

Review article

Staphylococcus aureus enterotoxins: a key in airway disease?

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Staphylococcus aureus (*S. aureus*), one of three pathogenic species of the Gram-positive cocci, is often found as part of the normal microflora of the human skin, the upper respiratory tract, especially the *vestibulum nasi*, and the intestinal tract. Transmission of the organism is frequently achieved by direct contact with an infected individual, although it may also be airborne or infect via fomites. It is thought that about 25% of the population are permanent carriers of *S. aureus* and whilst approximately 20% of all human staphylococcal infections are autogenous, several factors have been identified that predispose the host to increased susceptibility to infection by *S. aureus* (1). These include injury to skin or mucous membrane, abnormal leukocyte function, viral infections (e.g., influenza), metabolic abnormalities (e.g., diabetes mellitus and uraemia) and miscellaneous conditions (e.g., malnutrition, old age, malignancies, etc.). Under appropriate conditions, the organism leads to a variety of clinical conditions, affecting the skin, lungs, heart, central nervous system (CNS), bones and joints, gastrointestinal tract and the blood system (1). Skin infections, lung abscesses, pneumonia, osteomyelitis, food poisoning, fever, scalded skin syndrome and toxic shock syndrome are among the most common conditions. Although the pathogenicity of *S. aureus* is closely correlated with the production of coagulase enzymes, and many conditions result as a consequence of invasion by the bacterium, these organisms also contain a number of cellular antigens and produce a variety of toxins with superantigenic properties (2, 3). Recent studies of these staphylococcal superantigens have demonstrated that they can influence the activity of both immuno-

modulatory and pro-inflammatory effector cell types, and therefore may have a potentially important role in the pathogenesis of chronic inflammatory disease.

Staphylococcal superantigens

Staphylococcal superantigens are a group of high-molecular weight pyrogenic proteins that have in common an extremely potent stimulatory activity for T lymphocytes, including CD4+ , CD8+ , and gamma delta + T cells, of several species (4). Several staphylococcal superantigens have been described, of which the staphylococcal enterotoxins (SEs) are the most widely studied. The SEs are a family of structurally related heat-stable proteins of approximately 27 kDa molecular mass comprising several major serological types: five prototypic SEs (types A to E) and three newly characterized SEs (types G to I) (2, 5). More recently a novel 26 kDa molecular mass SE, serological type K, which has many of the biological activities associated with other SEs, including superantigenicity, pyrogenicity and enhancement of endotoxin lethality, has been shown to be secreted from clinical isolates of *S. aureus* (6). Other studies have suggested the existence of variants of some of these major serological types. Abe and colleagues (5) have cloned a superantigen variant of SEG (SEGV) from clinical isolates of *S. aureus* and, using the polymerase chain reaction (PCR), have demonstrated that SEG or SEGV is one of the most frequently produced superantigens by *S. aureus*. Similarly, variants of SEA, SEC and SEE with superantigenic properties have also been

demonstrated (7–9). More recently, the effect of a mutated variant of SEA with no measurable affinity for major histocompatibility complex (MHC) class II, has been shown to inhibit the wild type SEA-induced psoriasis in a severe combined immune-deficient (SCID)-hu xenogenic transplantation mouse model, suggesting that mutated variants of SEs with no superantigenic activity may have a therapeutic potential in the management of staphylococcal induced disease (10).

Toxic shock syndrome toxin (TSST-1), a causative agent of toxic shock syndrome, is another staphylococcal toxin that has been widely investigated and shown to possess superantigenic properties, as indicated by its ability to activate T cells to proliferate and secrete lymphokines and to act as a nominal antigen to induce proliferation and immunoglobulin (Ig) secretion in human B cells (11, 12). Similar to the SEs, studies have demonstrated that there are naturally occurring variants of also this superantigen, which may or may not be fully effective as the wild type TSST-1 (13, 14).

In addition to these most commonly occurring superantigenic toxins, some strains of *S. aureus* have also been shown to produce exfoliative toxins (ETs), which are the causative agents of staphylococcal scalded skin syndrome, a blistering skin disorder that predominantly affects children (15). Although there have been conflicting reports on the superantigenic nature of these agents, a recent study has confirmed this by the use of highly purified ETA and ETB to demonstrate that these agents induce selective polyclonal expansion of several human T cells (16). Additionally, ETB was also shown to be moderately pyrogenic and enhanced susceptibility to lethal shock in rabbits. Studies of staphylococcal virulence factor, protein A, have suggested that this toxin also has superantigenic properties because it directly influences the activity of a subset of B cells and leads to a T-cell-independent depletion of these cells *in vivo* (17, 18).

Structural studies have demonstrated that pyrogenic superantigens produced by *S. aureus*, and indeed *Streptococcus pyogenes*, are all similar in size and have a conserved two domain tertiary fold, of which the structure of domain 1 is similar to the Ig-binding motif of streptococcal proteins G and L, and the structure of domain 2 is similar to the oligosaccharide/oligonucleotide-binding motifs of the AB[5] heat-labile enterotoxins, cholera toxin, pertussis toxin, and verotoxin (19). The close structural homology amongst the bacterial superantigens suggests that they may have evolved through the recombination of two smaller beta-strand motifs.

Mechanistic studies have shown that superantigens stimulate the T cells by cross-linking the variable part on the beta chain of the T-cell receptor (TCR) with MHC class II molecules on accessory or target T cells, outside the peptide-binding groove area (20, 21),

although binding to MHC class I molecule has also been observed in a MHC class II-negative epidermal cell line (22). This leads to stimulation of up to 20–25% of the naive T-cell population in a nonspecific way, compared with stimulation of only about 0.1% of the T-cell population via the conventional allergen-specific MHC-restricted route utilizing both TCR-V α and β chains (23, 24). Additionally, mutational analysis of the TCR variable beta domain, combined with the crystal structure of the TCR beta chain, have demonstrated that the recognition sites for these bacterial superantigens are distinct from those recognized by viral superantigens, which also exaggerate T-cell activity in a similar MHC-unrestricted manner (25).

Crystallographic studies have also shown that there is considerable homology between the superantigen-binding site and peptide/MHC-binding sites of the TCR, which allows the superantigen to act as a 'wedge' between the TCR and MHC (8). This leads to displacement of the antigenic peptide away from the TCR-binding site and results in the superantigen circumventing the normal mechanism for T-cell activation by specific peptide/MHC complexes. Similarly, a study employing the use of a plasmon resonance biosensor to characterize the kinetics of the trimolecular interaction between TCR, MHC class II and the superantigen SEA has demonstrated that although the binding between the individual components is very weak, the stability of the trimolecular complex is significantly increased, to a level similar to that seen for the interaction between TCR and MHC:peptide ligand (26). Consequently, it has been suggested that the potency of the SEA, and likely the other SEs, in activating the T cell is owing to the co-operative effect of interactions in the TCR-superantigen-MHC class II trimolecular complex rather than as a result of particularly strong affinities between the various proteins. A study investigating the interaction between TSST-1 and MHC class II, however, has suggested that this is controlled by the carboxyl (C)-terminal residues of the class II associated peptides (27). It appears that binding/presentation of TSST-1 to MHC class II does not involve cognate interaction between the peptide and TSST-1, but is dependent on the length of the C-terminal region. Thus, many naturally processed class II/peptide complexes with 'inappropriate' C-terminal regions may not necessarily present TSST-1 to T-cells.

Effect of staphylococcal superantigens on immuno-modulatory and pro-inflammatory cell types

T lymphocytes

Studies investigating the effect of staphylococcal superantigens, in particular enterotoxins A to E (SEA to SEE) and TSST-1, on T-cell function have been pivotal in understanding the etiology and pathogenesis of

S. aureus-induced disease. They, particularly in transgenic KO mice, have demonstrated that injection of these superantigens lead to T-cell proliferation/polyclonal expansion, pro-inflammatory cytokine production, and programmed cell death (apoptosis), as well as T-cell anergy *in vivo* (28). Compared with normal IL-(interleukin)-4 + mice, SEB increased the synthesis of interferon-(IFN γ) and decreased the synthesis of IL-5 in IL-4 knockout (KO) mice, in which the IL-4 gene was deleted (29). Additionally, this was accompanied by a partial deletion and anergy of the remaining SEB-reactive T cells, in particular the CD8Vbeta8 + T cells, and increased susceptibility to low-dose D-galactosamine-induced shock in the KO mice, but not the normal mice, suggesting that IL-4 is essential in the rescue of CD8 + T-cells from SEB-induced apoptosis and anergy. Indeed, studies of patients who develop glomerulonephritis following methicillin-resistant *S. aureus* infection have also demonstrated that there is a marked increase in CD4 + , CD8 + , and TCR Vbeta + T cells and a variety of cytokines, including IL-1 β , IL-2, IL-6, IL-8, IL-10, and tumour necrosis factor-alpha (TNF α), compared with patients not developing glomerulonephritis or normal healthy subjects (30).

These findings for staphylococcal superantigen-induced human T-cell proliferation and pro-inflammatory cytokine release from these cells have been confirmed *in vitro* by several groups of workers. Many of these studies have additionally provided useful information with respect to the mechanisms underlying these effects. Investigation of cell-surface molecules that can mediate staphylococcal TSST-1 and enterotoxin SEB/SEC1/SED-induced T-cell proliferation and production of cytokines have indicated that CD2, CD11a, CD18, CD28, CD44, CD58 and ICAM-1 are likely to be involved, because these effects could be inhibited by the treatment of the cells with monoclonal antibodies (MoAbs) to these molecules (31–33). A more recent study has suggested that T-cell anergy may be mediated by the direct effect of IL-10 on the CD28-signaling pathway (34). The study demonstrated that IL-10 inhibited the phosphorylation of CD28 and subsequently inhibited the production of the Th1 and Th2 cytokines, including IL-2, IFN- γ , IL-4, IL-5 and IL-13. In contrast, the treatment of the cells with anti-IL-10 MoAbs significantly increased both the T-cell proliferation and the secretion of Th1 and Th2 cytokines. Furthermore, the high viability and the unresponsive state of T cells tolerized to IL-10 was reversed by the stimulation with anti-CD3 MoAb and IL-12, but not by stimulation with anti-CD28 antibody.

B lymphocytes

Although a large number of studies investigating the effects of staphylococcal superantigens have focused on the T-cell function, there is increasing evidence that the

staphylococcal superantigens can also directly affect the frequency and activation of B cells, and subsequently influence the expression of the B-cell repertoire. Studies, predominantly with *S. aureus* protein A, have demonstrated that this superantigen influences the activity of B cells by binding the Fab region of human Igs whose heavy chains are encoded by V (H) clan III genes (17, 35). More recently, the interaction between *S. aureus* protein A and the Fab fragment of human IgM antibody has been investigated by crystallography, and shown to occur between specific regions in domain D of protein A and the variable region of the Fab heavy chain, without involvement of the hypervariable regions (36). Similarly, sequence analysis of VH3 gene products and binding with the Ig VH3 domain have indicated that there are several sites that can interact with SEA to stimulate B-cell activity (37).

Functional studies in B cells have shown that *S. aureus* protein A induces proliferation of these cells, following the signal transduction involving protein kinase C (PKC), mitogen-activated protein kinase (MAP kinase), serum responsive factor (SRF) and Bcl-2 gene expression (38). SEB and SED, however, stimulate human B cells expressing VH3- and VH4-containing IgM, respectively (37, 39). Whilst SEB does not induce T-cell independent proliferation nor differentiation of VH3-expressing cells, it enhances the survival of these B cells. In contrast, SED induces the T-cell dependent polyclonal proliferation and differentiation of VH4-expressing cells, and additionally enhances the T-cell-independent survival of these cells, suggesting that both these staphylococcal enterotoxins may function as unique B-cell superantigens by rescuing them from apoptosis. Studies with TSST-1 have shown that this staphylococcal superantigen may play an important role in the modulation of allergic disease, because it can augment isotype switching and synthesis of IgE both *in vitro* (40, 41) and *in vivo*, in a SCID-mouse model (42). Although TSST-1-induced activation of B cells *in vitro* is indirect and dependent on the increased expression of CD40 ligand on T cells, a more recent study has provided additional evidence for a direct effect by demonstrating TSST-1-induced expression on B cells of B7.2 (43), a molecule that has been shown to enhance Th2 responses and to be involved in IgE regulation.

Pro-inflammatory cells

Compared with studies on lymphocytes, there are comparatively fewer studies investigating the effects of staphylococcal superantigens on other pro-inflammatory cell types, in particular eosinophils, macrophages, mast cells, and epithelial cells, which are known to play key roles in the pathogenesis of inflammatory airway disease.

Studies on eosinophils have mostly described the role

of these cells as accessory cells, which stimulate T cells by presenting staphylococcal superantigens via the MHC class II molecules expressed on their cell surface. Incubation of human eosinophils in the presence of granulocyte macrophage-colony stimulating factor (GM-CSF), leads to an increased expression of HLA-DR (a MHC class II molecule) and a subsequent proliferation of resting CD4+ T cells, in response to SEA, SEB and SEE (44). Although eosinophil-mediated T-cell proliferation is correlated with the proportion of HLA-DR-expressing eosinophils, eosinophils are not as efficient as macrophages in inducing proliferation of T cells in response to the staphylococcal superantigens (44). A preliminary study has recently suggested that SEA, SEB, SEC, SED and TSST-1 may also directly affect the eosinophil activity by inhibiting apoptosis and modulating important cell-surface antigens, including the upregulation of CD11b, CD45 and CD69, the and downregulation of CD34 and CD54 expression (45).

Macrophages also act as accessory cells for a T-cell activation/proliferation response to staphylococcal superantigens (44, 46, 47), and direct effects of superantigens on macrophage activity have been documented, in particular the production of cytokines. Studies of human alveolar macrophages have shown that the incubation with SEA leads to a concentration-dependent increase in synthesis and secretion of IL-8 and mRNA encoding IL-8 (48). Incubation of a monocytic cell line with SEA, however, led to the release of lower concentrations of SEA-induced IL-8, which were increased 50-fold by prior treatment of the cell line with PMA, suggesting that maturation of the undifferentiated cell to the mature macrophage facilitates the release of IL-8, and likely other cytokines (48). Cross-linking of MHC class I molecules, with either anti-MHC class I antibodies or MHC class I bound SEA with anti-SEA antibody, in MHC class II-deficient macrophages, also leads to the release of IL-6 and TNF α from these cells (49), suggesting that SEA may activate macrophages via both MHC class I and MHC class II molecules. Studies with SEB have also demonstrated that this superantigen induces IL-12 production in macrophages (50). Activation of the gene encoding IL-12 in macrophages involves signal transduction pathways resulting from binding of SEB with MHC class II molecules, which lead to the activation of PKC and PKA, followed by the activation and nuclear translocation of nuclear factor-kappaB (NF κ B) (51).

Mast cells can be activated directly by staphylococcal superantigens and also act as accessory cells for the activation of T cells, similar to eosinophils and macrophages. Staphylococcal protein A is capable of cross-linking IgE molecules on mast cells by a structure within the constant portions of the Fab'_{2c}-region (52). Genovese and colleagues (53) have investigated the

effect of staphylococcal protein A on the release of several mediators from mast cells isolated from human heart tissue, and demonstrated that this superantigen led to the increased release of histamine, tryptase and leukotriene C₄ (LTC₄). Pre-treatment of the cells with monoclonal IgM V (H)3 + inhibited the protein A-induced response, while pretreatment with protein A resulted in complete cross-desensitization to a subsequent challenge with anti-IgE, suggesting that an interaction between protein A and IgE on the mast cells was important in mediating the effect of the superantigen. In contrast, SEB was not shown to release histamine from a human mast-cell line (MHC-1), and indeed led to a dose-dependent inhibition of IL-4 release (54). More recently, however, one study of mature human cord blood-derived mast cells and a leukemic immature mast-cell line expressing MHC class II antigens demonstrated that SEB and TSST-1 activated CD4+ T-cell hybridomas and induced the release of IL-2 from the cord-blood mast cells (55). The superantigen-induced release of IL-2 was dependent on cell-to-cell contact between the mast cells and T cells and was inhibited by MHC class II antibodies, suggesting an indirect activation of mast cells by the superantigens.

Epithelial cells have also been shown to release pro-inflammatory cytokines, including IL-1 β and IL-8, following treatment with SEB (56). Co-culture of epithelial cells with T cells also leads to indirect superantigen-mediated effects on epithelial cell activity and function, as a result of epithelial accessory cell function for superantigen-induced T-cell activation. Possibly, the most notable and important effects of this interaction are on epithelial ion transport and barrier functions, which are adversely affected by SEB and thought to be owing to the increased release of IFN- γ and TNF α from the T cells (57–59). It has been demonstrated that these adverse epithelial effects may be attenuated by nitric oxide, IL-10 and transforming growth factor (TGF)- β 2 (56–59).

Staphylococcal superantigens and airway disease

In view of the ever increasing evidence for the effects of staphylococcal superantigens on immuno-modulatory and pro-inflammatory cells, it is likely that there is an association between staphylococcal infection and pathogenesis of diseases such as dermatitis, rhinitis, nasal polyposis, and possibly asthma, which is greatly influenced by these cells. The putative role of staphylococcal superantigens in atopic dermatitis, according to the new nomenclature referred to as atopic eczema/dermatitis Syndrome (AEDS) (60), has recently been reviewed by Breuer and colleagues (23), and will therefore be discussed only briefly in the present review to draw parallels with airway disease.

Overall, studies of patients with AEDS have shown

that there is much greater *S. aureus* colonization in the skin of patients (80–100%) than in the skin of normal healthy subjects (5–30%), and that the organism often constitutes up to 80% of the normal flora in the atopic individuals (23). *S. aureus* isolated from the skin of at least 65% of the AEDS patients secrete superantigens SEA, SEB, SEC, SED and TSST-1, which penetrate the epidermis and dermis, where they interact with different cells of the immune system, leading primarily to a T-cell-dependent inflammation. The cutaneous inflammation is exacerbated by the superantigens primarily by one of two pathways: (i) polyclonal activation of T cells and (ii) induction of antigen-specific T cells that are able to promote the generation of antigen-specific IgE antibodies, which subsequently play a role in 'conventional' allergen-mediated reactions. The superantigens also influence cutaneous inflammation by influencing the activity of both professional and nonprofessional antigen-presenting cells (APCs) by binding to MHC class I and class II molecules, which results in the increased expression of adhesion molecules and inflammatory cytokines in these cells.

Compared with AEDS, few studies have documented the role of *S. aureus* or its superantigens in allergic or nonallergic airway disease. Earlier investigations suggested an allergy to different bacteria as an important cause of exacerbation of disease in patients suffering from allergic airway disease (61–63).

However, the tests involved whole bacterial lysates and were highly unspecific, and no correlations were found among these results. We have recently investigated nasal tissue from patients with bilateral nasal polyps, which are characterized by a severe eosinophilic inflammation and often coexist with asthma, for specific IgE to a variety of inhalant allergens and *S. aureus* enterotoxins (64), in comparison to controls. We demonstrated that in about 50% of the polyp homogenates, specific IgE to *Staphylococcus aureus* enterotoxins SEA and/or SEB was present, and was linked to a polyclonal IgE formation against inhalant allergens. Of interest is that, in SEA/SEB-specific IgE positive polyp samples compared to controls, the eosinophilic inflammation was significantly stronger in terms of synthesis of IL-5, eotaxin and Cys-leucotrienes, and these patients more often suffered from asthma and/or aspirin sensitivity.

Mechanistic studies have shown that *S. aureus* can induce the release of histamine from leukocytes of both house-dust mite (*Dermatophagoides pteronyssinus*)- and birch pollen-allergic individuals (65, 66), and additionally potentiate *D. pteronyssinus*-induced release of histamine in asthmatics sensitized to *D. pteronyssinus* (67). It appears that the expression of TCR-Vbeta8 (+) T cells obtained in bronchoalveolar lavage (BAL) of patients with poorly controlled

asthma is also significantly increased, compared with cells in BAL of patients with well-controlled asthma and normal control subjects, suggesting that the staphylococcal superantigens are a potential trigger of T-cell activation in poorly controlled asthma (68). More recent studies, however, have suggested that the staphylococcal superantigens may lead to poor disease control because they can induce steroid insensitivity in peripheral blood mononuclear cells (PBMCs) (69). Compared with the level of dexamethasone-induced inhibition in proliferation of PBMCs in response to PHA treatment, the level of dexamethasone-induced inhibition in proliferation of PBMCs in response to treatment with SEB-, SEE-, and TSST-1 was reduced 3–5-fold. Furthermore, SEB also significantly increased the expression of glucocorticoid receptor beta, compared with PHA and control cells. Similarly, a comparison of normal control B cells vs. cystic fibrosis B cells has shown that although both cell types were equally efficient in presenting *S. aureus* superantigen to an immortalized T-cell line, the antigen-presenting activity of the control B cells, but not cystic fibrosis B cells, was inhibited by the treatment with dexamethasone (70).

Whilst the effect of superantigens has not been investigated on airway responsiveness in asthmatics for obvious reasons, studies in animals have shown that SEB triggers airway recruitment of several pro-inflammatory cell types (including TCR Vbeta (+) and TCR Vbeta (–) T cells, eosinophils, neutrophils and TNF- α (+) macrophages) and release of cytokines (including IL-4 and TNF- α , but not IFN- γ), which are associated with increased airway responsiveness in these animals (71, 72). Importantly, the increase in airway responsiveness was particularly CD4+T-cell-dependent and was observed in the absence of either allergen or allergen-specific IgE/IgG.

Although studies, particularly in patients suffering from rhinosinusitis, have documented the presence of *S. aureus* as one of the more common and predominating bacteria in the nasal airways (73–76), there is a very marked paucity of studies documenting an association between *S. aureus* or its superantigens and exacerbation of upper airways/nasal disease, particularly allergic rhinitis or chronic sinusitis. One recent study, however, has provided some information, which suggests that *S. aureus* superantigens may aggravate disease in patients suffering from perennial allergic rhinitis (PAR) (77). The rates of nasal carriage of total and superantigen-producing *S. aureus* were found to be significantly higher in PAR patients, compared with nonallergic control subjects. More importantly perhaps, nasal symptom scores were significantly higher in *S. aureus*-positive patients, compared with *S. aureus*-negative patients, although no significant correlation was observed

between nasal symptom scores and superantigen production. Furthermore, *in vitro* evaluation of the response of peripheral blood lymphocytes to SEB or TSST-1, indicated that the lymphocytes of PAR patients proliferated to a significantly greater degree than the lymphocytes of control subjects, and also produced significantly greater amounts of IL-4 and IL-5 in a dose-dependent manner. In contrast, the control lymphocytes were seen to produce significantly greater amounts of IFN- γ , compared with lymphocytes derived from PAR subjects.

Although the presence of *S. aureus* has also been documented as a common pulmonary pathogen in cystic fibrosis (78, 79), there is similarly little or no information on the possible role of staphylococcal superantigens in this disease. Animal studies, however, have demonstrated that the intratracheal administration of SEA- and SEB-induced development of interstitial pneumonia in both autoimmune and nonautoimmune mice strains, although the severity of disease was milder in the nonautoimmune strains (80). Pneumonia, however, was manifested by the infiltration of mononuclear cells into the alveolar septal walls and periarterial space and an increase in pulmonary interstitial collagen fibres, characteristic in idiopathic pulmonary fibrosis.

Conclusion

There is now increasing evidence that staphylococcal toxins act as both superantigens and 'conventional' allergens and therefore play a role in modulating chronic inflammatory airway disease, both via non-IgE- and IgE-mediated mechanisms. Studies of patients predominantly suffering from AEDS have increasingly demonstrated associations between the incidence of disease and the colonization with *S. aureus* or presence of staphylococcal superantigens, thus proposing a putative causative role for *S. aureus* bacterium in the pathogenesis of AEDS. Similarly, there is increasing evidence that *S. aureus* superantigens may also have the potential to influence the etiopathogenesis of upper and lower airway disease, including bronchial asthma, rhinitis and sinusitis. The evidence for the role of staphylococcal superantigens in the etiology of allergic airways disease, however, is preliminary and at the best of times circumstantial. Consequently, more basic studies and properly controlled clinical trials are clearly required to elucidate the role of *S. aureus* and its superantigens in the etiology of these diseases unequivocally. Additionally, understanding the superantigen function may also elucidate novel therapeutic strategies for these disorders.

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