IL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice

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*Staphylococcus aureus* is the most common cause of skin and soft tissue infections, and rapidly emerging antibiotic-resistant strains are creating a serious public health concern. If immune-based therapies are to be an alternative to antibiotics, greater understanding is needed of the protective immune response against *S. aureus* infection in the skin. Although neutrophil recruitment is required for immunity against *S. aureus*, a role for T cells has been suggested. Here, we used a mouse model of *S. aureus* cutaneous infection to investigate the contribution of T cells to host defense. We found that mice deficient in γδ but not αβ T cells had substantially larger skin lesions with higher bacterial counts and impaired neutrophil recruitment compared with WT mice. This neutrophil recruitment was dependent upon epidermal Vγ5+ γδ T cell production of IL-17, but not IL-21 and IL-22. Furthermore, IL-17 induction required IL-1, TLR2, and IL-23 and was critical for host defense, since IL-17R–deficient mice had a phenotype similar to that of γδ T cell–deficient mice. Importantly, γδ T cell–deficient mice inoculated with *S. aureus* and treated with a single dose of recombinant IL-17 had lesion sizes and bacterial counts resembling those of WT mice, demonstrating that IL-17 could restore the impaired immunity in these mice. Our study defines what we believe to be a novel role for IL-17–producing epidermal […]

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Introduction

Staphylococcus aureus is a Gram-positive extracellular bacterium that is the most common cause of skin and soft tissue infections, such as cellulitis, impetigo, and folliculitis (1, 2). Although these infections usually originate in the skin, invasive and life-threatening infections such as bacteremia, pneumonia, meningitis, endocarditis, and sepsis may ensue (1, 2). In recent years, S. aureus skin infections have become an enormous public health problem, resulting in approximately 11.6 million outpatient and emergency room visits and 464,000 hospital admissions per year in the US (1). Moreover, these infections have been increasingly caused by virulent antibiotic-resistant strains, such as methicillin-resistant S. aureus (MRSA; refs. 2, 3). In 2005, MRSA caused 94,360 severe invasive infections and resulted in 18,650 deaths in the US, exceeding the annual number of deaths in the US caused by HIV/AIDS, tuberculosis, and viral hepatitis combined (4, 5). Given this rapidly emerging epidemic and the decreased efficacy of currently available antibiotic therapy, immune-based antimicrobial therapy may provide a novel alternative approach to combat these infections. For this to be possible, a clear understanding of the protective immune response against S. aureus infection in the skin is vital.

A key correlate to an effective immune response against S. aureus involves neutrophil recruitment and abscess formation, which are required for bacterial clearance (6, 7). The importance of neutrophils in host defense against S. aureus infection has been demonstrated by recurrent S. aureus infections in patients with chronic granulomatous disease, who have a defect in neutrophil NADPH oxidase and respiratory burst (8). In addition, a large study found that neutropenic patients with cancer were particularly susceptible to S. aureus infections, which resulted in increased morbidity and mortality (9). The essential role of neutrophils in S. aureus infection has also been observed in mouse models of S. aureus infection: neutrophil-depleted mice developed nonhealing skin lesions and were unable to clear the bacteria (6, 7).

In addition to neutrophil recruitment, a number of different observations in humans have provided evidence that T cells play a critical role in host defense against S. aureus skin infections. First, patients with HIV disease, especially those with low peripheral blood CD4+ T cell counts, are highly susceptible to S. aureus colonization and skin infection (10, 11). Second, patients with the inflammatory skin disease atopic dermatitis have increased colonization and superinfection with S. aureus (12), which has been attributed to the Th2 cytokine profile (i.e., IL-4, IL-13, and IL-10) and decreased antimicrobial peptides observed in this disease (13, 14). Finally, human subjects with hyper-IgE syndrome (also known as Job’s syndrome), who suffer from recurrent and severe S. aureus skin infections (15), have STAT3 mutations that render them deficient in Th17 cells (16–18).

In mouse models, there have also been reports that T cells contribute to host defense against S. aureus cutaneous infection. A previous study found that αβ T cell–deficient mice had defective neutrophil abscess formation compared with WT mice after subcutaneous S. aureus challenge (19). However, contrary to the well-established importance of neutrophils in host defense against S. aureus infections, the defective neutrophil abscess formation in the αβ T cell–deficient mice was associated with decreased bac-

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γδ but not αβ T cell–deficient mice develop markedly larger skin lesions compared with WT mice in response to cutaneous challenge with *S. aureus*. γδ T cell–deficient, αβ T cell–deficient, and WT mice were inoculated intradermally with *S. aureus* SH1000 strain. (A) Mean total lesion size (cm²) ± SEM. (B) Representative lesions for each mouse strain. Shown are entire dorsal backs (top, mm ruler shown for scale) and close-ups of lesions (bottom). (C) Mean total flux (photons/s) ± SEM. (D) Representative in vivo bioluminescence. Data are from 2 experiments with at least 6 mice/group per experiment. *P < 0.05, †P < 0.01, ‡P < 0.001, γδ T cell–deficient versus WT (Student’s *t* test).
terial burden within the infected lesions through an unknown mechanism (19). Another study demonstrated that γδ T cell–deficient mice had substantially higher bacterial counts within the infected skin lesions than did WT mice after intradermal S. aureus challenge, but the mechanism was not well defined (20).

Taken together, the results of these studies in humans and mice suggest a role for T cells in host defense against S. aureus; nevertheless, the mechanism by which T cells promote protective immunity against S. aureus skin infection is not clear. Thus, we chose to evaluate the contribution and mechanism by which αβ and γδ T cells participate in cutaneous host defense using an in vivo mouse model of S. aureus cutaneous infection.

Results

γδ T cell–deficient mice, but not αβ T cell–deficient mice, developed markedly larger skin lesions with higher bacterial counts compared with WT mice in response to cutaneous challenge with S. aureus. As a first step toward identifying mechanisms by which T cells contribute to host defense against S. aureus infection in skin, we investigated the contribution of αβ and γδ T cells using an in vivo mouse model. This model uses a bioluminescent SH1000 strain of S. aureus and techniques of in vivo bioluminescence imaging to track the bacterial burden within the infected lesions of anesthetized mice in real time (21, 22). The S. aureus SH1000 strain was chosen for this study because it has a complete virulence repertoire due to a restored rsbU gene in the sigB stress response pathway of S. aureus (23), and it has been previously used to study S. aureus cutaneous infection in mouse models in vivo and in human culture systems (21, 24). WT mice and mice deficient in either γδ or αβ T cells were inoculated intradermally with the bioluminescent S. aureus SH1000 strain (unless otherwise indicated, inoculations consisted of 2 × 10⁶ CFUs in 100 μl saline). Lesion sizes and in vivo bioluminescence of live actively metabolizing bacteria within the lesions over time were evaluated (Figure 1). WT mice developed visible skin lesions, which had a maximum size of 0.41 ± 0.05 cm² by day 3, and healed by day 14 (Figure 1, A and B). αβ T cell–deficient mice developed skin lesions that did not significantly differ in size from those of WT mice. In contrast, γδ T cell–deficient mice developed lesions approximately 3-fold larger than those of WT or αβ T cell–deficient mice that failed to completely heal by day 14 after inoculation.

To determine whether the larger lesions of γδ T cell–deficient mice were associated with impaired bacterial clearance, we anesthetized mice and determined bacterial counts within the lesions in real time (Figure 1, C and D) using in vivo bioluminescence imaging techniques (see Methods). We previously demonstrated that in vivo bioluminescence signals closely estimate bacterial CFUs harvested from the skin lesions at various time points after infection (21). After S. aureus infection, WT and αβ T cell–deficient mice had similar bioluminescent signals that decreased over 14 days. In contrast, γδ T cell–deficient mice had increased bioluminescent signals (Figure 1D) that were up to 20-fold higher than those of WT mice (Figure 1C) at all time points after inoculation. Similar to in vivo bioluminescence, numbers of S. aureus CFUs recovered from 8-mm punch biopsies were up to 10-fold higher in γδ T cell–deficient mice than in WT mice on days 1 and 3 after infection (Figure 2). Thus, γδ T cell–deficient mice have a marked defect in bacterial clearance, which likely explains the increased size and persistence of the skin lesions. These data suggest that γδ T cells, but not αβ T cells, are required for mediating host defense and bacterial clearance against cutaneous S. aureus infection.
γδ T cell–deficient mice have markedly impaired neutrophil recruitment and production of neutrophil chemokines and granulopoiesis factors in response to cutaneous challenge with *S. aureus*. γδ T cell–deficient and WT mice were inoculated intradermally with *S. aureus*. (A) Representative photomicrographs of sections labeled with H&E stain and anti–Gr-1 mAb (immunoperoxidase method) and Gram stain of lesional skin at 1 day after inoculation. Original magnification, ×40 (H&E, top, and Gram); ×200 (H&E, bottom, and Gr-1). (B) Mean MPO activity (U/mg tissue weight) ± SEM of lesional skin. (C) Mean level of mRNA (AU) ± SEM of KC, MIP2, GM-CSF, and IFN-γ from lesional skin homogenates of skin biopsies performed by Q-PCR 8 hours after *S. aureus* inoculation. (D) Mean protein levels (pg/mg tissue weight) ± SEM of KC, MIP2, GM-CSF, and IFN-γ from lesional skin homogenates of skin biopsies performed at 8 hours after *S. aureus* inoculation. Data are from 2 experiments with at least 5 mice/group per experiment. *P* < 0.05, γδ T cell–deficient versus WT (Student’s *t* test).
against S. aureus skin infection, we investigated the production of T cell–derived cytokines known to promote neutrophil recruitment. These include IL-17 (i.e., IL-17A and IL-17F) and IL-22, which trigger epithelial cells and fibroblasts to produce neutrophil recruitment factors, including the neutrophil chemokines KC and MIP2 and the granulopoiesis factor GM-CSF (25–30). For comparison, we also evaluated levels of IL-21, which, along with IL-17 and IL-22, is produced by Th17 cells (31, 32). We found that WT mice exhibited an early burst of both IL-17A and IL-17F gene expression, which peaked at 8 hours and returned to baseline by 24 hours after infection in vivo (Figure 4A). In contrast, γδ T cell–deficient mice lacked this early induction of IL-17A and IL-17F. IL-22 was induced in both γδ T cell–deficient and WT mice, which suggests that IL-22 is produced by cells other than γδ T cells. WT mice did not induce IL-21 at all time points evaluated. However, γδ T cell–deficient mice had detectable levels of IL-21 at 4 hours after infection. Because γδ T cell–deficient mice had a defect in IL-17 induction, but not IL-21 or IL-22 induction, we investigated the cellular source of IL-17 in lesional skin samples of WT mice harvested 8 hours after inoculation (Figure 4B). IL-17A and IL-17F were highly expressed in the epidermal compartment and were virtually absent from the dermal compartment. Furthermore, at 8 hours after infection, IL-17A and IL-17F induction was detected exclusively in the T cell fraction (CD3+ cells) after enrichment of epidermal T cells using a specific mAb directed against the pan–T cell marker CD3 (Figure 4C). In contrast, at 8 hours after infection, IL-17A and IL-17F induction was not observed in either the T cell–enriched fractions or the negative fractions from the epidermis of γδ T cell–deficient mice (Figure 4D). Thus, there was no evidence of IL-17–producing compensatory cells in γδ T cell–deficient mice after infection. Finally, at 8 hours after infection, IL-17A and IL-17F induction was detected exclusively in the γδ T cell–positive fraction after enrichment of epidermal γδ T cells using the γδ T cell–specific mAb GL3 (Figure 4E). Taken together, these findings demonstrate that epidermal γδ T cells are the major source of IL-17 production in response to S. aureus cutaneous challenge. Furthermore, epidermal γδ T cells are not required for induction of IL-22 or IL-21.

Figure 4

Impaired induction of IL-17, but not IL-22 or IL-21, in γδ T cell–deficient mice after S. aureus cutaneous challenge. Mice were inoculated intradermally with S. aureus, and the mean level of mRNA (AU) ± SEM of IL-17A and IL-17F by Q-PCR were determined from (A) lesional skin homogenates of skin biopsies from γδ T cell–deficient and WT mice performed 0, 4, 8, and 24 hours after inoculation (levels of IL-22 and IL-21 also evaluated); (B) epidermal and dermal split specimens of skin biopsies from WT mice performed 8 hours after inoculation; (C and D) positively selected cells (CD3+) and negative fractions (CD3–) of epidermal cell suspensions after enrichment for CD3+ cells (MACS) from skin biopsies taken from WT (C) or γδ T cell–deficient (D) mice performed 8 hours after inoculation; and (E) positively selected cells (γδ pos) and negative fractions (γδ neg) of epidermal cell suspensions after enrichment for γδ T cells (MACS) from skin biopsies taken from WT mice 8 hours after inoculation. Values in D were at or below the limit of detection. Data are from 2 experiments with at least 5 mice/group per experiment. *P < 0.05 between experimental groups (Student’s t test).

Figure 4

γδ T cells expressing the Vγ5 chain are present in the epidermis of WT mice after cutaneous challenge with S. aureus and produce IL-17 after in vitro stimulation. To determine the precise location of the γδ T cells within the infected skin lesions, we evaluated histological sections of lesional biopsy specimens taken 8 and 24 hours after skin inoculation (Figure 5A; isotype controls are provided in Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI40891DS1). We observed CD3+ T cells within the epidermal compartment of WT mice at 8 and 24 hours after inoculation. The vast majority of these T cells were positively selected cells (CD3+CD4–CD8–) or γδ T cells (MACS) from skin biopsies taken from WT (C) or γδ T cell–deficient (D) mice performed 8 hours after inoculation; and (E) positively selected cells (γδ pos) and negative fractions (γδ neg) of epidermal cell suspensions after enrichment for γδ T cells (MACS) from skin biopsies taken from WT mice 8 hours after inoculation. Values in D were at or below the limit of detection. Data are from 2 experiments with at least 5 mice/group per experiment. *P < 0.05 between experimental groups (Student’s t test).
γδ T cells expressing the Vγ5 chain are present in the epidermis of WT mice after skin challenge with *S. aureus* and produce IL-17 after in vitro stimulation. (A) Representative photomicrographs labeled with specific mAbs for CD3+ (arrows) and GL3+ (i.e., γδ T) cells (arrows; immunoperoxidase method) of histologic sections from skin biopsies from γδ T cell–deficient and WT mice performed 8 and 24 hours after skin inoculation with *S. aureus*. Insets show positively labeled cells. Isotype controls are shown in Supplemental Figure 2. Original magnification, ×400; ×800 (insets). (B) Epidermal cell suspensions of skin biopsies from normal uninfected WT mouse skin were labeled with specific mAbs for CD3, γδ T cells (GL3+), and Vγ5, and expression was analyzed by flow cytometry. Cells were gated on CD3+ cells, and the percentage of cells in each quadrant is indicated. (C) Purified GL3+ epidermal γδ T cells (10⁶ cells/ml) from uninfected C57BL/6 mice were left unstimulated or were activated with PMA/ionomycin and cultured in the presence or absence of 20 ng/ml IL-1β, 20 ng/ml IL-23, or 20 ng/ml of both IL-1β and IL-23. Supernatants were collected after 24 hours for analysis of IL-17 protein levels (pg/ml) by ELISA. Data are from 2 experiments with at least 4 mice/group per experiment. *P < 0.05 (Student’s t test).
mRNA production in response to skin of WT and γδ T cells present in normal skin of S. aureus, and the mean level of mRNA (AU) ± SEM of IL-17A and IL-17F by Q-PCR were determined from lesional skin homogenates of skin biopsies from (A) MyD88-deficient, IL-1R–deficient, TLR2-deficient, and WT mice and (B) IL-12/23p40–deficient, IL-23p19–deficient, IL-12p35–deficient, and WT mice, performed 8 hours after inoculation. (C) Mean mRNA level (AU) ± SEM of IL-1β and IL-23p19 from lesional skin homogenates of skin biopsies performed 8 hours after inoculation by Q-PCR. Data are from 2 experiments with at least 5 mice/group per experiment. ^P < 0.05, **P < 0.01 versus appropriate WT or 0-hour control (Student’s t test).

TCR remained unchanged 8–24 hours after cutaneous challenge with S. aureus (Supplemental Figure 3).

To confirm the ability of resident epidermal γδ T cells to produce IL-17 protein, we isolated γδ T cells from the epidermis of uninfected mice. These γδ T cells were enriched using positive selection and then stimulated with PMA/ionomycin (Figure 5C). PMA/ionomycin stimulation resulted in a 3-fold induction of IL-17 protein compared with unstimulated cells in culture supernatants, as determined by ELISA. In addition, since previous reports have demonstrated that IL-1β and IL-23 promote production of IL-17 by CD4+ γδ Th17 cells (33–39), we also evaluated whether addition of exogenous recombinant IL-1β and/or IL-23 could promote IL-17 production by epidermal γδ T cells. We found that addition of IL-1β, IL-23, or both in combination all resulted in 2- to 3.5-fold enhancement of IL-17 protein production over PMA/ionomycin alone, demonstrating that IL-1β and/or IL-23 is required for optimal production of IL-17 by epidermal γδ T cells. Taken together, these data indicate that the resident epidermal γδ T cells expressing the Vγ5 chain represent the predominant T cell subset present in mouse skin at baseline and during the early immune response to S. aureus skin infection. Furthermore, these resident epidermal γδ T cells have the capacity to readily produce IL-17 upon activation in vitro.

It should be noted that we also examined whether other CD3+ T cells present in normal skin of γδ T cell–deficient mice have the capacity to produce IL-17. After enriching CD3+ T cells from the skin of WT and γδ T cell–deficient mice, CD3+ cells were cultured (5 x 10^5 cells/ml) in the presence of PMA/ionomycin with or without IL-1β and IL-23 (20 ng/ml each, in combination), and IL-17 protein levels in culture supernatants were measured by ELISA (Supplemental Figure 4). IL-17 was only slightly induced in cultures of CD3+ T cells from γδ T cell–deficient mice compared with the substantial production observed in cultures of CD3+ T cells from WT mice. Because approximately 90% of the T cells from the skin of WT mice were γδ T cells (Figure 5B), this finding further demonstrates that the major population of cells responsible for IL-17 production after S. aureus cutaneous infection is epidermal γδ T cells, not other compensatory T cells present in the skin of γδ T cell–deficient mice.

IL-17 production in response to S. aureus cutaneous challenge is dependent upon IL-1, TLR2, and IL-23. We next evaluated whether IL-1 and IL-23 are required for IL-17 production in response to S. aureus cutaneous challenge in vivo. In addition, we also evaluated whether TLR2 is required, since TLR2 has recently been shown to be expressed by γδ T cells (40). To determine whether the induction of IL-17A and IL-17F was dependent upon IL-1 and/or TLR2, we inoculated WT mice and mice deficient in IL-1R, TLR2, or MyD88, the signaling adapter for IL-1R and TLR family members (Figure 6A). At 8 hours after challenge, IL-1R–, TLR2–, and MyD88-deficient mice had impaired induction of IL-17A and IL-17F compared with WT mice. The impaired induction of IL-17 in IL-1R– or MyD88-deficient mice was not caused by reduced numbers of γδ T cells, since similar numbers of Vγ5 γδ T cells were found in the epidermis between WT mice and mice deficient in IL-1R or MyD88 (Supplemental Figure 5). In addition, to determine whether IL-17A and IL-17F induction in the skin was also dependent upon IL-23, we inoculated WT mice and mice deficient in p40 (the shared subunit for IL-12 and IL-23), p19 (the IL-23–specific subunit), and p35 (the IL-12–specific subunit). At 8 hours after inoculation, IL-12/23p40– and IL-23p19–deficient mice, but not IL-12p35–deficient mice, had impaired induction of IL-17A and IL-17F compared with WT mice (Figure 6B), demonstrating an important role for IL-23, but not IL-12, in induction of IL-17A and IL-17F. Finally, to confirm that IL-1β and IL-23 were induced after infection, IL-1β and IL-23p19 gene induction was measured at 0 and 8 hours after infection (Figure 6C). We found that both IL-1β and IL-23p19 were significantly upregulated in response to infection. Taken together, these findings suggest that the induction of IL-17A and IL-17F by γδ T cells is dependent upon IL-1 and IL-23, which corresponds to our findings with purified epidermal γδ T cells in vitro and is similar to the characteristics of CD4+ γδ Th17 cells.
IL-17 receptor–deficient mice, but not IFN-γ receptor–deficient mice, have defects in immunity against S. aureus infection similar to those of γδ T cell–deficient mice. To evaluate whether IL-17 contributes directly to the impaired neutrophil recruitment and host defense against S. aureus cutaneous challenge observed in γδ T cell–deficient mice, WT mice and mice deficient in the IL-17 receptor (IL-17R) were inoculated intradermally with S. aureus. IL-17R–deficient mice developed significantly larger lesions and higher bacterial counts (as assessed by in vivo bioluminescence signals) compared with WT mice (Figure 7, A and B). For comparison, WT and IFN-γ receptor–deficient (IFN-γR–deficient) mice were also inoculated intradermally. In contrast to IL-17R–deficient mice, IFN-γR–deficient mice had lesion sizes and bacterial burdens similar to those of WT mice (Figure 7, C and D), which suggests that IFN-γ is not required for cutaneous host defense against S. aureus skin infection in our model. Taken together, these data demonstrate that IL-17R–deficient mice have an impairment in host defense against S. aureus cutaneous challenge similar to that of γδ T cell–deficient mice. These data provide additional evidence that IL-17–producing γδ T cells are required for host defense against S. aureus skin infection.

Administration of recombinant murine IL-17A with the S. aureus inoculum rescues γδ T cell–deficient mice. Given the critical role of γδ T cells and IL-17 in host defense against cutaneous S. aureus challenge, we evaluated whether the addition of exogenous recombinant murine IL-17A (rIL-17) can rescue the increased lesion sizes and bacterial counts observed in γδ T cell–deficient mice. Administration of a single dose of rIL-17 (1,000 ng in 100 μl saline) given with the S. aureus inoculum resulted in significantly reduced lesion sizes and bioluminescent signals compared with γδ T cell–deficient mice inoculated with S. aureus plus vehicle alone (Figure 8, A and B). In addition, administration of rIL-17 to γδ T cell–deficient mice resulted in lesion sizes and bacterial counts that were indistinguishable from those of WT mice, demonstrating the rIL-17 alone was sufficient to restore the immune defects in these mice. Thus, these data indicate that IL-17 is the essential mediator by which γδ T cells contribute to host defense against S. aureus cutaneous challenge. To confirm the requirement of IL-17 for clearance of infection, a neutralizing IL-17A mAb (50 μg) or an isotype control mAb was administered...
to WT mice along with the bacterial inoculum (Figure 8, C and D). Consistent with our demonstration that IL-17R–deficient mice had impaired bacterial clearance (Figure 7, A and B), administration of a single dose of a neutralizing IL-17A mAb with the inoculum to WT mice resulted in larger lesions and higher bacterial counts similar to those of γδ T cell–deficient mice, further demonstrating the key role for IL-17 in host defense against cutaneous S. aureus infection.

It should be mentioned that we attempted to administer rIL-17 to WT mice to evaluate whether additional IL-17 has any impact for host defense in animals with a normal endogenous IL-17 response. We found no difference in bacterial counts, as assessed by in vivo bioluminescence, between the IL-17–treated and –untreated mice (data not shown). Despite this finding, the fact that a single dose of IL-17 was sufficient to rescue the immune defects of the γδ T cell–deficient mice suggests that immunomodulatory therapy or vaccine development to enhance IL-17 responses may help in the treatment of S. aureus cutaneous infections in patients with altered T cell function with deficient or dysregulated Th17 responses.

Discussion

Neutrophil recruitment is an essential component of the host innate immune response against S. aureus skin infection and is required for bacterial clearance (6, 7). However, humans with diseases characterized by altered T cell function, including HIV/AIDS, atopic dermatitis, and hyper-IgE syndrome, are highly susceptible to S. aureus cutaneous infection (10, 12, 15), which suggests that T cells also contribute to host defense. Studies in mouse models have also indicated a key role for T cells, although the mechanism by which they contribute to host defense is not clear (19, 20). In this study, we report that resident epidermal γδ T cells, but not αβ T cells, were required for neutrophil recruitment in host defense against S. aureus cutaneous challenge, directly linking cutaneous T cell function with neutrophil recruitment. Furthermore, we demonstrated a mechanism by which skin γδ T cells contributed to host defense, via the IL-17–, TLR2–, and IL-23–dependent production of S. aureus granulopoiesis factors (25–27, 30), which is ultimately required for the production of antimicrobial peptides (e.g. β-defensins and cathelicidin) or by inducing epithelial proliferation, thereby increasing epithelial resistance to infection (28, 29, 41, 46, 47).

Although these human subjects with diseases with altered T cell responses have increased susceptibility to S. aureus colonization and infection (10–15), the mechanisms have been thought to be unrelated. However, emerging data suggests that these unrelated clinical conditions share in common a deficiency in Th17 cells. For example, there is significant loss of Th17 cells in the blood and mucosa of HIV-infected patients (48). Similarly, skin lesions from patients with atopic dermatitis have recently been shown to be deficient in Th17 cells (49). Finally, human subjects with hyper-IgE syndrome have STAT3 mutations that render them deficient in Th17 cells (16–18). Our findings demonstrating that IL-17 was required for host defense against S. aureus skin infections thus point to a mechanism by which Th17 dysregulation leads to increased susceptibility to S. aureus skin infections among these different human diseases.

We found that IL-17–producing resident epidermal γδ T cells were required for host defense against S. aureus skin infection. In contrast, humans with altered T cell function and susceptibility to S. aureus skin infections have been shown to be deficient in αβ Th17 cells (16–18, 48, 49). In human skin, a large proportion of αβ Th17 cells are present in inflamed skin (50), and γδ T cells have been reported to be recruited to a site of skin infection (51). However, it is not clear whether αβ Th17 cells, γδ T cells, or other functionally equivalent cells are important in producing IL-17 during S. aureus infections in human skin. In the mouse, our findings are supported by previous studies demonstrating that γδ T cell–deficient mice had a severe defect in their ability to clear intradermal S. aureus inoculation (20), that mice deficient in both IL-17A and IL-17F develop spontaneous S. aureus skin infections (52), and that a large proportion of the resident epidermal γδ T cells constitutively express the retinoid-related orphan receptor–γt (RO RTE) transcription factor, which is required for IL-17 production (53).

In the present study, we found that after S. aureus cutaneous challenge, there was little IFN-γ production, which was equivalent in γδ T cell–deficient and WT mice. Furthermore, IFN-γ–deficient mice had no impairment in host defense against S. aureus skin infection. In contrast, humans with altered T cell function and susceptibility to S. aureus skin infections among these different human diseases. The critical role of vaccine-induced IL-17 at epitHELial γδ T cells may depend on the site of infection: IL-17 for skin infections (and perhaps other epithelia; refs. 52, 57), and IFN-γ for invasive and systemic infections (55, 56). This key role for IL-17 against S. aureus skin infection has direct implications in future vaccine development, which may depend on the generation of IL-17–producing memory T cells at cutaneous sites. The critical role of vaccine-induced IL-17 at epithelial sites has been previously demonstrated against Helicobacter pylori gut infection and Mycobacterium tuberculosis, Streptococcus pneumoniae, and Pseudomonas aeruginosa lung infections (58–61). However, both IL-17– and IFN-γ–producing memory T cells may be required for vaccine protection against S. aureus at all sites of infection. It should be mentioned that any therapeutic strategy aimed at modulating...
IL-17 responses would need to be tightly controlled, since a previous study demonstrated that a neutrophil-rich environment, which may be induced by IL-17, could potentiate S. aureus pathogenesis by facilitating bacterial survival within the neutrophils (54).

Finally, we have demonstrated that IL-17 production by skin γδ T cells required IL-1R/MyD88 signaling, TLR2/MyD88 signaling, and IL-23. We have previously demonstrated that IL-1R/MyD88 is a more critical determinant than TLR2/MyD88 for neutrophil recruitment to a site of S. aureus cutaneous infection (21). Therefore, we have defined a key host defense circuit, whereby IL-1 (along with TLR2 and IL-23) promotes neutrophil recruitment to a site of cutaneous S. aureus infection by inducing IL-17 production by resident skin γδ T cells. A similar host defense circuit may exist in humans, since pediatric patients deficient in signaling molecules downstream of IL-1R and TLR2, including MyD88 and IRAK4, are highly susceptible to S. aureus skin infection (62, 63). These human subjects and patients with hyper-IgE syndrome all share in common increased susceptibility to S. aureus skin infection, providing further evidence of a possible link among IL-1, TLR2, and IL-17 in the human immune response against S. aureus cutaneous infection. From a therapeutic point of view, augmentation of the downstream IL-17 portion of this host defense circuit may provide a basis for novel therapeutic strategies to combat S. aureus cutaneous infections. This may be particularly relevant for providing immunomodulatory therapy or vaccine development to treat S. aureus infections in humans, especially in patients with altered T cell function with deficient or dysregulated Th17 responses.

Methods

S. aureus strains. All data were obtained using the bioluminescent S. aureus SH1000 strain ALC2906, which possesses the shuttle plasmid pSK236 with the penicillin-binding protein 2 (pBP2) promoter fused to theluxABCDE reporter cassette from Photobacterim luminescens as previously described (21, 22). This strain emits bioluminescent signals from live, actively metabolizing bacteria in all stages of theS. aureus life cycle (21, 22).

Preparation of S. aureus for skin inoculation. The S. aureus SH1000 strain has a chloramphenicol resistance plasmid selection marker, and all cultures were performed in the presence of chloramphenicol (10μg/ml; Sigma-Aldrich). S. aureus was streaked onto tryptic soy agar (tryptic soy broth plus 1.5% bacto agar; BD Biosciences) and grown overnight at 37°C in a bacterial incubator. Single colonies of S. aureus were placed into tryptic soy broth and grown overnight at 37°C in a bacterial shaking incubator. Midlogarithmic phase bacteria were obtained after a 3-hour subculture of a 1:50 dilution of the overnight culture. Bacterial cells were pelleted, resuspended, and washed 3 times in PBS. Bacterial concentrations were estimated by measuring the absorbance at 600 nm (A600; DU 640B spectrophotometer; Beckman Coulter). CFUs were verified by plating dilutions of the inoculum overnight.

Mice. Male congenic mice on a C57BL/6 genetic background were used in all experiments. γδ T cell–deficient mice (Tcrδ−/−; B6.129P2-Tcrδtm1Mom/J), αβ T cell–deficient mice (Tcrβ−/−; B6.129P2-Tcrβtm1Hon/J), IL-1R–deficient mice (B6.129S1-Il1r1tm1Ael/J), IL-12p35–deficient mice (B6.129S1-Ii12atm1J/J), IL-12/23p40–deficient mice (B6.129S1-Ii122tm1J/J), and WT C57BL/6 control mice were obtained from Jackson Laboratories. MyD88–deficient mice were a gift from S. Akira (Osaka University, Osaka, Japan). IL-23p19–deficient mice were obtained from N. Ghilardi (Genentech Inc., San Francisco, California, USA). IL-17–deficient mice (Iil17atm1−/−) have been previously described (64). All mouse colonies were maintained at UCLA in autoclaved cages under specific pathogen–free conditions.

Mouse model of cutaneous S. aureus infection. All procedures were approved by the UCLA Animal Research Committee. The mice were shaved on the back and inoculated intradermally with midlogarithmic growth phase S. aureus SH1000 strain (2 × 106 CFUs) in 100 μl sterile pharmacy-grade saline (0.9%) using a 27-gauge insulin syringe (Abbott Laboratories). Measurements of total lesion size (cm2) were made by analyzing digital photographs (Nikon Coolpix S400) of mice taken every 1–3 days using the software program Image J (http://rsbweb.nih.gov/ij/) and a millimeter ruler as a reference.

Quantification of in vivo S. aureus by in vivo bioluminescence and CFUs. Mice were anesthetized via inhalation of 2% isoflurane, and in vivo bioluminescence imaging was performed using the Xenogen IVIS imaging system (Caliper Life Sciences) at the Crump Institute for Molecular Imaging, UCLA, as previously described (21, 22). Data are presented on color scale overlaid on a grayscale photograph of mice and were quantified as total flux (photons/s) within a circular region of interest (1 × 105 pixels) using Living Image software (Xenogen), with a lower limit of detection of 1 × 104 photons/s. In some experiments, to confirm that the in vivo bioluminescence signals accurately reflected bacterial burden in vivo, S. aureus CFUs were determined after overnight cultures of homogenized (Pro200 Series homogenizer; Pro Scientific) 8-μm punch biopsy (Acuterm) specimens of lesional skin taken at days 1 and 3 after inoculation.

Tissue embedding and staining. For histological analysis, lesional 8-μm punch biopsy (Acuterm) skin specimens were bisected; one-half was fixed in 10% formalin and embedded in paraffin, and the other half was embedded in Tissue-Tek OTC compound (Sakura Finetek) and frozen in liquid nitrogen. H&E and Gram stains were performed on paraffin sections (4 μm) by the Tissue Procurement and Histology Core Laboratory and Histopathology Laboratory, UCLA, according to guidelines for clinical samples.

Immunoperoxidase labeling. Detection of Gr-1–positive (Ly-6G+), CD3+, and γδ T cell–positive (GL3+) cells on frozen cryostat specimens of lesional skin punch biopsy specimens was performed using the immunoperoxidase method, as previously described (21, 22), with the following mAbs: biotinylated rat anti-mouse Gr-1 (1 μg/ml; clone RB6-8C5; IgG2b isotype; BD Biosciences—Phar mingen), hamster anti-mouse CD3e (5 μg/ml; clone 145-2c11, IgG1 isotype; BD Biosciences—Phar mingen), hamster anti-mouse TCRγδ complex (5 μg/ml; clone GL3, IgG2a isotype; BD Biosciences—Phar mingen), and corresponding isotype controls. In some cases, prima ry antibody binding was detected using biotin-labeled mouse anti-ham ster IgG cocktail (5 μg/ml; clone G70-204, G94-56, IgG1, and IgG2b isotypes; BD Biosciences—Phar mingen).

MPO assay. MPO enzymatic activity was determined from homogenized (Pro200 Series homogenizer; Pro Scientific) 8-μm punch biopsy (Acuterm) lesional skin specimens using an MPO assay kit according to the manufacturer’s recommendations (Cytostore). The amount of MPO activity was normalized to the weight of the tissue specimen, and data were presented as μg/mg tissue.

Protein arrays. Protein levels of K, MIP2, GM-CSF, and IFN-γ were determined from homogenized (Pro200 Series homogenizer; Pro Scientific) 8-μm punch biopsy (Acuterm) lesional skin specimens using SearchLight Chemiluminescent Assays according to the manufacturer’s recommendations (Aushon Biosystems). The levels of these cytokines and chemokines were normalized to the weight of the tissue specimen, and data were presented as pg/mg tissue.

Separation of epidermis and dermis and ex vivo epidermal CD3+ T cell or γδ T cell enrichment. To separate the epidermis from the dermis, whole skin biopsy specimens were incubated in RPMI containing dispase II (2.4 U/ml; Roche) for 1 hour at 37°C, according to the manufacturer’s recommendations. For Q-PCR analysis, epidermal and dermal specimens were separated and placed into TRIzol (Invitrogen) for RNA extraction as described below. In some experiments, epidermal cell suspensions were generated by incubation of the epidermal specimens in 0.05% trypsin-EDTA (Invitrogen) for 30 minutes at 37°C. A single-cell suspension was obtained by mechanical
Before 90 minutes at 37 °C with an anti-FITC Alexa Fluor 488–conjugated anti-body (Invitrogen). After extensive washing, epidermal sheets were mount-ed onto slides with Prolong Gold mounting medium (Invitrogen) and were examined with a Leica Microsystems inverted confocal microscope equipped with a x63 oil immersion objective.

Administration of rIL-17. A single dose of rIL-17 (1,000 ng/100 μl; R&D Systems) in sterile pharmacy-grade saline, or vehicle alone (saline), was mixed and administered intradermally along with the inoculum of S. aureus (2 × 10^6 CFUs in 100 μl PBS) to γδ T cell–deficient mice. Similar doses of rIL-17 (500–1,000 ng) were previously shown to be biologically active in mouse skin in vivo (68, 69). We demonstrated that rIL-17 did not have any direct bactericidal or bacteriostatic activity against S. aureus in vitro (Supplemental Figure 6).

Monitoring growth of S. aureus in the presence of rIL-17. To determine whether rIL-17 has a direct effect in the growth of S. aureus, the growth responses of S. aureus were monitored in RPMI (with 10% serum) in the presence of rIL-17 at various concentrations as previously described (70). Briefly, RPMI cultures of 10^6 CFU midlogarithmic growth phase S. aureus were supplemented with 0, 20, 100, and 500 ng rIL-17 and incubated at 37 °C in a humidified incubator with 5% CO_2; for 5 hours, with intermittent shaking. Culture aliquots were serially diluted and cultured overnight on tryptic soy agar plates (tryptic soy broth plus 1.5% Bacto-agar; BD Biosciences) supplemented with 10 μg/ml chloramphenicol. The resulting bacterial colonies were counted manually and expressed as total CFU number.

Statistics. Data were compared using 2-tailed Student’s t test. All data are expressed as mean ± SEM as indicated. P values less than 0.05, 0.01, and 0.001 were considered statistically significant.

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IL-23 and IL-17 in the establishment of infection

Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome.


