**Staphylococcus** δ-toxin induces allergic skin disease by activating mast cells

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Atopic dermatitis is a chronic inflammatory skin disease that affects 15–30% of children and approximately 5% of adults in industrialized countries1. Although the pathogenesis of atopic dermatitis is not fully understood, the disease is mediated by an abnormal immunoglobulin-E immune response in the setting of skin barrier dysfunction2. Mast cells contribute to immunoglobulin-E-mediated allergic disorders including atopic dermatitis3. Upon activation, mast cells release their membrane-bound cytotoxic granules leading to the release of several molecules that are important in the pathogenesis of atopic dermatitis and host defence. More than 90% of patients with atopic dermatitis are colonized with *Staphylococcus aureus* in the lesional skin whereas most healthy individuals do not harbour the pathogen. Several staphylococcal exotoxins can act as superantigens and/or antigens in models of atopic dermatitis. However, the role of these staphylococcal exotoxins in disease pathogenesis remains unclear. Here we report that culture supernatants of *S. aureus* contain potent mast-cell degranulation activity. Biochemical analysis identified δ-toxin as the mast cell degranulation-inducing factor produced by *S. aureus*. Mast cell degranulation induced by δ-toxin depended on phosphoinositide 3-kinase and calcium (Ca2+) influx; however, unlike that mediated by immunoglobulin-E crosslinking, it did not require the spleen tyrosine kinase. In addition, immunoglobulin-E enhanced δ-toxin-induced mast cell degranulation in the absence of antigen. Furthermore, *S. aureus* isolates recovered from patients with atopic dermatitis produced large amounts of δ-toxin. Skin colonization with *S. aureus*, but not a mutant deficient in δ-toxin, promoted immunoglobulin-E and interleukin-4 production, as well as inflammatory skin disease. Furthermore, enhancement of immunoglobulin-E production and dermatisis by δ-toxin was abrogated in KitW-sh/W-sh mast-cell-deficient mice and restored by mast cell reconstitution. These studies identify δ-toxin as a potent inducer of mast cell degranulation and suggest a mechanistic link between *S. aureus* colonization and allergic skin disease.

Because mast cells (MCs) may play a critical role in the pathogenesis of atopic dermatitis4, we asked first whether *S. aureus* can release factors that induce MC degranulation. We found that the culture supernatant of *S. aureus* induced rapid and robust MC degranulation in a dose-dependent manner (Fig. 1a and Supplementary Fig. 1a, b). Analysis of a panel of *Staphylococcus* isolates showed that the culture supernatant of several *S. aureus* strains as well as of that from *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*, but not of several *Staphylococcus* species, elicited MC degranulation (Supplementary Fig. 1c). Toll-like receptor 2 (TLR2) stimulation by lipoproteins has been shown by some studies, but not others, to induce MC degranulation5,6. However, neither the culture supernatant of *S. aureus* deficient in lipoproteins (Δlgt), which lacks TLR2-stimulating activity5, nor that from bacteria deficient in α-, β- and γ-haemolysins (Δα,β,γ) were impaired in MC degranation activity (Supplementary Figs 1c and 3c). The MC degranulation activity was enriched in the culture supernatant of *S. aureus* and was sensitive to heat, phenol/chloroform extraction and protease K treatment (Supplementary Fig. 2a). Furthermore, the MC degranulation-inducing factor bound to both diethylaminoethyl and carboxymethyl cellulose matrices and was present in the void fraction on gel filtration at neutral pH (Supplementary Fig. 2b). On the basis of these observations, we developed a many-step strategy for biochemical purification of the MC degranulation-inducing factor (Supplementary Fig. 2c). Liquid chromatography–mass spectrometry analysis showed that δ-toxin (also called δ-haemolysin or phenol-soluble modulin (PSM)-δ), a 2.9 kDa peptide secreted by *S. aureus* that belongs to the peptide toxin family of PSMs, was the most abundant and significant protein identified in the purified sample (Supplementary Fig. 2c). Mutant analyses in two *S. aureus* strains showed that MC degranulation induced by *S. aureus* culture supernatant required expression of δ-toxin whereas deficiency of related PSM-α or PSM-β peptides had minimal or no effect on MC degranulation (Fig. 1b and Supplementary Fig. 3a). Complementation of the *AhlD* mutant strain with δ-toxin-producing plasmid, but not control plasmid, restored the ability of the culture supernatant to induce MC degranulation (Fig. 1b). Stimulation of MCs with 30 μg ml–1 of synthetic δ-toxin peptide, a concentration of δ-toxin normally found in *S. aureus* culture supernatants (Supplementary Fig. 3b), also induced rapid release of histamine (Fig. 1c). Furthermore, transmission electron microscopy showed classic features of MC degranulation without loss of plasma membrane integrity upon δ-toxin stimulation (Fig. 1d). These results indicate that δ-toxin is the MC degranulation-inducing factor released by *S. aureus*.

PSMs, especially PSM-α2 and PSM-α3, induce cell death and interleukin (IL)-8 release in human neutrophils10,11. In accord with these results10, PSM-α2 and PSM-α3 induced robust loss of cell viability in MCs (Supplementary Fig. 4a). Non-toxic concentrations of PSM-α2 did not possess any MC-degranation activity (Supplementary Fig. 4b). In contrast, stimulation with a concentration of δ-toxin that induces robust MC degranulation did not induce detectable cell death in MCs (Supplementary Fig. 4a, c). Furthermore, formylation of the amino (N) terminus of the δ-toxin peptide was not required for MC degranulation activity, whereas it was essential for the ability of δ-toxin to induce the release of IL-8 from human neutrophils (Supplementary Fig. 4c, d). Consistent with previous results, stimulation of human neutrophils with formylated PSM-α2, PSM-α3 or δ-toxin induced robust IL-8 release (Supplementary Fig. 4d). Moreover, stimulation of primary mouse macrophages and keratinocytes with PSM-α2, but not δ-toxin, triggered robust cell death (Supplementary Fig. 5). Thus, the MC degranulation activity induced by δ-toxin is not associated with cell death and is different from other activities triggered by PSM-α2 and PSM-α3. Immunoblotting confirmed that the presence of δ-toxin in *S. aureus* supernatants correlated with MC degranulation activity (Fig. 1e). Notably, the supernatant

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The administration of a toxicin by the vascular leakage of Evan’s blue dye into the extravascular space between experimental and control groups (Fig. 6). Furthermore, deficiency of weak MC degranulation which correlated with smaller amounts of degranulation in vitro and in vivo.

To assess whether δ-toxin induces MC degranulation in vitro and in vivo. Ca2+ influx in human neutrophils is triggered by δ-toxin through FPR2 (ref. 11). Because Ca2+ influx is an essential step in MC degranulation, we analysed whether δ-toxin induces Ca2+ influx in MCs. Stimulation of MCs with ionicycin or dinitrophenol (DNP) plus anti-DNP immunoglobulin-E (IgE) induced rapid Ca2+ influx (Fig. 2a). Likewise, δ-toxin triggered Ca2+ influx and this was abrogated by treatment with the Ca2+ chelator ethylene glycol tetra-acetic acid (EGTA) (Fig. 2a). EGTA also blocked MC degranulation induced by ionicycin, DNP plus anti-DNP IgE or δ-toxin (Fig. 2b). Similarly, MC degranulation induced by DNP plus anti-DNP IgE or δ-toxin was inhibited by the phosphoinositide 3-kinase inhibitor LY294002 (Fig. 2c).

Unlike antigen plus IgE, MC degranulation induced by δ-toxin did not require spleen tyrosine kinase (Syk) (Fig. 2d). Fpr1, Fpr2 and related family members were expressed in mouse MCs although their expression was higher in neutrophils (Supplementary Fig. 8). WRW4, a peptide antagonist of formyl peptide receptor 2 (FPR2), blocks human and mouse neutrophil activation induced by PSMs including δ-toxin. Notably, WRW4 inhibited mouse MC degranulation induced by δ-toxin both in vitro and in vivo (Supplementary Fig. 9a, b). Cyclosporin H, an antagonist of human FPR1, also partly inhibited mouse MC degranulation induced by δ-toxin (Supplementary Fig. 9c). However, human FPR2 ligands, MMK1 and lipoxin A4 did not induce mouse MC degranulation (Supplementary Fig. 10a). Furthermore, treatment with pertussis toxin (PTX), an inhibitor of G-protein coupled receptors, partly reduced MC degranulation induced by δ-toxin (Supplementary Fig. 10b). However, MCs from wild-type and Fpr2+/− mice showed comparable MC degranulation induced by δ-toxin (Supplementary Fig. 10c). Collectively, these results indicate that δ-toxin induces MC degranulation through a signalling pathway that is different from that induced through antigen and IgE.

Stimulation with IgE and antigen, but not monomeric IgE, induces robust MC degranulation. Notably, pre-incubation of MCs with anti-DNP or anti-trinitrophenyl (TNP) IgE alone markedly increased the degranulation activity of δ-toxin (Fig. 3a). The synergistic effect of monomeric IgE and δ-toxin was abrogated in MCs deficient in Syk (Fig. 3b). To test whether the synergism between monomeric IgE and δ-toxin could be observed in vivo, we injected monomeric IgE and δ-toxin (at concentrations not inducing MC degranulation) into the skin of mice and monitored MC degranulation in vivo with the PCA assay. At these inactive concentrations, δ-toxin induced Evans blue dye leaking at the site of injection in mice pre-treated with anti-DNP (Fig. 3c). These results indicate that IgE increases the MC degranulation activity of δ-toxin in the absence of antigen.

RNAIII, a regulatory RNA that is induced by the agr quorum-sensing system of Staphylococcus aureus, encodes δ-toxin. Additionally, supernatants from 26 S. aureus strains isolated from the lesional skin of patients with atopic dermatitis produced δ-toxin (Supplementary Fig. 11a). Moreover, RNAIII expression was detected in lesional skin colonized with S. aureus, but not normal skin, of patients with atopic dermatitis (Supplementary Fig. 11b, c). To test whether δ-toxin plays a role in allergic skin disease, we used a modified epidermograph disease model in which the skin of BALB/c mice was colonized with wild-type or δ-toxin-deficient S. aureus and then challenged once with ovalbumin (OVA) to assess antigen-specific IgE production (Fig. 4a). One week after colonization with wild-type S. aureus, the mice developed severely inflamed reddened skin at the site of application (Fig. 4b, c). Expression of hld was detected in the skin on day 4 after bacterial colonization using a bioluminescent reporter S. aureus strain (Supplementary Fig. 12). Histological analysis showed spongiosis, parakeratosis and marked neutrophil-rich inflammatory infiltrates in the skin of mice colonized with wild-type S. aureus (Fig. 4c, d). In contrast, mice colonized with S. aureus lacking δ-toxin showed a significantly reduced skin inflammatory cell infiltrate and
**Research**

At 3 weeks, there was a slight increase in IgG1 production in mice colonized with the wild-type bacterium (Supplementary Fig. 13). Furthermore, mice colonized with a δ-toxin-producing S. aureus developed greater amounts of total serum IgE and IgG1 production dependent on S. aureus but independent of δ-toxin. In addition, pre-colonization with wild-type, but not the δ-toxin-deficient, S. aureus enhanced the production of OVA-specific IgE (Fig. 4f). Colonization with S. aureus without disrupting the skin barrier by stripping also induced inflammatory disease and enhanced IgE responses (Supplementary Fig. 16). Pre-colonization with δ-toxin-producing S. aureus was important to elicit antigen-specific IgE because administration of OVA before or concurrent with S. aureus colonization did not enhance OVA-specific IgE production (Supplementary Fig. 17). To test whether δ-toxin is sufficient to trigger allergic skin disease, we epicutaneously sensitized the skin of mice with OVA in the presence and absence of δ-toxin and challenged the mice with OVA alone or OVA plus δ-toxin 3 weeks later. We found that δ-toxin triggered inflammatory skin disease including OVA-specific IgE and IgG1 production whereas challenge with OVA alone did not (Supplementary Fig. 18).

**Figure 3** | Antigen-independent IgE signalling enhances δ-toxin-induced MC activation. **a**, Activity of β-hexosaminidase in culture supernatants of FSMCs stimulated with or without anti-DNP-IgE or TNP-IgE and then re-stimulated with δ-toxin (0.01 μg ml⁻¹), DNP-HSA (DNP) or TNP-HSA (TNP). **b**, Activity of β-hexosaminidase in culture supernatants of FSMCs derived from Syk⁻/⁻ and wild-type (WT) mice pre-treated with or without anti-DNP-IgE, then stimulated with the indicated concentration of δ-toxin (μg ml⁻¹). Representative of at least three independent experiments. **P < 0.01, ***P < 0.001, two-tailed t-test (**, ***).**

**Figure 2** | MC degranulation induced by δ-toxin depends on Ca²⁺ influx/phosphoinositide 3-kinase pathway, but is independent of Syk. **a**, FSMCs loaded with the fluorescent Ca²⁺ indicator Fluo-4AM with or without EGTA were stimulated for 50 s. Baseline fluorescence (red) was measured, then the MCs were stimulated with indicated stimuli and fluorescence shift (green) was measured. RFU, relative fluorescence units. **b**, c, Activity of β-hexosaminidase in culture supernatants of FSMCs pre-treated with EGTA (b) or LY294002 (c) stimulated with medium alone (control), ionomycin, DNP-HSA (DNP) plus anti-DNP-IgE or δ-toxin (10 μg ml⁻¹). **b**, Activity of β-hexosaminidase in culture supernatants of FSMCs derived from Syk⁻/⁻ and wild-type (WT) mice stimulated with the indicated concentration of δ-toxin (micrograms per millilitre). Data represent means ± s.d. of triplicate cultures and are representative of at least three independent experiments (b–d). NS, not significant; **P < 0.05, ***P < 0.01; ****P < 0.001, two-tailed t-test.

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*NS*, not significant; *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed t-test. **Figure 2** | MC degranulation induced by δ-toxin depends on Ca²⁺ influx/phosphoinositide 3-kinase pathway, but is independent of Syk. **a**, FSMCs loaded with the fluorescent Ca²⁺ indicator Fluo-4AM with or without EGTA were stimulated for 50 s. Baseline fluorescence (red) was measured, then the MCs were stimulated with indicated stimuli and fluorescence shift (green) was measured. RFU, relative fluorescence units. **b**, c, Activity of β-hexosaminidase in culture supernatants of FSMCs pre-treated with EGTA (b) or LY294002 (c) stimulated with medium alone (control), ionomycin, DNP-HSA (DNP) plus anti-DNP-IgE or δ-toxin (10 μg ml⁻¹). **d**, Activity of β-hexosaminidase in culture supernatants of FSMCs derived from Syk⁻/⁻ and wild-type (WT) mice stimulated with the indicated concentration of δ-toxin (μg ml⁻¹). Representative of at least three independent experiments. **P < 0.01, ***P < 0.001, two-tailed t-test (**, ***).

**d**}
**Figure 4** | Staphylococcus δ-toxin promotes IgE production and inflammatory skin disease by mast cells. a. S. aureus (S. a.) colonization and OVA sensitization protocol. Mice were colonized epicutaneously with 10^8 colony-forming units of S. aureus using a gauze patch for 1 week. For OVA sensitization, a patch containing OVA or PBS was applied to the same skin site 2 weeks after S. aureus inoculation. b. Skin disease score 1 week after colonization with wild-type and δ-toxin mutant (Δhld) S. aureus or treated with PBS. **P < 0.01; ***P < 0.001, Kruskal–Wallis test with post-hoc Dunn’s test for multiple comparisons. c. Skin phenotype and histopathology of BALB/c mice colonized with wild-type and S. aureus or treated with PBS. Skin sections were stained with haematoxylin and eosin. Scale bar, 100 μm. Inset shows high-power image with neutrophil-rich inflammation. Representative of 14 mice per group. d. Number of inflammatory cells in skin of BALB/c mice colonized with S. aureus or treated with PBS. Results are depicted as the number of inflammatory cells per high-power field. Error bars, means ± s.e.m. e. Concentrations of serum IgE in BALB/c mice colonized with S. aureus or treated with PBS at 1 and 3 weeks after colonization with S. aureus. f. Concentrations of serum OVA-specific IgE after OVA sensitization in BALB/c mice colonized with S. aureus or treated with PBS. A405, absorbance at 405 nm. g. Skin disease score in C57BL/6 (B6), MC-deficient (Kit^W-sh/W-sh^) and MC-deficient (Kit^W-sh/W-sh^) mice reconstituted with MCs at 1 week after the inoculation with S. aureus. h. Concentrations of serum IgE 1 week after colonization of B6, Kit^W-sh/W-sh^ and Kit^W-sh/W-sh^ mice reconstituted with MCs with wild-type and δ-toxin mutant (Δhld) S. aureus or treated with PBS. Dots represent individual mice pooled from two independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001, one-way analysis of variance with Tukey post-hoc test for multiple comparisons (e–h).

with wild-type S. aureus also developed higher concentrations of serum IgE and more severe inflammatory skin disease than mice inoculated with the bacterium deficient in δ-toxin (Fig. 4g, h). MC-deficient Kit^W-sh/W-sh^ mice inoculated with wild-type S. aureus showed reduced concentrations of IgE serum and skin inflammation than wild-type mice (Fig. 4g, h). Adoptive transfer of MCs into the skin of Kit^W-sh/W-sh^ mice restored skin disease and increased IgE production in mice colonized with wild type, but not S. aureus lacking δ-toxin (Fig. 4g, h and Supplementary Fig. 19). There were increased numbers of S. aureus and total bacteria in the skin of Kit^W-sh/W-sh^ mice (Supplementary Fig. 19), suggesting that mast cells can regulate bacterial colonization under our experimental conditions. Microscopic analysis showed that the dermal MC densities in the skin of Kit^W-sh/W-sh^ recipient mice were approximately 50% of those found in age-matched C57BL/6 mice (Supplementary Fig. 19). Furthermore, toluidine-positive granules associated with MC degranulation were present in the skin of mice colonized with wild-type, but not δ-toxin-deficient, S. aureus (Supplementary Fig. 19). Taken together, these results indicate that δ-toxin from S. aureus promotes allergic skin disease through activation of MCs.

The δ-toxin transcript is contained in RNAIII, a regulatory RNA that governs S. aureus virulence genes^{13,14}. The role of δ-toxin in the growth of S. aureus is not understood. Because δ-toxin can form pores
on the surface of certain bacteria, one possibility is that it promotes pathogen colonization by killing competing bacteria. Our results indicate that the host senses *S. aureus* through the detection of δ-toxin to promote innate and adaptive Th2 immune responses by MC degranulation. Although clinical studies are needed to determine the role of δ-toxin in atopic dermatitis, our results in mouse models suggest that in the setting of genetic defects associated with the disease, δ-toxin may promote allergic immune responses and that strategies to inhibit δ-toxin might be beneficial for the treatment of atopic dermatitis.

**METHODS SUMMARY**

**Culture of mast cells and degranulation.** Preparations of BMCMCs and fetal skin-derived mast cells (FSMCs) were previously described. The purity of MCs was greater than 95% as assessed by surface expression of FcεRI and CD117 (e Bioscience). Degranulation of MCs was assessed by β-hexosaminidase assay as described. PCA assay. PCA assay was performed as described with minor modifications.

**Epicutaneous sensitization with *S. aureus***. The dorsal skin of 6- to 8-week-old female mice was shaved and stripped using a transparent bio-occlusive dressing (Tegaderm; 3M). One hundred million colony-forming units of *S. aureus* female mice was shaved and stripped using a transparent bio-occlusive dressing (Tegaderm; 3M). Each mouse was exposed to *S. aureus* for 1 week through the patch. After a 2 week interval, each mouse was challenged once with 100 μg ovalbumin epicutaneously for 1 week and the animals then killed for analyses.

**Animal study.** All animal studies were performed according to approved protocols by the University of Michigan Committee on the Use and Care of Animals.

**Statistical analysis.** All analyses were performed using GraphPad Prism. Differences were considered significant when *P* < 0.05.

**Online Content** Any additional Methods, 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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**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** Y.N., N.I. and G.N. designed the research. Y.N. conducted the experiments and analyzed data with the help of R.M.-P., S.M.C. and M.H. J.O., K.B.C., J.B.T. and M.J.M. generated and provided critical reagents or material. A.E.V, G.Y.C.C. and M.O. engineered bacterial strains. Y.N. and G.N. wrote the manuscript. All authors discussed the results and commented on the manuscript.

**Author Information** 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 G.N. (gabriel.nunez@umich.edu).
**METHODS**

**Bacterial strains.** *S. aureus* strain 8325-4 and its isogenic toxin mutant (Δ*fus*) have been previously described.

*S. aureus* strains SA113 and Newman, and isogenic mutants deficient in lipoprotein diaalcglycerol transferase (*AgtI*), have also been previously described.

*S. epidermidis* strain 3220 (NI335), *Protein purification from* *S. aureus* culture supernatant.

manufacturer's protocol.

lity. Polyclonal anti-

ing. We used 4- to 12-week-old age-matched female mice for

procedure.

Tandem were then searched for variants in the Tandem samples.

**Culture of mast cells and degranulation.** Preparations of BMCMCs and fetal skin-derived mast cells (FSMCs) were previously described.

Bone marrow cells from *P. ergosterol* mice were provided by J. M. Wang. The purity of MCs was greater than 95% as determined by surface expression of FcεRI and CD117 (eBioscience).

**PCR reconstitution in mice.** Thorough BMCMC reconstitution experiments.

10^8 BMCMCs (cell purity was greater than 95%) were injected into the ear skin. Four million BMCMCs in 50 μl × eight injections were injected into the shaved back skin of non-randomized *Knw-ab*-mice as described.

Four to six weeks later, the mice were subjected to experimental PCA assay or epicutaneous *S. aureus* sensitization. The number of animals per group (n = 5–8) was chosen as the number probably required for conclusions of biological significance, established from previous experience. The reconstitution rate of cutaneous MCs was quantified blindly by an independent observer and scored as the number of MCs per low-power field in toluidine blue stained tissue slides by microscopy.

The average rate of reconstituted MCs was approximately 40% in the ear pinnal and 50% in the back skin (Supplementary Figs 19 and 20).

**PCA assay.** PCA assay was performed as previously described with minor modifications.

Ears of non-randomized mice were injected intradermally with or without Δ-DNP-IgE (40 μg/ml saline; 15 h later, mice were challenged with 20 μl saline with or without synthetic δ-toxin (100 μg/ml saline) for 1 min).

**Extravasation of Evans blue dye was measured by fluorescence microscopy**

as described. Quantitative analysis of extracts was determined by measuring the absorbance at 600 nm.

**Cat**^−^** influx assay.** FSMCs (2 × 10^6 ml^−1) were pre-loaded with or without anti-DNP-IgE (0.3 μg/ml) in RPMI with IL-3 for 15 h. Cells were washed and then labeled with 5 μM (5 μM). Cells were washed again and further incubated in Tyrode’s buffer with or without EGTA (1 mM) for 30 min. DNP-HSA (30 ng/ml), ionomycin (1 μM), and/or δ-toxin (30 μg/ml) were used to induce calcium flux in these cells. **Cat**^−^** fluorescence was measured using a flow cytometer (FCM) in the absorbance at 600 nm.

**Epicutaneous sensitization with *S. aureus* or OVA.** We performed epicutaneous colonization with *S. aureus* by shaving the dorsal skin of non-randomized 6- to 8-week-old female mice and three-time stripping using a transparent bio-occlusive dressing (Tegaderm; 3M). Sample size (n = 5–8 per group) was based on previous experience as the minimum probably required for conclusions of biological significance and adequate statistical analysis. After overnight culture at 37 °C with shaking, *S. aureus* were cultured in fresh TSB medium for 4 h at 37 °C with shaking, washed and re-suspended in PBS at 10^6 colony-forming units of *S. aureus* LAC or LAC (*Δfus*) strains. One hundred microlitres of the *S. aureus* suspension was placed on a patch of sterile gauze (1 cm x 1 cm) and attached to the shaved skin with transparent bio-occlusive dressing. Each mouse was exposed to *S. aureus* for 1 week through the patch. After a 2-week interval, each mouse was challenged once with 100 μg OVA (Grade V, Sigma) epicutaneously for 1 week and the animals were killed for analyses.

For OVA sensitization model, BALB/c mice were sensitized epicutaneously with 5 μg OVA (Sigma) for 10 days.

**Skin disease score.** The severity of skin lesions was scored according to defined macroscopic diagnostic criteria in a blind fashion.

In brief, the total clinical score
of skin lesions was designated as the sum of individual scores, graded as 0 (none), 1 (mild), 2 (moderate) and 3 (severe) for thickness, erythema, oedema, erosion and scaling.

**Histology.** Skin tissue was formalin fixed, paraffin embedded and sectioned for haematoxylin and eosin and toluidine blue staining.

**Cytokine and immunoglobulin concentrations.** Chemokines and cytokines were measured with enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems). For tissue cytokines, the skin tissue (5 mm × 10 mm area) was removed and homogenized. The skin homogenates were centrifuged and supernatants were collected for cytokine measurements by ELISA. Serum IgG1 and IgG2a were measured with ELISA kits (Bethyl Laboratories). ELISA for OVA-IgE was described previously31.

**RNA isolation from human skin samples.** Wash fluid derived from lesional and normal skin of patients with atopic dermatitis was collected using a 2.5-cm-diameter polypropylene chamber as reported31. One hundred microlitres of the samples were mixed with an equal volume of RNAprotect Bacteria Reagent (QIAGEN) and RNA extracted with a Bacterial RNA Kit (OMEGA). The human studies were approved by the Indiana University Institutional Review Committee31. Informed consent was obtained from all participants.

**Quantitative real-time PCR with reverse transcription.** Complementary DNA was synthesized using a High Capacity RNA-to-cDNA Kit (Applied Biosystems), according to the manufacturer’s instructions. Quantitative real-time RT–PCR (qPCR) was performed using a SYBR green PCR master mix (Applied Biosystems) and StepOne Real-time PCR system (Applied Biosystems). Primers to amplify mouse Fpr genes32 and bacterial genes (rRNAIII, gyrB, 16S rRNA) have been described33,34. Expression of mouse Fpr genes was normalized to that of Gapdh (F: 5'-CCTCCTGC CCGTAGACAAATG-3', R: 5'-TCTCCACTTTGCCACCTGCAA-3') and expression was analysed by the 2^ΔΔCT method. RNAIII expression in human skin samples was normalized to that of S. aureus gyrB and that of gyrB to universal bacterial 16S rRNA, and relative expression calculated by the 2^ΔΔCT method. RNAIII and gyrB expression in some human skin samples was below the detection limit and arbitrarily given a value of zero for statistical analysis. LAC wild type and LAC Aagr cultured for 24 h were used as reference controls.

**Measurement of P3-lx expression.** To determine the amounts of P3-lx expression in culture, 10^6 ml^-1 LAC P3-lx strain was suspended in TSB and luminescence emitted from P3-lx-expressing bacteria was measured using a LMax luminometer (Molecular Devices). For in vivo bioluminescence imaging, mice were killed, the skin dressing removed and immediately placed into the light-tight chamber of the CCD (charge-coupled device) camera system (IVIS200, Xenogen). Luminescence emitted from lux-expressing bacteria in the tissue was quantified using the software program Living Image (Xenogen).