

Abbreviations used

AD: Atopic dermatitis
AMP: Antimicrobial peptide
ILC: Innate lymphoid cell
WT: Wild-type

promoting a healthy skin barrier.¹⁷ Indeed, IL-22 induces the secretion of antimicrobial peptides by keratinocytes *in vitro*,¹⁸ and is necessary for the expression of neutrophil-attracting chemokines in a mouse model of psoriasis driven by topical application of the TLR7/8 agonist imiquimod.¹⁹

AD skin lesions exhibit increased *IL22* expression and infiltration by IL-22-expressing T cells.²⁰ The role of IL-22 in controlling *S aureus* infection in the skin is not well understood. We demonstrate that scratching human skin or tape stripping mouse skin, a surrogate for scratching, induces local IL-22 expression, and that IL-22 is important for limiting the growth of *S aureus* on mouse skin mechanically injured by tape stripping. These findings have important implications for AD; they caution that IL-22 blockade in patients with AD may enhance susceptibility to staphylococcal skin infection.

METHODS**Mice**

C57BL/6J wild-type (WT) and *Rorc*^{-/-} mice were purchased from the Jackson Laboratory (Bar Harbor, Me). *Rag2*^{-/-} mice were purchased from Taconic (Rensselaer, NY). BALB/c WT mice were purchased from Charles River Laboratory (Wilmington, Mass). *IL22*^{-/-} mice were previously described.²¹ *Tcrd*^{-/-} mice were provided by Dr Joonsoo Kang (University of Massachusetts Medical School, Worcester) and also purchased from Jackson Laboratory. *IL23*^{-/-} (*p19*^{-/-}) mice were a gift of Dr Georges Tsokos (Beth Israel Deaconess Medical Center, Boston, Mass). All mice were kept in a pathogen-free environment. All procedures performed on the mice were in accordance with the Animal Care and Use Committee of the Children's Hospital Boston.

Skin cell preparation and flow cytometry

Skin pieces (1 cm²) from unmanipulated or tape-stripped mice were obtained. Skin pieces were finely chopped using scissors after fat removal and digested for 90 minutes in the media containing Liberase (0.2 mg/mL; Roche, Indianapolis, Ind) and DNase II (Sigma, St Louis, Mo), with continuous shaking at 37°C. Digested skin homogenates were filtered, washed, and resuspended in PBS and used for flow cytometry. Cells were preincubated with FcγR-specific blocking mAb (2.4G2) and washed before staining with the following mAbs: APC-anti-CD45, BV605-anti-CD11b (Biolegend), FITC - anti-Gr1 (Biolegend), PercpCy5.5 γδ TCR (ebioscience), and CD3 efluor-450 (ebioscience). Cells were analyzed on LSR Fortessa (BD Biosciences), and the data were analyzed with FlowJo software (TreeStar, Ashland, Ore).

IL-22 detection

For IL-22 detection by flow cytometry, isolated skin cells were incubated with PMA (10 ng/mL) and ionomycin (500 ng/mL) and IL-23 (50 ng/mL) with Golgi-stop and Golgi-plug for 5 hours in Iscove's modified Dulbecco modified Eagle medium. Intracellular staining was performed using BD cytokine detection kit. APC-anti-IL-17A (ebioscience) and PE-anti-IL-22 (Biolegend) were added to permeabilized cells after surface staining with antibodies against CD3, CD11b, CD19, CD4, and γδ TCR.

For quantitative PCR, skin cells were isolated from tape-stripped skin using Liberase and DNase as described earlier. Single-cell suspensions were stained with antibodies against CD45, CD3, CD49b, FcεRIα, CD90, CD117, and

TCRδ. Antibodies against CD11b, CD11c, class II, F4-80, and Gr1 were added in a single color to define the lineage gate. Mast cells were identified as CD45⁺CD3⁻Lin⁻CD117⁺FcεRIα⁺ population, innate lymphoid cells (ILCs) were identified as CD45⁺CD3⁻Lin⁻CD49b⁺FcεRIα⁻CD90⁺ cells, and γδ T cells were identified as CD45⁺CD3⁺TCRδ⁺ cells. All populations were sorted on FACS Aria II with purity of more than 95%. Total RNA was extracted from sorted cells using RNeasy Micro Kit (Qiagen, Valencia, Calif). cDNA preparation and quantitative PCR details are described below.

Tape stripping of mouse skin and quantitative RT-PCR

Ears or shaved back skin of anesthetized mice was tape stripped 6 times. At indicated times after tape stripping, total skin RNA was extracted with Total RNA Isolation Kit (Ambion; purchased through Thermo Fisher Scientific, Franklin, Mass). cDNA was prepared with iscript cDNA synthesis kit (Biorad, Hercules, Calif). PCR reactions were run on ABI Prism 7300 (Applied Biosystems) sequence detection system platform. Taqman primers and probes were obtained from Life Technologies (Thermo Fisher Scientific). The housekeeping gene β₂-microglobulin was used as an internal control. Relative mRNA expression was quantified using the 2^{-ΔΔC_t} method.

Skin explant culture and *in vitro* cytokine expression

Tape-stripped skin explants (1 cm²) were chopped with scissors and cultured in complete RPMI. The supernatants were harvested after 18 hours and IL-22 was measured by an ELISA kit according to the manufacturer's instructions (sensitivity, 8 pg/mL; eBioscience).

Quantification of IL-22 levels in scratched human skin

After obtaining informed consent, the inner side of the forearm of 2 healthy nonallergic adult male subjects aged 35 and 69 years was scratched 30 times with a #11 sterile blade, with care to not draw any blood. Six hours later, a 4-mm punch biopsy was obtained from the scratched site and another one from an unscratched skin site on the contralateral forearm. The skin sample was chopped with scissors and cultured in complete RPMI medium for 18 hours and IL-22 protein released in the supernatants was measured by ELISA (eBioscience).

***S aureus* infection and quantification of skin infection**

The community-acquired methicillin-resistant *S aureus* USA300 SF8300 strain, a kind gift of Dr Binh Diep (University of California at San Francisco), was cultured in tryptic soy broth as previously described.¹⁵ Briefly, an *S aureus* inoculum was streaked onto tryptic soy agar plate and grown overnight at 37°C. Single colonies were picked and inoculated into a 5-mL tube containing tryptic soy broth and cultured overnight in a shaking incubator. The following morning, 1:50 dilution of bacterial suspension was inoculated in 5 mL of tryptic soy broth and cultured for another 2 hours. Bacterial concentrations were estimated by measuring absorbance at 600 nm. The bacteria were concentrated to 10⁸ colony-forming units/50 μL of PBS, and used for cutaneous infection. Colony-forming units were verified by overnight culturing of inoculum on Chrom-agar plates. To enumerate the bacterial load from the skin, two 8-mm² skin biopsies were obtained. After mechanical homogenization, serial dilutions of skin homogenates were cultured on Chrom-agar plates. The growth of USA300 strain was quantified by counting only pink colonies after overnight incubation.

Neutrophil depletion

Neutrophils were depleted 24 hours before tape stripping by intraperitoneal injection of anti-Ly6G antibody (1A8, BioXcell, West Lebanon, NH). The depletion was confirmed by FACS analysis for neutrophils in the blood and skin of tape-stripped mice.

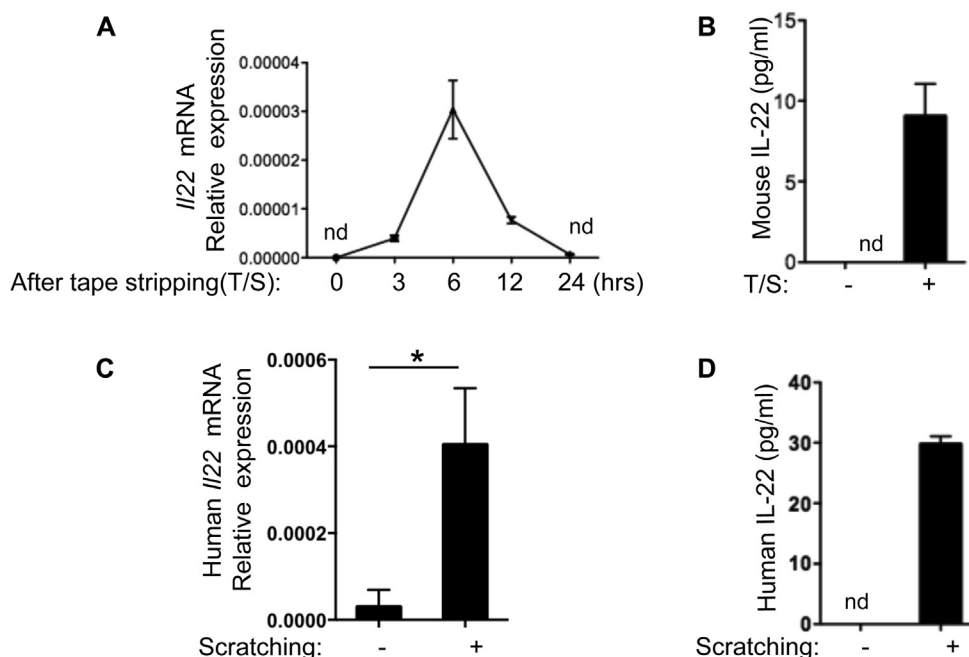


FIG 1. Mechanical injury induces IL-22 expression in the skin. **A**, IL-22 mRNA expression levels in the skin from WT (Balb/c) mice at 0, 3, 6, 12, and 24 hours after tape stripping ($n = 3$ per group). The expression can be detected at 3 hours, peaks at 6 hours, and is undetectable (nd) at 24 hours. **B**, IL-22 protein levels in the supernatants were quantitated after culturing the tape-stripped skin for 18 hours. **C**, IL-22 mRNA expression in scratched versus unscratched human skin biopsies. One representative experiment out of 2, with a total of 3 samples, is shown. **D**, IL-22 protein levels quantitated by ELISA in supernatants from cultured scratched and control skin. Mean with SEM is shown. * $P < .05$.

Histological analysis

Hematoxylin and eosin staining was performed using the formalin-fixed skin biopsies by the Histology core of Children's Hospital. ImageJ was used for the quantification of the epidermal thickness.

Statistical analysis

Depending on the number of sample groups and sample size, statistical significance was determined by Mann-Whitney test, Student t test, or 1-way ANOVA analysis on graph-pad prism. A P value of less than .05 was considered statistically significant.

RESULTS

Mechanical injury induces IL-22 expression in the skin

We examined IL-22 mRNA expression in mouse skin mechanically injured by tape stripping. IL-22 mRNA was not detectable in unmanipulated mouse skin, but became readily detectable in the skin 6 hours after tape stripping, and was no longer detectable 24 hours later (Fig 1, A). More importantly, IL-22 was released into the supernatants by explants of tape-stripped skin, but not unmanipulated control skin (Fig 1, B).

We also investigated whether mechanical injury inflicted by scratching causes IL-22 expression in human skin. IL-22 mRNA expression was barely detectable in unmanipulated human skin, but was readily detected 6 hours postscratching (Fig 1, C). IL-22 was released into the supernatants by explants of scratched human skin, but not control unmanipulated skin (Fig 1, D). These results demonstrate that mechanical skin injury induces IL-22 expression in both mouse and human skin.

$\gamma\delta$ T cells are the major IL-22-producing cells in response to mechanical skin injury

IL-22 is produced by adaptive $\alpha\beta$ T_H17/22 cells as well as by innate lymphocytes such as $\gamma\delta$ T cells and type-3 innate lymphoid cells.¹⁶ To determine the source of IL-22 released following tape stripping, we examined *Rag2*^{-/-} mice, which lack all mature T and B cells. IL-22 mRNA induction in the skin by tape stripping was virtually absent in *Rag2*^{-/-} mice (Fig 2, A), demonstrating an absolute requirement for mature T cells. IL-22 mRNA induction by tape stripping the skin was strikingly reduced in *Tcrd*^{-/-} mice, indicating that $\gamma\delta$ T cells contribute to the IL-22 induced by tape stripping (Fig 2, B). We further determined whether $\gamma\delta$ T cells are the primary producers of IL-22 upon tape stripping in murine skin. Flow-cytometric analysis of skin cells after stimulation with PMA and ionomycin and IL-23 for 5 hours demonstrated that Lineage-positive, but not Lineage-negative cells, stained intracellularly for IL-22 (see Fig E1, A, in this article's Online Repository at www.jacionline.org). Importantly, intracellular IL-22 expression was restricted to CD3⁺ $\gamma\delta$ T cells (Fig E1, A). Detection of intracellular IL-22 in the Lineage-positive cells from tape-stripped skin required *in vitro* stimulation with IL-23 along PMA and ionomycin. We therefore also sorted $\gamma\delta$ T cells, ILCs, and mast cells from tape-stripped skin and examined their expression of IL-22 mRNA without *in vitro* stimulation. Real-time PCR analysis of RNA prepared from these cells confirmed the finding that $\gamma\delta$ T cells were the major source of IL-22 (Fig E1, B). ILCs, which represent a much smaller proportion of skin immune cells, compared with $\gamma\delta$ T cells (50-fold lower number) demonstrated only weak expression of IL-22 mRNA, whereas mast cells had no detectable IL-22 mRNA.

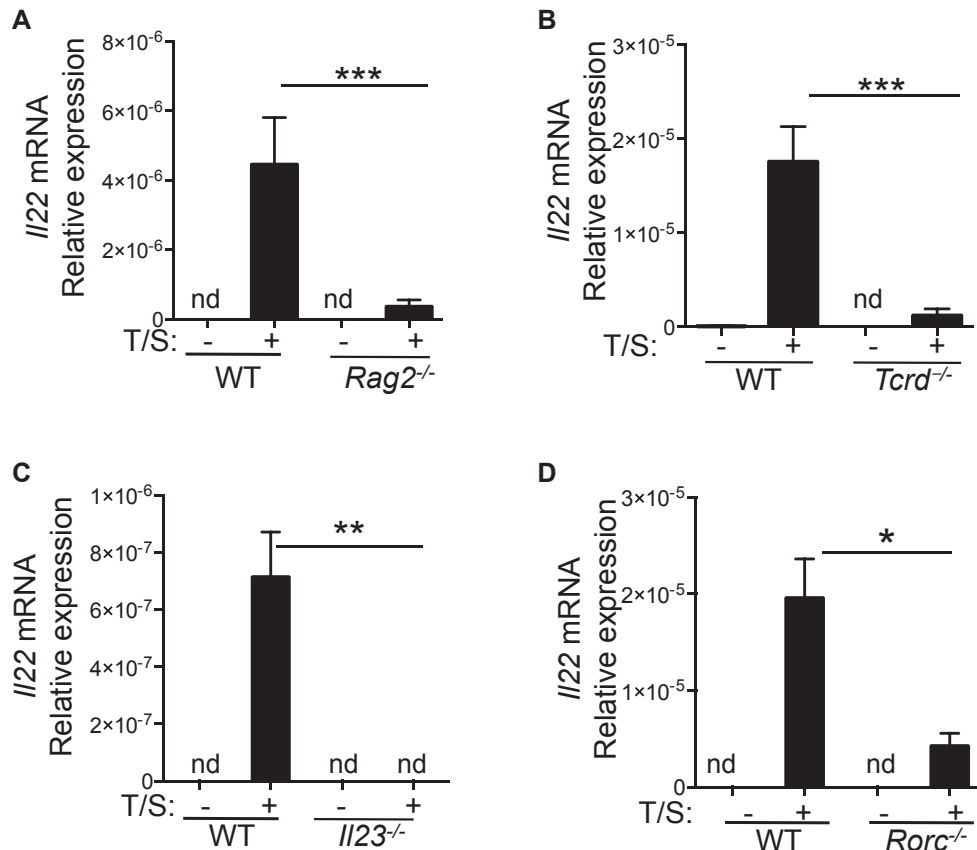


FIG 2. $\gamma\delta$ T cells are the major IL-22-producing cells in response to mechanical skin injury. **A**, *Il22* mRNA expression levels in the skin from unmanipulated WT (Balb/c), tape-stripped WT (Balb/c), unmanipulated *Rag2*^{-/-}, and tape-stripped *Rag2*^{-/-} mice (n = 7 per group). *Il22* levels remain undetectable (nd) in unmanipulated skin. **B**, *Il22* mRNA expression levels in the skin from unmanipulated WT (C57BL/6), tape-stripped WT, unmanipulated *Tcrd*^{-/-}, and tape-stripped *Tcrd*^{-/-} mice (n = 6 per group). **C**, *Il22* mRNA expression levels quantitated in unmanipulated WT (C57BL/6), tape-stripped WT, unmanipulated *Il23* (*p19*)^{-/-}, and tape-stripped *Il23* (*p19*)^{-/-} mice (n = 3 per group). **D**, *Il22* mRNA expression levels in unmanipulated WT (C57BL/6), tape-stripped WT, unmanipulated *Rorc*^{-/-}, and tape-stripped *Rorc*^{-/-} mice (n = 3 per group). T/S, Tape stripping. Mean with SEM is shown. **P* < .05. ***P* < .01. ****P* < .001.

$\gamma\delta$ T cells express *Il22* rapidly following stimulation with IL-23; this is dependent on the transcription factor ROR γ t, which is activated following IL-23 stimulation.^{22,23} Induction of *Il22* mRNA expression following tape stripping was markedly decreased in *Il23*^{-/-} and *Rorc*^{-/-} mice, respectively (Fig 2, C and D). These results suggest that IL-23 induces IL-22 expression in $\gamma\delta$ T cells by activating the transcription factor ROR γ t.

IL-22 limits cutaneous *S aureus* infection

To test the potential role of IL-22 in host defense against *S aureus* infection at sites of mechanical skin injury, we applied *S aureus* (USA300 SF8300 strain) to the skin of WT and *Il22*^{-/-} mice 24 hours after tape stripping (Fig 3, A). At the time of *S aureus* application, *Il22* mRNA expression was no longer detected in tape-stripped skin and *S aureus* application to tape-stripped skin did not upregulate *Il22* mRNA expression (Fig 3, B). We monitored skin lesions daily and quantitated bacterial growth 5 days after infection. On day 5, no skin lesions were detected at the sites of *S aureus* application in any of the WT mice (n = 9). In contrast, erythematous skin lesions were detected at the sites of *S aureus* application in 7 of 9 *Il22*^{-/-}

mice (Fig 3, C). Hematoxylin and eosin staining of skin biopsies showed that *S aureus* infection of tape-stripped skin caused epidermal thickening in WT mice. Epidermal thickening in *S aureus*-infected tape-stripped skin was modestly reduced in the *Il22*^{-/-} mice (see Fig E2, A and B, in this article's Online Repository at www.jacionline.org), 3 days after *S aureus* infection. Reduced epidermal thickening may have contributed to the erythematous appearance of the skin lesions in *Il22*^{-/-} mice. To ascertain whether the skin lesions observed in the absence of IL-22 were associated with overgrowth of *S aureus*, we cultured skin homogenates on chrome agar, a selective medium that permits the growth of *S aureus* strain USA300 colonies, which can be identified by their pink coloration. Only sparse *S aureus* colonies were recovered from WT mice. In contrast, significantly higher numbers of bacterial colonies were recovered from *Il22*^{-/-} mice (Fig 3, D, left and right). These results suggest that IL-22 induction is important for efficient clearance of *S aureus* by mechanically injured skin.

IL-22 is often coexpressed with IL-17A in T cells, including $\gamma\delta$ T cells.¹⁶ The role of IL-17A in the clearance of *S aureus* infections has been established in several models.¹⁵ We tested whether IL-17A also plays a role in the clearance of *S aureus* in

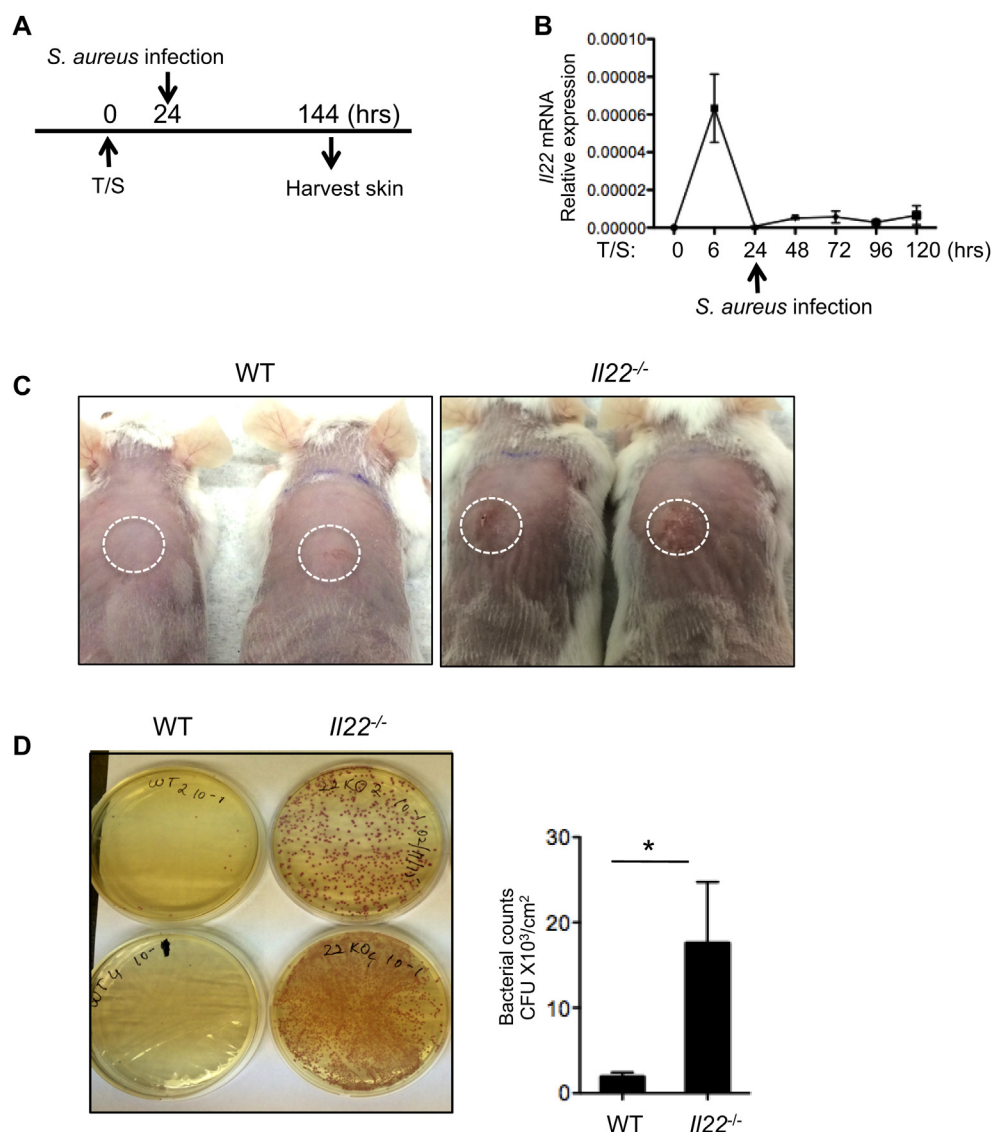


FIG 3. IL-22 limits cutaneous *S aureus* infection. **A**, Schematic of *S aureus* infection in tape-stripped mice. **B**, *Il22* mRNA expression determined at different times after tape stripping and after *S aureus* infection ($n = 3$ per time point). **C**, Representative pictures of WT and *Il22*^{-/-} mice, 5 days after *S aureus* infection. **D**, Left, Bacterial colonies obtained from culture of skin homogenates from WT or *Il22*^{-/-} mice on Chrom-agar. Right, Bacterial colony-forming unit (CFU) obtained from skin of WT or *Il22*^{-/-} mice ($n = 9$ per group). T/S, Tape stripping. Mean with SEM is shown. * $P < .05$.

our model of superficial infection of tape-stripped skin. As expected, we observed a significant increase in the bacterial skin burden in *S aureus*-infected *Il17a*^{-/-} mice compared with WT controls 5 days after *S aureus* infection (see Fig E2).

IL-22 is necessary for the early recruitment of neutrophils and the induction of *Cxcl1*, *Cxcl2*, and *Cxcl3* expression

Neutrophils are critical for the clearance of intradermally inoculated *S aureus*.²⁴ To ascertain the role of neutrophils in our model of epicutaneous *S aureus* inoculation of tape-stripped skin, WT mice were depleted of neutrophils by treatment with the anti-Ly6G mAb IA8 (Fig 4, A). Twenty-four hours later, the skin was tape stripped and 24 hours after tape stripping *S aureus*

was applied. Virtually complete neutrophil depletion was achieved in the skin and blood of mice treated with anti-Ly6G mAb IA8; there was no significant change in skin and blood neutrophil numbers in mice treated with IgG2a isotype control mAb (Fig 4, B; see Fig E3 in this article's Online Repository at www.jacionline.org). Neutrophil-depleted mice showed a significant defect in clearing *S aureus* from tape-stripped skin compared with mice treated with isotype control mAb (Fig 4, C). These results demonstrate that neutrophils are critical for the clearance of *S aureus* infection from mechanically injured skin.

We next determined whether IL-22 plays a role in neutrophil recruitment to mechanically injured skin. There was a robust infiltration of neutrophils in the skin of WT mice 24 hours after tape stripping, the time when *S aureus* was inoculated. In contrast,

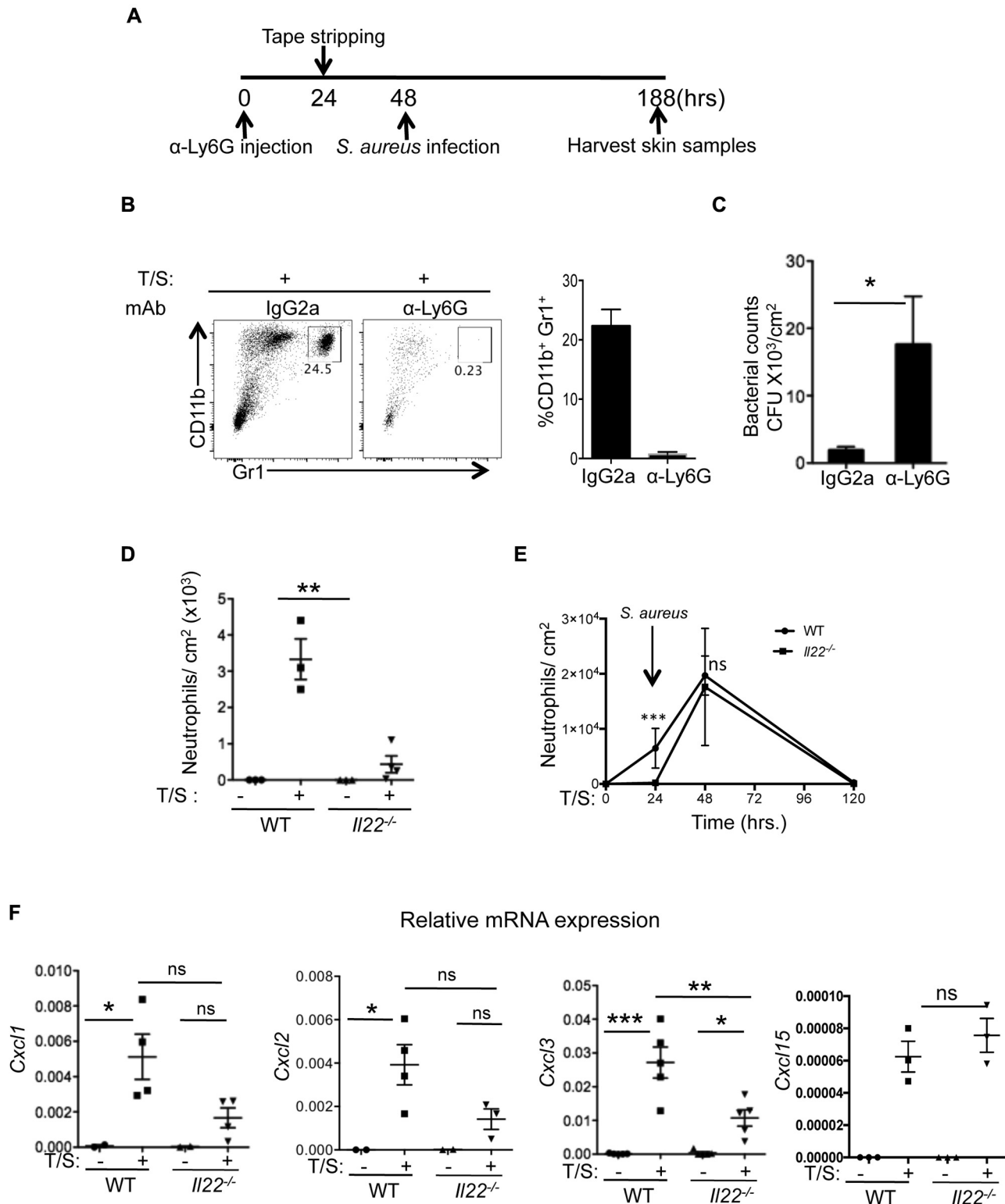


FIG 4. IL-22 is necessary for the early recruitment of neutrophils and the induction of *Cxcl1*, *Cxcl2*, and *Cxcl3* expression. **A**, Schematic of *S. aureus* infection in neutrophil-depleted mice. **B**, Left, Neutrophils, identified as CD11b⁺ Gr1^{hi} cells, are shown in the tape-stripped skin of isotype-treated or anti-Ly6G-treated mice. Right, Percentage of neutrophils in the 2 groups of mice (n = 4 per group). **C**, Bacterial colony-forming units (CFUs) enumerated from the skin biopsies of WT and neutrophil-depleted mice. **D**, Left, Neutrophils quantified in the skin of WT or *Il22*^{-/-} mice in unmanipulated skin and 24 hours after tape stripping (n = 4 per group). **E**, Neutrophil numbers in skin were quantitated in WT and *Il22*^{-/-} mice at indicated time points after tape stripping and following *S. aureus* infection (n = 3 per time point). **F**, mRNA expression levels of chemokines *Cxcl1*, *Cxcl2*, *Cxcl3*, and *Cxcl15* determined in unmanipulated and tape-stripped skin of WT or *Il22*^{-/-} mice (n = 4 per group). ns, Not significant; T/S, tape stripping. Mean with SEM is shown *P < .05. **P < .01. ***P < .001.

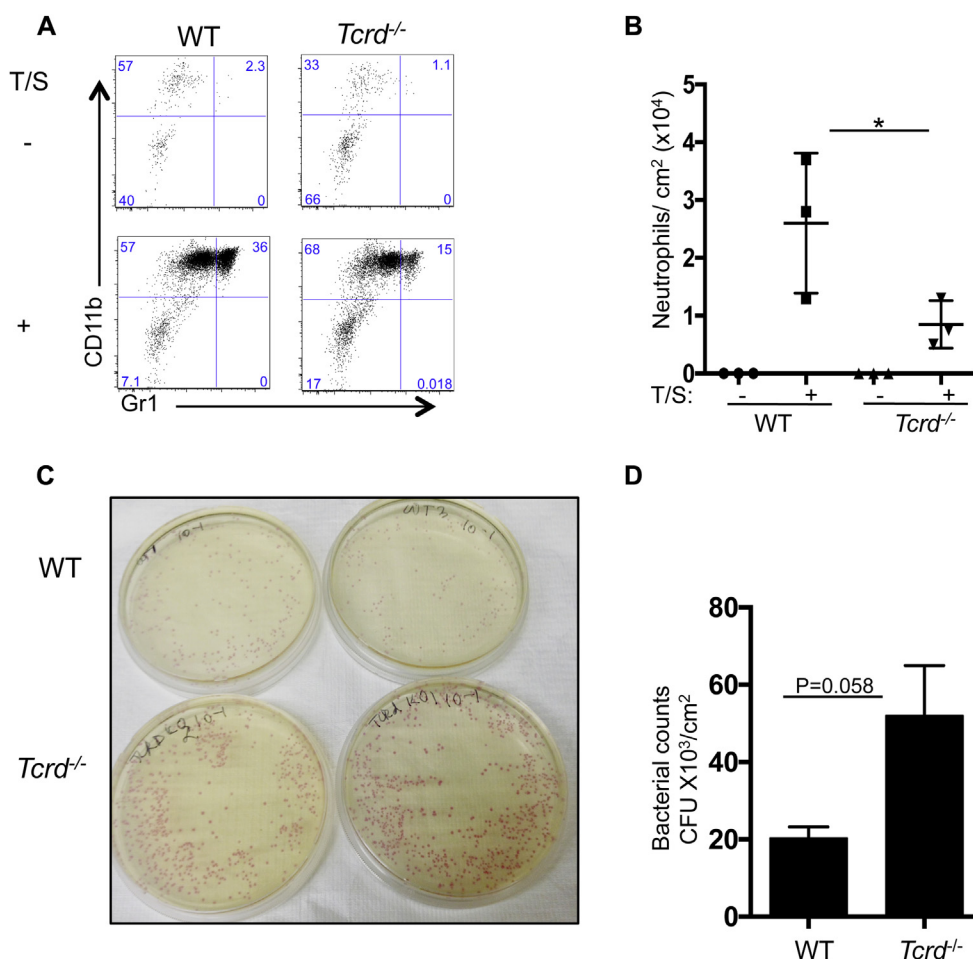


FIG 5. $\gamma\delta$ T cells are necessary for neutrophil recruitment and suppression of *S aureus* infection in tape-stripped skin of mice. **A** and **B**, Percentages and numbers of neutrophils in unmanipulated and tape-stripped skin of *Tcrd*^{-/-} mice and WT controls, 24 hours after tape stripping (n = 3 per group). *P < .05. **C**, Bacterial colonies obtained from homogenates of tape-stripped skin from *Tcrd*^{-/-} mice and WT controls cultured on Chrom-agar, 5 days after infection. **D**, Bacterial colony-forming units (CFUs) obtained from tape-stripped skin of *Tcrd*^{-/-} mice and WT controls (n = 6 per group). T/S, tape stripping. Mean with SEM is shown (P = .058).

the number of skin-infiltrating neutrophils 24 hours after tape stripping was strikingly diminished in *Il22*^{-/-} mice (Fig 4, D). We also examined neutrophil infiltration in skin inoculated with *S aureus* 24 hours after tape stripping. There were significantly less neutrophils in *Il22*^{-/-} mice at the time of *S aureus* inoculation, compared with WT controls; however, neutrophil infiltration 24 hours after inoculation was comparable in *Il22*^{-/-} mice and WT controls (Fig 4, E).

Expression of mRNA for the neutrophil-attracting chemokines *Cxcl1*, *Cxcl2*, and *Cxcl3* was not detectable in unmanipulated skin of WT or *Il22*^{-/-} mice. Tape stripping induced a significant increase in the expression of *Cxcl1* and *Cxcl2* in the skin of WT mice, but not in the skin of *Il22*^{-/-} mice (Fig 4, F). Tape stripping induced a significant increase in *Cxcl3* expression in both groups, but the increase in *Il22*^{-/-} mice was significantly less than in WT controls. Other chemokines such as *Cxcl15* were similarly induced in both WT and *Il22*^{-/-} mice upon tape stripping (Fig 4, F). Overall, these data indicate that IL-22 is important for the expression of neutrophil chemoattractants and neutrophil recruitment in mechanically injured skin.

$\gamma\delta$ T cells are necessary for neutrophil recruitment upon tape stripping and for suppressing *S aureus* infections in mice

Because $\gamma\delta$ T cells were the primary source of IL22 in tape stripping, we examined whether their absence reduced neutrophil recruitment and promoted *S aureus* growth in mechanically injured skin. Similar to *Il22*^{-/-} mice, we observed a 3-fold decrease in the number of neutrophils in the skin of *Tcrd*^{-/-} mice after tape stripping (Fig 5, A and B). We also observed increased growth of *S aureus* in tape-stripped skin of *Tcrd*^{-/-} mice compared with WT controls after 5 days of infection (Fig 5, C and D), with the difference approaching statistical significance (P = .058; n = 6 per group). This result is consistent with the demonstrated role of $\gamma\delta$ T cells in limiting the growth of intradermally delivered *S aureus*.¹⁵

IL-22 regulates the expression of antimicrobial peptides *Slurp1* and *Defensin-beta14*

Antimicrobial peptides (AMPs) play a role in the skin defense against *S aureus*.²⁵ IL-22 is reported to induce the expression of

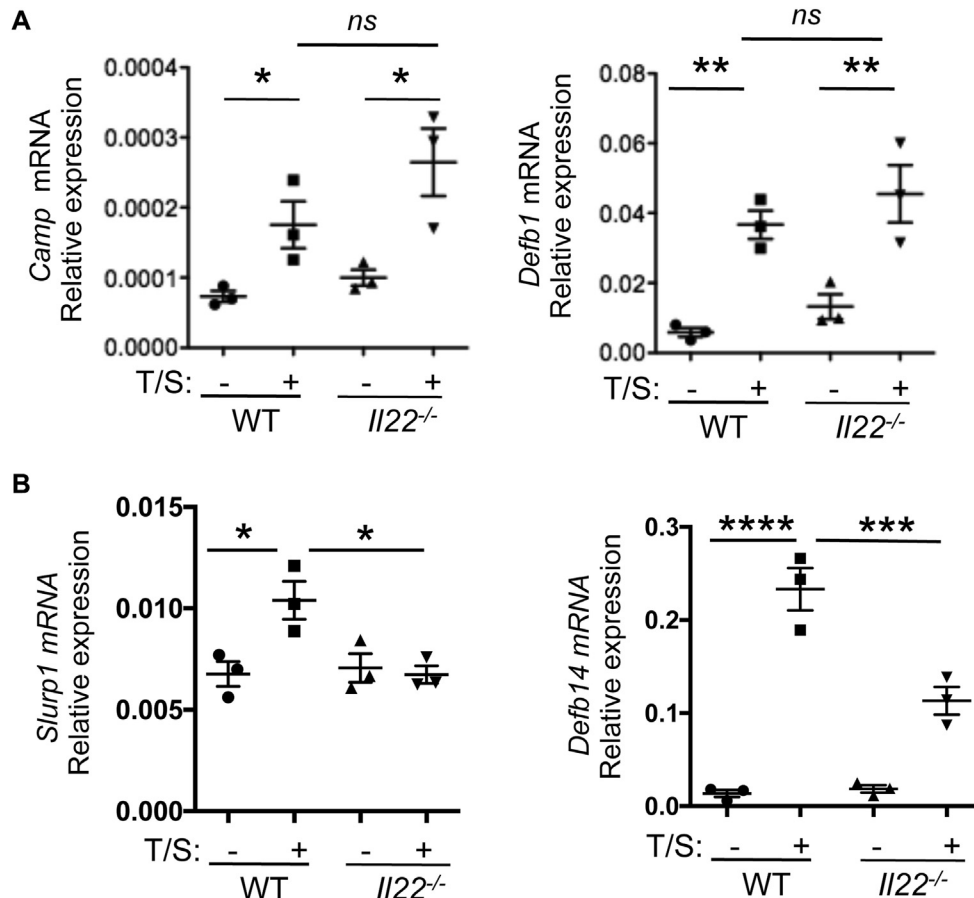


FIG 6. Reduced induction of *Slurp1* and *Defb14* antimicrobial peptides in WT and *Il22*^{-/-} mice upon tape stripping (T/S). **A**, mRNA expression levels of genes encoding antimicrobial peptides *Camp* and *Defb1* from unmanipulated versus tape-stripped WT and *Il22*^{-/-} mice. **B**, mRNA expression levels of genes encoding antimicrobial peptides *Slurp1* and *Defb14* from unmanipulated versus tape-stripped WT and *Il22*^{-/-} mice. ns, Not significant. Mean with SEM is shown. **P* < .05. ***P* < .01. ****P* < .001. *****P* < .0001.

AMPs by keratinocytes.¹⁸ Tape stripping upregulated the expression of multiple AMPs in both WT and *Il22*^{-/-} mice. We observed a significant reduction in the induction of *Slurp1* and *Defb14*, but not *Camp*, *Defb1*, *Lcn2*, and *Reg3g* in the tape-stripped skin of *Il22*^{-/-} mice as compared with WT controls (Fig 6, A and B, and data not shown). Mouse β -DEFENSIN 14 is the orthologue of human β -DEFENSIN 3. The expression of *Defb3* mRNA is strikingly diminished in patients with recurrent *S aureus* infections,²⁶ suggesting its importance in controlling the severity of *S aureus* infections. The secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor related protein-1 encoded by *Slurp1* has been shown to induce proliferation of keratinocytes and suppression of growth of *S aureus*.²⁷ Hence, our finding that the induction of *Slurp1* and *Defb14* in tape-stripped skin is regulated by IL-22 demonstrates that IL-22 possesses a pleiotropic role in the control of *S aureus* infection in mechanically injured skin of mice.

DISCUSSION

We demonstrate a critical role for IL-22 in limiting *S aureus* infection of mechanically injured skin. We show that mechanical injury induces IL-22 release in the skin that is dependent on IL-23 and $\gamma\delta$ T cells, and demonstrate that IL-22 is critical for the rapid

mobilization of neutrophils to the skin and the containment of *S aureus* infection.

Mechanical injury to mouse skin inflicted by tape stripping induced a rapid burst of *Il22* mRNA expression and the release of IL-22 from skin explants. $\gamma\delta$ T cells were the major source of IL-22 released in the skin following mechanical injury. The release of IL-22 in the skin was dependent on both IL-23 and on the transcription factor ROR γ t. *Il23* mRNA expression is induced rapidly (within 3 hours) by the application of an antigen patch to shaved skin.²⁸ We have observed a similarly rapid induction of *Il23* mRNA in tape-stripped skin (data not shown). ROR γ t is activated by IL-23R signaling to drive *Il22* gene expression. We demonstrated that ROR γ t is critical for the induction of *Il22* expression in tape-stripped skin. ROR γ t is expressed in $\gamma\delta$ T cells and type-3 innate lymphoid cells. We demonstrated that $\gamma\delta$ T cell are the major source of IL-22 in tape-stripped skin. $\gamma\delta$ T cells are present in both the dermis and the epidermis.²³ IL-23R and ROR γ t are constitutively expressed by dermal $\gamma\delta$ T cells, whereas there is conflicting data regarding their expression by epidermal $\gamma\delta$ T cells.²⁹ Thus, dermal $\gamma\delta$ T cells are likely the major contributor to IL-22 production in mechanically injured skin.²³ Apart from $\gamma\delta$ T cells, other innate cells such as type-3 innate lymphoid cells and mast cells have been shown to be important sources of IL-22 in mice and

humans.^{29,30} We observed no IL-22 induction upon tape stripping in *Rag2*^{-/-} mice, which lack T cells but have normal numbers of ILCs and mast cells. Furthermore, we observed normal *Il22* induction in *Kit*^{-Wsh/Wsh} mice, which lack mast cells (data not shown). Mechanical injury to human skin caused by scratching also induced *Il22* mRNA and protein expression. This suggests that the results we have obtained in the mouse model are applicable to humans. Potential sources of IL-22 in human skin include $\alpha\beta$ T cells, $\gamma\delta$ T cells, and mast cells.^{29,31,32} We have not been able to obtain the necessary samples to determine the source of *Il22* mRNA in the scratched skin of human volunteers.

We show that IL-22 plays a protective role in suppressing the growth of *S aureus* on tape-stripped mouse skin. This was evidenced by a 1-log increase in the bacterial burden in *Il22*^{-/-} mice compared with WT controls. Notably, *Il22* mRNA expression at the time of *S aureus* application to tape-stripped skin had returned to undetectable levels and was not induced by *S aureus* application. This suggests that the IL-22 induced by tape stripping is responsible for the protective effect against the growth of *S aureus* in our model. This is consistent with the importance of bacterial replication early after inoculation in the establishment of *S aureus* infection. Consistent with $\gamma\delta$ T cells being the major source of IL-22 in tape-stripped skin, we demonstrated a critical role for $\gamma\delta$ T cells in limiting the growth of *S aureus* on tape-stripped mouse skin.

The receptor for IL-22 is highly expressed on keratinocytes.¹⁷ IL-22 signaling induces keratinocytes to release AMPs and neutrophil-attracting chemokines.¹⁸ We observed a decrease in the expression of *Slurp1* and *Defb14*. Both these proteins have been shown to suppress the growth of *S aureus*.^{26,27} These findings suggest that IL-22 acts pleiotropically to inhibit *S aureus* infections. Neutrophils were essential for limiting *S aureus* infection of tape-stripped skin, consistent with their well-established role in protecting against the growth of intradermally injected *S aureus* bacteria.²⁴ Twenty-four hours after tape stripping, which corresponds to the time when *S aureus* was inoculated, there was a robust influx of neutrophils and significant induction of *Cxcl1*, *Cxcl2*, and *Cxcl3* in the skin of WT mice, which were severely attenuated in *Il22*^{-/-} mice. However, 24 hours after *S aureus* inoculation of tape-stripped skin, neutrophil infiltration was comparable in *Il22*^{-/-} mice and WT controls. These findings suggest that IL-22-driven neutrophil infiltration in tape-stripped skin at the time of bacterial application is important for the containment of cutaneous infection by *S aureus*. They imply that IL-22 is important for closing a critical window that restricts early the replication of *S aureus* in mechanically injured skin.

In summary, we show a protective role of IL-22 in limiting the growth of *S aureus* in mechanically injured skin of mice. Induction by IL-22 of AMP gene expression, neutrophil-attracting chemokines, neutrophil infiltration, and epidermal thickening may all contribute to this protective role. Using *Il22*^{-/-} mice, it was previously reported that IL-22 is dispensable for the protection against the growth of intradermally injected *S aureus*.³³ However, recent studies show that neutralization of IL-22 increases the bacterial burden following intradermal inoculation of *S aureus*.³⁴ Our model of epicutaneous application of *S aureus* to skin mechanically injured by tape stripping mimics the natural route of *S aureus* infection of skin mechanically injured by scratching in patients with AD. Our demonstration of the role of IL-22 in limiting *S aureus* infection of mechanically

injured skin suggests that IL-22 expression in AD skin lesions may counterbalance the effect of IL-4 and IL-13 in promoting cutaneous *S aureus* infection.¹² Our findings have also important clinical implications because they caution that IL-23 and IL-22 blockade in patients with AD may aggravate staphylococcal skin infection. Hence, careful monitoring for cutaneous *S aureus* infection should accompany the use of these biological therapeutic approaches.

We thank Dr Binh Diep, University of California at San Francisco, for his kind gift of *S aureus* USA300, SF8300 strain.

Key message

- Mechanical injury induces IL-22 expression in the skin. This is dependent on IL-23 and $\gamma\delta$ T cells. IL-22 is important for the expression of AMPs and neutrophil-attracting chemokines, neutrophil recruitment, and containment of *S aureus* infection in tape-stripped mouse skin

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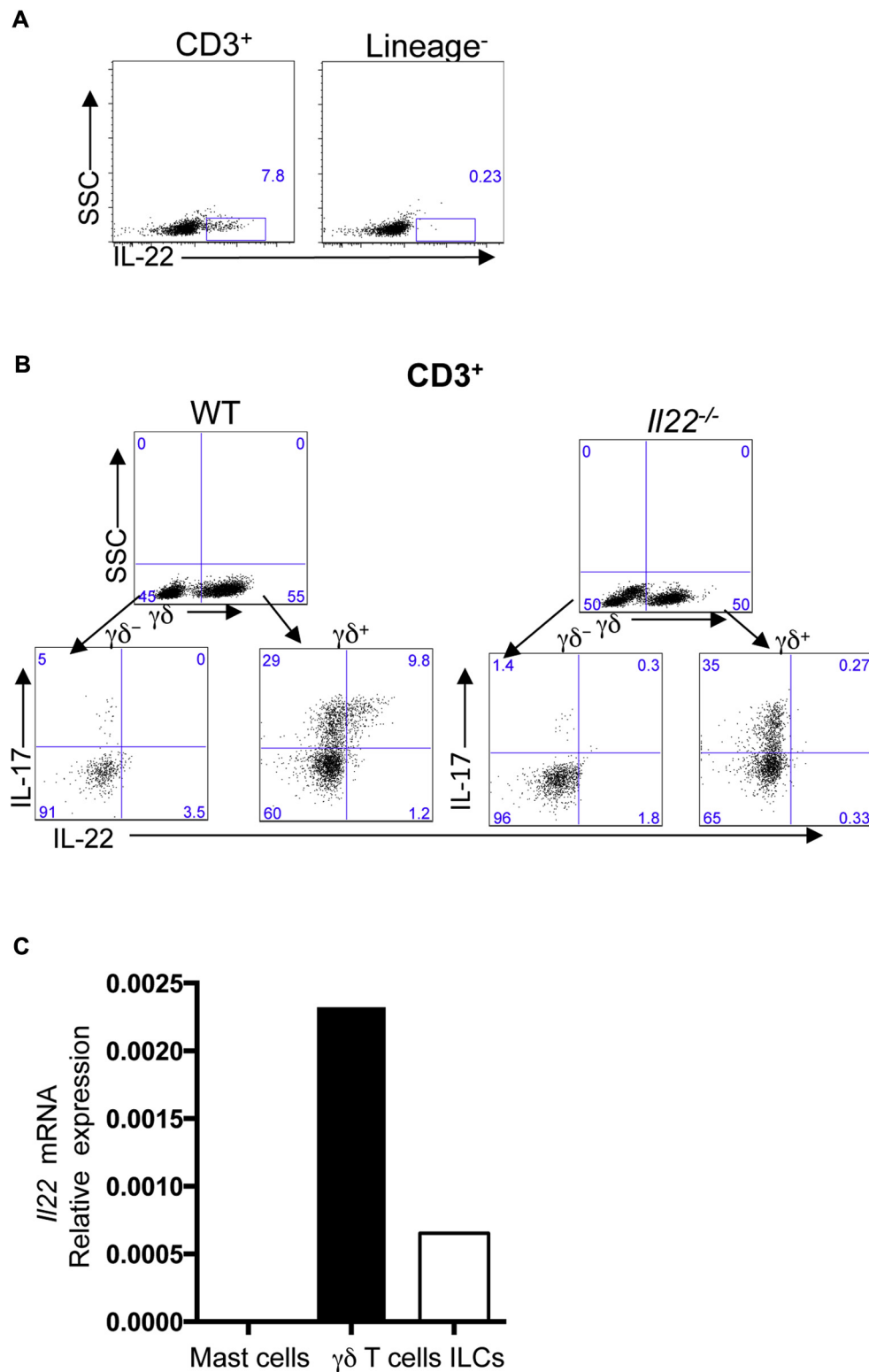


FIG E1. $\gamma\delta$ T cells are the major source of IL-22 in murine tape-stripped skin. **A**, Flow-cytometric analysis of intracellular IL-22 in CD3⁺ and Lineage⁻ (CD3⁺CD11b⁻CD19⁻) CD45⁺ cells in the skin. **B**, Intracellular IL-22 and IL-17A in $\gamma\delta$ T cells obtained from tape-stripped skin of WT mice. *Il22*^{-/-} mice were used as a negative control for IL-22 antibody staining. **C**, Quantitative PCR analysis of *Il22* mRNA levels in $\gamma\delta$ T cells ILCs and sorted mast cells, sorted from tape-stripped skin of WT mice. One of the 2 representative experiments is shown. SSC, Side scatter.

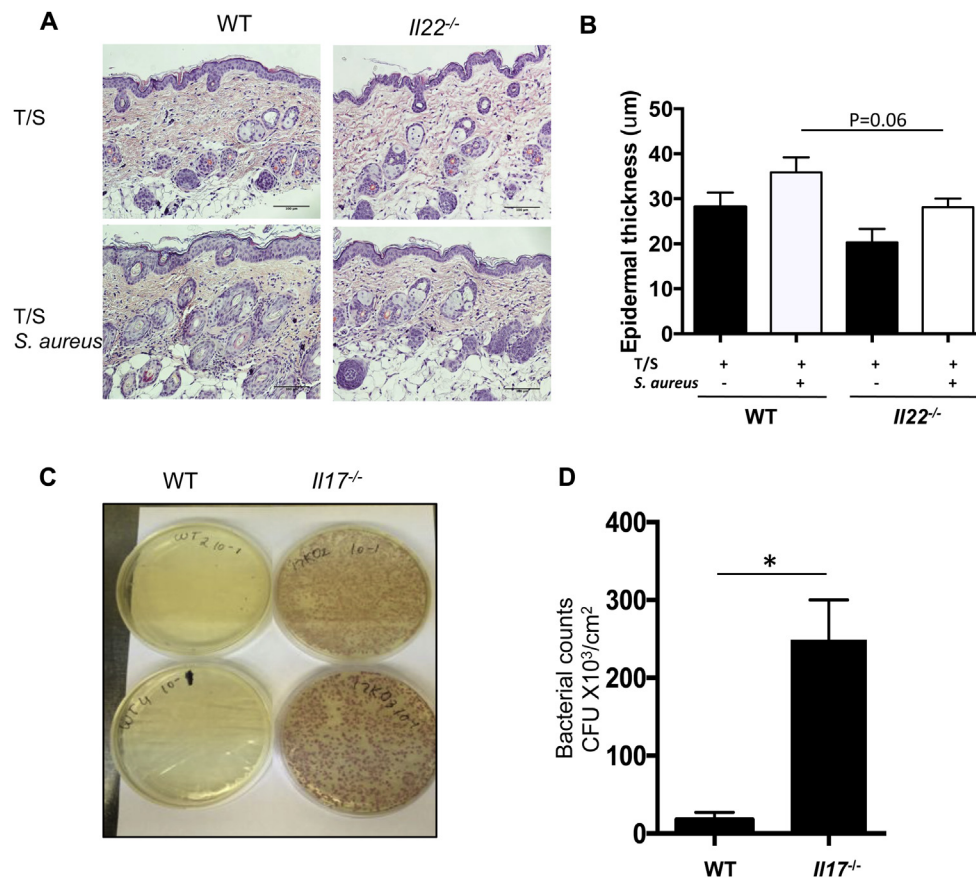


FIG E2. **A**, Hematoxylin and eosin staining of tape-stripped or tape-stripped and *S. aureus*-infected skin of WT and *Il22*^{-/-} mice, shown at 20× magnification. **B**, Measurement of epidermal thickness performed using ImageJ software is shown (n = 3 mice per group). **C**, Bacterial colonies obtained from skin homogenates from WT or *Il17a*^{-/-} mice cultured on Chrom-agar. **D**, Bacterial colony-forming unit (CFU) obtained from skin of WT or *Il17a*^{-/-} mice (n = 3 mice per group). Assays were performed 5 days after *S. aureus* infection. T/S, tape stripping. Mean with SEM is shown. * $P < .05$.

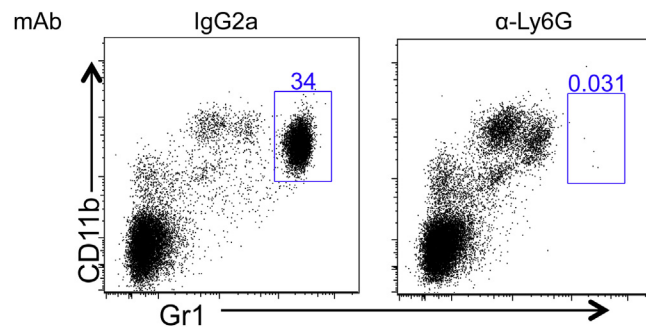


FIG E3. Depletion of circulating neutrophils in the blood of anti-Ly6G-treated mice. Proportions of neutrophils identified as CD11b^{hi}Gr1^{hi} in the blood of tape-stripped mice that were treated with isotype antibody or anti-Ly6G antibody are shown 24 hours after depletion.