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Original article

The receptor for advanced glycation end products promotes bacterial growth at distant body sites in *Staphylococcus aureus* skin infection

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Abstract

The receptor for advanced glycation endproducts (RAGE) has been implicated in the regulation of skin inflammation. We here sought to study the role of RAGE in host defense during skin infection caused by *Staphylococcus (S.) aureus,* the most common pathogen in this condition. Wild-type (Wt) and RAGE deficient ($rage^{-/-}$) mice were infected subcutaneously with *S. aureus* and bacterial loads and local inflammation were quantified at regular intervals up to 8 days after infection. While bacterial burdens were similar in both mouse strains at the primary site of infection, $rage^{-/-}$ mice had lower bacterial counts in lungs and liver. Skin cytokine and chemokine levels did not differ between groups. In accordance with the skin model, direct intravenous infection with *S. aureus* was associated with lower bacterial loads in lungs and liver of $rage^{-/-}$ mice. Together these data suggest that RAGE does not impact local host defense during *S. aureus* skin infection, but facilitates bacterial growth at distant body sites.

Keywords: *Staphylococcus aureus*; Skin infection; Innate immunity; Receptor for advanced glycation endproducts

1. Introduction

Staphylococcus (S.) aureus is both a common commensal bacterium and an important pathogen responsible for a wide array of human infectious diseases [17]. The vast majority of staphylococcal infections involve skin and soft tissue [19]. In recent years, the incidence of staphylococcal skin infection has remarkably increased due to the emergence of more virulent and antibiotic resistant strains [5,9]. This urges the need to gain more understanding of protective immune responses during *S. aureus* infections, which could help in the development of new therapeutic strategies.

The cutaneous immune response against invading staphylococci encompasses a variety of mechanisms, which are essential for bacterial clearance, including the production of proinflammatory cytokines, recruitment of innate and adaptive immune cells and formation of neutrophil abscesses [18,20,13]. A protein that may aid in the control of bacterial skin infection is the Receptor for Advanced Glycation End products (RAGE). RAGE is expressed on various cell types [12,11,14,16] and binds several damage associated molecular patterns (DAMPs) such as high mobility group box (HMGB)-1 and S100 proteins, which are released during invasive diseases [12,3]. Engagement of RAGE activates the NF-κB pathway, which in turn upregulates expression of RAGE, perpetuating the inflammatory response [3,12]. Previous studies have shown that RAGE contributes to the establishment and maintenance of sterile cutaneous inflammation [11,14]. In addition, RAGE contributes to infiltration of neutrophils, as it upregulates adhesive molecules [3] and acts as an adhesive molecule itself by binding

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to β_2-integrins on neutrophils [6,10,12]. Previous investigations have pointed to a complex role for RAGE in the outcome of infectious diseases, depending on the pathogen or site of infection [1,24,26]. In the current study we sought to investigate the role of RAGE during *S. aureus* skin infection.

2. Methods

2.1. Mice

C57Bl/6 wild-type (Wt) mice were purchased from Charles River Laboratories Inc. (Maastricht, the Netherlands). RAGE deficient (*Rage*$^{-/-}$) mice, backcrossed > 10 times to a C57Bl/6 background were generated as described [8] and bred in the animal facility of the Academic Medical Center (Amsterdam, the Netherlands). Experiments were carried out in accordance with the Dutch Experiment on Animals Act and approved by the Animal Care and Use Committee of the University of Amsterdam (Permit number: DIX101223 and DIX102335).

2.2. Design

Abscess formation in mice was induced as previously described [4]. In short mice were lightly anesthesized by inhalation of isoflurane (Abbot Laboratories, Queensborough, Kent, UK), shaved at the right flank and subcutaneously injected with a suspension of 1×10^5 colony forming units (CFU) of *S. aureus* (Newman strain) in phosphate buffered saline (PBS) that was mixed with an equal volume of autoclaved dextran beads in PBS (Cytodex-1 microcarrier beads; Sigma, St. Louis, Missouri) which was prepared according to the manufacturer's instructions, in a total volume of 100 µl ($n = 7-8$ per strain). Abscesses were serially measured with a digital caliper for 8 days. In addition, mice were sacrificed at 6 h or 1, 2, 4 or 8 days post infection. After euthanization blood was drawn into heparinized tubes and livers and lungs were removed aseptically and homogenised in 4 volumes of sterile isotonic saline using a tissue homogenizer (Biospec Products, Bartlesville, UK). Abscesses were excised using 8 mm punch biopsies (Stiefel, Wächtersbarg, Germany) and homogenised in 4 ml sterile isotonic saline. In separate experiments bacteremia was induced by intravenously injecting 1×10^6 of *S. aureus* (Newman strain) in a 200 µl saline solution in the tail vein ($n = 7-8$ per strain). Mice were sacrificed 6, 24 or 48 h thereafter. Blood was obtained and organs were collected and homogenised as described above. To determine bacterial loads, ten-fold dilutions were plated on blood agar plates and incubated at 37 °C for 16 h.

2.3. Assays

Homogenates were processed for cytokine measurements as described [27]. Tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, keratinocyte-derived chemokine (KC), macrophage inflammatory protein (MIP)-2 (all R&D systems, Minneapolis, MN) and myeloperoxidase (MPO, Hycult Biotechnology BV, Uden, the Netherlands) concentrations were measured in skin homogenates using ELISAs according to manufacturer's recommendations. Plasma TNF-α and IL-6 were measured by cytometric bead array flex set assay (BD Biosciences, San Jose, CA) in accordance to the manufacturer's instructions.

2.4. Statistical analysis

Data are expressed as medians and interquartile ranges. Differences between *rage*$^{-/-}$ and Wt mice were analyzed by Mann–Whitney U test. Analyses were done using GraphPad Prism version 5.0 (Graphpad Software, San Diego, CA). Values of $p < 0.05$ were considered statistically significant different.

3. Results

3.1. RAGE does not influence local growth of S. aureus in the skin or abscess size, but facilitates bacterial growth at distant body sites

To study the role of RAGE in S. *aureus* skin infection we used an abscess model in which staphylococci (10^5 CFU) were injected subcutaneously together with dextran beads [4] in Wt and *rage*$^{-/-}$ mice [8]. We determined bacterial loads in standardized punch biopsies taken from the infection site at 6 h or 1, 2, 4 or 8 days after infection. Bacterial loads were similar in both mouse strains at all time points (Fig. 1A). In accordance, while the infection caused abscesses in all mice, the abscess size was similar in Wt and *rage*$^{-/-}$ mice at all time points (Fig. 1B). This model of skin infection resulted in bacterial dissemination to distant sites. Remarkably, *rage*$^{-/-}$ mice displayed reduced bacterial burdens in liver (at 4 days; $p < 0.05$) and lung (8 days; $p < 0.05$) (Fig. 1C–D). Bacteria were hardly found in blood (data not shown). These data suggest that RAGE does not impact on local host defense in the skin but enhances growth of *S. aureus* at distant sites in localized skin infection.

3.2. Impact of RAGE on neutrophil influx and cytokine responses in cutaneous skin infection

Previous studies have shown that RAGE promotes neutrophil emigration [6,10,12,3], a hallmark for abscess formation in staphylococcal skin disease [18]. To determine local influx of neutrophils, we measured MPO concentrations, which correlate with the degree of infiltrated neutrophils [7], in skin biopsies taken at the infection site. Wt and *rage*$^{-/-}$ mice displayed similar MPO levels at all time points, except at 2 days after infection when *rage*$^{-/-}$ mice had lower values (Table 1, $p < 0.01$). RAGE activation leads to a sustained NF-κB activation thereby perpetuating inflammatory responses [3,12]. To determine the role of RAGE in cytokine and chemokine responses we measured the concentrations of these inflammatory mediators in skin biopsies obtained at different time points. We found no differences in cytokine or chemokine concentrations between Wt and *rage*$^{-/-}$ mice (Table 1).