Preferential Binding of *Staphylococcus aureus* to Skin Sites of Th2-Mediated Inflammation in a Murine Model

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Staphylococcus aureus is found on over 90% of atopic dermatitis skin lesions and is thought to contribute to skin inflammation via the production of potent exotoxins. In contrast, less than 5% of normal subjects harbor *S. aureus*. This suggests that an atopic immune response itself may play a role in preferential binding of *S. aureus* to the skin. To examine this issue more directly, we analyzed the *S. aureus* binding characteristics of skin in mice undergoing different T helper type 1 cell *versus* T helper type 2 cell inflammatory responses using a novel *in vitro* bacterial binding assay. BALB/C female mice were first sensitized to ovalbumin with alum or ovalbumin with complete Freund's adjuvant to induce T helper type 2 or T helper type 1 responses, respectively. Mice were then challenged intradermally with either saline (control) or ovalbumin. Forty-eight hours later, skin specimens were obtained from the challenge sites, and the number of *S. aureus* binding to each skin section was quantitated. Bacterial binding was found to be significantly greater at skin sites of BALB/C mice that had been ovalbumin/alum sensitized compared with ovalbumin/complete Freund's adjuvant sensitized ($p \leq 0.01$). When compared to the ovalbumin sensitized/challenged skin of wild type BALB/C mice or interferon-γ gene knockout mice, interleukin-4, but not interferon-γ, gene knockout mice had significantly less *S. aureus* binding at their ovalbumin sensitized/challenged skin sites. Mutant *S. aureus* strains that lacked either fibronectin- or fibrinogen-binding protein expression showed significantly reduced *S. aureus* binding compared with the parent wild type strain ($p < 0.005$). Moreover, preincubation of the wild type bacteria with fibronectin or fibrinogen, but not collagen, resulted in significantly less skin binding of *S. aureus* ($p < 0.01$). Incubation of skin with interleukin-4, and less so with interferon-γ, led to more binding of wild type *S. aureus* but not of an *S. aureus* mutant deficient in fibronectin binding protein expression. After interleukin-4 incubation, but not interferon-γ, epidermal immunoreactivity for fibronectin was observed in murine skin explants. These results show that a T helper type 2 inflammatory environment can promote skin binding by *S. aureus* and that this binding is mediated by fibronectin and fibrinogen. *Key words: atopic dermatitis/collagen/fibronectin/fibrinogen. J Invest Dermatol 116:658–663, 2001*

Atopic dermatitis (AD) is a chronic inflammatory skin disease affecting over 10% of children, and is a major cause of occupation-related disability caused by skin disease (Leung, 2000). More than 90% of AD patients are colonized with *Staphylococcus aureus* on their skin lesions and on unaffected skin as well (Leyden *et al*, 1974). In contrast, only 5% of normal subjects carry *S. aureus* on their skin and it is localized mainly in the nose and intertriginous areas. Numerous reports suggest that this bacterium has an important role in the exacerbation and chronicity of AD through the release of toxins (Leung *et al*, 1993a; Bunikowski *et al*, 1999; Nomura *et al*, 1999). Some of these toxins are superantigenic in nature and deliver strong activating signals to macrophages and T cells, thus augmenting skin inflammatory responses (Kotzin *et al*, 1993). The excellent response to antistaphylococcal antibiotics in the treatment of poorly controlled AD strongly suggests that this bacterium can be involved in the perpetuation of chronic AD (Lever *et al*, 1988). Due to increasing concerns over the emergence of antibiotic-resistant *S. aureus*, it is imperative to understand the mechanisms by which *S. aureus* binds to atopic skin and identify potential therapeutic targets for reduction of *S. aureus* colonization.

The mechanism by which *S. aureus* is able to successfully colonize the skin of AD patients is largely unknown. There is considerable evidence, however, that acute skin inflammation in AD is primarily mediated by T helper type 2 (Th2) cells (Hamid *et al*, 1994, 1996). Treatment with topical steroids has been shown to reduce skin colonization with *S. aureus* suggesting that it is the

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Abbreviations: AD, atopic dermatitis; CFA, complete Freund's adjuvant; Clf, fibrinogen binding protein or clumping factor; Cna, collagen binding protein; DU5883, *S. aureus* FnbpA- and FnbpB-negative mutant strain; DU5944, *S. aureus* ClfA- and ClfB-negative mutant strain; Fnbp, fibronectin binding protein; KO, knockout; MFI, mean fluorescence intensity; MSCRAMMs, microbial surface components recognizing adhesive matrix molecules; OVA, ovalbumin; PH100, *S. aureus* Cna-negative mutant strain; Th1, T helper type 1; Th2, T helper type 2; WT, wild type.

[1]Cho and Strickland contributed equally to the successful completion of experiments described in this manuscript. They should both be considered first authors on the manuscript.

inflammatory process that drives attachment of *S. aureus* to atopic skin (Nilsson *et al*, 1992). In psoriasis, which is a chronic inflammatory skin disease mediated by Th1 cells, *S. aureus* colonization occurs in only approximately 50% of patients and generally involves several log-fold fewer numbers of *S. aureus* per square centimeter of skin than in AD (Leung *et al*, 1993a,b). Thus, it would be of interest to know whether *S. aureus* attachment to Th1- *versus* Th2-mediated skin lesions differs.

Recent studies have demonstrated that *S. aureus* strains express a distinct array of surface proteins, termed microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), that recognize human extracellular matrix (ECM) proteins. This family of microbial cell surface proteins has been shown to specifically bind ECM proteins such as fibronectin and collagen (Foster and Höök, 1998), implicating these proteins as potential ligands for *S. aureus* binding to AD skin. In addition, all *S. aureus* strains bind fibrinogen. The potential role of these molecules in *S. aureus* binding to inflamed skin has not previously been explored.

To address these questions, two previously established murine models of skin inflammation were utilized in this study: first, a model of Th1-induced, interferon-γ (IFN-γ) skin inflammation where BALB/C mice were sensitized to ovalbumin (OVA) in the presence of complete Freund's adjuvant (CFA) and then OVA challenged; second, a model of Th2-induced interleukin-4 (IL-4) mediated skin inflammation in which an OVA/alum sensitization was followed by an OVA challenge regimen (Beck and Spiegelberg, 1989; Ke *et al*, 1995; Sawada *et al*, 1997; Spergel *et al*, 1998; 1999). We also took advantage of the availability of mice with targeted deletions of the IL-4 gene and IFN-γ gene and *S. aureus* isogenic mutant strains that were selectively deficient in specific MSCRAMM genes to explore the role of cytokines and MSCRAMMs in the binding of *S. aureus* to sites of allergic skin inflammation.

MATERIALS AND METHODS

Bacterial strains Two sources of *S. aureus* strains were utilized in this study: *S. aureus* cultured from the lesions of AD patients and isogenic mutants of *S. aureus* that had been generated by allele replacement or transposon mutagenesis to eliminate the expression of specific MSCRAMMs, i.e., fibronectin binding protein (FnbpA and FnbpB), fibrinogen binding protein (ClfA and ClfB), or collagen binding protein (Cna), together with their corresponding wild type (WT) parent strains. The isogenic mutant strains were: (i) strain DU5883, which is the FnbpA- and FnbpB-negative mutant (8325–4 *fnbA*::Tc^R *fnbB*::Em^R) of *S. aureus* strain 8325–4 kindly provided by Professor Timothy J. Foster, Trinity College, Dublin, Ireland (Greene *et al*, 1995); (ii) strain DU5944, which is the ClfA- and ClfB-negative mutant (Newman *clfA2*::Tn*917* *clfB*::Tc^R) of the Newman *S. aureus* strain, also provided by Dr. Foster (Ni Eidhin *et al*, 1998); (iii) strain PH100, which is a collagen adhesin-negative mutant (Phillips *cna*::Gm^R) made in *S. aureus* strain Phillips, generously provided by Dr. Magnus Höök (Texas A & M University, Houston, TX) (Patti *et al*, 1994). For each of these mutant strains of *S. aureus*, we had the corresponding WT *S. aureus* parent strain 8325–4 and Newman (from Dr. Foster) and Phillips (from Dr. Höök) for comparison of baseline binding to skin sections.

Sensitization of mice and preparation of skin sections BALB/C female mice (Jackson Laboratories, Bar Harbor, ME), IL-4 knockout (IL-4 KO) female mice with a BALB/C background, and IFN-γ knockout (IFN-γ KO) female mice with a C57BL/6 background were sensitized by intraperitoneal injection of 20 μg OVA (grade V, Sigma) emulsified in 2.25 mg aluminum hydroxide (AlumImuject: Pierce, Rockford, IL) (to produce a Th2 response) or CFA (50% vol/vol, final concentration; Sigma) (to produce a Th1 response) in a total volume of 100 μl on days 1 and 14 as previously described (Sawada *et al*, 1997). On day 21, the abdomens of the sensitized mice were shaved, then challenged intradermally with 100 μl injections of OVA solution [500 μg per ml in phosphate-buffered saline (PBS)] or vehicle alone (PBS) at single sites and left for 48 h to allow the development of a Th2 inflammatory reaction at the OVA site. After antigen skin challenges, the mice were sacrificed by cervical dislocation and skin biopsies were taken from both the saline control and challenge sites, snap frozen in OCT (Triangle Biomedical Sciences, Durham, NC), and then stored at −70°C until use. Frozen sections 10 μm in cross-section were then prepared and used for bacterial binding experiments as described below. The level of inflammation was found to be comparable between groups as assessed by hematoxylin and eosin histologic staining.

In a further attempt to confirm the effect of Th1 *versus* Th2 immune responses on bacterial adherence to the skin, mouse skin explants were incubated in mouse recombinant IL-4 or recombinant IFN-γ (R&D Systems, Minneapolis, MN). In these experiments, fresh skin specimens from BALB/C mice were cut into 4 mm pieces. Each piece of skin tissue was incubated in RPMI 1640 culture medium with recombinant IL-4 (1000 U per ml) or IFN-γ (1000 U per ml), or medium alone at 37°C for 72 h. After these incubations, skin tissues were washed three times with PBS and snap frozen in OCT, and then cryostat sections 10 μm in cross-section were prepared for assessment of *S. aureus* binding.

Bacteria binding assay *Staphylococcus aureus* were streaked for isolation on blood agar plates (Remel, Lenexa, KS) and incubated overnight at 37°C. One colony from each plate was picked, inoculated into 1% human serum albumin (HSA)/tryptic soy broth (Becton Dickinson, Sparks, MD), and incubated for 4 h at 37°C with gentle shaking to achieve early logarithmic growth phase. Bacteria were harvested by 10 min of centrifugation at 3000 rpm and washed twice with sterile PBS. Bacteria were re-suspended in sterile PBS to a concentration of about 10^7 colony-forming units per ml. Before every experiment, bacteria were incubated with 1% HSA for 1 h at room temperature to block nonspecific binding sites. Bacteria were then pelleted by centrifugation, washed twice with sterile PBS, and then re-suspended at a final concentration of 1×10^7 per ml in sterile PBS before use.

One hundred microliters of each bacterial suspension (10^6 bacteria) were added to each skin specimen previously washed with PBS to remove any residual serum antibody. The skin sections were then incubated with the bacteria for 60 min at room temperature on a rotating table in a humidified atmosphere to allow bacterial adherence to the skin sections. After this incubation period, sections were washed in PBS twice to remove unbound bacteria from the skin sections, treated with 1% (wt/vol) paraformaldehyde (Sigma) in PBS for 10 min at room temperature, washed twice in PBS, and then air-dried. Adherent bacteria were then visualized by a standard Gram staining procedure. In brief, slides were fixed by passing through a flame, immersed in crystal violet for 1 min, and then gently washed in water. Slides were immersed in Gram iodine for 1 min, washed in water, then decolorized with acetone-alcohol and washed in water. Slides were blotted dry with filter paper and mounted in a permanent mounting medium (VectaMount, Vector, Burlington, CA). The number of bacteria bound to each skin section was quantitated microscopically by a blinded assessor and then expressed as number of bacteria bound per high power field (× 1000); a minimum of three fields were counted. Bacterial adherence was also quantitated in relation to the epidermal strata they were bound to, the stratum corneum or subcorneal epithelium.

To further demonstrate the role of ECM proteins in bacterial adherence to epidermis in selected experiments, *S. aureus* were incubated with 10 μg per ml of fibronectin, fibrinogen, or type I collagen (Sigma), or 1% HSA as a control for 2 h at room temperature. Bacteria were harvested by 10 min of centrifugation, washed twice with PBS, and re-suspended at a concentration of 10×10^6 per ml in sterile PBS. One hundred microliters of each bacterial strain was pipetted sequentially onto individual skin sections derived from each subject group. The *S. aureus* binding and staining procedure was carried out as described above.

Fibronectin staining of murine skin Normal BALB/C mouse skin was excised, washed in PBS to remove any residual serum, and then incubated for 48 h at 37°C, in either medium alone (RPMI, 10% fetal bovine serum) or medium with 1000 U per ml of either mouse IL-4 or IFN-γ (R&D Systems). After 48 h the explants were washed twice in PBS and snap frozen, and frozen sections 5 μm in cross-section were prepared for immunostaining. Sections were blocked for 10 min at room temperature with a commercial blocking solution (Superblock, Scytek, Logan, UT). The blocking solution was then aspirated and sections were incubated overnight at 4°C with a 1:50 dilution of antifibronectin rabbit polyclonal antibody (Sigma). After this incubation period, sections were washed in 1 × PBS, and incubated at room temperature for 1 h with an antirabbit fluorescein isothiocyanate conjugated antibody (Dako, Carpinteria, CA) at a final dilution of 1:50 in PBS. Sections were again washed in 1 × PBS and then mounted using a 50% (vol/vol) solution of glycerol (Sigma) in 1 × PBS and assessed by fluorescence microscopy. Intensity of staining was quantified by image analysis (IP Laboratory Spectrum, Signal Analytics, Vienna, VA) and expressed as mean fluorescence intensity (MFI). To ensure specific quantification of staining

within the epithelium, multiple small gates were created using the image analysis software. This ensured that readings were taken solely within the epithelial layer.

Statistical analysis The number of bacteria bound/hpf was expressed as the mean ± SEM. Differences observed between groups were determined by using an unpaired, two-tailed Student *t* test. Probability (p) values of less than 0.05 were considered statistically significant.

RESULTS

Adherence of *S. aureus* to Th1- and Th2-mediated sites of skin inflammation *Staphylococcus aureus* isolated from AD skin lesions of 37 different patients were analyzed by polymerase chain reaction for MSCRAMM gene expression. The majority of *S. aureus* expressed genes for FnBP, Clf, and Cna (data not shown). These *S. aureus* all had similar binding characteristics to mouse experiments. We therefore selected one representative AD *S. aureus* strain to examine differential binding to mouse epidermis that was associated with lack of inflammation (saline control) *versus* Th1-mediated inflammation (OVA/CFA-sensitized) *versus* Th2-mediated inflammation (OVA/alum-sensitized). Similar data were observed with other strains of *S. aureus*.

As shown in **Figs 1** and **2**, bacterial binding was found to be significantly greater to the stratum corneum of BALB/C mice that had been OVA/alum sensitized (Th2 response) than to OVA/CFA-sensitized mice. OVA/CFA-sensitized mice (Th1 response) showed no increase in bacterial binding compared with saline-challenged skin. The *S. aureus* binding to challenged sites from the OVA/alum-sensitized mice, however, was significantly higher than that of OVA/CFA-sensitized mice ($p \leq 0.01$). Furthermore, binding to subcorneal epithelium was significantly higher in the OVA/alum-sensitized mice than in OVA/CFA-sensitized mice ($p \leq 0.01$).

Baseline *S. aureus* binding to the skin of OVA-sensitized/challenged mice was not significantly different between BALB/C mice, IL-4 KO mice, and IFN-γ KO mice (**Fig 3**). Compared with the OVA-sensitized/challenged skin of BALB/C mice, however, the IL-4 KO mice showed significantly less binding than WT animals ($p < 0.0001$). The IFN-γ KO mice also showed less *S. aureus* binding ($p < 0.0005$), but the binding was significantly higher than found in the IL-4 KO mice ($p < 0.0001$).

The effect of blocking MSCRAMMs on *S. aureus* adherence To determine which MSCRAMMs are involved in the binding of *S. aureus* to Th2-mediated inflamed skin, *S. aureus* from AD were incubated with fibronectin, collagen, or fibrinogen in an attempt to block MSCRAMM receptors and thus inhibit bacterial binding (**Fig 4**). The reduction in binding of fibronectin-treated *S. aureus* compared with untreated *S. aureus* was as follows: OVA-sensitized/saline-challenged skin of BALB/C mice was from 57.94 ± 8.23 to 26.17 ± 3.93 ($p < 0.005$), and OVA-sensitized/challenged skin of BALB/C mice was from 103.67 ± 12.50 to 56.39 ± 10.90 ($p < 0.01$). The respective reductions in binding of the fibrinogen-treated *S. aureus* compared to the untreated *S. aureus* were as follows: OVA-sensitized/saline-challenged skin of BALB/C mice was from 57.94 ± 8.23 to 24.50 ± 2.98 ($p < 0.001$), and OVA-sensitized/challenged skin of BALB/C mice was from 103.67 ± 12.50 to 53.78 ± 11.59 ($p < 0.01$). In contrast, collagen-treated *S. aureus* did not show significant reduction in binding to the stratum corneum under any experimental conditions. *S. aureus* incubated with 1% HSA as a control, under the same incubation conditions as ECM proteins, showed no significant difference in adherence of bacteria to the skin. Thus, the reduction in binding of *S. aureus* to the stratum corneum following preincubation with fibronectin or fibrinogen appeared secondary to the specific blocking of MSCRAMM receptors.

Adherence of *S. aureus* mutant strains to mouse skin To confirm these results, an independent approach to identify the key MCRAMMS involved in the binding of *S. aureus* to Th2-induced skin inflammation was used. *Staphylococcus aureus* mutants with selective deficiencies in specific MCRAMMS were examined for their ability to bind inflamed mouse skin sites. As shown in **Fig 5**, binding of *S. aureus* strain DU5883, which is an FnbpA- and FnbpB-negative mutant, to OVA-sensitized/challenged skin of BALB/C mice was markedly reduced compared to its parent 8325–4 strain ($p < 0.0005$). Similarly, binding to OVA-sensitized/challenged skin of *S. aureus* strain DU5944, which is a ClfA- and ClfB-negative mutant, was significantly less than its parent Newman strain ($p < 0.05$). In contrast, the difference in binding of *S. aureus* strain PH100, which is a Cna-negative mutant, to the OVA-sensitized/challenged skin of BALB/C mice was not significantly less than its parent Phillips strain. These data confirm that fibronectin and fibrinogen contribute to *S. aureus* binding to inflamed skin induced by Th2 cytokines.

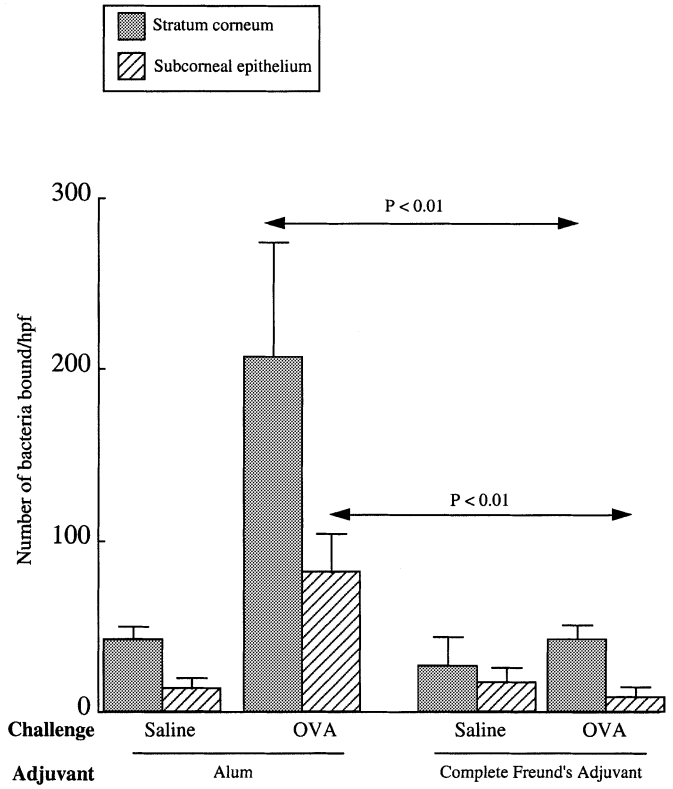

Figure 1. *Staphylococcus aureus* binding to Th1- *versus* Th2-mediated skin inflammation. Quantification of AD *S.* aureus adherence to the epidermis of BALB/C mouse skin [OVA/alum ($n = 5$) and OVA/CFA sensitized ($n = 4$), OVA challenged], using an *S. aureus* strain derived from AD that expressed FnBP, Can, and Clf. Consistent with previous reports (Beck *et al*, 1989; Ke *et al*, 1995), we found that CFA/OVA sensitization resulted in IFN-γ production (18 pg per ml) but no IL-4 (not detectable) production, whereas alum/OVA sensitization resulted in IL-4 but little IFN-γ (22 pg per ml *vs* 7 pg per ml, respectively) production. The means ± SD in bacterial binding between OVA and saline challenged controls are shown for both sensitization protocols (OVA/alum for the Th2 model and OVA/CFA for the Th1 model).

Effects of IL-4 *versus* IFN-γ on *S. aureus* binding To obtain independent evidence that *S. aureus* binding is preferentially increased to IL-4-stimulated skin, BALB/C mice skin explants were incubated with recombinant IL-4 or recombinant IFN-γ for 72 h. As shown in **Fig 6**, a significantly greater number of *S. aureus* from AD bound to IL-4-treated skin than IFN-γ-treated skin ($p < 0.05$) or skin that had been incubated in medium alone ($p < 0.0001$). In contrast, the *S. aureus* DU5883 Fnbp-negative mutant strain did not show any increased binding to IL-4-treated skin.

Induction of fibronectin expression by IL-4 The failure of the DU5883 *S. aureus* strain to show increased binding to IL-4-

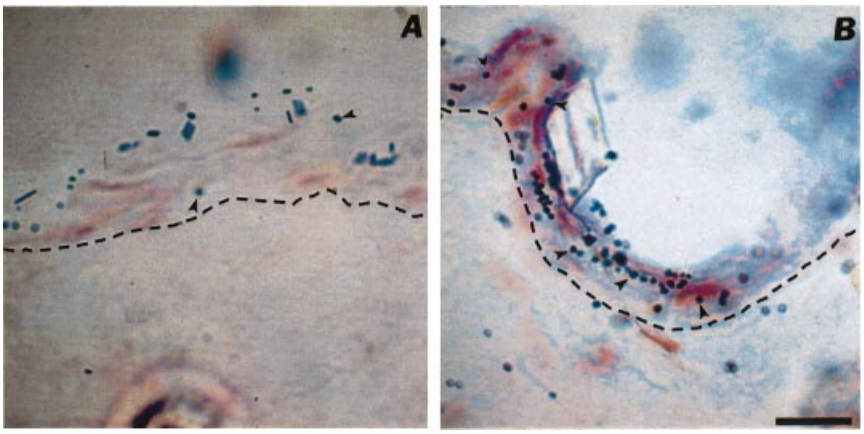

Figure 2. *Staphylococcus aureus* binding to skin sections. Visualization of AD *S. aureus* (*arrow*) adherence to epidermis in OVA-sensitized, saline-challenged skin of BALB/C mice (*A*), and OVA/alum-sensitized, OVA-challenged skin of BALB/C mice (*B*). A broken black line differentiates the dermal/epidermal junction.

treated skin suggested that IL-4-induced fibronectin synthesis was required for enhanced *S. aureus* binding. We therefore directly examined fibronectin expression in IL-4 *versus* IFN-γ *versus* medium control treated skin from WT BALB/C mice. As shown in **Fig 7**, IL-4-treated skin showed a significant increase in fibronectin staining in the epidermis (mainly the stratum corneum) compared with skin treated with medium control alone (mean MFI = 1310 ± 126 and 687 ± 49, respectively; $p = 0.02$). Interestingly, IFN-γ-treated skin actually showed a significant decrease in fibronectin expression compared to medium alone (mean MFI = 453 ± 48 and 687 ± 49, respectively; $p = 0.01$).

DISCUSSION

S. aureus colonization and infection of the skin is thought to play an important role in the pathogenesis of a variety of chronic skin diseases including AD, psoriasis, and cutaneous T cell lymphoma (Leung *et al*, 1993a,b; Tokura *et al*, 1995; Bunikowski *et al*, 2000; Leung, 2000). This relationship has been most convincingly demonstrated in AD where the number of *S. aureus* colonizing the skin correlates with disease severity and success of antistaphylococcal antibiotic therapy used in combination with topical corticosteroids for treatment of poorly controlled AD (Leyden *et al*, 1974; Lever *et al*, 1988). The mechanism by which *S. aureus* triggers the exacerbation of AD is an area of active investigation and includes a role for the release of toxins known to act as superantigens that stimulate marked activation of T cells and macrophages (Kotzin *et al*, 1993; Leung *et al*, 1993b; Bunikowski *et al*, 1999; Nomura *et al*, 1999).

One aspect of this disease that has largely been overlooked, however, is the mechanism by which this bacterium is able to preferentially colonize the skin of atopic individuals. In this regard, the numbers of *S. aureus* reached on AD skin are frequently over 10×10^6 per cm^2, which is far greater than seen in other forms of skin inflammation (Hauser *et al*, 1985). Although multiple factors are likely to control this process, the initial step in colonization is binding of *S. aureus* to the skin. It was therefore of interest to determine whether the atopic state itself is contributory to the adherence process.

IL-4 is known to play a key role in the pathogenesis of atopic diseases and is highly expressed in unaffected skin as well as acute AD skin lesions (Hamid *et al*, 1994). It is important for IgE isotype switching, development of Th2 cells, and induction of adhesion molecules on endothelial cells that recruit eosinophils (Oettgen and Geha, 1999). Indeed, a recent study of OVA-sensitized skin from BALB/C mice demonstrated that deletion of the IL-4 gene results in a remarkable decrease in eosinophil infiltration at cutaneous sites of allergen challenge (Spergel *et al*, 1999).

Our study reports a novel action of IL-4, i.e., the induction of *S. aureus* binding to the skin. The critical role of IL-4 in this process is supported by two key observations. First, *S. aureus* binding to

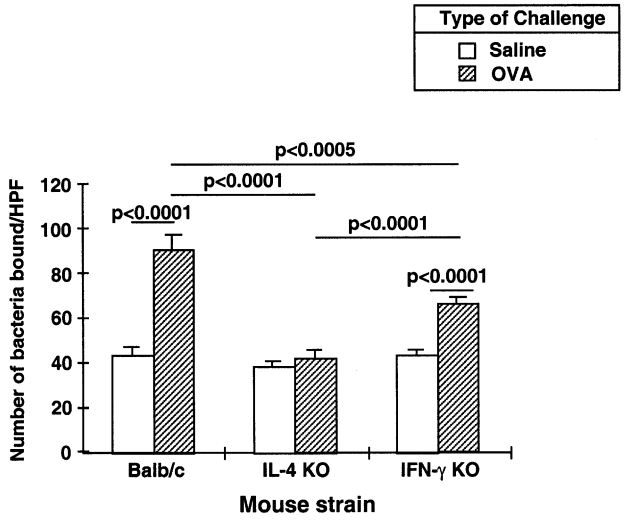

Figure 3. AD *S. aureus* binding is dependent on IL-4. Comparison of AD *S. aureus* binding to stratum corneum in BALB/C mice *versus* IL-4 KO mice *versus* IFN-γ KO mice that were either OVA sensitized/saline challenged or OVA sensitized/challenged using *S. aureus* derived from AD. The means ± SEM in bacterial binding are shown for four separate experiments.

OVA-sensitized/challenged skin of BALB/C mice is reduced to baseline in IL-4 KO mice (**Fig 3**). Second, incubation of normal mouse skin with IL-4 induces increased *S. aureus* skin binding (**Fig 6**). Although IFN-γ can also increase *S. aureus* skin binding, it was significantly less effective than IL-4. This may partially account for the observation that AD is associated with significantly higher levels of *S. aureus* skin colonization than found in psoriasis, which is primarily a Th1-mediated disease.

An important issue that needs to be addressed is the mechanism by which IL-4 induces *S. aureus* binding to the skin. It is known that *S. aureus* express an array of adhesins termed MSCRAMMs that allow it to bind ECM components and also certain plasma proteins that can exudate into sites of skin inflammation (Foster and Höök, 1998). By utilizing these adhesins, initial adherence to the skin occurs and the colonization process can begin. Therefore this study also sought to examine the role of these MSCRAMMs and their ligands (ECM and plasma proteins) on *S. aureus* binding to atopic skin.

Our findings indicate that fibronectin and fibrinogen, but not collagen, are the key MSCRAMMs used in the binding of *S. aureus* to Th2-induced skin inflammatory reactions. This is supported by the following observations. First, isogenic mutants of *S. aureus* that were selectively deficient in fibronectin- or fibrinogen-binding

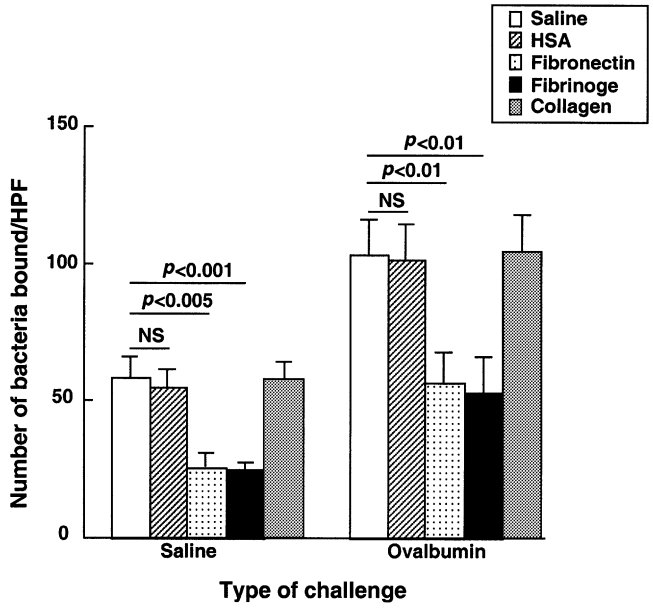

Figure 4. *Staphylococcus aureus* binding is blocked by fibronectin and fibrinogen. The effect of blocking MSCRAMM receptors by preincubation of AD *S. aureus* with purified fibronectin, fibrinogen, and collagen on the binding of *S. aureus* to the epidermis of BALB/C mice. The means ± SEM in bacterial binding are shown for four separate experiments.

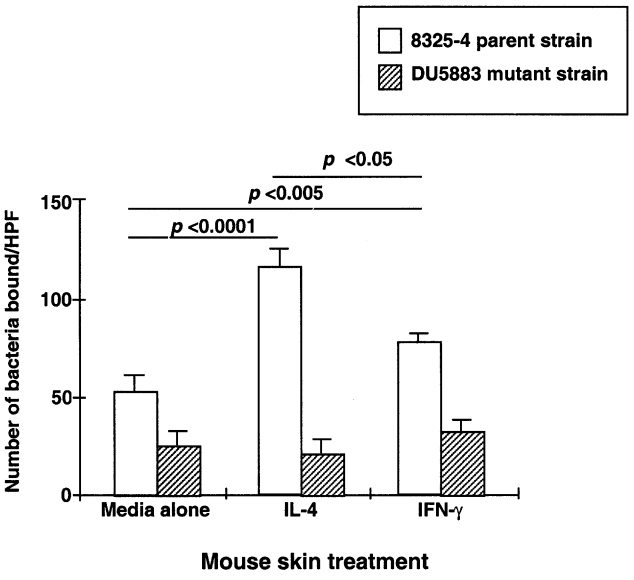

Figure 6. Treatment of skin with IL-4 leads to enhanced *S. aureus* binding. Quantification of DU 5883 (Fnbp-negative) mutant strain and its parent 8325–4 strain of *S. aureus* binding to the stratum corneum of BALB/C mouse skin incubated with recombinant IL-4 (1000 U per ml) or recombinant IFN-γ (1000 U per ml) or medium alone at 37°C for 72 h. The means ± SEM in bacterial binding are shown for four separate experiments.

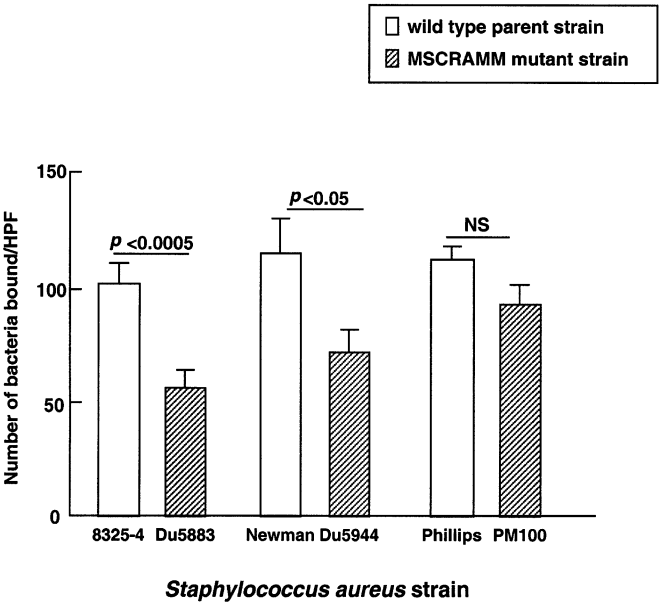

Figure 5. Quantification of MSCRAMM mutant *S. aureus* strain adherence to the stratum corneum of OVA-sensitized/challenged BALB/C mice. Binding of each bacterial mutant strain was compared to its corresponding WT parent strain. The means ± SEM in bacterial binding are shown for four separate experiments.

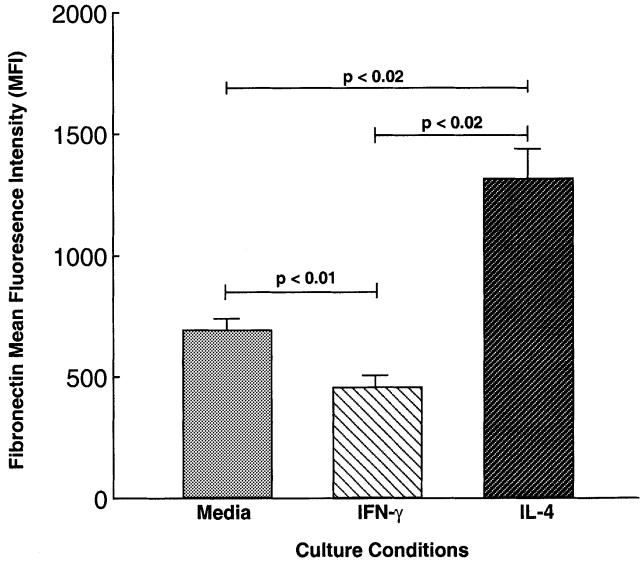

Figure 7. Visualization of fibronectin staining in control medium *versus* IFN-γ *versus* IL-4 treated skin explants from BALB/C mice. Marked differences can be seen in the epidermis of the three subject groups, with IL-4-treated skin showing the greatest epidermal staining for fibronectin.

proteins demonstrated reduced binding to OVA-sensitized/challenged skin reactions (**Fig 5**). In contrast, a collagen adhesin-negative mutant did not show decreased binding to Th2-mediated inflamed skin. Second, when *S. aureus* were preincubated with either saline, HSA, fibronectin, collagen, or fibrinogen in an attempt to block the MSCRAMM receptors and thus interfere with bacterial adherence, only fibronectin and fibrinogen, but not saline, collagen, or HSA, significantly reduced the level of *S. aureus* binding to Th2-induced skin inflammation sites (**Fig 4**). Of note, the bacterial binding sites were mainly confined to the stratum corneum.

Although fibronectin and fibrinogen are plasma proteins that can exudate from the blood at sites of skin inflammation, it is unlikely that this represents the exclusive process by which *S. aureus* binding is increased to atopic skin. *In vitro* incubation of skin explants from normal mouse skin with recombinant IL-4 increased *S. aureus* binding to a level significantly greater than incubation of skin from the same skin sites with either medium control or IFN-γ.

Furthermore, IL-4 but not IFN-γ also increased suprabasal epidermal fibronectin deposition. This suggests a selective mechanism by which Th2, as compared to Th1, responses can enhance *S. aureus* binding to the skin. This is consistent with previous *in vitro* studies that have also demonstrated that IL-4, but not IFN-γ, can induce fibronectin production by skin fibroblasts (Clark, 1990; Postlethwaite *et al*, 1992). Thus, IL-4-induced fibronectin synthesis, in combination with plasma exudation of fibrinogen, could provide a mechanism by which the atopic/inflammatory environment mediates enhanced *S. aureus* attachment to the skin. Interestingly, a recent study by Wann *et al* (2000) demonstrated that the *S. aureus* fibronectin-binding MSCRAMM FnbpA is a bifunctional protein that also binds to fibrinogen. This observation is consistent with our observations suggesting that blocking the binding of *S. aureus* to fibrinogen and fibronectin may be an important therapeutic target for reduction of *S. aureus* colonization in atopic skin.

In summary, using *S. aureus* mutant strains, deficient in the expression of selective MSCRAMMs, and mouse strains with targeted deletions of cytokine genes, it has been possible to demonstrate for the first time a direct link between Th2-mediated cutaneous inflammation and increased colonization of the skin by *S. aureus*. Although fibronectin and fibrinogen provide abundant adherent sites for bacterial adherence, other adhesins may also be involved as both ECM bacterial incubation and utilization of MSCRAMM mutants of *S. aureus* did not totally abrogate the binding of *S. aureus*. Nevertheless, our study provides an approach for further investigation into the interactions between MSCRAMMs and ECM components that initiate colonization of host tissues. Additional studies are needed to identify other biophysiologic factors that support and perpetuate this colonization process, as well as further growth of bacteria, once they attach to inflamed skin.

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REFERENCES

Beck L, Spiegelberg HL: The polyclonal and antigen-specific IgE and IgG subclass response of mice injected with ovalbumin in alum or complete Freund's adjuvant. *Cell Immunol* 123:1–8, 1989

Bunikowski R, Mielke M, Skarabis H, Herz U, Bergmann RL, Wahn U, Renz H: Prevalence and role of serum IgE antibodies to the *Staphylococcus aureus*-derived superantigens SEA and SEB in children with atopic dermatitis. *J Allergy Clin Immunol* 103:119–124, 1999

Bunikowski R, Mielke MEA, Skarabis H, *et al*: Evidence for a disease-promoting effect of *Staphylococcus aureus*-derived exotoxins in atopic dermatitis. *J Allergy Clin Immunol* 105:814–819, 2000

Clark RAF: Fibronectin matrix deposition and fibronectin receptor expression in healing and normal skin. *J Invest Dermatol* 94:128S–134S, 1990

Foster TJ, Höök M: Surface protein adhesins of *Staphylococcus aureus*. *Trends Microbiol* 6:484–488, 1998

Greene C, McDevitt D, Francois P, Vaudaux PE, Lew DP, Foster TJ: Adhesion properties of mutants of *Staphylococcus aureus* defective in fibronectin-binding proteins and studies on the expression of fnb genes. *Mol Microbiol* 17:1143–1152, 1995

Hamid Q, Boguniewicz M, Leung DYM: Differential *in situ* cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 94:870–876, 1994

Hamid Q, Naseer T, Minshall EM, Song YL, Boguniewicz M, Leung DYM: *In vivo* expression of IL-12 and IL-13 in atopic dermatitis. *J Allergy Clin Immunol* 98:225–231, 1996

Hauser C, Wuethrich B, Matter L, Wilhelm JA, Schopfer K: Immune response to *Staphylococcus aureus* in atopic dermatitis. *Dermatologica* 170:114–120, 1985

Ke Y, Li Y, Kapp JA: Ovalbumin injected with complete Freund's adjuvant stimulates cytolytic responses. *Eur J Immunol* 25:549–553, 1995

Kotzin BL, Leung DYM, Kappler J, Marrack P: Superantigens and their potential role in human disease. *Adv Immunol* 54:99–166, 1993

Leung DYM: Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 105:860–876, 2000

Leung DYM, Harbeck R, Bina P, *et al*: Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. *J Clin Invest* 92:1374–1380, 1993a

Leung DYM, Walsh P, Giorno R, Norris DA: A potential role for superantigens in the pathogenesis of psoriasis. *J Invest Dermatol* 100:225–228, 1993b

Lever R, Hadley K, Downey D, Mackie R: Staphylococcal colonization in atopic dermatitis and the effect of topical mupirocin therapy. *Br J Dermatol* 119:189–198, 1988

Leyden JE, Marples RR, Kligman AM: *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br J Dermatol* 90:525–530, 1974

Ni Eidhin D, Perkins S, Francois P, Vaudaux P, Höök M, Foster TJ: Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of *Staphylococcus aureus*. *Mol Microbiol* 30:245–257, 1998

Nilsson EJ, Henning CG, Magnusson J: Topical corticosteroids and *Staphylococcus aureus* in atopic dermatitis. *J Am Acad Dermatol* 27:29–34, 1992

Nomura I, Tanaka K, Tomita H, *et al*: Evaluation of the staphylococcal exotoxins and their specific IgE in childhood atopic dermatitis. *J Allergy Clin Immunol* 104:441–446, 1999

Oettgen HC, Geha RS: IgE in asthma and atopy: cellular and molecular connections. *J Clin Invest* 104:829–835, 1999

Patti JM, Bremell T, Krajewska-Pietrasik D, Abdelnour A, Tarkowski A, Ryden C, Höök M: The *Staphylococcus aureus* collagen adhesin is a virulence determinant in experimental septic arthritis. *Infect Immun* 62:152–161, 1994

Postlethwaite AE, Holness MA, Katai H, Raghow R: Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin 4. *J Clin Invest* 90:1479–1485, 1992

Sawada K, Nagai H, Basaki Y, *et al*: The expression of murine cutaneous late phase reaction requires both IgE antibodies and CD4 T cells. *Clin Exp Allergy* 27:225–231, 1997

Spergel JM, Mizoguchi E, Brewer JP, Martin TR, Bhan AK, Geha RS: Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. *J Clin Invest* 101:1614–1622, 1998

Spergel JM, Mizoguchi E, Oettgen H, Bhan AK, Geha RS: Roles of TH1 and TH2 cytokines in a murine model of allergic dermatitis. *J Clin Invest* 103:1103–1111, 1999

Tokura Y, Yagi H, Ohshima A, *et al*: Cutaneous colonization with staphylococci influences the disease activity of Sezary syndrome: a potential role for bacterial superantigens. *Br J Dermatol* 133:6–12, 1995

Wann ER, Gurusiddappa S, Höök M: The fibronectin-binding MSCRAMM FnbpA of *Staphylococcus aureus* is a bifunctional protein that also binds to fibrinogen. *J Biol Chem* 275:13863–13871, 2000