Nonredundant Roles of Interleukin-17A (IL-17A) and IL-22 in Murine Host Defense against Cutaneous and Hematogenous Infection Due to Methicillin-Resistant *Staphylococcus aureus*

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Staphylococcus aureus is the leading cause of skin and skin structure infections (SSSI) in humans. Moreover, the high frequency of recurring SSSI due to *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA) strains, suggests that infection induces suboptimal anamnestic defenses. The present study addresses the hypothesis that interleukin-17A (IL-17A) and IL-22 play distinct roles in immunity to cutaneous and invasive MRSA infection in a mouse model of SSSI. Mice were treated with specific neutralizing antibodies against IL-17A and/or IL-22 and infected with MRSA, after which the severity of infection and host immune response were determined. Neutralization of either IL-17A or IL-22 reduced T cell and neutrophil infiltration and host defense peptide elaboration in lesions. These events corresponded with increased abscess severity, MRSA viability, and CFU density in skin. Interestingly, combined inhibition of IL-17A and IL-22 did not worsen abscesses but did increase gamma interferon (IFN- γ) expression at these sites. The inhibition of IL-22 led to a reduction in IL-17A expression, but not vice versa. These results suggest that the expression of IL-17A is at least partially dependent on IL-22 in this model. Inhibition of IL-17A but not IL-22 led to hematogenous dissemination to kidneys, which correlated with decreased T cell infiltration in renal tissue. Collectively, these findings indicate that IL-17A and IL-22 have complementary but nonredundant roles in host defense against cutaneous versus hematogenous infection. These insights may support targeted immune enhancement or other novel approaches to address the challenge of MRSA infection.

Staphylococcus aureus is the most frequent etiologic agent of skin and skin structure infections (SSSI), including cellulitis, folliculitis, and furunculosis (1–4). The skin is a primary portal of entry for deeper infections and subsequent hematogenous dissemination. For example, SSSI is a frequent prelude to invasive infections, such as osteomyelitis or endocarditis (5, 6). At present, *S. aureus* is the second most common bloodstream isolate in health care settings (7). Methicillin-resistant *S. aureus* (MRSA) strains predominate in health care- and community-acquired staphylococcal infections (8). Regardless of prior exposure or antibody status, patients having SSSI due to MRSA exhibit 1-year recurrence rates as high as 27 to 45%, (9–11), and these infections often require surgical debridement (12). Despite reports of modest declines in rates of MRSA infection in some populations (6), as many as one-third of *S. aureus* bacteremia patients succumb to this infection, even with gold-standard therapy. As a result, infections due to MRSA represent a leading cause of infection-induced mortality in the United States, ranging from 11,000 to 18,650 deaths per year (13–15). Compounding the concerns noted above, the increasing use of antibiotics has accelerated the emergence of multidrug-resistant *S. aureus* strains (16–19). Consequently, resistance to even the most modern antistaphylococcal agents is rising at an increasing rate (20, 21). The public health impact of this trend is of urgent concern, especially given the 15 to 40% mortality rates associated with invasive MRSA infection (22, 23).

Beyond causing infection in otherwise healthy individuals, MRSA is a principal cause of disease in patients with certain immune dysfunctions (24). For example, patients with chronic gran-

ulomatous disease (CGD) have deficient Th1 responses and poor phagocyte oxidative burst. These individuals are at increased risk of both localized and disseminated staphylococcal infections. In comparison, autosomal dominant hyper-IgE syndrome patients have deficient Th17 response and are at increased risk for SSSI but not for disseminated *S. aureus* infection (25). Furthermore, patients with *acne inversa* have deficits in cutaneous interleukin-22 (IL-22) responses and exhibit staphylococcal persistence in skin abscesses (26, 27). These patterns suggest that innate and adaptive immune responses must cooperate for optimal host defense against MRSA.

Host immune defenses against *S. aureus* SSSI are likely differ-

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ent from those against invasive infection. Prior studies have shown that IL-17A and IL-22 are involved in protection against SSSI in mice (25, 28, 29). In humans, IL-17A and IL-17F induce the production of the neutrophil-attracting chemokines CXCL8, CXCL1, and CXCL2 (i.e., equivalent to keratinocyte chemoattractant [KC] and macrophage inflammatory protein 2 [MIP2], respectively, in mice) and other proinflammatory cytokines that enhance neutrophil responses to promote clearance of *S. aureus* (29). However, the relative roles of IL-17A and IL-22 in defense against SSSI and ensuing invasive *S. aureus* infections have not been well studied. Thus, the present investigation aimed to test the hypothesis that IL-17A and IL-22 play nonredundant roles that contribute to defense against skin versus invasive MRSA infections in a relevant model of SSSI.

MATERIALS AND METHODS

MRSA strain. This study utilized MRSA strain Xen 30 (Xenogen/Caliper Life Sciences, Inc.), which was derived from *S. aureus* strain 16 and contains a *luxABCDE* operon in the chromosome, allowing for detection via bioluminescence *in vivo* (30). Although MRSA strains can vary in alpha-toxin production (31–33), the virulence of the study strain was confirmed as equivalent to that of USA300 in the model as used in our prior studies (25). The virulence of strain Xen 30 was assessed in a previous study and demonstrated to be equivalent to that of other clinically relevant MRSA strains in the SSSI murine model (25). Cells were cultured from virulence-validated master cell banks and grown to log phase in brain heart infusion (BHI) medium at 37°C. The resulting cells were harvested, washed, suspended in phosphate-buffered saline (PBS), sonicated, quantified by spectrophotometry, and diluted to the desired CFU inoculum.

Mouse model of SSSI. Male BALB/c mice (20 to 25 g) were purchased from Harlan Laboratories and allowed to acclimate prior to each experiment. A subcutaneous skin/skin structure abscess model was utilized as previously described (34, 35). In brief, mice were randomized into appropriate study groups and given general anesthesia, their flanks were shaved and sterilized, and 2×10^7 CFU MRSA in 100 μ l PBS was injected subcutaneously into each flank as previously described (25). Unless specified otherwise, a minimum of 15 mice were assigned to each group. Animal studies were performed in accordance with approved animal use policies of LABioMed at Harbor-UCLA following NIH guidelines.

Skin lesion magnitude. Dermonecrosis area and abscess volume were measured in each mouse flank during the postchallenge study period. Mice were anesthetized, and the length (*l*) and width (*w*) of each lesion site measured to quantify the dermonecrosis area (mm^2).

Bioluminescence imaging. At selected time points during the postinfection period, control and antibody-treated mice underwent bioluminescence signal detection using an *in vivo* imaging system (IVIS; Caliper Life Sciences, Inc.). Bioluminescence signals from regions of interest of each animal were captured for a 5-min time period and analyzed using the Living Image software for quantification in photons per second per abscess.

MRSA tissue burden. At preselected endpoints postinfection, control and antibody-treated mice were sacrificed, and the abscesses and kidneys were processed for quantitative culture. Each flank was aseptically dissected and the abscess removed, homogenized, and quantitatively cultured on sheep blood agar. One kidney from each mouse was excised and processed for quantitative culture to assess hematogenous dissemination. All cultures were incubated at 37°C for 24 h, and the resulting colonies enumerated as $\log_{10}$ CFU/abscess or $\log_{10}$ CFU/kidney. The lower limit of detection in quantitative cultures was 50 CFU per gram of abscess tissue or one whole kidney.

Cytokine inhibition. Monoclonal antibodies were used to neutralize IL-17A and/or IL-22 to assess their roles in natural immunity to MRSA SSSI. Purified monoclonal anti-mouse IL-17A antibody and/or anti-mouse IL-22 antibody (see Table S1 in the supplemental material) was

administered intraperitoneally (i.p.) (100 μ g in a 100- μ l volume) at study days -2 and $+2$ relative to infection (day 0). Control groups received nonspecific isotype control IgG i.p. (100 or 200 μ g) in an otherwise identical dosing regimen.

Immunohistochemistry. After 7 days of infection, immunological determinants associated with host defense against MRSA were assessed in skin abscesses and kidneys (36). In brief, samples were aseptically dissected from animals, fixed in 10% zinc formalin, and embedded in paraffin. Next, 3- μ m sections were cut, dewaxed, and rehydrated, followed by heat-induced antigen retrieval (Dako, Carpinteria, CA). Samples were blocked with dual endogenous enzyme block (Dako) and 2 to 10% normal serum corresponding to the secondary antibody. Sections were then incubated with a primary antibody targeting a specific molecule or cell of interest, followed by a secondary antibody conjugated to horseradish peroxidase or biotin (Santa Cruz Biotechnology, Santa Cruz CA) (see Table S1 in the supplemental material). Colorimetric development was achieved by reaction with streptavidin-horseradish peroxidase (Dako) and 3,3'-diaminobenzidine (DAB; Vector Laboratories, Burlingame, CA), and the samples were counterstained with hematoxylin and eosin. Images were obtained using a Zeiss BX43 microscope with a DP21 digital camera. Cytokine, immune cell, and host defense peptide expression in tissues were quantified using the Image J software (<http://imagej.nih.gov/ij/>), and results determined by densitometric image analysis of stained tissue sections (see Fig. S1 in the supplemental material). Outcomes were enumerated as the percentages of the areas that were stained in samples from antibody-neutralized mice compared to the results for the control mice. To optimize the objectivity of image analysis, at least 10 (range, 10 to 30) images with identical total areas were quantified for each stain in each study group; areas containing no tissue were subtracted as background. As described above, immunohistochemistry images were analyzed and outcomes quantified as the percentage of the area that was stained in antibody-neutralized-mouse samples compared to the results for the control mice. Renal T cell and neutrophil populations were assessed in immunohistochemically treated sections using anti-CD3 and anti-Ly6G antibodies, respectively. Random fields at $\times 10$ magnification were captured, and brown cells were counted. Ten fields of CD3⁺ cells were counted to enumerate T cells, and statistical analysis was performed. Similarly, 20 fields of Ly6G⁺ cells were counted to enumerate neutrophils, and statistical analysis was performed. Data were expressed as the average number of cells per field.

Statistical analyses. Differences in experimental results were compared with the Mann-Whitney U test. Data are represented as the median values and 25%-to-75% interquartile ranges (IQR). *P* values of <0.05 were considered statistically significant.

RESULTS

In our model of MRSA SSSI, *S. aureus* was inoculated subcutaneously and the lesions were measured over 7 days. At 7 days postinfection, the CFU burden was assessed in tissue samples and immune responses to the infection were analyzed by immunohistochemistry.

(i) Cutaneous infection. Natural immune response to MRSA SSSI. In control mice, dermonecrotic skin lesions developed rapidly and reached a maximal size at 5 to 7 days. The evolution of infection paralleled bacterial proliferation in abscesses (see bioluminescence signals). After 7 days of infection, quantitative cultures of the abscesses verified high bacterial burdens (Fig. 1).

IL-17A and IL-22 contribute to control of abscess severity. Inhibition of either IL-17A or IL-22 alone resulted in significantly larger lesion sizes (dermonecrosis area) and conferred stronger *S. aureus* bioluminescence signals *in situ*. After 7 days of infection, mice treated with anti-IL-17A antibody had increased abscess CFU burdens (Fig. 1 and 2). While there was a trend toward in-

FIG 1 Qualitative and quantitative effects of IL-17A and/or IL-22 on SSSI lesions. Mice were treated with anti-IL-17A antibody, anti-IL-22 antibody, both, or neither (control), and lesions monitored over time with MRSA viability assessment and subsequent quantitative bacterial burden analysis. The areas of dermonecrosis were significantly greater in neutralizing-antibody-treated mice than in control mice, while IL-17A neutralization resulted in greater CFU burdens in abscesses. (A) Each image shows dermonecrotic lesions from a single mouse that was representative of the group. (B) The areas of dermonecrosis (mm²) were measured at selected time points for 7 days postinfection. The mean values at the time point with the greatest degree of lesion severity (day 6 postinfection) are shown. (C) Bioluminescence-visualized MRSA *in vivo* in SSSI abscesses of IL-17A-neutralized and/or IL-22-neutralized mice and untreated control mice on the indicated days postinfection. The color scale represents luminescence photons/second (p/s) per unit area (flux). Each group of 10 mice is represented by four mice that were representative of the group. (D) Luminescence was quantified as photons/s at days 2, 4, and 7 postinfection. Interquartile range (IQR) error bars were intentionally omitted for clarity. (E) CFU burdens in skin abscesses of comparative treatment groups were determined by quantitative culture at 7 days postinfection. *, $P < 0.05$; **, $P < 0.01$ (versus control mice; $n = 10$ per group). Data are represented as the median values, and error bars represent IQR. The lower limit of detection of bacteria in abscess tissue samples is 50 CFU/g.

creased abscess CFU burdens in mice treated with anti-IL-22 antibody, this increase did not achieve statistical significance ($P = 0.26$). However, the aggregate data, including lesion area and volume, as well as bioluminescence signal, strongly suggest that IL-22 is necessary for optimal host defense against SSSI. Surprisingly, in mice with dual inhibition of IL-17A and IL-22, the dermonecrosis areas and amounts of viable *S. aureus* bacteria (Fig. 1) were similar to those in control mice. Collectively, these results indicated that both IL-17A and IL-22 are necessary for local control of *S. aureus* SSSI. These data also suggest that dual inhibition of IL-17A and IL-22 may induce a compensatory response that reduces the extent of SSSI (see below).

IL-17A and IL-22 influence T cell and neutrophil infiltration.

To investigate the effects of cytokine neutralization on the local host immune response, the levels of lymphocyte and neutrophil trafficking into cutaneous abscess sites in the various treatment groups were compared. Hematoxylin-and-eosin (H&E) and Gram stains of skin cross sections showed the location of bacteria within skin abscesses (Fig. 2A to H). By histopathology, CD3⁺ T lymphocytes and Ly6G⁺ neutrophils were found to have infiltrated within proximity to skin lesions. The results seen in high-magnification fields (Fig. 3A) correspond with significant reductions in infiltration of CD3⁺ T cells and Ly6G⁺ neutrophils into abscess foci (Fig. 3B) in the setting of IL-17A and/or IL-22 inhibition ($P < 0.05$).

FIG 2 Influence of IL-17A or IL-22 inhibition on histopathology of SSSI lesions. Histopathology profiles of skin lesions were used to show the pathology at 7 days postinfection. Tissue staining of the epidermal surface (top) and deeper tissue parenchyma is shown for control and neutralizing-antibody-treated mice at $\times 5$ and $\times 20$ magnification. Gram (A to D) and hematoxylin-and-eosin (H&E) (E to H) stains show the locations of macroabscesses and bacteria (*S. aureus* cells are seen as cocci stained violet in Gram-stained sections).

IL-17A and IL-22 differentially affect host defense peptide elaboration. IL-17A and IL-22 are known to influence the expression of host defense peptides, which likely play a key role in the local defense against staphylococcal infection (37). Therefore, we analyzed the effects of neutralizing IL-17A and/or IL-22 on representative host defense peptides, murine β -defensin 3 (m β D-3), psoriasin (S100A7), and CRAMP. In control mice, all three host defense peptides were detected in close proximity to the abscesses (Fig. 4A, panels A, E, and I). Inhibition of IL-17A or IL-22 individually or together decreased the expression of m β D-3 and psoriasin in skin lesions compared to their expression in the control mice (Fig. 4A, panels A to H, and B). In contrast, CRAMP levels were significantly reduced in skin lesions of anti-IL-22 antibody-treated mice but not those in which IL-17A was neutralized (Fig. 4A, panels I to L, and B). Collectively, these results demonstrate that in the context of *S. aureus* SSSI, IL-17A and IL-22 have nonredundant effects on the expression of different host defense peptides. More specifically, the present data indicate that IL-17A and IL-22 are necessary for maximal expression of m β D-3 and psoriasin, whereas IL-22 is required for maximal expression of CRAMP.

IL-17A and IL-22 cooperate to influence IFN- γ expression. Next, we used immunohistochemistry to assess the effects of cytokine neutralization on the expression of cytokines at abscess sites. As anticipated, IL-17A was significantly diminished in subcutaneous abscesses of mice treated with anti-IL-17A antibody (Fig. 5A, panels A to D, and B). Likewise, IL-22 elaboration was substantially decreased in skin lesions of mice treated with anti-IL-22 antibody (Fig. 5A, panels E to H, and B). These results verified that the antibody neutralization regimens were sufficient to cause significant reductions in IL-17A and IL-22 during the course

of study. Neutralization of IL-22 reduced IL-17A expression by approximately 40%. However, neutralization of IL-17A did not alter IL-22 expression. This pattern of results suggests that IL-22 is required for protective levels of IL-17A expression in this model. Also, neutralization of either IL-17A or IL-22 alone had no effect on the expression of interferon (IFN- γ). In contrast, combined neutralization of IL-17A and IL-22 resulted in a significant increase in IFN- γ elaboration (Fig. 5A, panels I to L, and B). Moreover, only the dual-neutralization group exhibited significantly higher IFN- γ /IL-17A ratios than the control mice (Fig. 5C). The IL-4 expression level was not changed in any treatment group compared to its level in the control group (Fig. 5A, panels M to P, and B).

(ii) Invasive infection. Natural immune response to disseminated MRSA infection. After 7 days of infection, quantitative culture of kidney homogenates indicated that modest hematogenous dissemination and proliferation of *S. aureus* had occurred. Immunohistochemical analyses revealed T cell and neutrophil infiltration into the kidneys in response to infection, even though macroabscesses were not visible (Fig. 6).

IL-17A protects against hematogenous infection. A frequent complication of invasive MRSA infections is hematogenous seeding of target organs, such as the kidney (38, 39). Thus, the kidney CFU burden was measured at 7 days postinfection to assess the extent of hematogenous dissemination. In control mice, although the kidney bacterial burden reached a median of 2.6 log CFU/kidney by quantitative culture (Fig. 6A), no abscess foci were observed on renal tissue histology. Neutralization of IL-17A alone resulted in a 15-fold increase in the kidney bacterial burden. In contrast, neutralization of IL-22 alone or of IL-17A and IL-22 in

FIG 3 Effect of IL-17A or IL-22 inhibition on immune cell infiltration into SSSI lesions. (A) Immunohistochemistry of skin sections from control and antibody-neutralized mice at day 7 postinfection show cells stained for CD3⁺ (A to D) and Ly6G⁺ (E to H), indicating T cell and neutrophil infiltration, respectively (immune cells are stained brown). The images shown are $\times 5$ and $\times 20$ magnifications of tissue sections that are representative of the respective groups. Note the reduced infiltration of immune cells in the skin of antibody-neutralized animals. (B) CD3⁺ and Ly6G⁺ staining quantify T cell and neutrophil infiltration, respectively. Stained regions with identical total areas in a minimum of 10 fields were quantified using ImageJ software, as described in the text. Data represent the percentages of cells stained in antibody-neutralized-mouse skin lesions compared to the results for control mice (100%). ***, $P < 0.001$ (versus control). Data are represented as the median values, and error bars represent IQR.

combination did not significantly increase the kidney CFU burden. These results suggest that IL-17A plays a key role in protecting target organs against hematogenously disseminated infection due to *S. aureus*.

IL-17A influences immune cell trafficking to kidneys. To investigate potential mechanisms of IL-17A-mediated defense against hematogenous dissemination, the extent of lymphocyte and neutrophil infiltration into the kidneys was assessed in control and cytokine-neutralized mice. Inhibition of IL-17A resulted in decreased T cell accumulation in the kidneys (Fig. 6B). The total neutrophil infiltration into the kidneys was not changed in any treatment group. However, there was a small but significant de-

crease in neutrophil infiltration into the peritubular compartment with anti-IL-17A antibody treatment (Fig. 6C). Neither inhibition of IL-22 alone nor dual inhibition of IL-17A and IL-22 significantly affected neutrophil or T cell trafficking into kidneys (see Fig. S2A in the supplemental material). We also examined the renal levels of prototypic host defense peptides and cytokines in the various treatment groups. Interestingly, the levels of these molecules in mice treated with anti-IL-17A antibody and/or anti-IL-22 antibody did not appear to differ from the levels in control animals (see Fig. S2B and C). Taken together, these results suggest that neutralization of IL-17A but not of IL-22 increases intrinsic vulnerability to hematogenous dissemination to the kidney by de-

FIG 4 Effect of IL-17A or IL-22 inhibition on host defense peptide elaboration in SSSI lesions. Immunohistochemistry (A) and quantitative (B) analyses of host defense peptides in skin sections from control and antibody-neutralized mice were performed at day 7 of the infection model. Stained regions with identical total areas in a minimum of 10 fields were quantified using ImageJ software, as described in the text. (A) Immunohistochemical sections show the profiles of antimicrobial peptide elaboration in skin abscesses. Immunohistochemistry was used to detect murine β -defensin 3 (m β D-3) (A to D), psoriasin (E to H), and CRAMP (I to L) expression (brown cells) in skin lesions of control and antibody-treated mice. Note that the expression levels of all antimicrobial peptides are decreased in abscesses of animals treated with anti-IL-17A antibody and/or anti-IL-22 antibody compared to their levels in control mice. The images shown are $\times 5$ and $\times 20$ magnifications of tissue sections that are representative of the respective groups. (B) The levels of host defense peptides murine β -defensin 3 (m β D-3), psoriasin, and CRAMP were quantified. Data represent the percentages of host defense peptide expression in antibody-neutralized-mouse skin lesions compared to the results for control mice (100%). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (versus control). Data are represented as the median values, and error bars represent IQR.

FIG 5 Effect of IL-17A or IL-22 inhibition on cytokine profiles in SSSI lesions. Immunohistochemistry (A) and quantitative (B) analyses of cytokines in skin sections from SSSI lesions of control and antibody-neutralized mice were performed 7 days postinfection. The specific cytokines measured, representing prototypic T helper subpopulation polarization, were IL-17A, IL-22, IFN- γ , and IL-4 (Th17, Th22, Th1, and Th2, respectively). (A) Immunohistochemistry was used to localize the extent of cytokine expression (brown color) for IL-17A (A to D), IL-22 (E to H), IFN- γ (I to L), and IL-4 (M to P). The images shown are $\times 5$ and $\times 20$ magnifications of tissue sections that are representative of the respective groups. (B) The levels of cytokines IL-17A, IL-22, IFN- γ , and IL-4 were quantified. Stained regions with identical total areas in a minimum of 10 fields were quantified for overall cytokine expression using ImageJ software, as described in the text. Data represent the percentages of cytokine expression in antibody-neutralized-mouse skin lesions compared to the results for the control (100%). ***, $P < 0.001$ (versus control). (C) IFN- γ /IL-17A ratios were determined by comparing IFN- γ and IL-17A expression levels shown in panel B. *, $P < 0.05$ (versus control). Data are represented as the median values, and error bars represent IQR.

creasing T cell and neutrophil accumulation in this target organ. However, neither IL-17A nor IL-22 had a detectable effect on renal expression of host defense peptides or cytokines during the study period in this model of kidney infection.

DISCUSSION

The relative contributions of IL-17A and IL-22 in host defense against cutaneous and invasive infection were investigated in our established mouse model of SSSI due to MRSA (25). After mice were treated with monoclonal antibodies to neutralize one or both of these cytokines or with an irrelevant isotype-matched control antibody, their flanks were inoculated subcutaneously with *S. aureus* and key aspects of infection were monitored for 7 days. In control mice, tissues adjacent to the abscesses exhibited increased infiltration of T cells and neutrophils, expression of IL-17A and IL-22, and elaboration of host defense peptides, including mouse β defensin-3 (mBD-3), psoriasin (S100A7), and CRAMP. However, neutralization of IL-17A and/or IL-22 resulted in increased

pathology and correlated decreased host defense profiles. Furthermore, while the inhibition of either IL-17A or IL-22 alone worsened the severity of lesions, only the inhibition of IL-17A and not inhibition of IL-22 affected the CFU density in skin. These findings indicate that both IL-17A and IL-22 are necessary for host defense against this pathogen but that they play nonidentical roles. Thus, the present data indicate that these cytokines make distinct and nonredundant contributions to protection against skin versus invasive *S. aureus* infection in this model.

The coordinated roles of IL-17A and IL-22 in host defense against *S. aureus* cutaneous infection are not completely understood. We (25, 40), and others (29, 41) have demonstrated the importance of the Th17 pathway in defense against *S. aureus* skin infection. For example, Cho et al. reported that T cell production of IL-17 but not of IL-22 promoted neutrophil recruitment in defense against *S. aureus* in a mouse model of SSSI (29). However, Montgomery et al. found that IL-17A was only protective against *S. aureus* SSSI in BALB/c mice that were B cell deficient (42). Our

FIG 6 Comparative MRSA burdens and quantitative assessment of immune cell infiltration in kidneys. Neutralization of IL-17A resulted in increased bacterial burdens in kidneys, correlating with decreased T cell and neutrophil infiltration. (A) MRSA burdens in kidneys were determined by quantitative culture at day 7 of the infection model. Logarithmic increases in CFU associated with inhibition of IL-17A but not of IL-22 are congruent with the observed increase in skin abscess CFU (Fig. 1). ***, $P < 0.001$ (versus control). The lower limit of detection is 50 CFU/kidney. (B and C) Levels of CD3⁺ lymphocyte (B) and Ly6G⁺ neutrophil (C) infiltration were determined by immunohistochemical analysis of kidney sections from control or antibody-neutralized mice 7 days postinfection. The numbers of CD3⁺ cells per field were imaged at $\times 10$ magnification, and the results (10 fields) enumerated. For Ly6G⁺ cells, infiltration was stratified into intraglomerular and peritubular regions. Twenty fields were imaged at $\times 10$ magnification, and the numbers of Ly6G⁺ cells enumerated. *, $P < 0.05$; **, $P < 0.01$ (versus control). Data are represented as the median values, and error bars represent IQR.

data suggest that IL-17A and IL-22 contribute to host defense of the skin through multiple mechanisms, including induction of host defense peptides, as well as recruitment of T cells and neutrophils. The present data also suggest a novel epistatic relationship between IL-17A and IL-22 in the host defense against MRSA. The current results indicate that the expression of IL-17A is at least

partially dependent on IL-22 but that the converse is not true. This interesting finding may afford novel insights into immune mechanisms optimized to defend against cutaneous versus hematogenous compartments.

IL-17A and IL-17F, members of the IL-17 cytokine family, are produced by subsets of $\alpha\beta$ CD4⁺ T helper 17 (Th17) cells, as well as $\gamma\delta$ T cells, natural killer (NK) cells, NK T cells, CD8⁺ T cells, and innate lymphoid cells (iLCs). The receptors for IL-17A, IL-17F, IL-17RA, and IL-17RC (43) are expressed on a wide array of cells, including epithelial cells, immune cells, adipocytes, hepatocytes, etc. (43–46). IL-22, a member of the IL-20 family of proinflammatory cytokines (47), is primarily secreted by $\alpha\beta$ CD4⁺ T helper 22 (Th22) cells (48) but can also be produced by Th17 cells, $\gamma\delta$ T cells, and iLCs. Receptors for IL-22, IL-10R2 and IL-22R, are expressed on epithelial cells (i.e., keratinocytes) and hepatocytes but not immune cells (49, 50). Unlike IL-17A, IL-22 does not signal immune cells directly. Rather, IL-22 regulates skin barrier integrity and protection from pathogens by acting on epithelial cells by several mechanisms. First, it prompts epithelial cells to secrete proinflammatory cytokines for leukocyte recruitment and activation (41, 51). Second, it induces epithelial cell proliferation during wound healing (52, 53). Third, it stimulates epithelial cells of skin, mucosa, and organ parenchyma to secrete host defense peptides (12, 37, 54). Moreover, IL-22, alone or with IL-17A, can act synergistically to induce keratinocytes to secrete host defense peptides in the skin (37).

Our studies also identified new insights into the apparent compensatory interactions among IL-17A, IL-22, and IFN- γ in host defense against cutaneous *S. aureus* infection. While neutralization of either IL-17A or IL-22 alone worsened the severity of lesions, only the inhibition of IL-17A permitted significantly greater CFU burdens, although IL-22 neutralization trended higher. It is recognized that the bioluminescence signal reflects the total metabolic activity of a *lux*-expressing organism and, therefore, can vary somewhat from the total number of recoverable CFU. Nonetheless, the present bioluminescence and CFU data were consistent in supporting the interpretation that IL-22 and IL-17A influence host defense against MRSA in distinct ways. Of great interest, dual neutralization of IL-17A and IL-22 yielded lesions of a severity that did not differ from the severity of the lesions in the control mice, despite host defense peptide expression and neutrophil recruitment being significantly reduced. Interestingly, significant increases in IFN- γ were observed in the dual treatment group. This pattern of results suggests that, in the setting of diminished IL-17A and IL-22, a compensatory Th1 response supports host defense against *S. aureus* and that this may rely upon effector cells other than neutrophils (24). This counterregulation of Th1 and Th17, also found in other mouse models (55, 56), may explain the lack of increased pathogenesis in the dual-neutralization group. In addition, despite decreased levels of neutrophils infiltrating into tissues, IFN- γ may provide protection through the potentiation of neutrophil microbicidal functions, as well as through other phagocytes that were not analyzed (e.g., monocyte/macrophage lineage) (39, 57–63). For example, IFN- γ increases the life span and oxidative microbicidal activities of neutrophils (64–66). Notably, Montgomery et al. reported that inhibition of IFN- γ had no impact on the severity of primary SSSI in mice (42). Thus, our data further suggest that the role of IFN- γ in host defense against SSSI may only be apparent when IL-17A and IL-22 activities are deficient. Future

studies addressing the role of IFN- γ in the context of suppressed IL-17A and IL-22 expression will elucidate the protective role of the Th1 response in MRSA SSSI.

The current data also support the concept that IL-17A and IL-22 play nonredundant roles in the host defense against SSSI, depending on whether the mice are immunologically naive or have been vaccinated against *S. aureus*. Previously (25), we found that in mice that received the NDV-3 vaccine prior to MRSA SSSI, vaccine-mediated efficacy in the skin was more dependent on IL-22 than on IL-17A. While there are several different potential explanations for this outcome, the simplest is that in naive mice, primary induction of IL-17A and IL-22 drives host defense peptide expression and leukocyte recruitment. However, the NDV-3 vaccine appears to induce more rapid and targeted IL-22 expression than would occur naturally, enhancing host protection (25). The kinetics and mechanistic pathways involved in IL-22 and IL-17A induction due to *S. aureus* are under investigation.

Both IL-17A and IL-22 are known to promote host defense peptide expression in human skin (37). The present findings in this mouse model support this concept. For example, mBD-3 (the murine homologue of human β -defensin 2) and psoriasin levels in the skin were reduced in mice treated with anti-IL-17A antibody and/or anti-IL-22 antibody, while CRAMP levels were only suppressed in mice treated with anti-IL-22 antibody. The dependence of psoriasin expression on IL-17A and IL-22 has also been seen in human keratinocytes (37, 67). Interestingly, in mice that were treated with both anti-IL-17A antibody and anti-IL-22 antibody, host defense peptide expression in the skin remained low even though there was a compensatory increase in IFN- γ . Although IFN- γ is known to increase host defense peptide expression (68), our results suggest that the presence of IL-17A and IL-22 is required for maximal host defense peptide response to IFN- γ in the skin.

The current studies further indicate that IL-17A and IL-22 have cooperative roles in defense against hematogenous dissemination from SSSI. There are two key phases in host defense against hematogenous dissemination of MRSA from a skin abscess. The first or proximal phase is local control of the infection to prevent the organism from accessing the bloodstream. The second or distal phase is preventing the organism from establishing a nidus of infection after it has traveled through the bloodstream to a target organ. We found that neutralization of IL-17A alone but not of IL-22 alone resulted in a significantly greater MRSA bacterial burden in the kidneys. This outcome was associated with decreased T cell and neutrophil infiltration in renal parenchyma. These results suggest that a key role of IL-17A is to enhance the host defense against MRSA in the kidneys and thereby control the infection after hematogenous seeding has occurred.

In summary, the current findings demonstrate that IL-17A and IL-22 are both required for optimal host defense against *S. aureus* SSSI but that they mediate different immune mechanisms in skin and hematogenous settings. Our results support the model that in naive mice, IL-22 induces the expression of IL-17A, which in turn stimulates the expression of host defense peptides and the recruitment of neutrophils and T cells to sites of infection. When both IL-17A and IL-22 activities are inhibited, there is a compensatory increase in IFN- γ , which may enhance the microbicidal activity of phagocytes independent of host defense peptide expression. Moreover, IL-17A has a role that is nonredundant to the role of IL-22 in controlling hematogenous MRSA infection in the kidney

by recruiting T cells and neutrophils. Further investigation of these outcomes in comparative infection models (e.g., MRSA pneumonia) may hold additional promise to yield new insights into host defense against *S. aureus*, as well as other pathogens.

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