common, whereas the wound healing time was significantly prolonged in RAGE$^{-/-}$ mice compared to WT controls. It is well known that the drainage of skin lesions during *S. aureus* skin infection is beneficial for host, leading to shorter healing time. Bacterial elimination was more efficient in RAGE$^{-/-}$ mice at the early phase, resulting in less robust local immune response, which is evidenced by the lower proinflammatory cytokine levels in local skin tissue. Therefore, significantly less skin necrosis and spontaneous drainage of skin abscess were observed in RAGE$^{-/-}$ mice, resulting in prolonged healing time. In the high-dose experiment, all infection sites became necrotic and spontaneously drained due to the overwhelming effect of massive bacterial infection. Therefore, no difference regarding the wound healing was found. There might be another explanation to higher incidence of abscess formation in RAGE$^{-/-}$ mice: RAGE binds to phosphatidylserine on cell surface and this facilitates to clear the apoptotic cells. It has been shown that RAGE$^{-/-}$ mice have defective clearance of apoptotic neutrophils [31], which might lead to more abscess formation in skin infections. Moreover, HMGB1 can interact with phosphatidylserine and diminish the phagocytosis of apoptotic neutrophils [32]. Elevated HMGB1 levels in skin tissue from healthy RAGE$^{-/-}$ mice might also result in diminished clearance of apoptotic cells and contribute to more abscess formation.

It is evident that RAGE deficiency leads to better bacterial elimination and milder skin infections. Importantly, neutrophils and macrophages from RAGE$^{-/-}$ mice expressed significantly higher phagocytic capacity compared to WT mice. Is this the only explanation to our finding? We found that MPO, MCP-1, and HMGB1 levels in the local healthy skin were significantly higher in RAGE$^{-/-}$ mice than controls, suggesting an enhanced proinflammatory status in the local tissue in RAGE$^{-/-}$ mice, which gave stronger first-line immune defense against invading bacteria. Indeed, higher proinflammatory cytokine levels were also found in blood from RAGE$^{-/-}$ mice compared to WT controls in our previous study [13]. It has been shown that RAGE mediates neutrophil adhesion to, and subsequent migration across, intestinal epithelial monolayers. This activity appears to be mediated by the binding of RAGE to the PMN-specific β2 integrin CD11b/CD18 [33]. Intriguingly, in the current study, we demonstrated that the neutrophil migration rate upon dead *S. aureus* stimulation was significantly increased in RAGE$^{-/-}$ mice, strongly suggesting that neutrophils are more active and respond better to chemoattractants in RAGE deficiency, resulting in the better bacterial control and milder infection when exposed to an inflammatory milieu. Our finding is in line with previous report that the administration of sRAGE worsened bacterial burden and neutrophils infiltration [29].

In conclusion, we have demonstrated that RAGE down-regulates cytokine and MPO levels in healthy skin, and impairs phagocytic capacity and recruitment of neutrophils and macrophages, thus resulting in higher bacterial burden in local skin and more severe skin lesions in mice with staphylococcal skin infection.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Disclaimer. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that

the editors consider relevant to the content of the manuscript have been disclosed.

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