

2. Superantigens, steroid insensitivity and innate immunity in atopic eczema

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There is now considerable evidence that colonization or infection with *Staphylococcus aureus* is an exacerbating factor in atopic dermatitis (AD) (1). Numerous studies have shown that the superantigens produced by staphylococci and streptococci are implicated in the pathogenesis of various inflammatory skin diseases. The skin lesions of patients with AD predominantly contain infiltrating T cells and monocyte/macrophages which can be activated by superantigenic toxins from these microbes.

This review focuses on two specific aspects of the involvement of *S. aureus* in AD. These are mechanisms by which superantigens can induce skin inflammation and impair the response of the inflammatory process to corticosteroids, and the inability of the innate immune system in atopic skin to counteract staphylococcal skin colonization and infection.

STAPHYLOCOCCAL SUPERANTIGENS

There are three primary actions of staphylococcal superantigens. Firstly, they readily penetrate the skin and react with activated keratinocytes. This can lead to the release of pro-inflammatory cytokines including IL-1 and TNF-alpha, which up-regulate adhesion molecules on vascular endothelial cells and initiate the process of tethering and adhesion required for extravasation of inflammatory cells into the skin. Secondly, superantigens cause depletion of Langerhans cells which can then migrate into regional lymph nodes, where they can produce IL-12, a cytokine that selectively up-regulates the expression of CLA (cutaneous lymphoid antigen) which is the skin-homing receptor on T cells. This process likely enhances the recirculation of T cells back into the skin where they can further augment the skin immune response. Finally, many patients with chronic presence of superantigen-producing *S. aureus* on their skin develop an IgE response to superantigens. IgE directed to superantigens can arm mast cells to respond to superantigens by degranulating and releasing histamine. The clinical consequence of these actions may be increased skin disease severity. Breuer et al. (2) found that the severity of AD (assessed as SCORAD index) in 28 patients with a positive IgE response to staphylococcal enterotoxin B (SEB) was significantly greater than that in the 43 patients who were not sensitized.

INTERACTION BETWEEN STAPHYLOCOCCI, STEROIDS AND ANTIBACTERIALS

One of the first studies to look at the clinical effect of topical steroids alone and the combination of steroids with antibacterials was that of Leyden & Kligman (3) who found that a combined antibacterial/corticosteroid preparation was more effective at reducing skin inflammation. Similar results have been seen with a fusidic acid/steroid combination (Table II) (4). A possible reason for this observation could be that the staphylococci are producing something, e.g. superantigens, which is negating the effect of the steroid in suppressing skin inflammation (5).

- Staphylococci produce superantigens, which negate the effect of steroids
- These observations provide a sound basis for using antibacterial/steroid combinations

In lymphocytes stimulated with PHA, corticosteroids are highly effective at suppressing T-cell proliferation. However, superantigens, such as SEB, SEE and TSST-1,

Table I. Comparison of steroid and antibacterial applied under occlusion to atopic dermatitis

Treatment	Patients (n)	Excellent clinical response (%)
Fluocinolone acetonide	21	30
Neomycin/polymyxin/gramicidin	25	0
Fluocinolone acetonide/neomycin/polymyxin/gramicidin	15	70*

* $p < 0.05$.

Taken from Leyden & Kligman (3).

Table II. Comparison of hydrocortisone, fusidic acid and the combination in atopic dermatitis

	Hydrocortisone 1%	Fusidic acid	Fucidin® H (fusidic acid 2% + hydrocortisone 1%)
Did not fail treatment	51%	34%	64%
Failed treatment	49%	66%	36%

Taken from Ramsay et al. (4).

cause a significant reduction in steroid responsiveness (Fig. 1) (5). In our experience, superantigens are the most potent inhibitors of steroid action in human T cells. These observations provide a sound basis for using antibacterial/steroid combinations in AD. Antibacterials shut off superantigen production before they exert any bactericidal effect.

One mechanism of steroid resistance could involve the human glucocorticoid receptor (hGR), of which there are two types, alpha and beta. The beta form does not bind corticosteroids. Therefore overexpression of hGR beta would reduce corticosteroid action. Indeed, hGR beta predominates in T lymphocytes exposed to staphylococcal superantigen. However, even at very high levels of hGR beta, complete inhibition of steroid response is not achieved, suggesting that there may be another factor contributing to corticosteroid resistance.

We have looked at the effect of various signalling pathway inhibitors on superantigen-activated T lymphocytes from normal subjects (6). The ability of various protein kinase inhibitors to reverse the steroid resistance induced by superantigens has been studied. The only inhibitor which has had any effect was U0126, which is a MEK/ERK (mitogen-activated protein kinase extracellular signal-regulated kinase) inhibitor that prevents SEB-induced steroid resistance (6). SEB stimulation induces rapid phosphorylation of ERK. *In vitro* studies have demonstrated that ERK can then in turn phosphorylate the glucocorticoid receptor, a molecular event known to inhibit nuclear translocation of the glucocorticoid receptor. Indeed, the glucocorticoid receptor nuclear translocation in T cells is inhibited after superantigen stimulation in the presence of dexamethasone. The receptor stays in the cytoplasm, it does not enter the nucleus and hence has no effect. MEK/ERK inhibitors restore glucocorticoid receptor

nuclear translocation, and therefore reverse steroid resistance. This appears to be the mechanism for steroid resistance at a molecular level.

THE PROBLEM OF *S. AUREUS* IN ATOPIC DISEASE

- *In vitro* *S. aureus* adheres more readily to the non-lesional skin of patients with AD than to the skin of normal individuals
- *S. aureus* multiplies readily in the skin of AD
- In normal skin there is a profound immune response to kill the bacteria, but this is lacking in atopic skin

S. aureus produces a wide range of virulence factors (Table III). *In vitro* *S. aureus* adheres more readily to the non-lesional skin of patients with AD than to the skin of normal individuals (7). This increased avidity for *S. aureus* is due to the underlying atopic inflammatory skin response. In a mouse model of AD, *S. aureus* binding was found to be dependent upon IL-4 (8). In atopic skin there are increased amounts of fibronectin, an adhesin to which *S. aureus* readily binds. The loss of skin barrier function and scratching, common in AD, can contribute to increased colonization by exposing underlying *S. aureus* adhesins such as fibronectin and fibrinogen, to which the bacteria bind. This accounts only in part for the several fold increase in the density of *S. aureus* found on atopic, as compared to normal or psoriatic, skin. Indeed, *S. aureus* multiplies rapidly in the skin of AD. In normal skin there is a profound immune response to kill the bacteria, but this is lacking in atopic skin. This suggests an innate immune defect in the skin of AD.

Antimicrobial peptides, a group of constitutively expressed or induced cationic molecules which lyse the outer membrane of microbes, are an important component of innate immunity (9). Each antimicrobial peptide has a specific antimicrobial action, giving the group a whole wide spectrum of activity against bacteria, fungi and viruses. In psoriasis, two of these peptides, human beta defensin 2 (HBD-2) and LL-37, are highly up-regulated. The peptides, HBD-2 and LL-37, have concentration-dependent synergistic cytotoxic activity

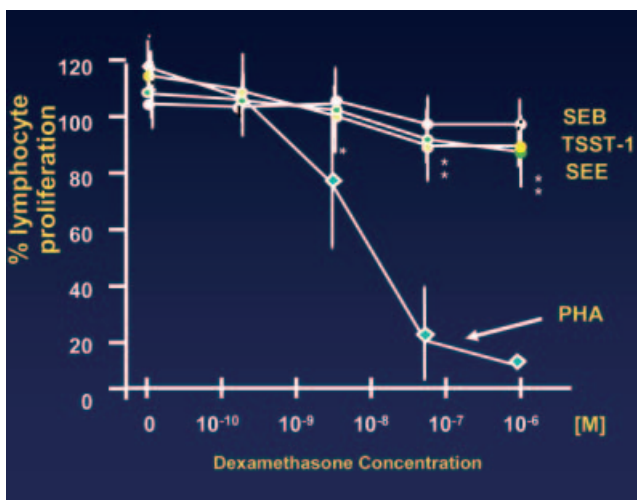


Fig. 1. Superantigens cause decreased steroid responsiveness to normal PBMC. Taken from Hauk et al. (5).

Table III. *S. aureus* virulence factors

Virulence factors
Attachment
Fibronectin-binding proteins (clfA, fbpA)
Fibronectin-binding proteins (fmbA, fmbB)
Evasion of host defences
Superantigens
Lipoteichoic acid
Protein A
Tissue penetration
Alpha toxin
Beta haemolysin

against *S. aureus*. However, their levels are very low in patients with acute and chronic AD (10). Genechip microassay of lesional skin has shown a wide range of innate immune effector molecules, but the inducible compounds such as HBD-2 and inducible nitric oxide synthase (INOS) are deficient in AD (11). Another of these inducible peptides is of particular interest. HBD-3 appears to be specific for *S. aureus*, displaying dose-dependent cytotoxic activity independent of other antimicrobial peptides (12). Immunochimistry has shown that the level of HBD-3 in the skin of AD is decreased significantly in comparison to psoriatic skin. There seems to be an innate inability of atopic skin to react to the presence of *S. aureus* by increasing HBD-3 production. There could be a genetic defect involved and this is under investigation, but there may be another explanation for its down-regulation. It could be acquired as a result of the Th2 response seen in atopics. HBD-2 and HBD-3 are induced by Th1 cytokines and are down-regulated by Th2 cytokines (Fig. 2). Taken together, reduced Th1 responses and increased Th2 responses in AD increase *S. aureus* attachment and decrease the innate immune response in atopic skin, thereby contributing to the increased prevalence of microbial infection.

As a consequence of all these interactions, a vicious cycle of *S. aureus* involvement in AD develops (Fig. 3). The initial step is the genetic predisposition to a deranged skin barrier, followed by increased colonization with *S. aureus*. *S. aureus* superantigens then trigger the immunological cascade leading to release of

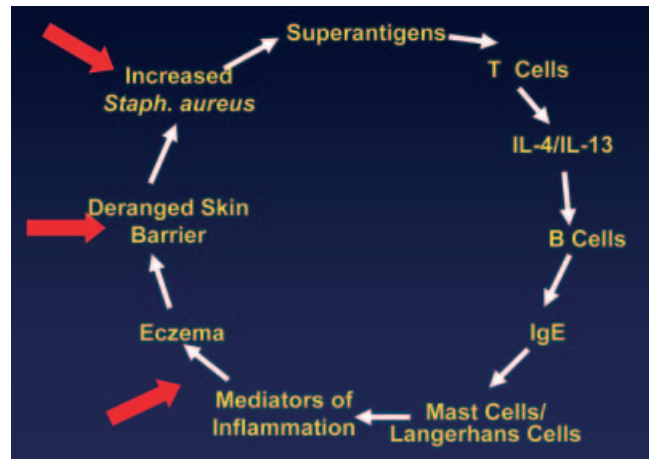


Fig. 3. The vicious cycle of *S. aureus* and atopic dermatitis.

mediators and the appearance of the inflammatory skin lesions of eczema accompanied by an impaired innate immune response which is ineffective at controlling the overgrowth of *S. aureus*. Interruption of this vicious cycle requires combination therapy. This includes skin hydration and restoration of skin barrier function, reduction of atopic skin inflammation and the judicious use of antibiotics to control *S. aureus* skin infection.

- A vicious cycle of *S. aureus* involvement in AD develops
- Interruption of this vicious cycle requires combination therapy, e.g. skin hydration and restoration of skin barrier function, reduction of atopic skin

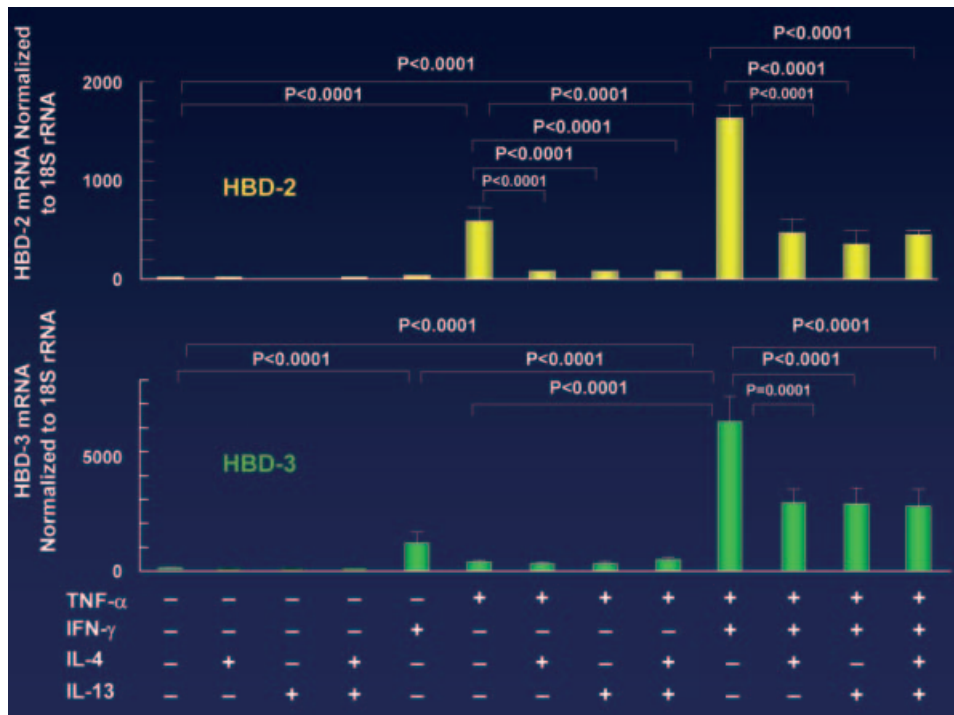


Fig. 2. Effect of Th1 and Th2 cytokines on human beta defensin (HBD)-2 and HBD-3. Taken from Nomura et al. (11).

inflammation and the judicious use of antibiotics to control the *S. aureus* skin infection

DISCUSSION

Thestrup-Pedersen; Was the antimicrobial peptide analysis on healthy or atopic skin?

Leung; All the studies were on lesional skin, but even normal-looking skin of atopics display a Th2 effect, so something is going on here. We hope to look at a keratinocyte model with other investigators in the near future.

Andersen; Are atopic patients colonized by the same strain of *S. aureus* in the long term?

Leung; Not necessarily.

Andersen; Does that affect the clinical picture?

Leung; That is not known. Most early studies were restricted to just a few superantigens, now we are up to 22 different ones. The early ones were identified because they caused explosive exacerbations, but now as the *S. aureus* genome has been characterized we can go back and look at other superantigens or toxins.

There are probably several different strains on the skin with varying antimicrobial susceptibilities. These will fluctuate as different treatments are employed.

Andersen; Has anyone looked at replacing these strains with non-pathogens?

Leung; We looked at this many years ago but had concerns about producing a more severe infection. We restricted ourselves to strains which did not express protein A. Despite several attempts with such a strain, we never replaced the natural ones, in part because you needed too many bacteria. We were never able to replace the original organism.

Agner; Do you think that relapses in patients are due to a new strain of *S. aureus* or the original one?

Leung; It is very difficult to eradicate the original strain. Baths eradicate around 90%, but within hours you are back to the starting number. Because atopic dermatitis patients have poor skin innate immunity, growth can continue almost indefinitely. If you wish to stop using antibiotics you can still control *S. aureus* levels with antiseptics.

McFadden; Do you know of any studies which show the superantigen induced cytokines entering the systemic circulation?

Leung; We have never looked for the relevant cytokines in the systemic blood and this would be difficult.

McFadden; Have you ever looked at nasal carriers of *S. aureus*?

Leung; No, my guess is it should be high, people with allergic rhinitis have high levels of cytokines. As the presence of *S. aureus* on the skin and in the nares is similar, one would expect the same process in both.

McFadden; Why do you think *S. aureus* produces all these superantigens?

Leung; No doubt to aid colonization, the superantigens augment the immune system leading to inflammation, which in turn leads to increased binding of the bacterium.

McFadden; Why should there be so many different superantigens?

Leung; Each superantigen provokes a different T-cell repertoire response. There are different families of superantigens and the number is probably due to genetic variation in the bacterium. For example methicillin-resistant strains are known to be virulent and produce a wide response from the immune system. Strains which produce few superantigens evoke less response and are more difficult to detect.

Langley; What percentage of patients have this steroid-suppressing effect?

Leung; Around 15–25% of the population have relative steroid insensitivity using the PHA test, this is believed to be the genetic population. Superantigen acquired steroid insensitivity is on top of this population and can result in 100% of patients, if enough superantigen is present. However, even in the case of severe steroid insensitivity you can probably overcome it clinically by applying large amounts of steroids. Kligman showed that the number of bacteria required to produce a clinically important *S. aureus* infection in AD is $10^6/\text{cm}^2$ and these patients respond very well to antimicrobials alone. When there are smaller numbers of organisms, antimicrobials alone may not be effective.

Langley; Our experience is that the number of patients with steroid insensitivity is quite small in mild to moderate cases.

Leung; Correct, you need the inflammation in the more severe cases to enable sufficient numbers of organisms to overgrow to produce the phenomenon. The superantigen model is more relevant to moderate to severe cases. On top of that there are around 15% of patients who do not respond well to steroids, regardless of the severity of the lesion.

Thestrup-Pedersen; Can you speculate on the role played by *S. aureus* in the development of dermatitis. The NGA mouse develops dermatitis unless it is kept in a totally

germ-free environment. If you give the mouse antibiotics you reduce the severity of the lesions. If you treat patients with *S. aureus* involvement very early in childhood, would this reduce the eczema development?

Leung; That is the holy grail of autoimmune disease. When you get polyclonal T-cell activation which recognizes an autoantigen in the skin you get disease. It is possible that the superantigens just activate large repertoires of T cells in the immune system and some of those T cells might be autoimmune. They may not normally get into the skin, but when they do they provoke reactions. Babies with food allergy have a lot of *S. aureus* colonization, it is possible that these superantigens can induce the gut to become educated to become skin-homing T cells, giving the link between AD and food allergy. Superantigens primarily affect Th1 and -2 cells whilst gram-negative bacteria involve Th1 types. In a genetically predisposed individual the involvement of both types including Th2 drives the allergic process.

Thestrup-Pedersen; If you consider adults, it is difficult to document the effect of systemic antibiotics in terms of reducing SCORAD, but with topical antibiotics you have a better response. Is that correct?

Leung; We have seen good results with systemic antibiotics, but it does depend upon the disease severity and its extent, a better response is seen with extensive rather than localized lesions.

Thestrup-Pedersen; Others have shown that there is no effect of antibacterial therapy in patients maintained on emollients and topical steroids.

Leung; That group does not respond, possibly because there are too few bacteria on the lesions. You need a fairly dense bacterial population to see any effect. We need a good technique for quantitative bacteriology for this reason to give good guidance on when to treat with antibiotics.

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