Commensal–dendritic-cell interaction specifies a unique protective skin immune signature

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The skin represents the primary interface between the host and the environment. This organ is also home to trillions of microorganisms that play an important role in tissue homeostasis and local immunity1–4. Skin microbial communities are highly diverse and can be remodelled over time or in response to environmental challenges5–7. How, in the context of this complexity, individual commensal microorganisms may differentially modulate skin immunity and the consequences of these responses for tissue physiology remains unclear. Here we show that defined commensals dominantly affect skin immunity and identify the cellular mediators involved in this specification.

In particular, colonization with Staphyloccocus epidermidis induces IL-17A+ CD8+ T cells that home to the epidermis, enhance innate barrier immunity and limit pathogen invasion. Commensal-specific T-cell responses result from the coordinated action of skin-resident dendritic cell subsets and are not associated with inflammation, revealing that tissue-resident cells are poised to sense and respond to alterations in microbial communities. This interaction may represent an evolutionary means by which the skin immune system uses fluctuating commensal signals to calibrate barrier immunity and provide heterologous protection against invasive pathogens. These findings reveal that the skin immune landscape is a highly dynamic environment that can be rapidly and specifically remodelled by encounters with defined commensals, findings that have profound implications for our understanding of tissue-specific immunity and pathologies.

We first assessed whether individual commensal species could modulate immunity in the context of pre-existing microbial communities. Despite the presence of a diverse microbiota, the skin of specific pathogen free (SPF) mice was permissive to long-term colonization with S. epidermidis at all skin sites analysed (Fig. 1a, b). At 2 weeks post topical association with as low as 1.3 × 10^6 colony-forming units (c.f.u.) per cm^2, levels of IL-17A- and IFN-γ-expressing T cells but not Foxp3+ regulatory T cells were significantly increased at several skin sites analysed (Fig. 1c, d and Extended Data Fig. 1a–d). Long-term accumulation of IL-17A-expressing T cells was not observed at sites distal to the skin and required colonization with live bacteria (Fig. 1c, d, h and Extended Data Fig. 1b). Furthermore, in contrast to responses to intradermal inoculation of S. epidermidis, commensal responses were not associated with inflammation (Fig. 1e–h and Extended Data Fig. 1e–h). Thus, an encounter with a new commensal can lead to a robust but non-inflammatory accumulation of effector T cells in the skin.

We next assessed the capacity of other constituents of the human (Corynebacterium pseudodiphtheriticum, Propionibacterium acnes and Staphylococcus aureus) and murine (Staphylococcus xylosus, Staphylococcus lentus, Rothia nasimurium and S. epidermidis 42E03) skin microbiota to influence T-cell responses (Extended Data Fig. 2a). Six out of eight bacteria tested increased the number of skin IL-17A+ T cells and half of the commensals also increased the number of IFN-γ-expressing T cells (Fig. 2a and Extended Data Fig. 2a, b). Thus, the induction of cytokines, and in particular IL-17A, is a relatively conserved response of the skin to an encounter with a new commensal.

The majority of γδ T cells found in murine skin are CD4+ T cells with few resident CD8+ T cells (Fig. 2b, c). Notably, S. epidermidis isolates were uniquely able to increase the number and frequencies of CD8+ T cells in the skin in both SPF and germ-free conditions and in response to an application dose as low as 1.3 × 10^6 c.f.u. per cm^2 (Fig. 2c and Extended Data Fig. 2c–h). Similarly to tissue-resident memory (TREM) cells induced by viral challenges9, clusters of CD8+ T cells preferentially localized to the basal epidermis or in close proximity to the epithelial layer and expressed CD103 and CD69 (Fig. 2c, d and Extended Data Fig. 3a, b). On the other hand, commensal-evoked CD8+ T cells have a distinct cytokine profile characterized by the production of either IL-17A or IFN-γ and in contrast to virally induced TREM cells that localize to the site of injury, commensal-induced CD8+ T cells accumulated at all skin sites analysed (Fig. 2c and Extended Data Fig. 1b). Although rarely seen at other body sites, Tc17 cells (a subset of CD8+ T cells) can be found in healthy non-human primate and human skin (Fig. 2e and Extended Data Fig. 3c). This discrete response provided us with the opportunity to explore the factors controlling a commensal-driven immune specification.

In germ-free mice, commensals promote T-cell responses through IL-1 (ref. 2). Consistently, mice deficient in IL-1R1 contained significantly fewer skin IL-17 A+ CD8+ T cells post S. epidermidis association, and in vitro stimulation of purified S. epidermidis-evoked CD8+ T cells with IL-1 boosted IL-17A release (Fig. 2f and Extended Data Fig. 3d). CD8+ T-cell response peaked at 2 weeks post association, at which point the number of cells slowly contracted, although increased frequencies were maintained up to 6 months post application (Fig. 2g, h).

Dendritic cells are exquisite sensors of their environment and previous studies uncovered a functional specialization of defined dendritic cell subsets in their capacity to drive unique immune modules10. What remains unexplored is how this specialization could account for the capacity of the host to regulate defined aspects of its relationship with the microbiota. In the skin, dendritic cells could potentially be exposed to the microbiota via emission of dendrites through epithelial cells and/or to commensal products passively diffusing at invaginations such as hair follicles, which have more permissive cell adhesions11. Supporting the idea that CD8+ T-cell accumulation post association depends on migratory dendritic cells12, the response was largely abolished in Cer7+ mice (Fig. 3a). Frequencies of skin dendritic cell subsets13 were not affected...
by *S. epidermidis* application, indicating that the induction of Tc17 cells did not result from altered dendritic cell frequencies (Extended Data Fig. 4a, b).

Mice constitutively deficient in Langerhans cells14 mounted T-cell responses to *S. epidermidis* in a manner comparable to their littermate controls (Fig. 3b and Extended Data Fig. 4c, and data not shown). Cross-presenting CD103+ dendritic cells depend on expression of IRF8 and BATF3 for their development10,16 while CD11b+ dendritic cells require CSF1 for their development and maintenance27,28 and IRF4 for the formation of peptide–MHC (major histocompatibility complex) class II complexes29. Making use of this differential requirement for transcription or survival factors, we assessed the relative contribution of these two dendritic cell subsets to CD8+ T-cell responses. The selective defect in skin-resident CD103+ dendritic cells (but not lymph-node-resident CD8x+ dendritic cells; Extended Data Fig. 4d) in our Batf3−/− colony allowed us to evaluate the direct contribution of these cells. Batf3−/− and Ifng−/− mice failed to develop CD8+ T-cell responses following colonization compared to control mice, highlighting a non-redundant role for CD103+ dendritic cells in the induction of CD8+ T-cell responses to *S. epidermidis* (Fig. 3c and Extended Data Fig. 4c, e).

Treatment of mice with an anti-CSF1R antibody led to a marked reduction in Langerhans cells and skin CD11b+ dendritic cells, as well as in skin IL-17A+ CD8+ T cells (Fig. 3d and Extended Data Fig. 4c). As the specific deletion of Langerhans cells had no consequence on T-cell responses to commensals (Fig. 3b), these results suggest that the immune effect observed was due to CD11b+ dendritic cells. A large fraction of *S. epidermidis*-evoked CD8+ T cells were in close contact with CSF1R+ CD11c+ cells in the skin and these cells were the most transcriptionally
altered (including increases in Il1a and Il1b transcripts and a decrease in Il1rn transcripts) following S. epidermidis application (Fig. 3e, f and data not shown). Furthermore, anti-CSF1R antibody treatment significantly reduced skin IL-1 levels post association (Extended Data Fig. 4f). In mice conditionally depleted of IRF4 in their dendritic cell compartment\(^{33}\), CD8\(^+\) T cells accumulated in the skin but failed to produce IL-17A (Extended Data Fig. 4g). Together, these results support the idea that through their capacity to produce IL-1, CD11b\(^+\) dendritic cells could promote the induction and/or maintenance of IL-17A expression by CD8\(^+\) T cells. Thus, cooperation between skin-resident dendritic cells promotes and tunes responses to a defined commensal (Fig. 4g).

Of note, healthy human skin contains approximately 20 billion effector lymphocytes of unknown specificity\(^{29}\). To address the possibility that T cells accumulating in murine skin in response to S. epidermidis were commensal specific, CD8\(^+\) T cells were purified from the skin or regional lymph nodes and exposed to dendritic cells loaded with S. epidermidis antigens. Exposure of S. epidermidis-loaded dendritic cells to CD8\(^+\) T cells promoted potent IL-17A and IFN-\(\gamma\) production (Fig. 4a, b and Extended Data Fig. 5a). In contrast, stimulation with IL-1\(\zeta\) or other bacterial antigens or ligands failed to induce cytokine production, and S. epidermidis-loaded dendritic cells did not promote cytokine release by activated T cells of irrelevant specificity (Fig. 4b and data not shown). A fraction of IFN-\(\gamma\) production by CD8\(^+\) T cells was still detectable in the absence of \(\beta2\)-microglobulin-dependent MHC class I presentation (Fig. 4a, b and Extended Data Fig. 5b), suggesting that some of the IFN-\(\gamma\)-producing CD8\(^+\) T cells may be responding in a bystander manner or in a \(\beta2\)-microglobulin-independent manner\(^{21}\). However, IL-17A was not observed following exposure to B2m\(^{-}\) dendritic cells loaded with S. epidermidis, demonstrating that Tc17 cells are commensal specific (Fig. 4a, b). Commensal-specific IL-17\(\alpha\) T cells were also found in the CD4\(^+\) T-cell compartment in both the skin and regional lymph nodes following S. epidermidis or S. xylosus application (Extended Data Fig. 5c–e). Our present results support the idea that, as in the gut\(^{22-24}\), the majority of IL-17A-producing T cells in the skin may be commensal specific.

To assess the consequence of adaptive immune responses to commensals for skin immunity we employed a model of epicutaneous infection with the fungal pathogen Candida albicans. Prior association with
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**Figure 4** | Commensal-driven CD8⁺ T cell response is specific for *S. epidermidis* antigen. **a**, CD8β⁺ effector T cells from the skin of *S. epidermidis*-associated mice were co-cultured with wild-type (WT) or B2m⁻/⁻ splenic dendritic cells (SpDC) untreated (Ctrl) or pre-incubated with heat-killed *S. epidermidis*, *S. xylosus* or *S. aureus*; LPS, Pam3Cys (P3C) or IL-1β. Flow plots illustrate the frequencies of IFN-γ⁺ CD8β⁺ and IL-17A⁺ CD8β⁺ T cells in overnight co-cultures. PMA, naïve SpDC + PMA/ionomycin. **b**, Frequencies of IFN-γ⁺ CD8β⁺ and IL-17A⁺ CD8β⁺ T cells in overnight co-cultures as described in **a** (mean ± standard deviation of triplicate cultures). Data are representative of 2–3 independent experiments. ****P < 0.001, **P < 0.01, ***P < 0.001 as calculated by Student’s *t*-test. **c**, Unassociated or *S. epidermidis*-topically associated mice were infected with *C. albicans* and treated with anti-CD8, anti-IL-17A or corresponding isotype control antibodies. **d**, Enumeration of *C. albicans* colony-forming units from dorsal skin biopsies from mice in 2 days post *C. albicans* infection. e, S100a8 and S100a9 gene expression (fold increase over naive unassociated) by interfollicular keratinocytes purified from the ears of mice 2 weeks after *S. epidermidis* application. f, S100a8 and S100a9 gene expression (fold increase over naive unassociated and uninfected) in dorsal skin biopsies from mice in 2 days post *C. albicans* infection. Results in **d**–**f** are representative of two independent experiments. **P < 0.05, **P < 0.01, ***P < 0.001 as calculated by Student’s *t*-test. **g**, Model of the response of the skin immune system to colonization with a new commensal. Skin-resident CD103⁺ dendritic cells (DC) may acquire commensals or commensal-derived antigens by reaching into skin appendages or via capture of soluble factors. CD8⁺ T cells primed by CD103⁺ dendritic cells in the lymph node, migrate to the skin and are locally tuned by IL-1 produced by CD11b⁺ dendritic cells. Commensal-specific CD8⁺ T cells can enhance antimicrobial defence of keratinocytes in an IL-17 dependent manner. Dotted lines indicate points that are not addressed in this work.

*S. epidermidis* significantly improved innate protection against *C. albicans*, an effect that was abolished by depletion of CD8⁺ T cells or neutralization of IL-17A (Fig. 4c, d). Microarray analysis of gene expression following *S. epidermidis* association revealed a significant upregulation of the alarmins S100A8 and S100A9 (data not shown), known to elicit microbicidal effects and as potent chemoattractants for neutrophils. Upregulation of these molecules was still detectable at an application dose as low as 1.3 × 10⁶ c.f.u. per cm², and 2 weeks past the peak of CD8⁺ T-cell response in the skin (Extended Data Fig. 5f and data not shown). In keratinocytes, induction of these molecules can be mediated by IL-17A. Analysis of gene expression by interfollicular keratinocytes from *S. epidermidis*-associated mice revealed a CD8⁺ T-cell- and IL-17A-dependent upregulation of S100A8 and S100A9 expression (Fig. 4e, f). Thus, *S. epidermidis* induces CD8⁺ T-cell responses able to promote skin innate responses in such a way that promotes heterologous protection against invasive microbes (Fig. 4g).

The skin immune system has evolved in the context of its constitutive exposure to a diverse microbiota that can be remodelled over time or in response to environmental challenges. Here we show that the skin is a highly dynamic immune environment that can be finely calibrated by defined commensals. In light of our present findings, it is intriguing to speculate that microbial diversity may also be required as a means to trigger and educate distinct aspects of the immune system. For instance, the capacity of defined commensals to promote CD8⁺ T-cell responses that can home to the epidermis may have evolved as a means to specifically reinforce the barrier function of this compartment. Here, we uncovered that tissue-resident dendritic cells are primary sensors of fluctuations in the commensal community and that these cells act in a highly coordinated manner to orchestrate the formation of commensal-specific T-cell responses. The result of this evolving interaction may represent a powerful protective mechanism of host defence by providing heterologous immunity against invasive microbes. In the context of highly perturbed communities, the cumulative cost of these responses may have severe consequences on tissue homeostasis. Indeed, commensal-specific responses are characterized by the production of IL-17A, a cytokine known to contribute to the aetiology and pathology of various skin inflammatory disorders, including psoriasis. Our work also presents a possible explanation for how variation in microbial communities at different skin sites may contribute to the site-specificity of dermatological disorders.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions S.N., N.B., and Y.B. designed the studies. S.N. and N.B. performed the experiments and analysed the data. J.L.L. assisted with in vitro co-culture studies and S.-J.H. with innate cell analysis and imaging. O.J.H. and C.W. provided technical assistance. S.C. and C.D. provided technical advice and performed 454 sequencing data are deposited in the Sequence Read Archive under accession number SRP039428. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to Y.B. (ybelkaid@niaid.nih.gov).
METHODS
Mice. C57BL/6 and BALB/c-specific pathogen free (SPF) mice were purchased from Taconic Farms. Germ-free C57BL/6 mice were bred at Taconic Farms and housed in the NIAID gnotobiotic facility. B6.SIL, C57BL/6-KO[IL1r1 (H1r1 −/−)] and C57BL/6-J[KO][B2m-KO][Abb (Abb−/−) B2m −/−] mice were obtained through the NIAID-Taconic exchange program. C57BL/6-Tg(Csf1r-EGFP-NFRG-FKBP1A-TNFRSF6)2Bcjk (CSF1R GFP reporter gene), B6.129P2(C)-Ccylm1003Czy/j (Ccr7 −/−), B6.129P2(C)-Batf3tm1Rkk/j (Batf3 −/−) mice and their C57BL/6 wild-type controls were purchased from The Jackson Laboratory. B6(Cg-Ify6tm1[lmig]) (Ify6 −/−), B6. FVB-Tg(CD207-DTA)12Dhka/j (Lan-DTA). B6, 129S2-Gzmm Therm1-cp/j (Gzmm −/−) mice were provided by R. Bosselut (National Cancer Institute, NIH). Irf4,6/f6 × Gdl1+/+ mice were obtained by breeding B6.129S1-Ify6tm1[lmig] mice with B6.Cg-Tg[ltagx-Cre]-1(cre) (ltagx-Cre) mice (both strains from The Jackson Laboratory). All mice were bred and maintained under pathogen-free conditions at an American Association for the Accreditation of Laboratory Animal Care (AAALAC)-accredited animal facility at the NIAID and housed in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals. All experiments were performed at the NIAID under an animal care and use proposal approved by the NIAID Animal Care and Use Committee. Gender- and age-matched mice between 6 and 12 weeks of age were used for each experiment. When possible, preliminary experiments were performed to determine requirements for sample size, taking into account resources available and ethical, reductionist animal use. In general, each mouse of the different experimental groups was reported. Exclusion criteria such as inadequate staining or low cell yield due to technical problems were pre-determined. Animals were assigned randomly to experimental groups.

Human and non-human primate skin tissue. Healthy human skin samples from anonymous patients were obtained as discarded material after cosmetic surgery, according to a protocol approved by the Institutional Review Board of NIAID. NIH. All subjects gave informed consent. Non-human primate skin tissue was obtained from the glabella of eight healthy rhesus (Macaca nemestrina) or pigtail (Macaca mulatta) macaques immediately post euthanasia. All animals were housed and cared in accordance with AAALAC standards in AAALAC-accredited facilities, and all animal procedures performed according to protocols approved by the NIAID Animal Care and Use Committee.

Topical association and intradermal infection. S. epidermidis strain 42E8031 and R. nasimurium were isolated from mouse colonies. S. epidermidis strain NIHMO687, S. epidermidis strain 42E803, S. lentus, S. xylosus (isolated from mice as previously described38), S. aureus strain NCTC 8325, C. pseudodiphtheriticum strain NIHLM086 and R. nasimurium were cultured for 18 h in tryptic soy broth at 37 °C. P. aerogenes ATCC 11827 was cultured for up to 72 h in tryptic soy broth in an anaerobic chamber. Bacteria were enumerated before topical application by assessing colony-forming units using traditional bacteriology techniques and by measuring optical density (OD) at 600 nm using a spectrophotometer.

For topical association of bacteria, each mouse was associated with bacteria by fresh digestion media and incubated at 37 °C with shaking. After 1 h, mice were killed by using 10% of normal mouse serum (Jackson Immunoresearch). Treatment schedule was as follows: 2 mg of either antibody was injected i.p. in each mouse 5 and 3 days before the first S. epidermidis topical application (days −5 and −3); each mouse then received 0.5 mg of either antibodies on days −2, −1 and days 0, 2, 4, 6, 9, 11 and 13 post topical application18. Mice were analysed two weeks after the first S. epidermidis topical application (or 19 days after the first antibody injection). For CD8 T-cell depletion, mice were treated i.p. with 0.5 mg of CD8 antibody (clone 2.43) or rat IgG2b isotype control antibody (clone LTF-2, BioXCell) every 2–3 days starting at day 6 after the first topical application of S. epidermidis. For IL-17A neutralization, mice were treated i.p. with 0.5 mg of anti-IL-17A antibody (clone 1F7, BioXCell) or mouse IgG1 isotype control antibody (clone MOPC-21, BioXCell) every 2 days starting at day 6 after the first topical application of S. epidermidis.

In vitro re-stimulation. For detection of basal cytokine potential, single-cell suspensions from various tissues were cultured directly ex vivo in a 96-well U-bottom plate in complete medium (RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 2 mM l-glutamine, 1 mM sodium pyruvate and nonessential amino acids, 20 mM HEPES, 100 U ml−1 penicillin, 100 μg ml−1 streptomycin, 50 μg ml−1 b-mercaptoethanol) and stimulated with 50 μg ml−1 phorbol myristate acetate (PMMAldrich) and 5 μg ml−1 (mouse) or 1 μg ml−1 (human) monokymycin (Sigma-Aldrich) for 2.5 h at 37 °C in 5% CO2. After stimulation, cells were assessed for intracellular cytokine production as described below.

Phenotypic analysis. Murine single-cell suspensions were incubated with fluorochrome-conjugated antibodies against surface markers CD3 (145–2C11), CD4 (clone RM4-5), CD8α (53–6–7), CD8β (eBioH13-17.2), CD11b (M1/70), CD11c (N418 or HL3), CD19 (6D5), CD45 (R45RA-6B2), CD49b (DX5), CD69 (H1.2F3), CD103 (2E7), DEC205 (NLDRC1.45L), KLRG1 (2FI1), MHCII (M5/11.152.5) and/or TCRβ (H5-57.97) in Hank’s buffered salt solution (HBSS) for 20 min at 4 °C washed. LIVE/DEAD Fixable Blue Dead Cell Stain Kit (Invitrogen Life Technologies) was used to exclude dead cells. Cells were then fixed for 15 min at 4 °C using 2% paraformaldehyde solution (Electron Microscopy Sciences) and washed twice. For simultaneous Foxp3 and intracellular cytokine staining, cells were stained with fluorochrome-conjugated antibodies against Foxp3 (FJK-16s), IFN-γ (XMG-16) and IL-17A (eBio17B7) in HBSS containing 0.5% saponin (Sigma-Aldrich) for 45 min at 4 °C. For detection of Langerin, cells were incubated with a fluorochrome-conjugated antibody against CD207 (929F3.01) in permeabilization buffer supplied with the BD Cytofix/Cytoperm kit (BD Biosciences) for 1 h at 4 °C. Each staining was performed in the presence of purified anti-mouse C16/32 (93.3), 0.2 mg ml−1 purified rat IgG and 1 mg ml−1 of normal mouse serum (Jackson Immunoresearch). Staining of cells from human or non-human primate skin tissue was performed using a similar protocol and the following antibodies against human proteins: anti-CD3 (SP3-42), anti-CD4 (L200), anti-CD8α (eBio87-T8), anti-IFN-γ (4S.B3) and anti-IL-17A (eBio64DEC17). All antibodies were purchased from eBioscience, BD Biosciences, Miltenyi Biotech or Dendritics. Cell acquisition was performed on an 15 Flow cytometer using FACSDiva software (BD Biosciences) and data were analysed using FlowJo software (TreeStar).

Analysis of skin microbiota after topical association. DNA extraction from skin and 454 pyrosequencing. Mouse ear skin samples were sterilely obtained and processed using a protocol adapted from ref. 31. For 16S RNA amplicon sequencing, the DNA from each sample was amplified using Accuprime High Fidelity Taq polymerase (Invitrogen Life Technologies) with universal primers flanking variable regions V1 (primer 27F 5′-AGATTTTGATCTCCTGCGAG-3′) and V3 (primer 534 R 5′-ATTACCGCGGTCTTCCTG-3′). For each sample, the universal primers were tagged with unique sequences (‘barcodes’) to allow for multiplexing/demultiplexing.39, PCR products were then purified using the Agencourt Ampure XP kit (Beckman Coulter Genomics) and quantitated using the QuantiFluor dsDNA High-Sensitivity Assay kit (Invitrogen Life Technologies). Approximately equivalent amounts of each PCR product were then pooled and purified on a column from the MinElute PCR Purification Kit (Qiagen) into 30 μl TE buffer before sequencing at the NIH Intramural Sequencing Center on a 454 GS FLX (Roche) instrument using titanium chemistry. Sequencing data were analysed as previously described39.

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**Bacteria quantitation.** The ear skin of topically associated or unassociated control mice was swabbed with a sterile cotton swab previously soaked in trypsic soy broth. Swabs were streaked on either tryptic soy agar or blood agar plates. Plates were then placed at 37 °C under aerobic or anaerobic conditions for 18 h. Colony-forming units on each plate were enumerated and the identity of the isolates was confirmed by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry.

**Purification of skin dendritic cell subsets.** Dendritic cell subsets were purified from the epidermis and dermis compartment of the ear skin tissue of unassociated mice and S. epidermidis-associated mice at days 5 and 12 post topical application. In brief, cell suspensions obtained from the dermis compartment were incubated with a mixture of antibodies containing anti-CD16/32 (93), anti-CD11b (M1/70), anti-CD11c (N418), anti-CD45.2 (104), anti-CD103 (2E7), anti-MHCII (MI/114.15.2) in the presence of DAPI (Sigma-Aldrich). Cells from the epidermis compartment were incubated with anti-CD16/32, anti-CD45.2, anti-MHCII and DAPI. The following two subsets of dendritic cells were sorted from the dermis by flow cytometry on a FACSARia (BD Biosciences): CD45.2+CD11c+MHCII+CD11b+CD103+ cells (CD103+ dendritic cells) and CD45.2+CD11c+MHCII+CD11b+CD11b- cells (CD11b+ dendritic cells). Langerhans cells were sorted from the epidermis as CD45.2+MHCII+ cells.

**Gene expression analysis by NanoString.** The nCounter analysis system (NanoString Technologies) was used to screen for the expression of signature genes associated with inflammation pathway in the different dendritic cell subsets. Two specific probes (capture and reporter) for each gene of interest were employed. In brief, RNA from each dendritic cell subset was obtained by lysing the sorted cells (10^6 cells per µl) in RLT buffer (Qiagen) and then hybridized with the customized Reporter CodeSet and Capture Probeset of the Mouse Inflammation Panel including 150 selected genes (NanoString Technologies), according to the manufacturer’s instructions. Messenger RNA molecules were counted on a NanoString nCounter, as previously described. Data analysis was performed according to NanoString Technologies recommendations. mRNA counts were processed to account for hybridization efficiency, background noise and sample content using the R package NanoStringNORM with arguments: CodeCount = ‘geo.mean’, Background = ‘mean.2sd’, SampleContent = ‘housekeeping.geo.mean’. Each sample profile was normalized to geometric mean before co-culture with CD8+ and CD4+ T cells and naive SpDC were supplemented with 5 µg ml^-1 ultra-pure lipopolysaccharide (LPS, InvivoGen), 100 nM N-α-palmitoyl-S-(2,3-bis[palmitoyloxy-(2RS)-propyl]-t-cysteine (Pam3Cys, InvivoGen) and/or 10 ng ml^-1 murine IL-12 (Peprotech). CD8+ and CD4+ T cells cytokine production following co-culture was determined by flow cytometry after intracellular cytokine staining using the following antibodies: anti-CD4, anti-CD8, anti-CD45.1 (clone A20), anti-CD45.2, anti-TCRβ, anti-IFN-γ and anti-IL-17A.

**Cytokine measurement.** Leukocytes isolated from the skin of S. epidermidis-associated mice were cultured in 100 µl of complete medium for 3 h at 37 °C in 5% CO2. FACs-purified CD8+ T cells from the skin of S. epidermidis-associated mice were cultured overnight at 37 °C in 5% CO2; in 30 µl of complete culture medium in a 96-well U-bottom plate coated with anti-CD3e (1 µg ml^-1, clone 145-2C11, BD Biosciences) in the presence or absence of IL-12 and IL-18 (10 ng ml^-1 each, Peprotech). Supernatants were collected and levels of inflammatory cytokines were assessed using a bead-based cytokine detection assay (FlowCytomix, EBioscience). Cytokine concentrations were adjusted to the plated density of 2 × 10^5 cells in 100 or 30 µl culture volume.

**Purification of keratinocytes.** Interfollicular keratinocytes were purified by cell sorting from the ear skin tissue of unassociated mice and S. epidermidis-associated mice at 14 days post topical application. In brief, cell suspensions obtained from ear skin tissue were incubated with the following antibodies: anti-CD16/32 (93), anti-CD45 (30-F11), anti-CD49f (eBioGoH3), anti-CD117 (2B8), anti-CD140a (APAS) and anti-Sca-1 (D7) in the presence of DAPI. Interfollicular keratinocytes were sorted by flow cytometry on a FACSARia (BD Biosciences) as CD45+CD117+CD49f+Sca-1- cells.

**Candida albicans infection.** Eleven days after the first topical application of S. epidermidis, mice were infected with C. albicans along the dorsal midline as previously described. In brief, C. albicans was grown in YPAD medium at 30 °C for 18 h and re-suspended at 10^8 C. albicans yeast per ml of sterile PBS. One hundred microliters of this suspension was applied to sandpaper (100 grit)-treated dorsal skin to achieve an infectious dose of 10^8 C. albicans. At 2 days post infection a 6 mm punch biopsy of skin was homogenized in sterile PBS containing penicillin and streptomycin before plating on YPAD plates. Colony-forming units were counted after culture at 30 °C for 48 h.

**RNA purification and quantitative PCR.** Total RNA was isolated from purified keratinocytes or skin tissue biopsies using the RNeasy Mini Kit (QIAGEN). Complementary DNA was prepared using the Omniscript Reverse Transcription Kit (QIAGEN) according to the manufacturer’s instructions. Quantitative real-time PCR was performed on a Bio-Rad iCycler using the iQ SYBR Green Supermix (BioRad) and predesigned Quantitect primers (QIAGEN) specific for the following genes: Hprt, S100a8 and S100a9.

**Histology.** Mice were euthanized 7 days after topical application or intraperitoneal injection of S. epidermidis LM087 in the ear. Unassociated mice were used as controls. The ears from each mouse were removed and fixed in PBS containing 10% formalin. Paraffin-embedded sections were cut at 0.5 mm, stained with haematoxylin and eosin and examined histologically.

**Statistics.** Data are presented as mean ± standard error of the mean or mean ± standard deviation. Group sizes were determined based on the results of preliminary experiments. No statistical method was used to predetermined sample size. Mice were assigned at random to groups. Mouse studies were not performed in a blinded fashion. Generally, each mouse of the different experimental groups is reported. Statistical significance was determined with the two-tailed unpaired Student’s t-test, under the untested assumption of normality. Within each group there was an estimate of variation, and the variance between groups was similar. All statistical analysis was calculated using Prism software (GraphPad). Differences were considered to be statistically significant when P < 0.05.

Extended Data Figure 1 | Assessment of Foxp3+ regulatory T cells and cytokine production by effector T cells after \( S. \) epidermidis topical application and/or intradermal inoculation. a, Frequencies and absolute numbers of skin regulatory (CD45\(^{–}\)CD4\(^{+}\) Foxp3\(^{+}\)) T cells in unassociated mice (Ctrl, \( n = 4 \)) and mice associated with \( S. \) epidermidis (\( n = 4 \)) at day 14 post first topical application. b, Absolute numbers of effector T cells producing IL-17A after PMA/ionomycin stimulation in the skin (ear pinnae) at day 14 post first topical application. c, Enumeration of colony-forming units and absolute numbers of neutrophils and monocytes in the skin of mice 14 days after the first topical application or intraderal inoculation with \( S. \) epidermidis (\( n = 4 \) per group). d, Assessment of cytokine production (mean \( \pm \) s.e.m., \( n = 3 \) per time point) by leukocytes from the ear skin tissue 24 and 48 h after topical association with \( S. \) epidermidis. Unassociated mice were used as controls. No significant amounts of IL-4, IL-5, IL-17A, IL-18, IL-21 or IL-22 could be detected at the time of analysis. g, IFN-\( \gamma \) and IL-17A production by skin effector T cells in mice 7 days after \( S. \) epidermidis topical application or intraderal inoculation. h, Frequencies of IFN-\( \gamma \) and IL-17A-producing effector T cells in the skin of mice 7 and 14 days after the first topical application or intraderal inoculation of \( S. \) epidermidis (\( n = 4–5 \) mice per group). All results shown are representative of 2–3 experiments with similar results. *\( P < 0.05 \); **\( P < 0.01 \); ***\( P < 0.001 \); NS, not statistically significant as calculated by Student’s t-test.
Extended Data Figure 2 | Assessment of CD8+ T-cell responses in the skin of specific pathogen-free and germ-free mice after topical application with skin commensals. 

**a**. Mice were left unassociated (Ctrl, n = 5) or topically associated with *S. epidermidis* human isolate (*n* = 5), *S. xylosus* (*n* = 5), *S. epidermidis* murine isolate (*S.epi* 42E03, *n* = 2), *S. lentus* (*n* = 2), *R. nasimurium* (*n* = 2), *S. aureus* (*n* = 5), *C. pseudodiphtheriticum* (*n* = 3) or *P. acnes* (*n* = 3). Quantification of colony-forming units from the ears after topical application is shown 2 weeks after first association. 

**b**. Frequencies and numbers of effector (CD45+ TCRβ+ CD4+ Foxp3−) T cells producing IFN-γ or IL-17A after PMA/ionomycin stimulation in the skin of mice from **a** at day 14 post first topical application. Bar graphs represent the mean value from two mice.

**c**. Frequencies of skin CD4+ and CD8β+ effector T cells in mice from **a** at day 14 post first topical application.

**d**. Absolute numbers of IFN-γ- and IL-17A-producing CD8β+ effector T cells in the skin of unassociated (Ctrl) mice or mice associated with different doses (107, 108 or 109 c.f.u. per ml) of *S. epidermidis* (*n* = 4 per group).

**e**. Absolute numbers of skin CD8β+ effector T cells in unassociated (Ctrl, *n* = 3) mice or mice associated with 1 ml (*n* = 5) or 5 ml (*n* = 5) of a suspension (109 c.f.u. per ml) of *S. epidermidis*. 

**f**. Flow cytometric assessment of the frequencies of CD4+ and CD8β+ effector T cells in unassociated (Ctrl) or *S. epidermidis*-associated SPF (*n* = 3 per group) and germ-free (GF, *n* = 4 per group) mice 2 weeks after *S. epidermidis* topical application.

**g**. Absolute numbers of IFN-γ- and IL-17A-producing CD8β+ effector T cells in the skin of unassociated (Ctrl) or *S. epidermidis*-associated C57BL/6 and BALB/c mice at 14 days post first topical application (*n* = 5 per group). For **d**–**g**, all results shown are representative of 2–3 independent experiments with similar results. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not statistically significant as calculated with Student’s t-test.

**h**. Quantification of colony-forming units from the ears of adult mice born from *S. epidermidis*-associated (*S. epi* 1, *n* = 3) or unassociated (Ctrl, *n* = 3) breeder pairs. Flow plots and bar graphs (mean ± s.e.m.) illustrate the frequencies of CD4+ and CD8β+ effector T cells and absolute numbers of CD8β+ effector T cells, respectively. n.d., not detected; **P < 0.01 as calculated with Student’s t-test.
Extended Data Figure 3 | CD8<sup>+</sup> T cells accumulate preferentially in the epidermis after topical application of S. epidermidis. a, Proportion of effector (CD45<sup>+</sup> TCR<sup>β</sup><sup>+</sup> Foxp3<sup>+</sup>) CD8<sup>β</sup><sup>+</sup> T cells in the epidermal and dermal compartments of the ear skin tissue 2 weeks after the first S. epidermidis topical application. b, Representative imaging volume projected along the x axis of ears from Langerin–GFP reporter mice at 14 days post first topical application with S. epidermidis. Scale bars, 30 μm. c, Numbers of CD3<sup>+</sup> CD8<sup>β</sup><sup>+</sup> cells producing IFN-γ or IL-17A (after PMA/ionomycin stimulation) from normal nonhuman primate (NHP) skin (n = 8). d, Assessment of IL-17A production in the supernatant of CD8<sup>β</sup><sup>+</sup> T cells purified from the skin of mice topically associated with S. epidermidis and cultured overnight in presence of anti-CD3e alone (Ctrl) or with IL-1α and IL-1β (+ IL-1). Bars represent the mean value ± s.e.m. (n = 3, **P < 0.01 as calculated with Student’s t-test). Results shown in a, c and d are representative of 2–3 experiments with similar results.
Extended Data Figure 4 | Depletion strategies for the different subsets of skin dendritic cells. a, Gating strategy for various dendritic cell subsets in the skin. Cells are first gated on live CD45+ CD11c+ MHCII+. Subsets of dendritic cells are then defined as follows: Langerhans cells (LC) are gated on CD11b+ CD207(Langerin)+ cells, CD103+ dendritic cells (CD103 DC) on CD11b+ CD207− cells and CD11b− dermal dendritic cells (CD11b DC) on CD11b+ CD207− cells. b, Comparative assessment by flow cytometry of Langerhans cell, CD103 DC and CD11b DC in the ear skin of unassociated mice (control) and mice first topically associated with S. epidermidis- or IL-17A−producing CD8+ effector T cells in S. epidermidis-associated Irf4β/β × CD11cCre+ (n = 3) and littermate control (n = 3) mice. All data shown in this figure are representative of 2–3 experiments with similar results. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not statistically significant as calculated with Student’s t-test.
Extended Data Figure 5 | Commensal-driven CD4+ and CD8+ T-cell responses in the skin tissue and the skin draining lymph nodes are specific for commensal antigens. a, Frequencies of IFN-γ- or IL-17A-producing CD8β+ T cells in overnight co-cultures of splenic dendritic cells (SpDC) and CD8β+ T cells purified from the skin draining lymph node (dLN) of mice first topically associated with S. epidermidis 2 weeks earlier. b, Frequencies of IFN-γ- and IL-17A-producing CD8β+ T cells in overnight co-cultures of SpDC and CD8β+ T cells purified from the skin of mice 14 days after the first S. epidermidis application. Dendritic cells were purified from either wild-type (WT) or Abb/−− B2m−−/− mice. c, d, Frequencies of IFN-γ- and IL-17A-producing CD4+ T cells in overnight co-cultures of SpDC and CD8β+ T cells purified from the skin ear tissue or the skin dLN of mice 14 days after the first S. epidermidis application. For a, b and d, Ctrl, naive SpDC, S. epi, SpDC + heat-killed S. epidermidis; Abb/B2m S. epi, Abb−−/B2m−−/−; SpDC + heat-killed S. epidermidis. e, Frequencies of IFN-γ- and IL-17A-producing CD4+ T cells in overnight co-cultures of SpDC and CD8β+ T cells purified from the skin ear tissue or the skin dLN of mice 14 days after the first S. xylosus application. Ctrl, naive SpDC, S. xylo, SpDC + heat-killed S. xylosus; Abb/B2m S. xylo, Abb−−/B2m−−/− SpDC + heat-killed S. xylosus. All data shown in a–d are representative of three independent experiments. Graph bars represent the mean ± standard deviation of triplicate cultures. **p < 0.01, ***p < 0.001, ****p < 0.0001 as calculated with Student’s t-test. f, S100a8 and S100a9 gene expression in dorsal skin biopsies of mice associated with different doses (107, 108 or 109 cfu ml−1) of S. epidermidis 2 weeks after the first topical application (n = 4 per group). Data are expressed as fold increase over gene expression in unassociated control mice.