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Protective immunity in recurrent *Staphylococcus aureus* infection reflects localized immune signatures and macrophage-conferred memory

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Staphylococcus aureus **is the leading cause of skin and skin structure infection (SSSI), a primary portal of entry for invasive infection. Our prior studies discovered a role for protective innate memory against recurrent methicillin-resistant *S. aureus* (MRSA) SSSI. In the present study, the dynamics and mechanisms of this response were explored in recurrent SSSI in WT mice. Priming by prior infection reduced skin lesion severity and MRSA burden, and protected against dissemination at day 7 but not day 2. Cytokine and cellular signatures in SSSI differed at day 2 versus 7, and were distinct in skin versus blood or spleen. Cytokines associated with protection in skin included increased IL-17, IL-6, monokine inducible by IFN-γ (MIG), and RANTES, while increased IP-10 correlated with protection from dissemination. Cellular signatures of protection included increased Th17, M1 macrophage, and dendritic cell populations in abscesses, and total macrophages in lymph nodes. Priming potentiated *S. aureus*-specific phagocytic killing by bone marrow-derived macrophages in vitro, and their adoptive transfer into naïve skin afforded protective efficacy in vivo. Present findings indicate that protective immunity in recurrent *S. aureus* infection is locally targeted, and involves specific memory conferred by macrophages. These insights provide targets for vaccine and immunotherapeutic development against MRSA.**

Staphylococcus aureus | innate immune memory | macrophage | recurrent skin infection | adoptive transfer

The most common cause of skin and skin structure infections (SSSI) is *Staphylococcus aureus* (1–5). Skin infection serves as a primary portal of entry for hematogenous dissemination (6, 7). Despite meritorious attempts, the failure of conventional vaccines targeting *S. aureus* infections has raised key questions regarding the determinants of protective immunity versus this pathogen (8, 9). Reinforcing this concern is the high rate of recurring *S. aureus* infection among otherwise healthy individuals who have no known immune deficiencies or risk factors (10–17). The emergence of *S. aureus* resistant to many gold-standard antibiotics further compounds these issues (18–21). Thus, there is an urgent need to understand the relative contributions of innate versus adaptive immunity in defense against recurrent SSSI and ensuing invasive *S. aureus* infection.

We have previously shown that innate immune memory contributes to host defense against recurrent murine methicillin-resistant *S. aureus* (MRSA) SSSI. Using $rag1^{-/-}$ mice, which lack mature T and B cells, we discovered that prior infection (priming) resulted in a protective host response involving innate memory. This protection was highly localized to the skin, and its effectors included macrophages and Langerin^{+} dendritic cells (DC), as well as host defense peptides. In the present study, we analyzed the efficacy and mechanisms of this protective immunity in recurrent MRSA infection in WT mice, focusing on cytokine signatures and cellular effectors of immune memory.

Results

Immune Protection Differs in Local vs. Disseminated Infection. Priming resulted in localized protection, as evidenced by smaller skin lesions over the course of infection compared with naïve-infected controls, as reported previously (Fig. 1*A*) (22–24). In primed mice, MRSA burden in skin lesions was similar to naïve mice at day 2, but was significantly reduced by day 7. Importantly, while priming achieved protection in both flanks at day 7, lesions on the flank previously infected had the greatest reduction in severity. Priming initially increased the MRSA burden of the kidney and spleen at day 2, but then reduced it in these organs and the liver by day 7 (Fig. 1 *B*–*E*). Thus, priming enhances the eventual control of infection in

Significance

Staphylococcus aureus (SA) is the leading cause of skin and skin structure infections (SSSI), which are the main portal of entry for life-threatening invasive infections. Treatment failures are increasingly common due to antibiotic resistance. Importantly, SA SSSI exhibit high 1-year recurrence, despite high antibody levels against staphylococcal antigens. Therefore, critical determinants of immune protection against recurrent SA SSSI are not well understood. The present study offers important insights in this area, including: (*i*) macrophages can confer protective memory transferrable to naïve hosts; (*ii*) SA SSSI can induce memory in bone marrow myeloid precursors; and (*iii*) specific memory against recurring SA SSSI is localized and distinct from disseminated infection. These findings will accelerate novel strategies to prevent and treat SA infections.

Author contributions: L.C.C., L.S.M., S.G.F., V.G.F., E.F.R., and M.R.Y. designed research; L.C.C., M.R., C.W.J., H.K.L., and H.W. performed research; L.C.C. and M.R.Y. contributed new reagents/analytic tools; L.C.C., M.R., L.S.M., S.G.F., D.G., E.F.R., M.R.Y., and M.S.I.G. analyzed data; and L.C.C., L.S.M., S.G.F., and M.R.Y. wrote the paper.

Conflict of interest statement: M.R.Y. and S.G.F. are cofounders of NovaDigm Therapeutics, which is developing novel vaccines and immunotherapeutics for infectious diseases, including methicillin-resistant *Staphylococcus aureus*.

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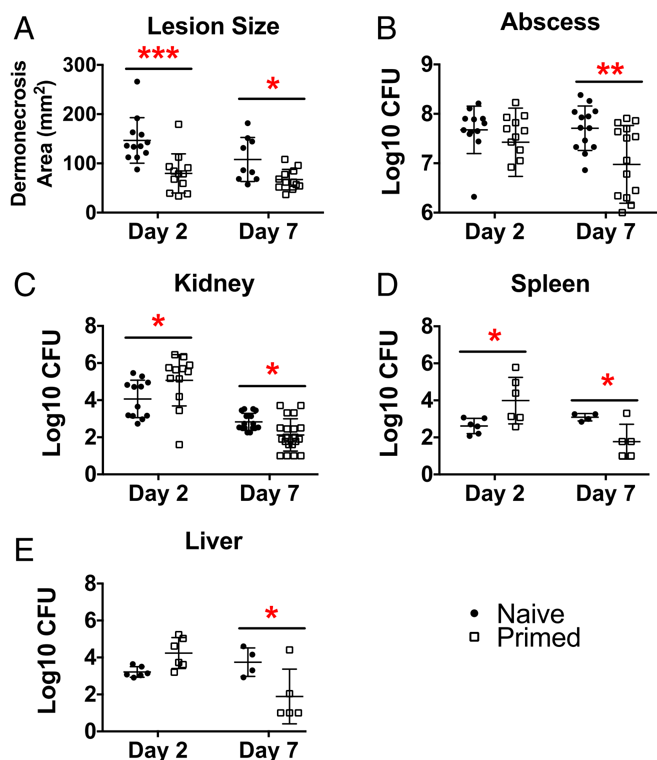

Fig. 1. Priming affords protection during recurrent MRSA SSSI. Lesion sizes of naïve and primed male C576BL/6 mice (*A*) were measured at days 2 and 7. MRSA burden in abscesses (*B*), kidney (*C*), spleen (*D*), and liver (*E*) were quantified at days 2 and 7. Bacterial burden in abscess, kidney, and spleen were quantified as CFU per abscess/organ. Burden in the liver was quantified as CFU per milligram liver. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ using Student's *t* test. At least four mice per group were analyzed.

the target organs. Collectively, these results suggest that priming affords protective immunity that is targeted, and manifests at later time points in the evolution of the infection.

Cytokine Signatures of Protection Differ in Spatial Contexts. Unsupervised proteomic analyses comparing cytokine patterns in skin, plasma, and spleen revealed distinct patterns during MRSA SSSI and invasive infection at day 2 vs. day 7. These patterns clustered into four groups: skin, plasma, spleen, and uninfected control skin (Fig. 2). In this analysis, the primed mice clustered with the naïve mice. As expected, uninfected control mice had minimal variations in the expression of most cytokines or chemokines detected. In supervised analyses, infection in skin resulted in increased proinflammatory cytokines (e.g., IL-1β, IL-6, TNF-α), chemokines (e.g., MIP-1α/β, MIP-2, KC), and markers of proliferation or differentiation (e.g., G-CSF, VEGF), compared with spleen or plasma (*SI Appendix*, Fig. S1). Notably, no specific pattern of IFN-γ response was detected. In comparison with skin, no specific patterns of cytokine responses were observed in the spleen or blood. Taken together, these results suggest that protective cytokine signatures are distinct for specific tissues during MRSA SSSI.

Priming Induces Specific Cytokine Signatures Corresponding to Protective Immunity. Specific cytokine signatures correlated with protective immunity. In skin, priming resulted in increased IL-6 expression at day 2, followed by increased IL-17 expression at day 7 (Fig. 3 *A* and *B*). Additionally, priming led to increased MIG (monokine inducible by IFN-γ) and RANTES (regulated upon activation, normal T cell expressed and secreted) in skin at day 7, cytokines that are involved in T cell recruitment and maintenance (Fig. 3 *C* and *D*). During disseminated infection, priming resulted in decreased IL-9 in the spleen and increased IP-10 in the plasma (Fig. 3 *E* and *F*). These results support a central role for Th17 responses in protective immunity of the skin, but not in hematogenous dissemination. More broadly, this pattern of findings is consistent with the hypothesis that protective immunity to MRSA involves molecular signals that correspond to specific tissue context and progression of infection.

Cellular Signatures of Protection Differ in Tissue-Specific Contexts. Cellular responses were analyzed at day 7 during recurrent MRSA infection, as this later time point corresponded with protection observed in primed mice (Fig. 1). Priming caused an increase of M1 macrophage and DC populations in skin abscesses, but a decrease in the total B cell population in skin (Fig. 4*A*). Concordant with increased IL-17 expression in skin abscesses, priming also increased the relative proportion of Th17 cells in skin, but not that of Th1 or Th2 cells, compared with naïve controls (Fig. 4*A*). Priming did not significantly change M2 macrophage, neutrophil, or NK cell populations in abscesses (Fig. 4*A*). Consistent with localized responses in the skin, draining inguinal lymph nodes (iLN) had an increased total macrophage population, but decreased B and NK cell populations (Fig. 4*B*). No significant changes were found in total T cell, polymorphonuclear leukocyte (PMN), or DC populations in the iLN of primed vs. naïve groups (Fig. 4*B*). Similar to cytokine results, no significant differences were detected in cell populations in the spleens of primed vs. naïve mice (*SI Appendix*, Fig. S2).

Macrophages Confer Protective Immunity Against MRSA. The preceding results suggested macrophages present in the skin and iLN of primed mice may be involved in protective immune memory against MRSA. To investigate this hypothesis, the role of bone marrow-derived macrophages (BMDM) from naïve and primed mice were analyzed using in vitro and in vivo models (*SI Appendix*, Fig. S3).

Priming increases macrophage staphylocidal activity in vitro. First, BMDM from naïve and primed mice were compared for their capacity to phagocytose and kill *S. aureus* with and without recall exposure to *S. aureus*. Phagocytosis of MRSA by BMDM from naïve and primed animals with and without exposure to *S. aureus* was equivalent (*SI Appendix*, Fig. S4). However, staphylocidal activity was greater in BMDM from primed mice vs. naïve mice as measured by survival of intracellular MRSA, if preexposed to heat-killed *S. aureus* (HKSA) (Fig. 5*A*). Interestingly, primed and naïve BMDMs preexposed to live *S. aureus* (LSA) were equivalent in their capacity to kill MRSA, as preexposure to LSA induced near maximal MRSA killing in BMDM. Furthermore, preexposure of BMDM to HKSA or LSA resulted in lower intracellular MRSA survival compared with untreated BMDM (Fig. 5*A*). Next, primed and naïve BMDM were tested for specific killing against comparative staphylococci, Gram-positive, and Gram-negative bacteria. Interestingly, BMDM primed with LAC-USA300 also exerted greater staphylocidal activity against a distinct *S. aureus* strain (SH1000) compared with untreated naïve BMDM (Fig. 5*B*). This protection was specific to *S. aureus*, as priming with *S. aureus* did not enhance macrophage efficacy against *Staphylococcus epidermidis* (distinct staphylococcal species), *Enterococcus faecalis* (Gram-positive bacterium of different genus), or *Escherichia coli* (Gram-negative bacterium) (Fig. 5 *C*–*E*). These in vitro data support the concept that priming of BMDM during in vivo infection affords greater antistaphylococcal activity specifically on recall to *S. aureus*.

Adoptive transfer of primed macrophages confers protective immunity in vivo. Next, we determined the in vivo relevance of primed macrophages in protection against recurring SSSI. BMDM from naïve or primed mice were recalled with either HKSA or LSA and then adoptively transferred into naïve recipient mice before

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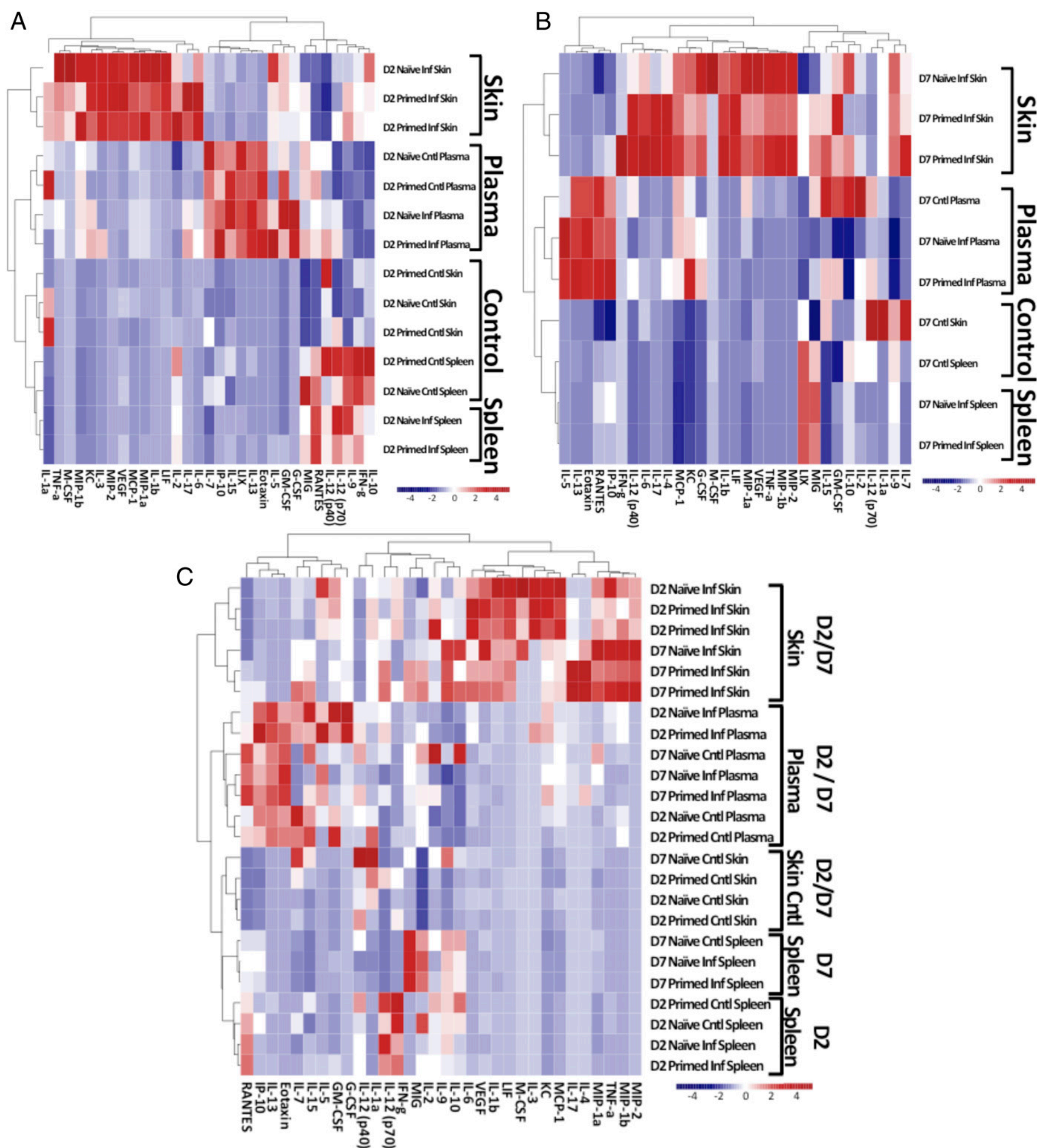

Fig. 2. MRSA SSSI results in distinct cytokine signatures in specific tissue contexts. Cytokine levels in skin, spleen, and plasma were analyzed using Luminex 32-plex assays. Data from day 2 (*A*), day 7 (*B*), and days 2 and 7 combined (*C*) were arranged via unsupervised clustering. Means of scaled values for each group were color-coded and plotted in heat maps. Unsupervised hierarchical clustering was performed on rows and columns using Euclidean distance as the similarity measure with Ward's linkage. At least four mice per group were analyzed.

MRSA SSSI challenge (*SI Appendix*, Fig. S3*B*). Transfer of BMDM from primed mice recalled by in vitro preexposure to LSA, but not untreated or HKSA, significantly reduced the abscess dermonecrosis area compared with transfer of BMDM from naïve mice (Fig. 6*A*). In the primed group, only mice receiving BMDM recalled with LSA achieved reduced abscess severity compared with controls receiving untreated BMDM (Fig. 6 *B* and *C*). Consistent with this outcome, only BMDM from primed mice recalled with LSA, but not HKSA, afforded reduced MRSA burden in abscesses compared with control (Fig. 6 *D* and *E*). Both naïve and primed BMDM recalled by exposure to either HKSA or LSA significantly reduced MRSA burden in kidney, compared with mice that did not receive

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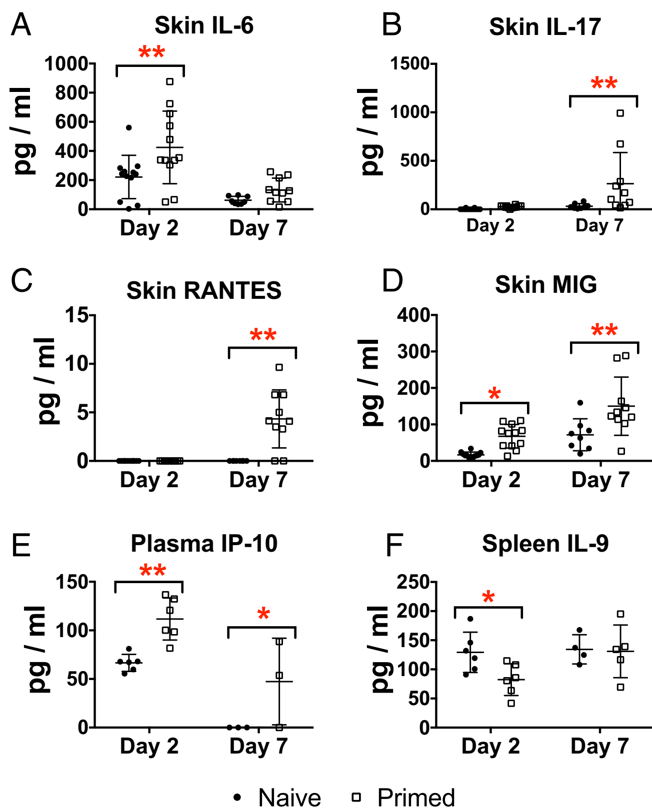

Fig. 3. Priming induces different cytokine responses in abscesses vs. blood vs. spleen during recurrent MRSA SSSI. Priming induced the expression of IL-6 and MIG in abscess at day 2 (*A* and *D*) while IL-17, RANTES, and MIG expressions were increased at day 7 (*B*–*D*). In comparison, priming induced IP-10 expression in plasma (*E*). Conversely, IL-9 expression in the spleen was decreased (*F*) at day 2. All other cytokines measured showed no difference between naïve and primed groups. $^*P < 0.05$, $^{**}P < 0.01$ using two-way ANOVA.

BMDM (Fig. 6 *F* and *G*). In contrast, neither naïve nor primed BMDM reduced the MRSA burden in the spleen (Fig. 6 *H* and *I*). Collectively, these data suggest that primary SSSI enhances protective immune memory by priming macrophages for enhanced staphylocidal activity in vivo.

Discussion

Innate immune memory in *S. aureus* infection is not well understood. The present study assessed four key aspects of protective immunity to MRSA during recurrent SSSI in a mouse model (22, 24): (*i*) time-dependent protection; (*ii*) cytokine signatures of protective immunity in skin vs. invasive infection; (*iii*) cellular correlates of protective immunity in these tissue contexts; and (*iv*) the impact of priming on the in vivo transferability of immune memory by primed macrophages. The present data suggest early cytokine responses (day 2) shape protective molecular and cellular responses later in the course of infection (day 7). A particularly significant finding was that macrophages can acquire memory during *S. aureus* infection and confer protective immunity to naïve recipient hosts.

Analysis of cytokines of primed vs. naïve infections at days 2 and 7 revealed time-dependent responses in different tissues. These responses suggest different mechanisms of protection in localized vs. disseminated infection during recurrent MRSA SSSI. Hence, the current model afforded a unique opportunity to study mechanisms of protective host responses in context. In skin, priming results in early induction of IL-6 by day 2 followed by induction of IL-17A by day 7, which correlates with increased Th17 cell presence. IL-17A, produced by Th17 and other cells, has been shown to be protective in MRSA SSSI (22, 24–27). Priming also resulted in increased DC populations in abscesses, which can produce IL-6 to mediate differentiation of Th17 cells (28–31). Furthermore, priming resulted in increased expression of T cell chemokines MIG and RANTES in the skin and IP-10 in the blood, ostensibly promoting T cell recruitment to abscesses (32–39). No changes in T cell populations were found in iLN, suggesting that the expansion of Th17 cells occurs proximate to sites of infection. In contrast, priming resulted in decreased proportions of B cells relative to total $CD45^+$ cells in the skin and draining iLN, suggesting a lesser role for humoral immunity during recurrent MRSA infection. This observation is consistent with recent staphylococcal vaccine studies targeting antibody production as the dominant protective mechanism, which did not prove effective (8, 9). Together, these results suggest that localized protective immune memory against recurring MRSA infection involves a robust and targeted Th17 response. In contrast, disseminated infection resulted in decreased IL-9 expression in the spleen, which is considered an autocrine cytokine for Th17 differentiation/expansion (40). This finding suggests that Th9-mediated immunity is not significantly involved in protection during dissemination. Moreover, no changes in cell population or other cytokine levels were detected in the spleen.

Our data strongly suggest that macrophages contribute to protection during recurrent MRSA infection. Priming resulted in increased M1 macrophage populations in the skin. In contrast to M2 macrophages, the M1 subtype is proinflammatory and involved in protection during MRSA infection (41–43). An increase in the total macrophage population, but not the proportion of M1 phenotype, was seen in the draining iLN. This finding is consistent with the concept that M1 macrophages in the skin undergo distinct polarization and functional differentiation from those in iLN. These data support the importance of IL-17A and M1 macrophages in localized protection during MRSA SSSI, as demonstrated by our prior studies and those of others (22, 24–27). The finding that primed macrophages confer protective immunity to naïve mice affirmed these cells as capable of retaining antistaphylococcal memory when recalled with *S. aureus*. This protection corresponded to greater intracellular killing of *S. aureus*, specifically by primed macrophages in vitro. Remarkably, BMDM primed with *S. aureus* conferred protection against multiple strains of *S. aureus*, but did not enhance macrophage efficacy against other bacteria, suggesting specific macrophage memory.

Memory originating from myeloid precursors in BM has recently been described by Mitroulis et al. (44), and may occur through two mechanisms: (*i*) antigenic dissemination to the BM, or (*ii*) primed immune cell traffic from periphery to BM and induced memory. Notably, BMDM underwent 14 d of differentiation in vitro before adoptive transfer and subsequent MRSA challenge, suggesting that their immune memory is durable. This concept was substantiated by the fact that only the adoptive transfer of primed BMDM recalled with LSA afforded protective immunity to naïve mice in skin during MRSA infection. These findings further imply that LSA boosts BMDM immune responses that are most relevant to localized efficacy in vivo, concordant with more robust stimulation of phagocytes by live vs. heat-killed organisms (45). Supporting the current findings, Schmaler et al. (46) also found no protective efficacy from priming mice with HKSA before LSA challenge in a mouse model of MRSA systemic infection. Similarly, Scumpia et al. (47) described differences in BMDM response to HKSA vs. LSA, through distinct Toll-like receptor (TLR)/MyD88/TRIF-dependent and -independent pathways. Collectively, our present studies support the concept that novel epigenetic mechanisms acquired during priming enable innate immune effectors, including

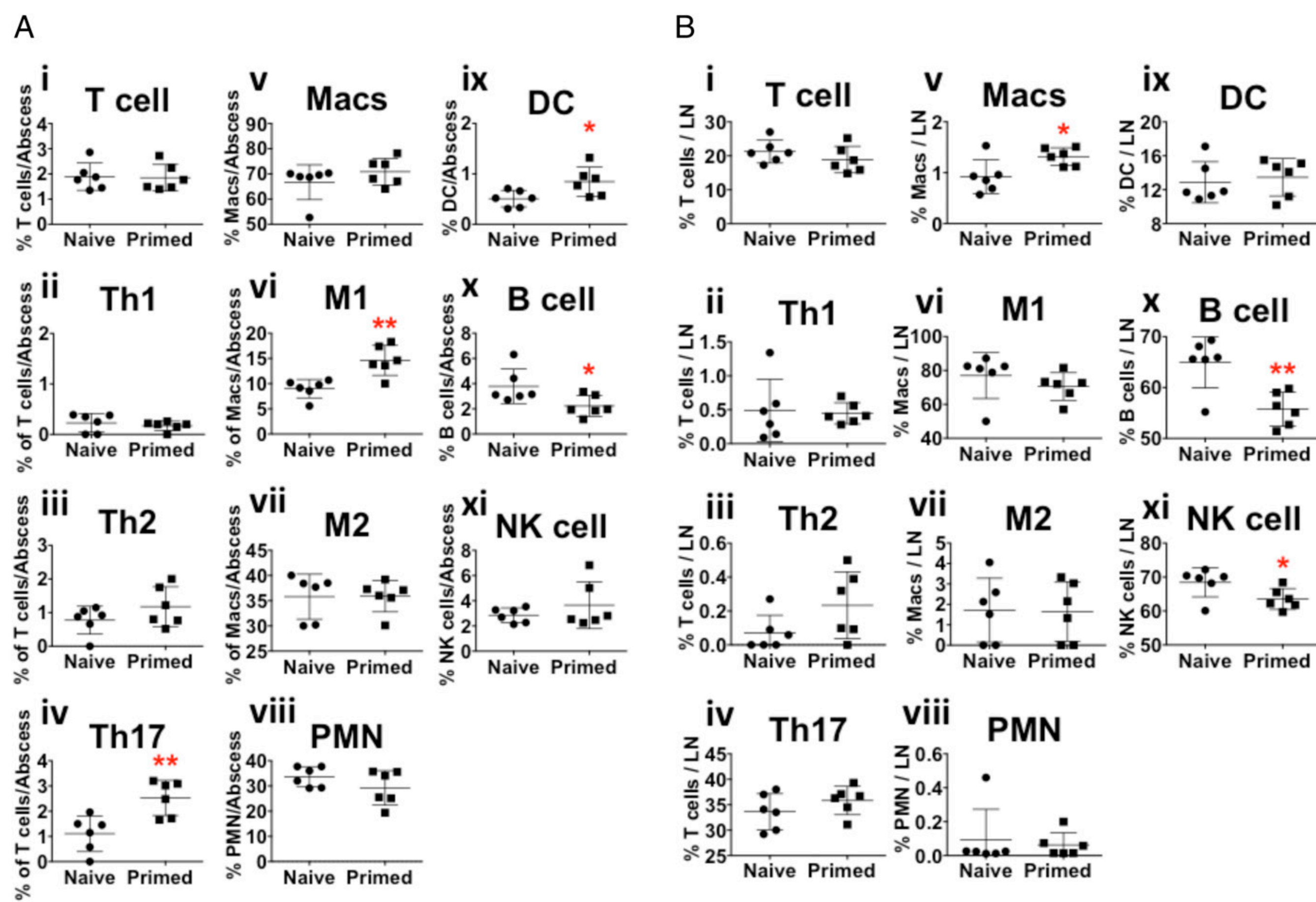

Fig. 4. Priming induces distinct changes in localized cell populations during recurrent MRSA SSSI. T cell, macrophage, neutrophil (PMN), DC, B cell, and NK cell populations were analyzed and expressed as percent of total $CD45^+$ cells in the skin (*A*) and draining iLNs (*B*). T cell subsets (Th1, Th2, and Th17) were expressed as percent of total $CD3^+$ T cells. Macrophage subsets (M1 and M2) were expressed as proportion of total macrophages. $^*P < 0.05$, $^{**}P < 0.01$ using Student's t test. At least six samples per group were analyzed.

macrophages, to develop memory that can be specifically recalled and transferred (48–50).

There are limitations to the interpretation of the present work. Mouse models are imperfect surrogates for studying human infection, and further studies are needed to understand protective immune memory in human *S. aureus* SSSI. Moreover, the extensive scope of investigations performed required a focus on one genetic background of mice. Parallel studies in other mouse strains will be informative to further dissect the specific genotypic and phenotypic determinants of protective immunity to *S. aureus*. Finally, we did not exhaust the cell types that may contribute to the observed immune memory. For example, the complete repertoire of cells responsible for production of increased IL-17A at the abscess site was beyond the scope of the present study. Dillen et al. (51) recently demonstrated that γδ T cells are important producers of IL-17A in protective immune responses against *S. aureus* infection of the skin. Our ongoing studies are further defining the molecular and cellular mechanisms essential to protective immune memory against *S. aureus* infection. This knowledge will enable new antiinfective, vaccine, and immunotherapeutics strategies to meet the challenge of antibiotic-resistant MRSA infections.

In conclusion, current studies support the hypothesis that immune memory is integral to protection against recurrent MRSA SSSI. The mechanisms through which this protection occurs involve early innate and adaptive cytokine responses that shape later Th17 polarization and M1 macrophage intensification in the skin. Furthermore, protective responses in the skin were localized and distinct from those of disseminated infection. Ultimately, the knowledge gained through this work and investigating epigenetic markers that may be essential to the protective mechanisms of primed BM cells and macrophages should provide important insights and strategies into new, targeted antiinfectives and vaccines to meet the challenge of antibiotic-resistant MRSA infections.

Methods

Bacterial Strains and Preparation. This study utilized MRSA strain LAC, a USA300 strain isolated from an outbreak at the Los Angeles County Jail and the phenotypic and genotypic profile of this strain has been thoroughly characterized previously (52). Studies also included methicillin-sensitive *S. aureus* strain SH1000 [a well-characterized laboratory strain (53)], *S. epidermidis* (ATCC 12228), *E. faecalis* (ATCC 29212), or *E. coli* (ATCC 43889). Bacteria were cultured from virulence-validated master cell banks and grown to log-phase in BHI medium at 37 °C. Resulting cells were harvested, washed, suspended in PBS, sonicated, quantified by spectrophotometry, and diluted to the desired CFU in PBS or RPMI for use in vitro or in vivo. To generate heat-killed LAC, bacteria were prepared as described above and boiled for 30 min.

Mouse of Model of Recurrent SSSI. Animal studies were performed in accordance with approved animal use policies of the Los Angeles Biomedical Research Institute at Harbor–University of California, Los Angeles following NIH guidelines. Male C57BL/6 (WT) mice (20–25 g; Jackson Labs) were studied using a subcutaneous SSSI model as we have previously detailed (22–24). For primary infection, SSSI was established via inoculation with ∼1×10^7 CFU

Fig. 5. Primed BMDM exhibit increased staphylocidal activity. Intracellular survival of bacteria in untreated (circles), HKSA-treated (triangles), and LSA-treated (squares) BMDM from naïve (filled symbols) and primed mice (open symbols) were measured 3-h postinfection (MOI 1:50). Naïve and primed BMDM were challenged with LAC (MRSA) (*A*), SH1000 (MSSA) (*B*), *S. epidermidis* (*C*), *E. faecalis* (*D*), or *E. coli* (*E*). $^{*}P < 0.05$, $^{***}P < 0.001$ using two-way ANOVA. Data are presented as the average and SD of points from a minimum of two independent experiments.

MRSA in 100 μL PBS by subcutaneous injection into the right flank. Control animals received an identical volume of PBS buffer alone. In infected animals, abscesses formed along with corresponding dermonecrosis, as anticipated, and were then permitted to completely resolve over 6 wk. For recurring SSSI, the same previously infected (primed) mice were then reinfected using an identical regimen in both the right and left flanks (6 wk postinitial infection). Lesions were analyzed over the study period. At days 2 or 7 postinfection, the mice were humanely killed for tissue analysis. Study design schematic can be found in *SI Appendix*, Fig. S3*A*.

Skin Lesion Severity. Dermonecrosis areas of each flank were measured in every mouse over the postchallenge study periods. To do so, mice were anesthetized and the dermonecrosis area [square millimeter; lesion length (*l*) and width (*w*)] was quantified for each lesion, as previously reported (22–24).

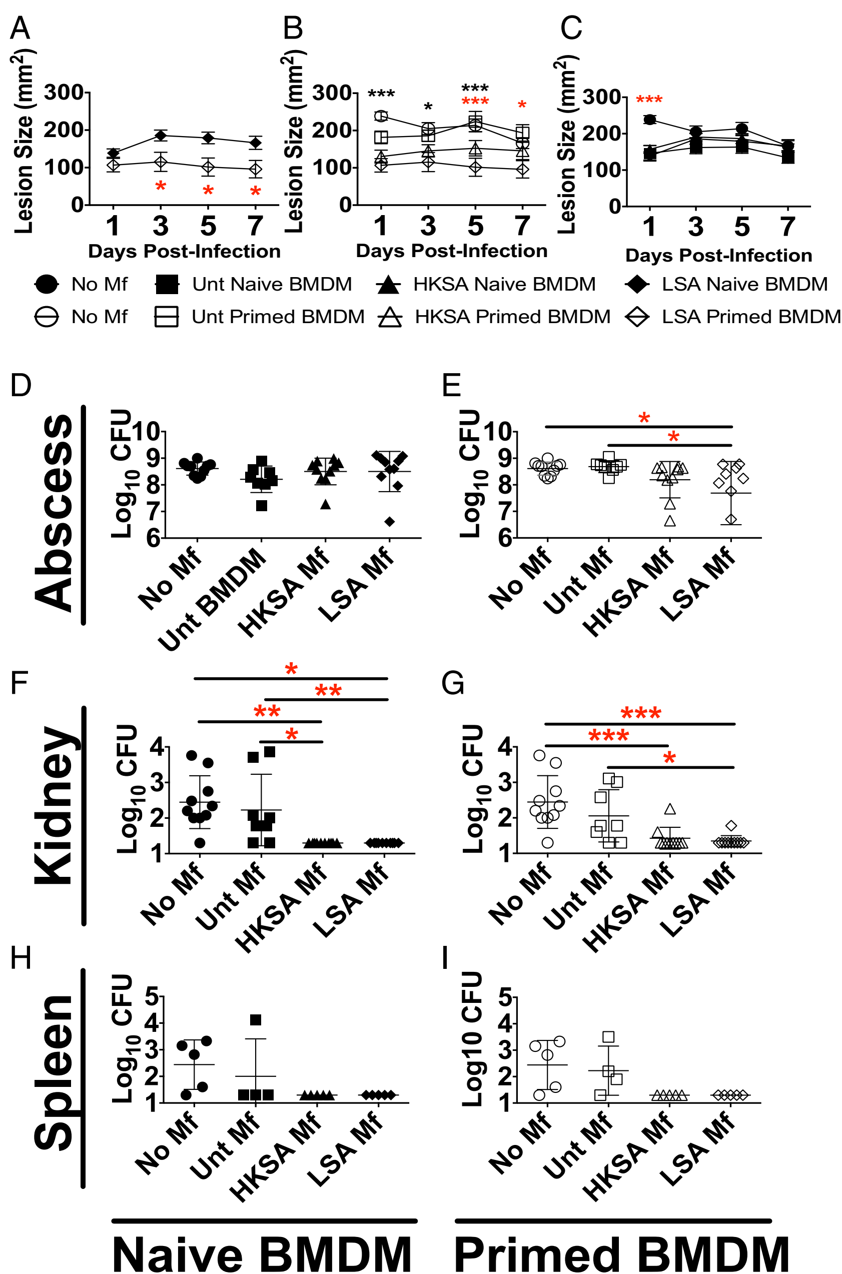

Fig. 6. Adoptive transfer of primed BMDM recalled with LSA protects naïve mice from localized and disseminated MRSA SSSI. BMDM from naïve (filled symbols) and primed (open symbols) mice treated with PBS (squares), HKSA (triangles), or LSA (diamonds) was adoptively transferred into the skin of naïve mice. Four-hours postadoptive transfer, mice were incoculate with MRSA subcutaneously (*SI Appendix*, Fig. S3*B*). Dermonecrosis area was measured at days 1, 3, 5, and 7 (*A*–*C*). Adoptive transfer of primed BMDM recalled with LSA resulted in smaller lesions compared with naïve BMDM recalled with LSA (*A*). Adoptive transfer of BMDM from primed (*B*) and naïve (*C*) mice (±SA treatment) were compared (red asterisk = LSA vs. no macrophage; black asterisk = LSA vs. untreated macrophage). At day 7, MRSA CFU was quantified from abscesses (*D* and *E*), kidneys (*F* and *G*), and spleens (*H* and *I*). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ using one-way ANOVA corrected for multiple comparison. At least four mice per group were analyzed.

MRSA Tissue Burden. At the 2- or 7-d study endpoint, mice were killed and skin abscesses, kidneys, spleen, and liver were enumerated ex vivo via CFU after overnight culture on plates (lower limit of detection = 20 CFU per sample). In parallel, a portion of tissue homogenate was reserved for cytokine analysis as detailed below.

Luminex xMAP Immunoassay. Mouse 32-plex magnetic cytokine/chemokine kits (EMD Millipore) were used per the manufacturer's instructions and fluorescence quantified using Luminex 200 instrument by the University of California, Los Angeles Immune Assessment Core. Cytokine/chemokine concentrations were calculated using Milliplex Analyst software v4.2 (EMD Millipore). Tissue homogenates were normalized by total protein content to 330 μg/mL by Coomassie Protein Assay Kit (Thermo Scientific).

Cytokine Heat Maps. For hierarchical clustering analysis, analyte abundances irrespective of conditions were normalized using *z*-scaling. Then, means of scaled values for each group were color-coded and plotted in heat maps. Unsupervised hierarchical clustering was performed on rows and columns using Euclidean distance as the similarity measure with Ward's linkage. R v3.3.1 was used (54).

Immune Cell Subset Profiles. iLNs and spleen of each mouse were mechanically dissociated and cell isolated (23). Skin abscesses were processed and cells isolated per protocol (55). Briefly, skin abscesses were aseptically dissected and placed into skin isolation buffer (HBSS, 10% FBS, 5 mM EDTA, 10 mM Hepes) for 30 min at 150 rpm. Abscesses were placed into fresh media containing collagenase D, chopped, and incubated for 30 min at 150 rpm.

Skin fragments were removed and cells isolated from media. Cells were stained with fluorochrome-conjugated antibodies (*SI Appendix*, Table S1) and analyzed by multicolor flow cytometric analysis using an LSRII cytometer (Becton-Dickinson). Intranuclear molecules were accessed for staining using an established protocol for cell permeabilization (Thermo Fisher). Gating strategies can be found in *SI Appendix*, Fig. S5.

BMDM. BMDM were differentiated as previously described (56). Briefly, BM cells were isolated from femurs and tibias of naïve or primed WT mice. Cells were differentiated in BMDM media [DMEM/F12, 10% FBS, 25 mM L-glutamate, penicillin/streptomycin, 500 U/mL recombinant mouse M-CSF (Biolegend)]. On day 10, cells were challenged with heat-killed MRSA or live MRSA [multiplicity of infection (MOI) 1:1] and permitted to acclimate for 4 d. The BMDM were used on day 14. Experimental design for BMDM differentiation and subsequent use in experiments can be found in *SI Appendix*, Fig. S3*B*.

BMDM Intracellular Survival Assay. The BMDM (5×10^5 cells) were plated on sterile 18-mm glass coverslips and challenged with MRSA. After 30 min, gentamicin (50 μg/mL final concentration) was added to kill extracellular bacteria. At indicated time points, BMDM were lysed and surviving MRSA CFU were enumerated after overnight culture on plates.

Macrophage Adoptive Transfer in MRSA SSSI. The BMDM (~5×10^5 BMDM/100 μL) were adoptively transferred into the right flank of naïve WT mice. Control animals received media alone. Four hours postadoptive transfer, SSSI was established via inoculation with ~1×10^7 CFU of MRSA by subcutaneous injection into both flanks. Lesions were monitored over 7 d, at which point the mice were killed and the abscesses were excised for quantitative culture. Schematic for experimental design can be found in *SI Appendix*, Fig. S3*B*.

Statistical Analyses. Differences in experimental results were compared by ANOVA or Student's *t* test as indicated. Data analyzed by GraphPad Prism software and reported as mean ± SD. *P* values of <0.05 were considered statistically significant.

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1. Grinberg A, et al. (2017) Genomic epidemiology of methicillin-susceptible *Staphylococcus aureus* across colonisation and skin and soft tissue infection. *J Infect* 75: 326–335.
2. Ahmad MK, Asrar A (2014) Prevalence of methicillin resistant *Staphylococcus aureus* in pyogenic community and hospital acquired skin and soft tissues infections. *J Pak Med Assoc* 64:892–895.
3. Borgundvaag B, Ng W, Rowe B, Katz K; EMERGency Department Emerging Infectious Disease Surveillance NeTwork (EMERGENT) Working Group (2013) Prevalence of methicillin-resistant *Staphylococcus aureus* in skin and soft tissue infections in patients presenting to Canadian emergency departments. *CJEM* 15:141–160.
4. Ellis MW, et al. (2014) Prevalence of nasal colonization and strain concordance in patients with community-associated *Staphylococcus aureus* skin and soft-tissue infections. *Infect Control Hosp Epidemiol* 35:1251–1256.
5. Kumar N, David MZ, Boyle-Vavra S, Sieth J, Daum RS (2015) High *Staphylococcus aureus* colonization prevalence among patients with skin and soft tissue infections and controls in an urban emergency department. *J Clin Microbiol* 53:810–815.
6. Tattevin P, et al. (2012) Concurrent epidemics of skin and soft tissue infection and bloodstream infection due to community-associated methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 55:781–788.
7. Landrum ML, et al. (2012) Epidemiology of *Staphylococcus aureus* blood and skin and soft tissue infections in the US military health system, 2005-2010. *JAMA* 308:50–59.
8. Fowler VG, Jr, Proctor RA (2014) Where does a *Staphylococcus aureus* vaccine stand? *Clin Microbiol Infect* 20(Suppl 5):66–75.
9. Proctor RA (2015) Recent developments for *Staphylococcus aureus* vaccines: Clinical and basic science challenges. *Eur Cell Mater* 30:315–326.
10. Shastry L, Rahimian J, Lascher S (2007) Community-associated methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections in men who have sex with men in New York City. *Arch Intern Med* 167:854–857.
11. Skiest D, et al. (2006) Community-onset methicillin-resistant *Staphylococcus aureus* in an urban HIV clinic. *HIV Med* 7:361–368.
12. Anderson EJ, Hawkins C, Bolon MK, Palella FJ, Jr (2006) A series of skin and soft tissue infections due to methicillin-resistant *Staphylococcus aureus* in HIV-infected patients. *J Acquir Immune Defic Syndr* 41:125–127.
13. Mascitti KB, Gerber JS, Zaoutis TE, Barton TD, Lautenbach E (2010) Preferred treatment and prevention strategies for recurrent community-associated methicillin-resistant *Staphylococcus aureus* skin and soft-tissue infections: A survey of adult and pediatric providers. *Am J Infect Control* 38:324–328.
14. Stevens DL, et al.; Infectious Diseases Society of America (2005) Practice guidelines for the diagnosis and management of skin and soft-tissue infections. *Clin Infect Dis* 41: 1373–1406, and erratum (2006) 42:1219.
15. Shallcross LJ, Hayward AC, Johnson AM, Petersen I (2015) Evidence for increasing severity of community-onset boils and abscesses in UK general practice. *Epidemiol Infect* 143:2426–2429.
16. Shallcross LJ, Hayward AC, Johnson AM, Petersen I (2015) Incidence and recurrence of boils and abscesses within the first year: A cohort study in UK primary care. *Br J Gen Pract* 65:e668–e676.
17. Del Giudice P, et al. (2011) Clinical manifestations and outcome of skin infections caused by the community-acquired methicillin-resistant *Staphylococcus aureus* clone ST80-IV. *J Eur Acad Dermatol Venereol* 25:164–169.
18. Amin AN, et al. (2014) Hospitalist perspective on the treatment of skin and soft tissue infections. *Mayo Clin Proc* 89:1436–1451.
19. Fernandez R, Paz LI, Rosato RR, Rosato AE (2014) Ceftaroline is active against heteroresistant methicillin-resistant *Staphylococcus aureus* clinical strains despite associated mutational mechanisms and intermediate levels of resistance. *Antimicrob Agents Chemother* 58:5736–5746.
20. Hanaki H, et al. (2014) Antibiotic susceptibility survey of blood-borne MRSA isolates in Japan from 2008 through 2011. *J Infect Chemother* 20:527–534.
21. Saravolatz SN, Martin H, Pawlak J, Johnson LB, Saravolatz LD (2014) Ceftaroline-heteroresistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 58:3133–3136.
22. Chan LC, et al. (2015) 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*. *Infect Immun* 83:4427–4437.
23. Chan LC, et al. (2017) Innate immune memory contributes to host defense against recurrent skin and skin structure infections caused by methicillin-resistant *Staphylococcus aureus*. *Infect Immun* 85:e00876-16.
24. Yeaman MR, et al. (2014) Mechanisms of NDV-3 vaccine efficacy in MRSA skin versus invasive infection. *Proc Natl Acad Sci USA* 111:E5555–E5563.
25. Miller LS, Cho JS (2011) Immunity against *Staphylococcus aureus* cutaneous infections. *Nat Rev Immunol* 11:505–518.
26. Maher BM, et al. (2013) Nlrp-3-driven interleukin 17 production by γδT cells controls infection outcomes during *Staphylococcus aureus* surgical site infection. *Infect Immun* 81:4478–4489.
27. Montgomery CP, et al. (2014) Protective immunity against recurrent *Staphylococcus aureus* skin infection requires antibody and interleukin-17A. *Infect Immun* 82: 2125–2134.
28. Chen Y, et al. (2014) Adipose tissue dendritic cells enhances inflammation by prompting the generation of Th17 cells. *PLoS One* 9:e92450.
29. Jung MY, Son MH, Kim SH, Cho D, Kim TS (2011) IL-32gamma induces the maturation of dendritic cells with Th1- and Th17-polarizing ability through enhanced IL-12 and IL-6 production. *J Immunol* 186:6848–6859.
30. Linehan JL, et al. (2015) Generation of Th17 cells in response to intranasal infection requires TGF-β1 from dendritic cells and IL-6 from CD301b+ dendritic cells. *Proc Natl Acad Sci USA* 112:12782–12787.
31. Zheng T, et al. (2018) p38α signaling in Langerhans cells promotes the development of IL-17-producing T cells and psoriasiform skin inflammation. *Sci Signal* 11:eaao1685.
32. Arai K, Liu ZX, Lane T, Dennert G (2002) IP-10 and Mig facilitate accumulation of T cells in the virus-infected liver. *Cell Immunol* 219:48–56.
33. Dufour JH, et al. (2002) IFN-gamma-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. *J Immunol* 168:3195–3204.
34. Eck M, et al. (2000) CXC chemokines Gro(alpha)/IL-8 and IP-10/MIG in *Helicobacter pylori* gastritis. *Clin Exp Immunol* 122:192–199.
35. Groom JR, Luster AD (2011) CXCR3 in T cell function. *Exp Cell Res* 317:620–631.
36. Lee JH, et al. (2017) Pathogenic roles of CXCL10 signaling through CXCR3 and TLR4 in macrophages and T cells: Relevance for arthritis. *Arthritis Res Ther* 19:163.
37. Liu M, et al. (2011) CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. *Cytokine Growth Factor Rev* 22:121–130.
38. Mikolajczyk TP, et al. (2016) Role of chemokine RANTES in the regulation of perivascular inflammation, T-cell accumulation, and vascular dysfunction in hypertension. *FASEB J* 30:1987–1999.
39. Tedla N, et al. (1996) Chemokines and T lymphocyte recruitment to lymph nodes in HIV infection. *Am J Pathol* 148:1367–1373.
40. Elyaman W, et al. (2009) IL-9 induces differentiation of TH17 cells and enhances function of FoxP3+ natural regulatory T cells. *Proc Natl Acad Sci USA* 106: 12885–12890.
41. Hanke ML, Heim CE, Angle A, Sanderson SD, Kielian T (2013) Targeting macrophage activation for the prevention and treatment of *Staphylococcus aureus* biofilm infections. *J Immunol* 190:2159–2168.
42. Krysko O, et al. (2011) Alternatively activated macrophages and impaired phagocytosis of *S. aureus* in chronic rhinosinusitis. *Allergy* 66:396–403.
43. Thurlow LR, et al. (2011) Staphylococcus aureus biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. *J Immunol* 186:6585–6596.
44. Mitroulis I, et al. (2018) Modulation of myelopoiesis progenitors is an integral component of trained immunity. *Cell* 172:147–161.e12.
45. DeChatelet LR, Mullikin D, Shirley PS, McCall CE (1974) Phagocytosis of live versus heat-killed bacteria by human polymorphonuclear leukocytes. *Infect Immun* 10: 25–29.

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46. Schmaler M, Jann NJ, Ferracin F, Landmann R (2011) T and B cells are not required for clearing *Staphylococcus aureus* in systemic infection despite a strong TLR2-MyD88-dependent T cell activation. *J Immunol* 186:443–452.
47. Scumpia PO, et al. (2017) Opposing roles of toll-like receptor and cytosolic DNA-STING signaling pathways for *Staphylococcus aureus* cutaneous host defense. *PLoS Pathog* 13:e1006496.
48. Leentjens J, et al. (2018) Trained innate immunity as a novel mechanism linking infection and the development of atherosclerosis. *Circ Res* 122:664–669.
49. Saeed S, et al. (2014) Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science* 345:1251086.
50. Quintin J, et al. (2012) *Candida albicans* infection affords protection against reinfection via functional reprogramming of monocytes. *Cell Host Microbe* 12:223–232.
51. Dillen CA, et al. (2018) Clonally expanded γδ T cells protect against *Staphylococcus aureus* skin reinfection. *J Clin Invest* 128:1026–1042.
52. Burlak C, et al. (2007) Global analysis of community-associated methicillin-resistant *Staphylococcus aureus* exoproteins reveals molecules produced in vitro and during infection. *Cell Microbiol* 9:1172–1190.
53. O'Neill AJ (2010) Staphylococcus aureus SH1000 and 8325-4: Comparative genome sequences of key laboratory strains in staphylococcal research. *Lett Appl Microbiol* 51: 358–361.
54. R Foundation (2016) R, Version 3.3.1. Available at https://www.r-project.org. Accessed on September 9, 2016.
55. Benck CJ, Martinov T, Fife BT, Chatterjea D (2016) Isolation of infiltrating leukocytes from mouse skin using enzymatic digest and gradient separation. *J Vis Exp,* e53638.
56. Pineda-Torra I, Gage M, de Juan A, Pello OM (2015) Isolation, culture, and polarization of murine bone marrow-derived and peritoneal macrophages. *Methods Mol Biol* 1339:101–109.