

Identification of Staphylococcal Protein A in Infected Atopic Dermatitis Lesions

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TO THE EDITOR

Staphylococcus aureus infection is a known trigger for skin inflammation and can modulate immune responses. Atopic dermatitis (AD), a chronic inflammatory pruritic skin disease, affects 10–20% of children and 1–3% of adults (De Benedetto *et al.*, 2009). Owing to the loss of skin integrity by scratching, as well as decreased levels of antimicrobial peptides in comparison with normal skin or other inflammatory diseases such as psoriasis (Ong *et al.*, 2002; Leung, 2003), patients with AD are particularly susceptible to staphylococcal skin infections, which can further worsen their skin disease (Bieber, 2008). Studies have suggested several underlying mechanisms for staphylococcus-mediated inflammation, which include production of inflammatory cytokines following either direct infection of keratinocytes or immune cells by the bacteria, or indirectly by bacterial products (Travers *et al.*, 2001; Leung, 2003; Sasaki *et al.*, 2003; Baker, 2006). We have demonstrated previously that lipoteichoic acid (LTA), a Gram-positive bacterial lipoprotein, may have an important role in the ability of *S. aureus* to exacerbate AD lesions (Travers *et al.*, 2010). In the present study, we report that staphylococcal protein A (SPA) could also contribute to this process.

SPA is a 40–60 kDa bacteria surface protein. It binds to the Fc region of IgG via interaction with the heavy chain, which disrupts opsonization and phagocytosis, contributing to the virulence of *S. aureus* (Foster, 2005). Studies have shown that SPA can also activate the tumor necrosis factor receptor-1, which leads to NF- κ B and activator protein 1 (AP-1) activation and subsequent pro-

duction of cytokines and chemokines, promoting the inflammatory response in staphylococcal pneumonia (Gomez *et al.*, 2004). Although SPA has potent biological effects, a potential pathologic role for this staphylococcal protein or the amounts found within infected skin lesions remain largely unknown.

To assess the SPA levels in infected AD lesions, we enrolled a total of 89 children (aged 3 months to 6 years) with clinically impetiginized AD diagnosed using the criteria of Hanifin and Rajka. Experiments in our protocol were approved by the Indiana University Institutional Review Committee, and adhered to the Helsinki Guidelines, including using written, informed patient consent in these human studies. (Travers *et al.*, 2010). Sixty-three patients returned for their second visit following a 2-week regimen of oral antibiotics (cephalexin or clindamycin if allergic to cephalosporins or penicillins) and topical corticosteroids. The experimental procedures were previously described (Travers *et al.*, 2010). At each visit, subjects underwent a clinical assessment of a clinically infected lesion of dermatitis using the eczema area and severity index score (Hanifin *et al.*, 2001). Lesional wash fluid was collected, aliquoted and stored at -80°C until use. *S. aureus*, *S. epidermidis* (coagulase-negative staphylococcus) and group A streptococcus colonies were quantified by limiting dilution assay. Quantitative measurement of SPA was performed using ELISA (Assay Designs, Ann Arbor, MI). Measurements of LTA and cytokines IL-6, IL-8 and tumor necrosis factor- α were done as previously described (Travers *et al.*, 2010). The levels of SPA, LTA and cytokines in

wash fluid were first calculated based on the area of the chamber (ng cm^{-2}), and then converted to volume (ng cm^{-3}) based on the estimation of 0.1 cm effective epidermal thickness.

S. aureus was detected from clinically impetiginized AD lesions in 88.8% (79/89) of the subjects at their first visit. Treatment with antibiotics resulted in decreased amounts of *S. aureus* on the lesional skin samples and improvement of the lesional eczema area and severity index scores (Travers *et al.*, 2010).

Measurable amounts of SPA were found in 91.0% (81/89) of the samples during the first visit and in 55.6% (35/63) during the second visit. Of note, 64.0% (57/89) of the first-visit samples but only 9.5% (6/63) of the second-visit samples had SPA levels $>0.5 \text{ ng cm}^{-3}$. As depicted in Figure 1a, there was a strong positive correlation between amounts of SPA and staphylococcal bacterial colony-forming unit (visit 1: $r=0.71$, $P<0.0001$; visit 2: $r=0.45$, $P=0.0002$). In addition, the level of SPA was positively correlated with the lesional eczema area and severity index score at visit 1 (visit 1: $r=0.36$, $P=0.0005$; visit 2: $r=0.15$, $P=0.23$) (Figure 1b).

S. aureus infection or SPA itself has been shown to induce the pro-inflammatory cytokine tumor necrosis factor- α in primary human keratinocytes (Ezpechuk *et al.*, 1996; Aufiero *et al.*, 2007). Our previous studies indicated that biologically relevant levels of pro-inflammatory cytokines can be measured in many wash fluid specimens derived from clinically impetiginized AD lesions (Travers *et al.*, 2010). As shown in Figure 1c, the level of SPA was positively correlated with the amounts of tumor necrosis factor- α for the first-visit samples ($r=0.41$, $P<0.0001$). Moreover, there was a positive correlation between SPA

Abbreviations: AD, atopic dermatitis; LTA, lipoteichoic acid; SPA, staphylococcal protein A

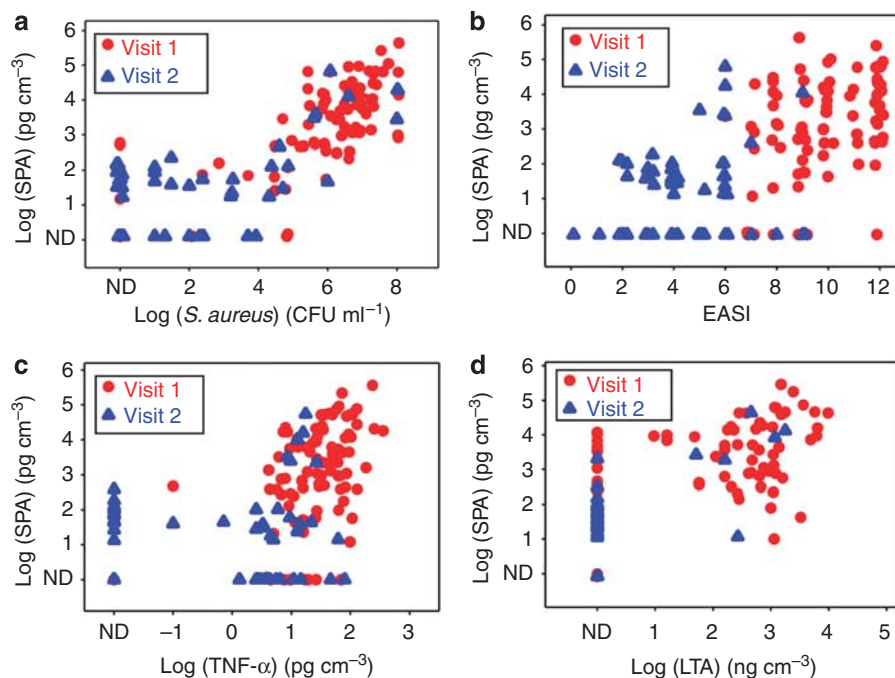


Figure 1. Correlations between amounts of SPA and amounts of *S. aureus*, levels of TNF- α and LTA in lesional wash fluids, and clinical assessment of inflammation in AD patients. Wash fluids were obtained from 89 first-visit (red circle) and 63 second-visit (blue triangle) subjects. (a) Amounts of SPA correlate with *S. aureus* bacteria. Spearman correlation coefficient visit 1 $r = 0.71$; $P < 0.0001$; visit 2 $r = 0.45$; $P = 0.0002$. (b) Amounts of SPA correlate with lesional EASI scores. Spearman correlation coefficient visit 1 $r = 0.36$; $P = 0.0005$; visit 2 $r = 0.15$; $P = 0.23$. (c) Amounts of SPA correlate with levels of TNF- α . Spearman correlation coefficient visit 1 $r = 0.41$; $P < 0.0001$; visit 2 $r = 0.04$; $P = 0.78$. (d) Amounts of SPA correlate with levels of LTA. Spearman correlation coefficient visit 1 $r = 0.55$; $P < 0.0001$.

and other pro-inflammatory cytokines, including IL-6 and IL-8 (data not shown).

The dose of SPA needed to induce cutaneous reactions in human skin has been reported to be in the ng ml⁻¹ range (White and Noble, 1985). Therefore, pharmacologically active (that is, >10 ng cm⁻³) levels of SPA are commonly encountered (in 30% of the first-visit samples) in subjects with impetiginized AD lesions. These data suggested that SPA might contribute to the worsening of AD lesions by *S. aureus* infection. In fact, it has been shown that topical application of SPA after destroying skin barrier function with a detergent can induce AD-like skin inflammation in mice (Terada et al., 2006). However, it is also possible that the increased skin inflammation might be simply caused by other coexisting bacterial products such as LTA and staphylococcal toxins that have been implicated in the worsening of AD in response to infection (Ezpechuk et al., 1996; Travers et al., 2001, 2010). Indeed, we observed a strong positive correlation between SPA and LTA among first-visit samples

($r = 0.55$, $P < 0.0001$) (Figure 1d). It also remains unclear at this point how SPA induces skin inflammation, for example, the cell type(s) and receptor(s) being involved. Further investigations are warranted to elucidate the role of SPA in the pathogenesis of *S. aureus*-mediated worsening of AD.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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