LETTER

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

Yuumi Nakamura[1], Jon Oscherwitz[2,3], Kemp B. Cease[2,3], Susana M. Chan[1], Raul Muñoz-Planillo[1], Mizuho Hasegawa[1], Amer E. Villaruz[4], Gordon Y. C. Cheung[4], Martin J. McGavin[5], Jeffrey B. Travers[6], Michael Otto[4], Naohiro Inohara[1] & Gabriel Núñez[1]

Atopic dermatitis is a chronic inflammatory skin disease that affects 15–30% of children and approximately 5% of adults in industrialized countries[1]. 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 dysfunction[2]. Mast cells contribute to immunoglobulin-E-mediated allergic disorders including atopic dermatitis[3]. Upon activation, mast cells release their membrane-bound cytosolic granules leading to the release of several molecules that are important in the pathogenesis of atopic dermatitis and host defence[4]. 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[5]. Several staphylococcal exotoxins can act as superantigens and/or antigens in models of atopic dermatitis[6]. 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 (Ca^{2+}) 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 dermatitis by δ-toxin was abrogated in $Kit^{W\text{-}sh/W\text{-}sh}$ 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 dermatitis[3], 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 lipopeptides has been shown by some studies, but not others, to induce MC degranulation[7,8]. However, neither the culture supernatant of *S. aureus* deficient in lipoproteins (*Δlgt*), which lacks TLR2-stimulating activity[9], nor that from bacteria deficient in α-, β- and γ-haemolysins (*Δαβγ*) were impaired in MC degranulation 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)-γ), 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 *Δhld* 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 neutrophils[10,11]. In accord with these results[10], PSM-α2 and PSM-α3 induced robust loss of cell viability in MCs (Supplementary Fig. 4a). Non-toxic concentrations of PSM-αs did not possess any MC-degranulation 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

[1]Department of Pathology and Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA. [2]Department of Internal Medicine, Division of Hematology/Oncology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA. [3]VA Ann Arbor Healthcare System, 2215 Fuller Road, Ann Arbor, Michigan 48105, USA. [4]Laboratory of Human Bacterial Pathogenesis, National Institute of Allergy and Infectious Diseases, US National Institutes of Health, Bethesda, Maryland 20892, USA. [5]Department of Microbiology and Immunology, and Centre for Human Immunology, Western University, University of Western Ontario, London, Ontario, N6A 5C1, Canada. [6]Department of Dermatology, Indiana University School of Medicine, Indianapolis, Indiana 46202, USA.

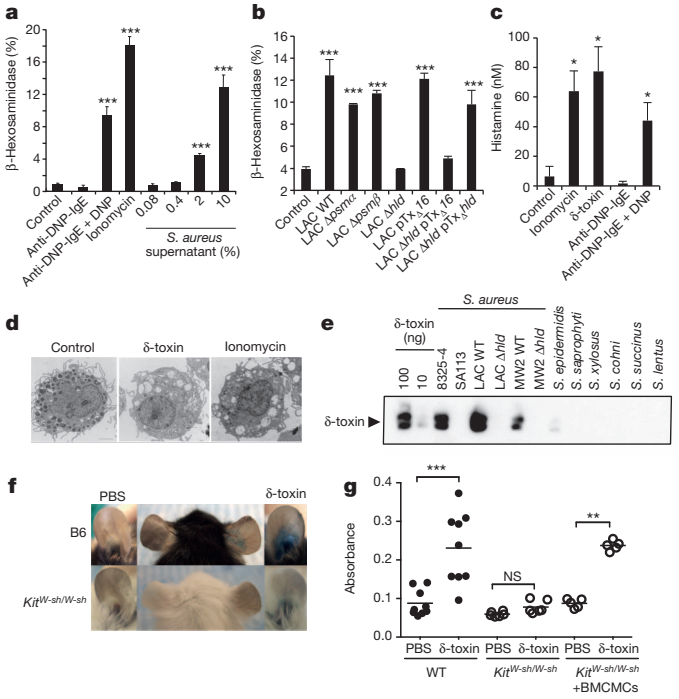

Figure 1 | *S. aureus* δ-toxin induces MC degranulation *in vitro* and *in vivo*. **a**, Activity of β-hexosaminidase released to the extracellular media of BMCMCs stimulated with medium alone (control) or indicated stimuli including different concentrations of culture supernatant of *S. aureus* 8325-4. **b**, Activity of β-hexosaminidase in supernatants of MC/9 cells stimulated with 10% of culture supernatant from LAC *S. aureus* wild type (LAC WT) or isogenic mutants deficient in PSM-α peptides (LAC *Δpsmα*), PSM-β peptides (LAC *Δpsmβ*), δ-toxin (LAC *Δhld*), LAC wild type expressing vector alone (LAC pTX$_{\Delta}$16), LAC deficient in δ-toxin expressing vector alone (LAC*Δhld* pTX$_{\Delta}$16) and strain complemented with δ-toxin plasmid (LAC*Δhld* pTX$_{\Delta}$*hld*). Control represents 10% tryptic soy broth (TSB) medium. **c**, Histamine concentrations in culture supernatant of FSMCs after stimulation with indicated stimuli including synthetic δ-toxin at 30 μg ml^{-1} for 15 min. Data represent means ± s.d. of triplicate cultures. Results are representative of at least three independent experiments (**a**–**c**). *P* value refers to comparisons between experimental and control groups (**a**–**c**). **d**, Electromicroscopic images of FSMCs stimulated with synthetic δ-toxin (30 μg ml^{-1}) for 15 min. Images of unstimulated (Control) and ionomycin-treated FSMCs are also shown. Representative of at least 20 images. **e**, Expression of δ-toxin in *Staphylococcus* culture supernatants (0.5 μl per well). Loading of lanes with synthetic δ-toxin (10 ng, 100 ng) is shown as reference. Representative of three experiments. **f**, C57BL6 (WT) and MC-deficient (*Kit*$^{w\text{-}sh/w\text{-}sh}$) mice were injected intradermally into the left and right ears with δ-toxin (100 μg) or PBS, respectively. One representative mouse for each group is shown. Representative of eight mice per group. **g**, Quantification of Evans blue extracted from skin tissue of WT, *Kit*$^{w\text{-}sh/w\text{-}sh}$ and *Kit*$^{w\text{-}sh/w\text{-}sh}$ reconstituted with BMCMCs is shown. Dots represent individual ear samples from two independent experiments. NS, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, two-tailed *t*-test.

from *S. epidermidis*, a bacterium that is present in normal skin, possessed weak MC degranulation which correlated with smaller amounts of δ-toxin than that from *S. aureus* strains (Fig. 1e and Supplementary Fig. 6). Furthermore, deficiency of δ-toxin had a larger effect on MC degranulation in *S. aureus* than in *S. epidermidis* (Supplementary Fig. 6). To assess whether δ-toxin induces MC degranulation *in vivo*, we injected synthetic δ-toxin into the skin of mouse ears and monitored MC degranulation by the vascular leakage of Evan's blue dye into the extravascular space using the passive cutaneous anaphylaxis (PCA) assay. Intradermal administration of δ-toxin induced Evan's blue dye leaking at the site of injection in wild-type mice, but not in MC-deficient *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice (Fig. 1f, g). Reconstitution of the skin of *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice with bone-marrow-derived cultured MCs (BMCMCs) restored leaking of the dye upon administration of δ-toxin (Fig. 1g). Moreover, the culture supernatant from the δ-toxin-positive LAC strain induced Evan's blue dye leaking whereas that from δ-toxin-negative LAC*Δhld* and SA113 strains did not (Supplementary Fig. 7). These results indicate that δ-toxin induces MC degranulation *in vitro* and *in vivo*.

Ca^{2+} influx in human neutrophils is triggered by δ-toxin through FPR2 (ref. 11). Because Ca^{2+} influx is an essential step in MC degranulation, we analysed whether δ-toxin induces Ca^{2+} influx in MCs. Stimulation of MCs with ionomycin or dinitrophenol (DNP) plus anti-DNP immunoglobulin-E (IgE) induced rapid Ca^{2+} influx (Fig. 2a). Likewise, δ-toxin triggered Ca^{2+} influx and this was abrogated by treatment with the Ca^{2+} chelator ethylene glycol tetra-acetic acid (EGTA) (Fig. 2a). EGTA also blocked MC degranulation induced by ionomycin, 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). However, 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[11]. 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[4]. 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 *S. aureus*, encodes δ-toxin[12]. Notably, 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 epicutaneous 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

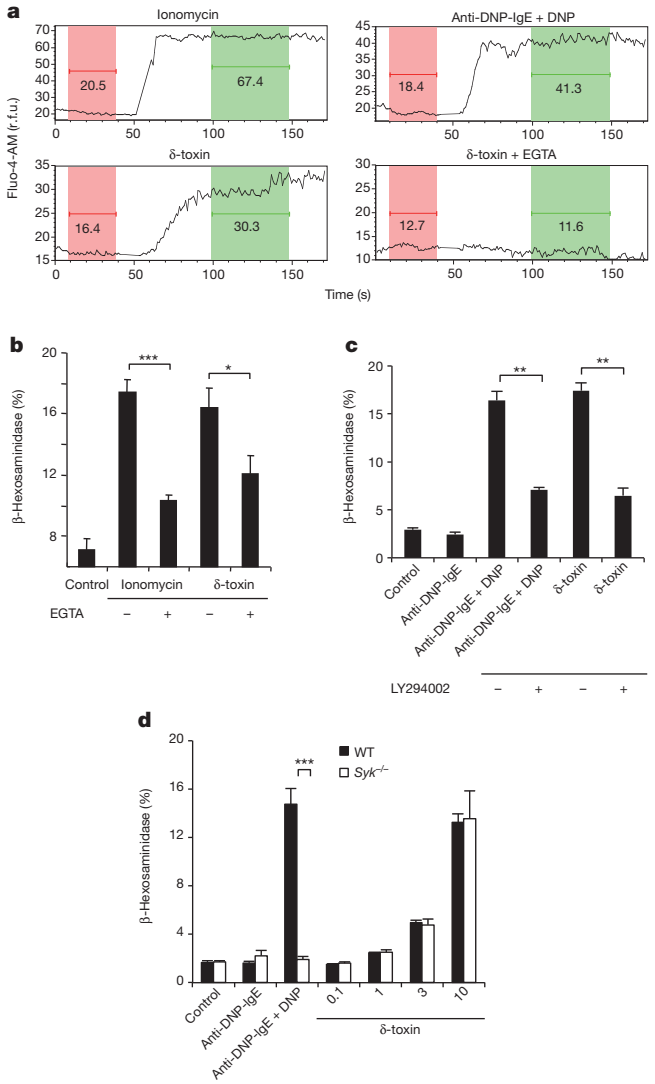

Figure 2 | MC degranulation induced by δ-toxin depends on Ca^{2+} influx/phosphoinositide 3-kinase pathway, but is independent of Syk. **a**, FSMCs loaded with the fluorescent Ca^{2+} indicator Fluo-4AM with or without EGTA were stimulated for 50 s. Baseline fluorescence (red) was measured, then the 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 (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.

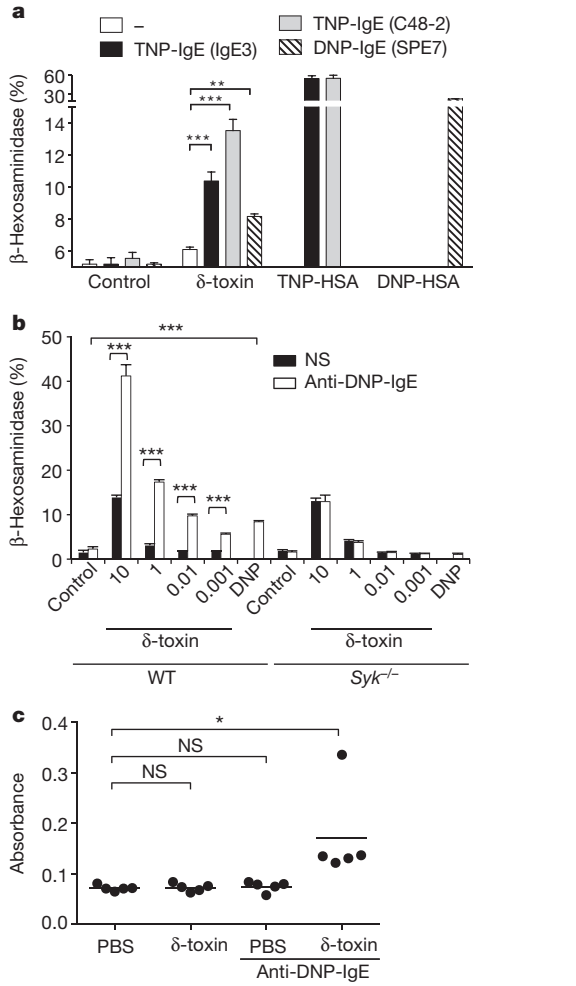

Figure 3 | Antigen-independent IgE signalling enhances δ-toxin-induced MC activation. **a**, Activity of β-hexosaminidase in culture supernatants of FSMCs stimulated with or without anti-DNP-IgE or TNP-IgE and then re-stimulated with δ-toxin (0.01 μg ml^{-1}), DNP-HSA (DNP) or TNP-HSA (TNP). **b**, Activity of β-hexosaminidase in culture supernatants of FSMCs derived from *Syk*$^{-/-}$ and wild-type mice (WT) 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 (**a**, **b**). **c**, Quantification of Evans blue extracted from skin tissue of C57BL6 mice injected intradermally into the left and right ears with δ-toxin (5 μg) or PBS, respectively. Data represent means ± s.d. of triplicate cultures and are representative of at least three independent experiments (**a**, **b**). Dots represent individual ear samples. Representative of two independent experiments (**c**). NS, not significant; $*P < 0.05$, one-way analysis of variance with Tukey post-hoc test for multiple comparisons.

disease score (Fig. 4b–d). Complementation of the *Δhld* mutant with a plasmid producing δ-toxin restored the disease scores comparable to those observed with the wild-type bacterium (Supplementary Fig. 13). The differential ability of wild-type and mutant *S. aureus* to promote inflammatory disease was not explained by differences in skin colonization (Supplementary Fig. 14a, b). Furthermore, mice colonized with wild-type *S. aureus* developed greater amounts of total serum IgE and IgG1, but not IgG2a, as well as IL-4 in the skin than mice inoculated with the δ-toxin mutant bacterium (Fig. 4e and Supplementary Figs 14c and 15). At 3 weeks, there was a slight increase in IgG1 production in mice colonized with the δ-toxin mutant bacterium compared with PBS control (Supplementary Fig. 15c), suggesting the existence of a minor pathway for 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). C57BL/6 mice colonized

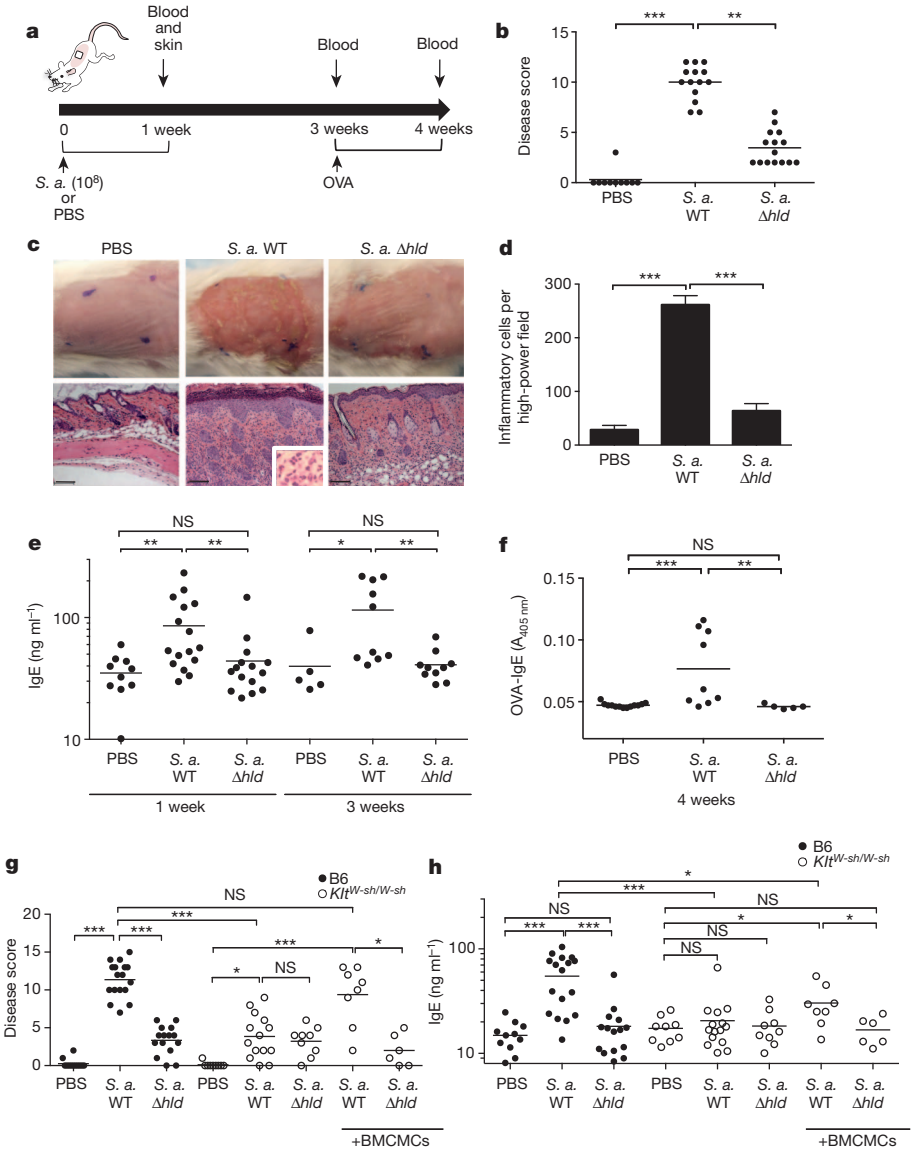

Figure 4 | *Staphyloccocus* δ-toxin promotes IgE production and inflammatory skin disease by mast cells. **a**, *S. aureus* (*S. a.*) colonization and OVA sensitization protocol. Mice were colonized epicutaneously with 10^8 colony-forming units of *S. aureus* using a gauze patch for 1 week. For OVA sensitization, 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 *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 ($Kit^{W\text{-}sh/W\text{-}sh}$) and MC-deficient ($Kit^{W\text{-}sh/W\text{-}sh}$) mice reconstituted with MCs at 1 week after the inoculation with *S. aureus*. **h**, Concentrations of serum IgE 1 week after colonization of B6, $Kit^{W\text{-}sh/W\text{-}sh}$ and $Kit^{W\text{-}sh/W\text{-}sh}$ 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 $Kit^{W\text{-}sh/W\text{-}sh}$ 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 $Kit^{W\text{-}sh/W\text{-}sh}$ 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 $Kit^{W\text{-}sh/W\text{-}sh}$ 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 $Kit^{W\text{-}sh/W\text{-}sh}$ 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[15], 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[2], 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[16]. The purity of MCs was greater than 95% as assessed by surface expression of FcεRI and CD117 (eBioscience). Degranulation of MCs was assessed by β-hexosaminidase assay as described[16].

PCA assay. PCA assay was performed as described with minor modifications[17].

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* strains were placed on a patch of sterile gauze and attached to the shaved skin with another 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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METHODS

Bacterial strains. *S. aureus* strain 8325-4 and its isogenic toxin mutant ($\Delta\alpha\beta\gamma$) have been previously described[18]. *S. aureus* strains SA113 and Newman, and isogenic mutants deficient in lipoprotein diacylglyceryl transferase (*Δlgt*), have also been previously described[19]. *S. aureus* strains LAC and MW2, their isogenic δ-toxin mutants (*Δhld*), the *psm* gene deleted mutants (*Δpsmα*, *Δpsmβ*) and LAC *agr* mutant (*Δagr)* have been previously described[10]. The isogenic *Δhld* mutant of *S. epidermidis* 1457, a clinical isolate[20], was produced by an allelic replacement procedure[21]. This was done in a way analogous to the *S. aureus Δhld* mutants used herein, abolishing translation by exchanging the third base in the *hld* start codon from ATG to ATA (to avoid interfering with the function of RNAIII). LAC P3-*lux* was constructed by integration of the *S. aureus* LAC *agr* P3 promoter fused to the *luxABCDE* operon of *Photorhabdus luminescens* with codon use optimized for staphylococci[22] into the *Φ11 attB* site of the *S. aureus* genome, using a procedure described by Luong and Lee[23]. Plasmid $pTX_{\Delta}hld$ was constructed by cloning the *hld* coding sequence containing the ribosomal binding site region in the BamH1/Mlu1 sites of plasmid pTX_{Δ}[10]. The *hld* gene was amplified from the genomic DNA of the respective strain, because the δ-toxin sequence differs in one amino acid in position 10 (serine or glycine) in these two strains. The δ-toxin is constitutively expressed in these plasmids. See Supplementary Table 1 for all oligonucleotides used in generation of the strains. Clinical isolates of *S. aureus* from children diagnosed with atopic dermatitis were obtained originally from the Department of Laboratory Medicine and Pathobiology at the University of Toronto[24]. *S. epidermidis* (NI335), *Staphylococcus cohnii* (NI446), *S. saprophyticus* (NI488), *Staphylococcus xylosus* (NI987), *Staphylococcus sciuri* (NI981), *Staphylococcus succinus* (NI534), *Staphylococcus lentus* (NI487) and *Staphylococcus fleuretti* (NI533) were isolated by plating on BHI after culturing at 37 °C for 2 days under aerobic conditions. Identification of bacterial species was verified by 16S rRNA gene sequencing as described[25]. Bacterial supernatants were produced by overnight culture with shaking in tryptic soy broth (TSB) followed by filtration through a 0.2 μm filter.

Mice. C57BL/6, C57BL/6-$Kit^{W\text{-}sh}$/$Kit^{W\text{-}sh}$ (B6.CG-$Kit^{W\text{-}sh}$/HNihrJaeBsmJ) and BALB/c mice were purchased from Jackson Laboratories. $Syk^{+/-}$ mouse breeders were a gift from S. Teitelbaum and $Syk^{-/-}$ embryos were generated by intercrossing. We used 4- to 12-week-old age-matched female mice for *in vivo* experiments. Mice were allocated randomly into experimental groups. All mouse strains were housed under pathogen-free conditions. The animal studies were conducted under approved protocols by the University of Michigan Committee on Use and Care of Animals.

Materials. The synthetic peptides fPSM-α2 (fMGIIAGIIKVIKSLIEQFTGK), fPSM-α3 (fMGIIAGIIKFIKGLIEKFTGK), fδ-toxin (fMAQDIISTIGDLVKWIIDTVNKFTKK), (WRWWWW-CONH2) and MMK-1 (LESIFRSLLFRVM) were purchased from American Peptide. Unformylated δ-toxin (MAQDIISTIGDLVKWIIDTVNKFTKK) was synthesized at The University of Michigan Protein Structure Facility. Polyclonal anti-δ-toxin antibody was produced in rabbits by immunization with a synthetic multiple antigenic peptide showing an 18 amino-acid peptide (IGDLVKWIIDTVNKFTKK) (Sigma-Genosys) from the full-length δ-toxin sequence. Rabbit IgG was purified from rabbit serum on Protein A (Pierce) according to the manufacturer's protocol.

Protein purification from *S. aureus* culture supernatant. *S. aureus* was cultured in 700 ml chemical defined medium supplemented with 2% yeast extract[26]. Filtrated cultured supernatant was incubated with carboxymethyl cellulose equilibrated with 10 mM sodium citrate (pH 5.5), and eluted with a linear gradient of 0–1 M NaCl. Fractions containing β-hexosaminidase activity were collected and adjusted at pH 7.4, 100 mM HEPES. The sample was concentrated using Amicon Ultra-15, 5 kDa filter (Millipore). Concentrated sample was further fractionated with a Superdex 200 10/300 GL column (GE). Final positive fractions were pooled and concentrated using an Amicon Ultra-15 filter (Supplementary Fig. 2b).

Protein identification by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Purified sample was denatured in 8 M urea, reduced by incubation with 10 mM DTT at 37 °C for 30 min and alkylated using 50 mM iodoacetamide at room temperature for 30 min. The protein sample was digested with sequencing grade trypsin (Promega) overnight at 37 °C. The reaction was terminated by acidification with trifluoroacetic acid (0.1% v/v) and peptides were purified using a SepPak C18 cartridge following the manufacturer's protocol (Waters Corporation). Eluted peptides were directly introduced into an ion-trap mass spectrometer (LTQ-XL, Thermo Fisher) equipped with a nano-spray source. The mass spectrometer was operated in data-dependent MS/MS mode to acquire a full MS scan (400–2000 *m/z*) followed by MS/MS on the top six ions from the full MS scan. Dynamic exclusion was set to collect two MS/MS spectra on each ion and exclude it for a further 2 min. Raw files were converted to mzXML format and searched against the *S. aureus* NCTC 8325 database appended with decoy (reverse) database using X! Tandem with k-score plug-in, an open-source search engine developed by the Global Proteome Machine (http://www.thegpm.org). Search parameters included a precursor peptide mass tolerance window of 1 Da and fragment mass tolerance of 0.5 Da. Oxidation of methionine (+16 Da), and carbamidomethylation of cysteines (+57 Da) were considered as variable modifications. The search was restricted to tryptic peptides with one missed cleavage. Results of the X! Tandem search were then subjected to Trans-Proteomic Pipeline (TPP) analysis, a suite of software including PeptideProphet and ProteinProphet. All proteins with a ProteinProphet probability of greater than 0.9 were considered positive and verified manually.

Culture of mast cells and degranulation. Preparations of BMCMCs and fetal skin-derived mast cells (FSMCs) were previously described[16]. Bone marrow cells from $Fpr2^{-/-}$ 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). Degranulation of MCs was assessed by β-hexosaminidase assay as previously described[16]. Briefly, MCs (2×10^6 ml^{-1}) were preloaded with or without IgEs (anti-DNP IgE(clone; SPE7); 0.3 μg ml^{-1}, anti-TNP IgE (clone; IgE3 and C48-2); 0.5 μg ml^{-1}) in RPMI with IL-3 for 15 h. The cells were re-suspended in Tyrode's buffer (Sigma) at 2×10^4 cells per 100 μl for FSMCs or 1×10^5 cells per 100 μl for BMCMCs and MC/9 cells, aliquoted in triplicate into a 96-well U-bottom plate and incubated with EGTA (1 mM, Sigma), LY294002 (100 μM, Sigma), WRW4 (10 μM) and Cyclosporine H (10 μM, Alexis Biochemicals) for 30 min, then stimulated with DNP-HSA (30 ng ml^{-1}), TNP-HSA (30 nM) for 30 min, ionomycin (1 μM, Sigma), δ-toxin (indicated concentrations), PSM-αs (indicated concentrations) or FPR2 ligands for 15 min. Results of various stimuli are given as a relative percentage, where freeze and thaw of total cell culture represents 100%.

MC reconstitution in $Kit^{W\text{-}sh/W\text{-}sh}$ mice. For BMCMC reconstitution experiments, 10^6 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 $Kit^{W\text{-}sh/W\text{-}sh}$ mice as described[27]. 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 minimum 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 microscope. The average rate of reconstituted MCs was approximately 40% in the ear pina and 50% in the back skin (Supplementary Figs 19 and 20).

PCA assay. PCA assay was performed as previously described with minor modifications[17]. Ears of non-randomized mice were injected intradermally with or without α-DNP-IgE in 40 μl saline; 15 h later, mice were challenged with 20 μl saline with or without synthetic δ-toxin (100 μg or 5 μg) or TSB bacteria supernatants. The number of animals per group ($n = 5$–8) was chosen on the basis of previous experience as the minimum probably required for conclusions of biological significance. After inoculation, 0.1 ml of 5 mg ml^{-1} Evans blue dye was injected intravenously. Extravasation of Evans blue dye was monitored for 30 min, and 4 mm of punched-out biopsies were incubated at 63 °C overnight in 200 μl formamide. Quantitative analysis of extracts was determined by measuring the absorbance at 600 nm.

Ca^{2+} influx assay. FSMCs (2×10^6 ml^{-1}) were preloaded with or without anti-DNP-IgE (0.3 μg ml^{-1}) in RPMI with IL-3 for 15 h. Cells were washed and then loaded with Fluo-4AM (5 μM, Life Technologies) for 30 min. 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^{-1}), ionomycin (1 μM) or δ-toxin (30 μg ml^{-1}) were used to induce calcium flux in these cells. Ca^{2+} flux was measured using a flow cytometer (FACSCalibur, BD Biosciences) to monitor relative fluorescence units (RFU) as described[28].

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 size necessary 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 10^8 colony-forming units of *S. aureus* LAC or LAC (*Δhld*) strains. One hundred microlitres of the *S. aureus* suspension was placed on a patch of sterile gauze (1 cm × 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 OVA (100 μg) with or without synthetic δ-toxin (100 μg) for 1 week. After a 2 week interval, mice were challenged with OVA (100 μg) with or without synthetic δ-toxin (100 μg) at the same skin site.

Skin disease score. The severity of skin lesions was scored according to defined macroscopic diagnostic criteria in a blind fashion[29]. 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 immunoabsorbent assay (ELISA) kits (R&D Systems). For tissue cytokines, 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 kit (Cayman chemical). Serum IgE was measured with ELISA kit (Bethyl Laboratories). ELISA for OVA-IgE was described previously[30].

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 reported[31]. 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 Committee[31]. 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* genes[32] and bacterial genes (*RNAIII*, *gyrB*, *16S rRNA*) have been described[33,34]. Expression of mouse *Fpr* genes was normalized to that of *Gapdh* (F; 5-CCTCGTCCCGTAGACAAAATG-3, R; 5-TCTCCACTTTGCCACCTGCAA-3) and expression was analysed by the $2^{-\Delta\Delta Ct}$ 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 $2^{-\Delta Ct}$ 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 *Δagr* cultured for 24 h were used as reference controls.

Measurement of *P3-lx* expression. To determine the amounts of *P3-lx* expression in culture, 10^5 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).

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