

Role of Staphylococcal Superantigens in Airway Disease

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Key Words

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Abstract

Staphylococcus aureus is a common human pathogen, which is regularly part of the normal microflora found in the nose and skin. It represents a significant threat to human health, not in the least because of its capability to produce exotoxins, which have superantigenic properties. These exotoxins, in particular the staphylococcal enterotoxins (SEs), are known to be involved in the modulation and aggravation of airway inflammation. Indeed, recent studies show an important impact of SEs on the natural course of allergic rhinitis, nasal polyposis, asthma and COPD. This review outlines the current knowledge on the influence of SEs on airway inflammation. We highlight, in particular, the recent evidence on their role in asthma.

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Introduction

Superantigens (SAGs) represent a growing family of bacterial and viral proteins that share the capacity of inducing massive activation of the immune system. White

et al [1] first described the concept of SAGs already in 1989, pointing out the ability of *Staphylococcus aureus* enterotoxin B to induce a remarkable expansion of T cells expressing T cell receptors with a specific subset of the T cell receptor β -chain variable region. SAGs are able to bind to the major histocompatibility complex (MHC) class II outside the peptide-binding groove, and to cross-link to the T cell receptor (TCR) via the variable region of the TCR β -chain (fig. 1).

This leads to a potent polyclonal activation of up to 25% of an individual's T cell population. SAGs differ from conventional antigens because they are not processed by antigen-presenting cells (APC) and present as short peptides. These conventional peptide antigens then require recognition by all five variable elements of the TCR ($V\beta$, $D\beta$, $J\beta$, $V\alpha$, $J\alpha$), resulting in a stimulation of about 0.001–0.0001% of naïve T cells.

As the number of different $V\beta$ regions in the human T cell repertoire is restricted to less than 50, and as most SAGs can bind more than one $V\beta$, a far more compelling activation of circulating T cells is achieved. Consequently, each SAG is associated with a characteristic $V\beta$ signature that is independent of the MHC polymorphism, as is shown in table 1.

This immune system stimulation of T cells and APC by SAGs initiates a cascade of proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and inter-

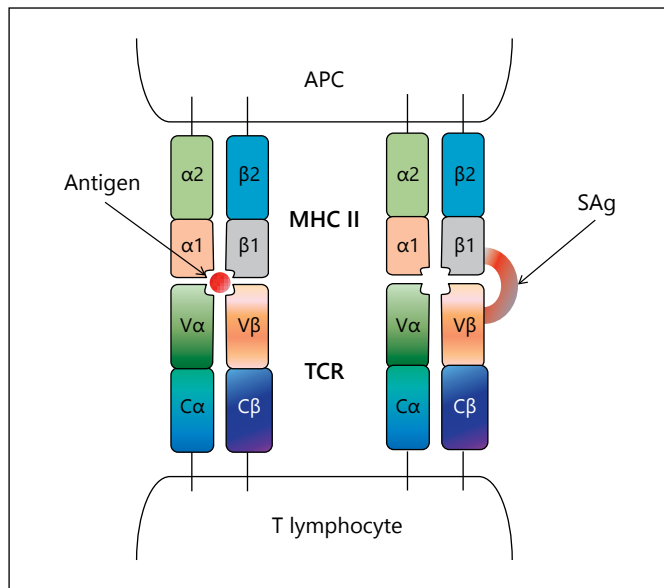


Fig. 1. SAg cross-linking of MHC class II receptor on APC and TCR on T lymphocyte. Left: conventional antigen presentation with processing and presentation by the MHC II within the peptide binding groove. Right: SAg cross-linking MHC II and TCR, without being processed.

leukin-1 beta (IL-1b) and T cell mediators, such as IL-2, leading to fever and shock. Concentrations of less than 0.1 pg/ml of a bacterial SAg are sufficient to stimulate T cells in an uncontrolled manner, making SAGs the most powerful T cell mitogens ever discovered [2].

Enterotoxin-producing *S. aureus* is probably the best studied source of SAGs. However, other bacteria like *Streptococcus pyogenes* [3], *Yersinia pseudotuberculosis* [4] or *Mycoplasma arthritidis* mitogen [5] have known SAg activities. Similarly, SAg formation is linked to some viruses like rabies [6], Epstein Barr virus [7], Cytomegalovirus [8] or HIV [9].

Staphylococcal SAGs

S. aureus is one of the most significant infectious threats to human health. It is a facultative anaerobic, Gram-positive coccus, which is frequently part of the normal microflora found in the nose and on the skin. Carriage of *S. aureus* appears to play a key role in the epidemiology and pathogenesis of infection. In healthy subjects, the anterior nares are most frequently colonized with *S. aureus*, and over time, three patterns of carriage can be distinguished: about 20% of people are persistent

Table 1. List of frequently studied bacterial SAGs

SAg	MW kDa	Organism	Human TCR Vβ specificity
SPE-A	26.0	<i>S. pyogenes</i>	2.1, 12.2, 14.1, 15.1
SPE-C	24.4	<i>S. pyogenes</i>	2.1, 3.2, 12.5, 15.1
SPE-G	24.6	<i>S. pyogenes</i>	2.1, 4.1, 6.9, 9.1, 12.3
SPE-H	23.6	<i>S. pyogenes</i>	2.1, 7.3, 9.1, 23.1
SPE-I	26.0	<i>S. pyogenes</i>	6.9, 9.1, 18.1, 22
SPE-J	24.6	<i>S. pyogenes</i>	2.1
SPE-K/L	27.4	<i>S. pyogenes</i>	1.1, 5.1, 23.1
SPE-L/M	26.2	<i>S. pyogenes</i>	1.1, 5.1, 23.1
SPE-M	25.3	<i>S. pyogenes</i>	1.1, 5.1, 23.1
SSA	26.9	<i>S. pyogenes</i>	1.1, 3, 15
SMEZ-1	24.3	<i>S. pyogenes</i>	2.1, 4.1, 7.3, 8.1
SMEZ-2	24.1	<i>S. pyogenes</i>	4.1, 8.1
SePE-H	23.6	<i>S. equi</i>	?
SePE-I	25.7	<i>S. equi</i>	?
SPE-LSe	27.4	<i>S. equi</i>	?
SPE-MSe	26.2	<i>S. equi</i>	?
SPE-Gdys	24.4	<i>S. dysgalactiae</i>	?
SDM	25.0	<i>S. dysgalactiae</i>	1.1, 23
SEA	27.1	<i>S. aureus</i>	1.1, 5.3, 6.3, 6.4, 6.9, 7.3, 7.4, 9.1, 23.1
SEB	28.4	<i>S. aureus</i>	1.1, 3.2, 6.4, 15.1
SEC1	27.5	<i>S. aureus</i>	3.2, 6.4, 6.9, 12, 15.1
SEC2	27.6	<i>S. aureus</i>	12, 13, 14, 15, 17, 20
SEC3	27.6	<i>S. aureus</i>	5.1, 12
SED	26.9	<i>S. aureus</i>	1.1, 5.3, 6.9, 7.4, 8.1, 12.1
SEE	26.4	<i>S. aureus</i>	5.1, 6.3, 6.4, 6.9, 8.1
SEG	27.0	<i>S. aureus</i>	3, 12, 13.1, 13.2, 14, 15
SEH	25.1	<i>S. aureus</i>	Vα27
SEI	24.9	<i>S. aureus</i>	1.1, 5.1, 5.3, 23
SEJ	28.5	<i>S. aureus</i>	
SEK	26.0	<i>S. aureus</i>	5.1, 5.2, 6.7
SEL	26.0	<i>S. aureus</i>	5.1, 5.2, 6.7, 16, 22
SEM	24.8	<i>S. aureus</i>	6, 7.1, 8, 9, 18, 21
SEN	26.1	<i>S. aureus</i>	5.1, 5.3, 9, 20
SEO	26.7	<i>S. aureus</i>	5.1, 7, 21.3
SEP	27.0	<i>S. aureus</i>	5.1, 8, 16, 18, 21.3
SEQ	25.0	<i>S. aureus</i>	2.1, 5.1, 6.7, 21.3
SER	27.0	<i>S. aureus</i>	3, 5.1, 8, 11, 12, 13.2, 14
SEU	27.1	<i>S. aureus</i>	12, 13.2, 14
TSST-1	22.1	<i>S. aureus</i>	2.1
MAM	25.2	<i>M. arthritidis</i>	6, 8 (murine)
YPM-A	14.5	<i>Y. pseudo-tuberculosis</i>	3, 9, 13.1, 13.2
YPM-B	14.6	<i>Y. pseudo-tuberculosis</i>	3, 9, 13.1, 13.2
YPM-C	14.6	<i>Y. pseudo-tuberculosis</i>	3, 9, 13.1, 13.2

The major TCR Vβ targets are underlined [10]. MW = Molecular weight.

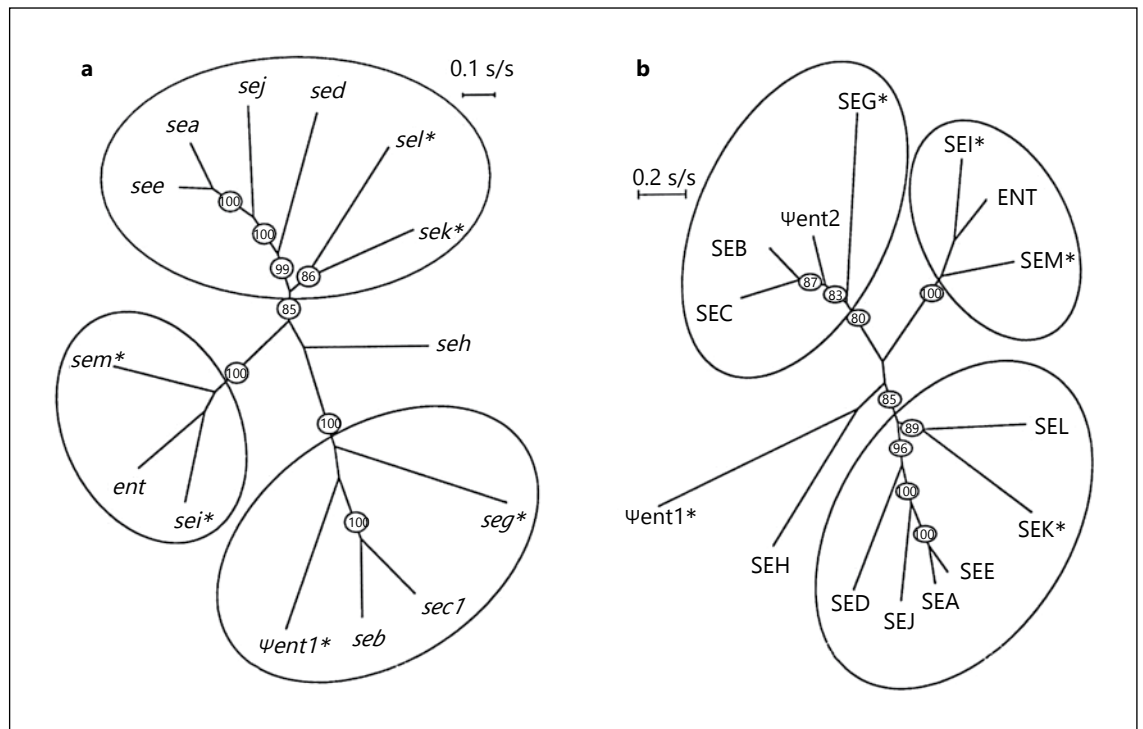


Fig. 2. Reconstruction of phylogenetic tree of staphylococcal enterotoxin genes (a) and toxins (b) [22].

carriers, 60% are intermittent carriers and approximately 20% almost never carry *S. aureus* [10]. This carriage is a major risk factor for infection, as the colonizing strains may serve as endogenous reservoirs for overt clinical infections or may spread to other patients. Indeed, the elimination of carriage in the anterior nares, the principal reservoirs of *S. aureus*, reduces the incidence of *S. aureus* infections [11].

S. aureus is responsible for an array of diseases ranging from minor skin and soft tissue infections, such as pimples, impetigo, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses, to more invasive and life-threatening infections, such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis. The ability of *S. aureus* to cause such diverse diseases is due primarily to an arsenal of virulence factors encoded in the staphylococcal genome. Amongst these bacterial pathogenicity or virulence factors, there are products whose role in the disease process is either clearly demonstrable, like toxins, or more or less obvious on the basis of biological properties, e.g. enzymes that degrade tissue components [12]. *S. aureus* is known for its production of potent toxins. Once introduced into a host's system,

these toxins can act to profoundly stimulate the immune system. The proteins are known to act on host systems in three distinct ways: as enterotoxins, they induce emesis and diarrhea in humans and nonhuman primates [13], as exotoxins, they have been implicated in the induction of toxic shock [14] and as SAg, they induce extensive V β -specific T cell stimulation followed by anergy and apoptosis, which results in immunosuppression [15]. For a long time, the production of SAg by *S. aureus* was thought to be a rare phenomenon, linked to the occurrence of staphylococcal toxemia and therefore restricted to isolates responsible for such syndromes. However, because of the structural and biological similarities that these staphylococcal toxins share, it is no surprise that phylogenetic analysis has identified an enterotoxin gene cluster (egc) in *S. aureus* which was probably generated from an ancestral gene through gene duplication and variation [16]. This is shown in figure 2. In fact, SAg production by *S. aureus* isolates appeared more frequent than initially expected, and it is more common for *S. aureus* to possess a SAg than not. Moreover, most of the strains harbor several SAg genes as illustrated in their genome sequences [17].

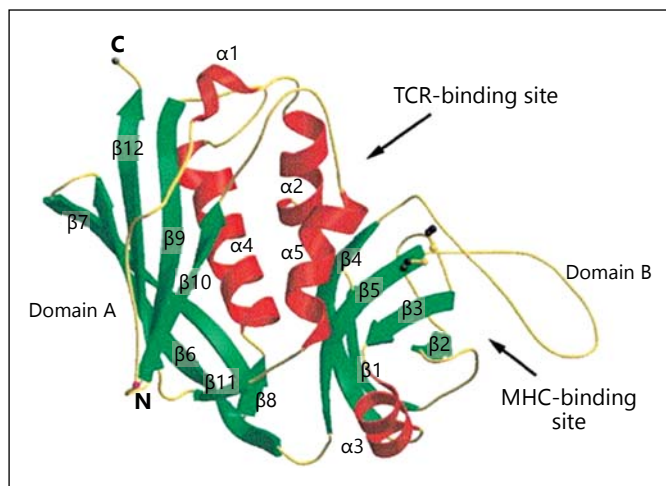


Fig. 3. Ribbon diagram of SEB as representative of SEs. The MHC class II and TCR recognition sites are indicated [25].

Staphylococcal Enterotoxins

Enterotoxins are short, secreted proteins (194–245 amino acids long) with a molecular mass of approximately 27 kDa. They share common biological and structural properties. Remarkably resistant to heat, the potency of these toxins can only be gradually degraded by prolonged boiling or autoclaving. Except for TSS toxin 1 (TSST-1), they are highly stable and resistant to most proteolytic enzymes, such as pepsin and trypsin, which explains the retention of their activity in the digestive tract after ingestion.

Staphylococcal enterotoxins (SEs) are able to induce high fever similar to bacterial endotoxin induction, lethal shock in animals resulting from excessive intravenous doses, enhanced host susceptibility to endotoxin lethality, cytokine production and the typical superantigenic feature of polyclonal T cell proliferation [18]. SEs can bind directly and unprocessed to MHC class II molecules on APCs. Figure 3 depicts this binding of the β -barrel of the SE domain A to the MHC II at a region distinct from the peptide-binding groove [19]. SEs are able to bind a wide variety of molecules, although there is some preference depending on the toxin involved: SEC prefers HLA-DQ and SEA, SEB, SED, SEE, SEH and TSST-1 prefer HLA-DR [20]. Subsequently, the α -helices of domain A can cobind and facilitate ligation between the MHC II molecules of APCs and certain TCRs. As described above, this binding is not dependent on T cell antigenic specificity, but occurs as a function of the vari-

able region of the TCR β - or α -chain [21]. Indeed, each SAg interacts with a defined TCR repertoire, resulting in massive cell activation of both cell types and the release of cytokines.

The reason bacteria produce SAGs and the advantage created by SAGs for the bacteria remain elusive. Most likely, the purpose is not to induce systemic toxic shock in the host. Probably, SAGs are rather inducing local T cell activation in the early stages of infection. This local T cell activation can result in the production of cytokines, such as IL-2, IFN- γ and TNF- α , which suppresses local inflammation [22].

Effects on Different Cells

Besides the activation of T cells, increasing evidence shows the ability of staphylococcal enterotoxins to interact with other cells, such as B cells, eosinophils, mast cells and epithelial cells. Similar to T cell binding, this unconventional type of B cell ligand can, in principle, trigger all B cells bearing the appropriate variable region, regardless of the other junctional and diversity segments. Interestingly, the concentrations of SEs and the surrounding cytokines determine the effect of SEs on B cells. Cross-linking of the MHC class II molecule on B cells with TCR on T cells results in IgE production, when low amounts of IFN- γ are combined with low SE concentration. However, IgE production is inhibited by IFN- γ upon high SE concentration [23]. Interestingly, the effect of SEs on B cell stimulation also depends on the type of SAg. SEA and SEB fail to induce proliferation of B cells in the absence of T cells. Survival of B cells, on the other hand, is enhanced by SEB. An elegant analysis of the distinct biological behavior of different staphylococcal enterotoxins has been made by Grumann et al. [24], concluding that the differential release of enterotoxins by *S. aureus* might explain the differences observed.

Eosinophils are mostly considered to be actors in the effector limb of the immune response, because of their origin in the myelocytic cell series and biochemical similarities to neutrophils. However, several biological features of human eosinophils suggest they may also perform functions in the afferent limb of the immune response. Human eosinophils become hypodense and express MHC class II molecules when activated by granulocyte-macrophage colony-stimulating factor (GM-CSF) in vitro or in vivo in pathological conditions such as allergic disorders. Studies have therefore been undertak-

en to examine the capacity of class II MHC-expressing eosinophils to serve as APC for resting and activated CD4+ T cells [25]. Eosinophils preincubated with GM-CSF induced resting T cells to proliferate in response to SEs (SEA, SEB and SEE). Furthermore, SAg-induced T cell proliferation correlated with the proportion of eosinophils expressing class II MHC molecules.

Compared to macrophages, however, eosinophils are not as efficient in acting as accessory cells for SE-induced T cell proliferation. The function of macrophages as accessory cells for SE-induced T cell proliferation has already been studied extensively in several research lines [26, 27]. On the other hand, the direct effects of SE on macrophage activity have been documented, in particular, the effect on the production of cytokines. IL-8 is synthesized and secreted by human alveolar macrophages upon incubation with SEA [28]. Furthermore, stimulation of macrophages with SEB causes release of chemotactic protein leading to neutrophil migration by a mechanism mediated by platelet-activating factor, histamine H(2) receptors, lipoxygenase products and substance P [29]. Interestingly, in patients suffering from chronic rhinosinusitis with nasal polyps (CRSwNP), we recently demonstrated that macrophages display a decreased phagocytotic capacity of enterotoxin-producing *S. aureus*, which potentially contributes to the persistence of chronic inflammation in CRSwNP [30].

Similar to eosinophils and macrophages, mast cells can be directly activated by SEs, but also act as accessory cells for T cell activation. Staphylococcal protein A is capable of cross-linking IgE molecules on mast cells [31]. Mast cells isolated from human heart tissue are able to release histamine, tryptase and leukotriene C4, when incubated with staphylococcal protein A [32].

The effect of SEs, in particular SEB, on epithelial cells has been described previously [33]. Briefly, SEB can activate the immune system after contact with epithelial cells by MHC class II binding and cross-linking. In this case, epithelial cells act as accessory cells for SAg-induced T cell activation [34, 35]. However, the involvement of non-MHC class II receptors has also been demonstrated [36]. Moreover, SEB is known to activate APCs like dendritic cells via Toll-like receptor (TLR)2, a receptor which plays an important role in pathogen recognition and innate immunity [37]. Interestingly, these TLRs are also present on nonprofessional APC these epithelial cells [38]. Figure 4 summarizes the effects of staphylococcal enterotoxins on different cell types.

Role of Staphylococcal Enterotoxins in Disease

Atopic Dermatitis

Atopic dermatitis (AD) is a chronically relapsing inflammatory disease of still unknown etiopathogenesis. A typical characteristic of AD is the infiltration of T cells, monocytes/macrophages and eosinophils into the skin lesions. AD patients show an enhanced susceptibility to cutaneous infections with certain viral, fungal and microbial pathogens. The first evidence that *S. aureus* might play a role in AD appeared already 30 years ago [39], and since then, the prominent role of *S. aureus* skin colonization as a factor contributing to the exacerbation of AD has been well established. In AD patients, colonization with *S. aureus* is found significantly more often (80–100%) than in healthy controls (5–30%) [40]. This is likely the result of a combination of host factors including skin barrier dysfunction as well as impaired host immune responses in AD [41]. The majority of these *S. aureus* isolates from AD skin secrete identifiable *S. aureus* enterotoxin A, SEB and TSST-1, suggesting a direct involvement of SAg in the disease mechanism. Furthermore, IgE to these toxins was found in the serum of 57% of patients in one study [42], and these IgE antibodies correlated with skin disease severity. This study also showed an increased number of T cell bearing a SAg-specific TCR V β repertoire in the diseased skin of AD patients, further supporting the role of SAg in AD pathophysiology.

SAg have been demonstrated to induce corticosteroid resistance of T cells in vitro [43]. This could contribute to difficulty in the management of AD, because topical corticosteroids are the most common medication used for treating AD. Indeed, *S. aureus* isolates from patients with steroid-resistant AD showed the ability to produce large numbers of SAg types per organism, significantly higher than those produced by other skin isolates. Furthermore, *S. aureus* isolates from patients with steroid-resistant AD have been selected for their production of greater numbers of SAg than those produced by isolates from a general population of patients with AD. Thirdly, in addition to having the potential to make more types of SAg, *S. aureus* isolates from patients with steroid-resistant AD also have dysregulated production of SAg and produce unusual SAg combinations [44].

Mouse models of AD have been developed using epicutaneous immunization with SEB. This elicits allergic skin inflammation accompanied by a systemic Th2 response to the SAg, suggesting that SAg might play a role in the pathogenesis of AD [45]. Indeed, epicutaneous exposure

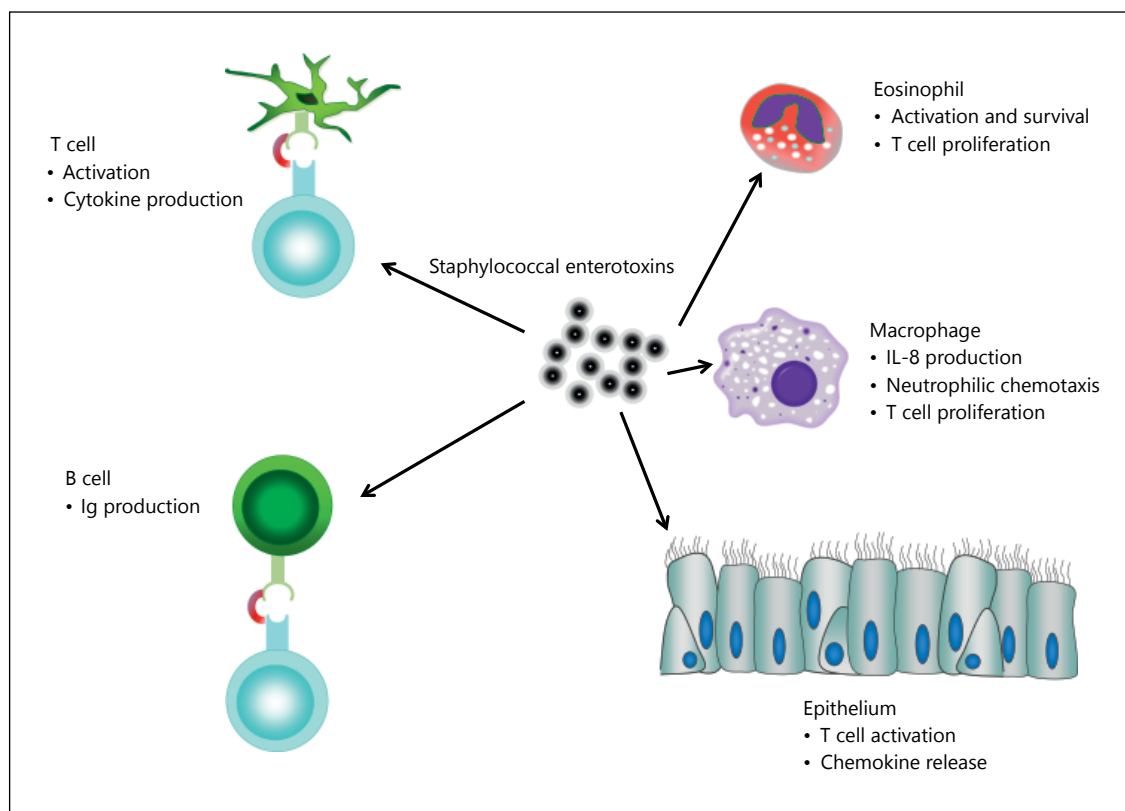


Fig. 4. Effects of staphylococcal enterotoxins on different cell types.

to SEB elicited a local, cutaneous, inflammatory response characterized by dermal infiltration with eosinophils and mononuclear cells and increased mRNA expression of the Th2 cytokine IL-4, sharing several features with AD skin lesions. In another model, topical SEB treatment provoked epidermal accumulation of CD8+ T cells, a mixed Th1/Th2 type dermatitis and vigorous production of SEB-specific IgE and IgG2a antibodies as well as significantly increasing the production of ovalbumin (OVA)-specific IgE and IgG2a antibodies [46]. These findings can be related to the chronic phase of atopic skin inflammation.

Food Allergy

SEs have an established association with several atopic conditions. *S. aureus* contamination is one of the most prevalent causes of food poisoning, and SEs are most frequently found in foods such as milk, dairy products, eggs, meat and fish – all potential food allergens [47]. As described above, SEB is resistant to breakdown by stomach acid and is transcytosed across the intestinal epithelium. This has led to the development of a new mouse model of

food allergy to oral antigen, using concomitant OVA and SEB application. In this model, orally administered SEB was able to elicit sensitization to low doses of OVA, promoting a dominant Th2 response [48]. However, this was only the case when SEB was administered together with the antigen, as SEB alone did not show any response. The authors hypothesize that SEB exposure leads to allergic sensitization to food allergens, perhaps via a breakthrough of oral tolerance. The development of Th2 immunity to oral antigen in this model likely occurred as a result of a loss in immune suppression, with decreased levels of FoxP3 mRNA and TGF- β levels in OVA/SEB mice.

Other experimental animal models have been described using SEB in the study for colitis [49]. In this model, the authors demonstrate alteration of regulatory T cell development by SEB, which contributes to the activation of effector T cells in a dysregulated environment. The observation that mucosal exposure to SEs activated the development of intestinal inflammation in immune-deficient hosts may explain how gut flora and bacterium-derived products could lead to the development of chron-

ic inflammatory bowel disease in immune-dysregulated intestinal mucosa.

One other interesting experimental model from Tilahun et al. [50] studied the superantigenic capacity of SEs in the field of TSS, a serious systemic illness. TSS is caused by SAgS which cause a massive acute T cell activation. The T cells activated by SAgS rapidly produce large amounts of cytokines and chemokines, resulting in a sudden surge in the systemic levels of these biological mediators. This process, called systemic inflammatory response syndrome, may lead to multiple organ dysfunction syndrome, where several vital organs within the body fail to perform their physiological functions. The usage of mouse models to study TSS is necessary in order to find new therapeutic strategies for this possible life-threatening disease, caused by the SAg exotoxins of *S. aureus*.

Role of Staphylococcal Enterotoxins in Airway Disease

S. aureus is a ubiquitous common human pathogen, which is most frequently found in the anterior nares of the nose. Producing enterotoxins with superantigenic properties, *S. aureus* is known to modulate airway disease. Most likely, it is not the purpose of these SAgS to induce systemic toxic shock in the host, but rather to act locally, probably suppressing local immune responses. However, transcytosis of SEB, a prototypic staphylococcal SAg, has been described in the intestinal epithelium [51, 52]. Furthermore, SEB can elicit robust systemic immune activation following exposure through nonenteric portals of entry such as the nasal, conjunctival or vaginal routes [53]. In order to study the systemic levels of SEB after endonasal application in our studies, we have demonstrated a time-dependent increase in the serum levels of SEB after endonasal application, demonstrating systemic absorption after local airway application (figure 5).

Allergic Rhinitis

Similar to AD, it has been suggested that staphylococcal enterotoxins may play a role in allergic rhinitis. It has been reported that the nasal carriage of *S. aureus* was higher in patients with perennial allergic rhinitis (44%) than in control subjects (20%). There was an even more significant association with enterotoxin-producing *S. aureus* (22 vs. 6.7% in control subjects) [54]. Rossi and Monasterolo [55] analyzed the prevalence of serum-specific IgE towards SEA, SEB, SEC, SED and TSST-1 in dust-mite-allergic patients with allergic rhinitis and found an

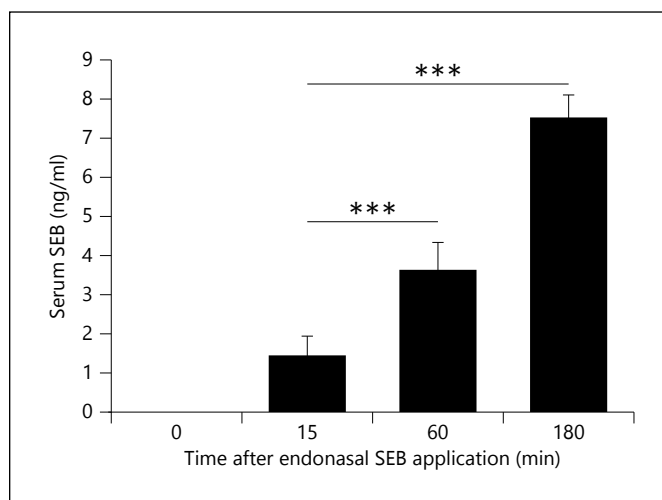


Fig. 5. Time-dependent increase in serum levels of SEB after endonasal application. Local upper airway application of SEB results in systemic absorption.

increase in serum eosinophil cationic protein in patients with antienterotoxin IgE compared with IgE-negative patients. This observation pointed to an involvement of SEs in local IgE production and a Th2-type biased eosinophil inflammation. Further evidence was obtained from animal experiments with BALB/c mice that were intranasally sensitized with *Schistosoma mansoni* egg antigen (SmEA) in the presence or absence of SEB. Nasal exposure to SEB resulted in the enhanced development of allergic rhinitis in SmEA-sensitized mice, shown by SmEA-specific IgE production, nasal eosinophilia and production of IL-4 and IL-5 by nasal mononuclear cells after antigen challenge [56].

Nasal Polyposis

Nasal polyps, also referred to as chronic rhinosinusitis with nasal polyps (CRSwNP), are characterized by an eosinophilic type of inflammation driven by IL-5 and eotaxin which together orchestrate the chemotaxis, activation and survival of eosinophils [57]. An increased colonization rate with *S. aureus* has been described in 60% of CRSwNP patients, and 87% of CRSwNP with comorbid asthma and aspirin sensitivity, versus 33% in control patients [58]. Furthermore, IgE antibodies to SEs were present in 28% of polyp samples, with rates as high as 80% in the subgroup with asthma and aspirin sensitivity, compared with 15% in control individuals. Moreover, an increased number of T cells expressing the TCR V β known to be induced by microbial SAgS was detected in CRSwNP

and correlated with the presence of specific IgE against SEs [59]. This SE-specific IgE serves as an indicator for the impact of SAgS on the mucosal inflammation. These specific IgE antibody level in the tissue is associated with high total IgE titers, and is polyclonal. Interestingly, this polyclonal IgE from mucosal tissue appears to be functional, and may induce mast cell degranulation to numerous inhalant allergens. Together with the fact that SEB may serve as an allergen, these findings may contribute to persistent inflammation by continuously activating mast cells [60]. These findings confirm the role played by *S. aureus* enterotoxins as disease modifiers in CRSwNP [61, 62]. As a proof of concept, we have recently demonstrated that anti-IgE treatment with omalizumab has clinical efficacy in the treatment of nasal polyps, which supports the importance and functionality of local IgE formation in the airways [63].

Asthma

The relation between SE-specific IgE and the severity of asthma has been suggested from the above-mentioned data in CRSwNP patients. Interestingly, it has been shown that serum from asthmatic patients more often contains SE-specific IgE than serum from controls. Furthermore, within the group of asthmatic patients, severe asthmatics more often have SE-specific IgE than those with mild asthma [64, 65]. A pathophysiological link between SE-specific IgE and bronchial inflammation has therefore been suggested. In mice, SEB enhanced the allergen-induced bronchial inflammation in experimental asthma, as reflected by more eosinophilic inflammation in the airway lumen and in bronchial tissue. Aggravation of experimental asthma correlated with higher expression of mRNA for IL-5, IL-4, IFN- γ , IL-12 p40, eotaxin-1 and TGF- β in bronchi. In addition, nasal SEB elevated concentrations of IL-4, IL-5 and IFN- γ in serum and bronchial SEB increased titers of OVA-specific and total IgE in serum [66].

Staphylococcal enterotoxins cause a Th2-biased inflammation and amplification in CRSwNP patients. In addition, in a specific phenotype of CRSwNP patients, namely the ones with high IL-5 expression, SEs significantly modify the severity of upper airway inflammation. Interestingly, these patients carry a considerable risk for comorbid asthma [62].

Based on the above-mentioned findings, a hypothesis was recently formulated, proposing a role for SAgS in the development of intrinsic asthma. *S. aureus* may be able to invade bronchial epithelial cells and release its enterotoxins. Subsequent stimulation of T and B cell proliferation may follow as well as the induction of class-switching to

IgE and the production of allergen-specific IgE in mucosal B cells. Furthermore, *S. aureus* may also degranulate mast cells and cause the release of cytokines. Patients with intrinsic asthma may be susceptible to colonization of the lower airways with *S. aureus*, which, through the local release of SAgS, drives an allergic inflammatory response and local IgE formation; this is associated with more severe asthma, as the anti-inflammatory response to corticosteroids is reduced [67]. This hypothesis is in line with murine data, where SEB induces lymphocytic inflammation and eosinophilia in the lungs with increased production of IL-4, together with airway hyperresponsiveness in both IgE- and non-IgE-producing mouse strains, demonstrating the capacity of SAgS to induce allergic inflammation independent of any allergen [68]. Recently, we have confirmed the involvement of staphylococcal SAgS in the pathophysiology of patients with severe asthma, demonstrating that staphylococcal enterotoxin IgE antibodies, but not IgE against inhalant allergens, are risk factors for asthma severity [69]. Indeed, the impact of staphylococcal SAgS has been shown to modify the immune response, contributing to persistent severe disease via the activation of T and B cells and the formation of local IgE [70].

In early childhood, SEs are potential modifiers of childhood wheeze and eczema. The levels of SE-specific IgE in children with eczema and wheeze are significantly increased compared to healthy 5-year-old children. Furthermore, the proportion of patients sensitized to SEs increases with increasing symptoms severity [71]. In a cohort of 1,380 teenagers, we were able to demonstrate a positive association between type 2 immunity to *S. aureus* and asthma phenotypes [72]. This association reflects IgE-mediated effector cell activation of SEs, which are secreted in soluble form. In this study, we could demonstrate significantly higher SE-specific IgE levels in atopic individuals compared to nonatopic individuals. Moreover, in univariate models, SE-specific IgE titer was a significant risk factor for asthma and bronchial hyperresponsiveness, particularly amongst atopics. In nonatopics, SE-specific IgE appeared to function as an independent risk factor for bronchial hyperresponsiveness, implying that, despite its low titer, this IgE may contribute to airway inflammation in subjects in whom significant IgE of other specificities is extremely rare. Interestingly, we found a contrasting benign nature of IgE to *Haemophilus influenzae* and *Streptococcus pneumoniae* antigens: IgE specific to these is inversely associated with asthma risk in healthy atopic teenagers, despite the fact that respective mean production levels are higher than corresponding responses to SEs which are positively associated with risk. This may reflect

their lower availability in soluble forms that can cross-link IgE receptors. We theorize that, instead, they may be processed by APC and presented to type-2 memory cells leading to the mucosal secretion of IL-4/IL-13, a mechanism widely recognized in other tissues to attenuate Th1-associated bacterial-induced inflammation [73].

COPD

Staphylococcal enterotoxins can modulate airway inflammation in COPD patients, similar to in unstable asthma. This was studied in smokers, stable COPD, exacer-

erbated COPD and healthy controls. The authors found a significant increase in serum SE-specific IgE levels in COPD patients compared to controls. This indeed reflects the SAg activity on the B and T cells of these SEs [74], which challenge the lower airways locally. This hypothesis is reinforced by the fact that bacterial colonization including that of *S. aureus* is increased in the lower airways of COPD patients. Moreover, during exacerbations of COPD, *S. aureus* has been found as a pathogen. Altogether, these findings indicate a possible role for SEs in the pathogenesis of COPD, similar to that in severe asthma. Further research is urgently needed to test this suggestion.

References

- ▶ 1 White J, Herman A, Pullen AM, Kubo R, Kappler JW, Marrack P: The V beta-specific superantigen staphylococcal enterotoxin B: Stimulation of mature T cells and clonal deletion in neonatal mice. *Cell* 1989;56:27–35.
- ▶ 2 Fraser J, Arcus V, Kong P, Baker E, Proft T: Superantigens – powerful modifiers of the immune system. *Mol Med Today* 2000;6:125–132.
- ▶ 3 Proft T, Fraser JD: Streptococcal superantigens. *Chem Immunol Allergy* 2007;93:1–23.
- ▶ 4 Abe J, Takeda T: Characterization of a superantigen produced by *Yersinia pseudotuberculosis*. *Prep Biochem Biotechnol* 1997;27:173–208.
- ▶ 5 Zhao Y, Li Z, Drozd SJ, Guo Y, Mourad W, Li H: Crystal structure of *Mycoplasma arthritis* mitogen complexed with HLA-DR1 reveals a novel superantigen fold and a dimerized superantigen-MHC complex. *Structure* 2004;12:277–288.
- ▶ 6 Lafon M, Lafage M, Martinez-Arends A, Ramirez R, Vuillier F, Charron D, Lotteau V, Scott-Algara D: Evidence for a viral superantigen in humans. *Nature* 1992;358:507–510.
- ▶ 7 Hsiao FC, Lin M, Tai A, Chen G, Huber BT: Cutting edge: Epstein-Barr virus transactivates the HERV-K18 superantigen by docking to the human complement receptor 2 (CD21) on primary B cells. *J Immunol* 2006;177:2056–2060.
- ▶ 8 Huber BT, Hsu PN, Sutkowski N: Virus-encoded superantigens. *Microbiol Rev* 1996;60:473–482.
- ▶ 9 Townsley-Fuchs J, Neshat MS, Margolin DH, Braun J, Goodlick L: HIV-1 gp120: a novel viral B cell superantigen. *Int Rev Immunol* 1997;14:325–338.
- ▶ 10 Kluytmans J, van Belkum A, Verbrugh H: Nasal carriage of *Staphylococcus aureus*: Epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* 1997;10:505–520.
- ▶ 11 von Eiff C, Becker K, Machka K, Stammer H, Peters G: Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study group. *N Engl J Med* 2001;344:11–16.
- ▶ 12 Novick RP: Pathogenicity factors and their regulation; in Fischetti VA, Novick RP, Ferretti JJ, Portnoy DA, Rood JI (eds): *Gram-Positive Pathogens*. Washington, ASM Press, 2000, pp 345–350.
- ▶ 13 Jett M, Brinkley W, Neill R, Gernski P, Hunt R: *Staphylococcus aureus* enterotoxin B challenge of monkeys: correlation of plasma levels of arachidonic acid cascade products with occurrence of illness. *Infect Immun* 1990;58:3494–3499.
- ▶ 14 Marrack P, Kappler J: The staphylococcal enterotoxins and their relatives. *Science* 1990;248:1066.
- ▶ 15 Rellahan BL, Jones LA, Kruisbeek AM, Fry AM, Matis LA: In vivo induction of anergy in peripheral V beta 8+ T cells by staphylococcal enterotoxin B. *J Exp Med* 1990;172:1091–1100.
- ▶ 16 Jarraud S, Peyrat MA, Lim A, Tristan A, Bes M, Mouguel C, Etienne J, Vandenesch F, Bonnevill M, Lina G: egc, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. *J Immunol* 2001;166:669–677.
- ▶ 17 Lindsay JA, Holden MT: *Staphylococcus aureus*: superbug, super genome? *Trends Microbiol* 2004;12:378–385.
- ▶ 18 Thomas D, Chou S, Dauwalder O, Lina G: Diversity in *Staphylococcus aureus* enterotoxins. *Chem Immunol Allergy* 2007;93:24–41.
- ▶ 19 Papageorgiou AC, Tranter HS, Acharya KR: Crystal structure of microbial superantigen staphylococcal enterotoxin B at 1.5 Å resolution: implications for superantigen recognition by MHC class II molecules and T-cell receptors. *J Mol Biol* 1998;277:61–79.
- ▶ 20 Herrmann T, Accolla RS, MacDonald HR: Different staphylococcal enterotoxins bind preferentially to distinct major histocompatibility complex class II isotypes. *Eur J Immunol* 1989;19:2171–2174.
- ▶ 21 Petersson K, Pettersson H, Skartved NJ, Walse B, Forsberg G: Staphylococcal enterotoxin H induces V alpha-specific expansion of T cells. *J Immunol* 2003;170:4148–4154.
- ▶ 22 Fraser JD, Proft T: The bacterial superantigen and superantigen-like proteins. *Immunol Rev* 2008;225:226–243.
- ▶ 23 Hofer MF, Lester MR, Schlievert PM, Leung DY: Upregulation of IgE synthesis by staphylococcal toxic shock syndrome toxin-1 in peripheral blood mononuclear cells from patients with atopic dermatitis. *Clin Exp Allergy* 1995;25:1218–1227.
- ▶ 24 Grumann D, Scharf SS, Holtfrete S, Kohler C, Steil L, Engelmann S, Hecker M, Volker U, Broker BM: Immune cell activation by enterotoxin gene cluster (egc)-encoded and non-egc superantigens from *Staphylococcus aureus*. *J Immunol* 2008;181:5054–5061.
- ▶ 25 Mawhorter SD, Kazura JW, Boom WH: Human eosinophils as antigen-presenting cells: relative efficiency for superantigen- and antigen-induced CD4+ T-cell proliferation. *Immunology* 1994;81:584–591.
- ▶ 26 Guan L, Eisenstein TK, Adler MW, Rogers TJ: Inhibition of T cell superantigen responses following treatment with the kappa-opioid agonist U50,488H. *J Neuroimmunol* 1997;75:163–168.
- ▶ 27 Zen K, Masuda J, Ogata J: Monocyte-derived macrophages prime peripheral T cells to undergo apoptosis by cell-cell contact via ICAM-1/LFA-1-dependent mechanism. *Immunobiology* 1996;195:323–333.
- ▶ 28 Miller EJ, Nagao S, Carr FK, Noble JM, Cohen AB: Interleukin-8 (IL-8) is a major neutrophil chemotaxin from human alveolar macrophages stimulated with staphylococcal enterotoxin A (SEA). *Inflamm Res* 1996;45:386–392.

- ▶29 Desouza IA, Hyslop S, Franco-Penteado CF, Ribeiro-DaSilva G: Evidence for the involvement of a macrophage-derived chemotactic mediator in the neutrophil recruitment induced by staphylococcal enterotoxin B in mice. *Toxicon* 2002;40:1709–1717.
- ▶30 Krysko O, Holtappels G, Zhang N, Kubica M, Deswarte K, Derycke L, Claeys S, Hammad H, Brusselle GG, Vandenabeele P, Krysko DV, Bachert C: Alternatively activated macrophages and impaired phagocytosis of *S. aureus* in chronic rhinosinusitis. *Allergy* 2011;66:396–403.
- ▶31 Inganas M, Johansson SG, Bennich HH: Interaction of human polyclonal IgE and IgG from different species with protein A from *Staphylococcus aureus*: demonstration of protein-A-reactive sites located in the Fab'2 fragment of human IgG. *Scand J Immunol* 1980;12:23–31.
- ▶32 Genovese A, Bouvet JP, Florio G, Lamparter-Schummert B, Bjorck L, Marone G: Bacterial immunoglobulin superantigen proteins A and L activate human mast cells by interacting with immunoglobulin E. *Infect Immun* 2000;68:5517–5524.
- ▶33 Huvenne W, Callebaut I, Reekmans K, Hens G, Bobic S, Jorissen M, Bullens DM, Ceuppens JL, Bachert C, Hellings PW: *Staphylococcus aureus* enterotoxin B augments granulocyte migration and survival via airway epithelial cell activation. *Allergy* 2010;65:1013–1020.
- ▶34 O'Brien GJ, Riddell G, Elborn JS, Ennis M, Skibinski G: *Staphylococcus aureus* enterotoxins induce IL-8 secretion by human nasal epithelial cells. *Respir Res* 2006;7:115.
- ▶35 Schulz H, Karau A, Filsinger S, Schoels M, Kabelitz D, Richter R, Hansch PW: Tubular epithelial cells as accessory cells for superantigen-induced T cell activation. *Exp Nephrol* 1998;6:67–73.
- ▶36 Peterson ML, Ault K, Kremer MJ, Klingelutz AJ, Davis CC, Squier CA, Schlievert PM: The innate immune system is activated by stimulation of vaginal epithelial cells with *Staphylococcus aureus* and toxic shock syndrome toxin 1. *Infect Immun* 2005;73:2164–2174.
- ▶37 Mandron M, Aries MF, Brehm RD, Tranter HS, Acharya KR, Charveron M, Davrinche C: Human dendritic cells conditioned with *Staphylococcus aureus* enterotoxin B promote Th2 cell polarization. *J Allergy Clin Immunol* 2006;117:1141–1147.
- ▶38 Sha Q, Truong-Tran AQ, Plitt JR, Beck LA, Schleimer RP: Activation of airway epithelial cells by Toll-like receptor agonists. *Am J Respir Cell Mol Biol* 2004;31:358–364.
- ▶39 Leyden JJ, Marples RR, Kligman AM: *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br J Dermatol* 1974;90:525–530.
- ▶40 Breuer K, Kapp A, Werfel T: Bacterial infections and atopic dermatitis. *Allergy* 2001;56:1034–1041.
- ▶41 Schlievert PM, Strandberg KL, Lin YC, Peterson ML, Leung DY: Secreted virulence factor comparison between methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*, and its relevance to atopic dermatitis. *J Allergy Clin Immunol* 2010;125:39–49.
- ▶42 Leung DY, Harbeck R, Bina P, Reiser RF, Yang E, Norris DA, Hanifin JM, Sampson HA: 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* 1993;92:1374–1380.
- ▶43 Hauk PJ, Hamid QA, Chrousos GP, Leung DY: Induction of corticosteroid insensitivity in human PBMCs by microbial superantigens. *J Allergy Clin Immunol* 2000;105:782–787.
- ▶44 Schlievert PM, Case LC, Strandberg KL, Abrams BB, Leung DY: Superantigen profile of *Staphylococcus aureus* isolates from patients with steroid-resistant atopic dermatitis. *Clin Infect Dis* 2008;46:1562–1567.
- ▶45 Laouini D, Kawamoto S, Yalcindag A, Bryce P, Mizoguchi E, Oettgen H, Geha RS: Epicutaneous sensitization with superantigen induces allergic skin inflammation. *J Allergy Clin Immunol* 2003;112:981–987.
- ▶46 Savinko T, Lauerma A, Lehtimäki S, Gombert M, Majuri ML, Fyhrquist-Vanni N, Dieu-Nosjean MC, Kemeny L, Wolff H, Homey B, Alenius H: Topical superantigen exposure induces epidermal accumulation of CD8+ T cells, a mixed Th1/Th2-type dermatitis and vigorous production of IgE antibodies in the murine model of atopic dermatitis. *J Immunol* 2005;175:8320–8326.
- ▶47 Le Loir Y, Baron F, Gautier M: *Staphylococcus aureus* and food poisoning. *Genet Mol Res* 2003;2:63–76.
- ▶48 Ganeshan K, Neilsen CV, Hadsaitong A, Schleimer RP, Luo X, Bryce PJ: Impairing oral tolerance promotes allergy and anaphylaxis: a new murine food allergy model. *J Allergy Clin Immunol* 2009;123:231–238.
- ▶49 Heriazon A, Zhou P, Borojovic R, Foerster K, Streutker CJ, Ng T, Croitoru K: Regulatory T cells modulate staphylococcal enterotoxin B-induced effector T-cell activation and acceleration of colitis. *Infect Immun* 2009;77:707–713.
- ▶50 Tilahun AY, Holz M, Wu T-T, David CS, Rajagopalan G: Interferon gamma-dependent intestinal pathology contributes to the lethality in bacterial superantigen-induced toxic shock syndrome. *PLoS One* 2011;6:e16764.
- ▶51 Hamad AR, Marrack P, Kappler JW: Transcytosis of staphylococcal superantigen toxins. *J Exp Med* 1997;185:1447–1454.
- ▶52 Shupp JW, Jett M, Pontzer CH: Identification of a transcytosis epitope on staphylococcal enterotoxins. *Infect Immun* 2002;70:2178–2186.
- ▶53 Rajagopalan G, Smart MK, Murali N, Patel R, David CS: Acute systemic immune activation following vaginal exposure to staphylococcal enterotoxin B – implications for menstrual shock. *J Reprod Immunol* 2007;73:51–59.
- ▶54 Shiomori T, Yoshida S, Miyamoto H, Makishima K: Relationship of nasal carriage of *Staphylococcus aureus* to pathogenesis of perennial allergic rhinitis. *J Allergy Clin Immunol* 2000;105:449–454.
- ▶55 Rossi RE, Monasterolo G: Prevalence of serum IgE antibodies to the *Staphylococcus aureus* enterotoxins (SAE, SEB, SEC, SED, TSST-1) in patients with persistent allergic rhinitis. *Int Arch Allergy Immunol* 2004;133:261–266.
- ▶56 Okano M, Hattori H, Yoshino T, Sugata Y, Yamamoto M, Fujiwara T, Satoskar AA, Satoskar AR, Nishizaki K: Nasal exposure to staphylococcal enterotoxin enhances the development of allergic rhinitis in mice. *Clin Exp Allergy* 2005;35:506–514.
- ▶57 Bachert C, Gevaert P, Holtappels G, Cuvelier C, van Cauwenberge P: Nasal polyposis: from cytokines to growth. *Am J Rhinol* 2000;14:279–290.
- ▶58 van Zele T, Gevaert P, Watelet JB, Claeys G, Holtappels G, Claeys C, van Cauwenberge P, Bachert C: *Staphylococcus aureus* colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. *J Allergy Clin Immunol* 2004;114:981–983.
- ▶59 Tripathi A, Kern R, Conley DB, Seiberling K, Klemens JC, Harris KE, Suh L, Huang J, Grammer LC: Staphylococcal exotoxins and nasal polyposis: analysis of systemic and local responses. *Am J Rhinol* 2005;19:327–333.
- ▶60 Zhang N, Holtappels G, Gevaert P, Patou J, Dhaliwal B, Gould H, Bachert C: Mucosal tissue polyclonal IgE is functional in response to allergen and SEB. *Allergy* 2011;66:141–148.
- ▶61 Bachert C, Zhang N, Patou J, van Zele T, Gevaert P: Role of staphylococcal superantigens in upper airway disease. *Curr Opin Allergy Clin Immunol* 2008;8:34–38.
- ▶62 Bachert C, Zhang N, Holtappels G, De Lobel L, van Cauwenberge P, Liu S, Lin P, Bousquet J, van Steen K: Presence of IL-5 protein and IgE antibodies to staphylococcal enterotoxins in nasal polyps is associated with comorbid asthma. *J Allergy Clin Immunol* 2010;126:962–968, 968 e961–e966.
- ▶63 Gevaert P, Calus L, van Zele T, Blomme K, De Ruyck N, Bauters W, Hellings P, Brusselle G, De Bacquer D, van Cauwenberge P, Bachert C: Omalizumab is effective in allergic and nonallergic patients with nasal polyps and asthma. *J Allergy Clin Immunol* 2013;131:110–116 e111.
- ▶64 Bachert C, Gevaert P, Howarth P, Holtappels G, van Cauwenberge P, Johansson SG: IgE to *Staphylococcus aureus* enterotoxins in serum is related to severity of asthma. *J Allergy Clin Immunol* 2003;111:1131–1132.
- ▶65 Perez-Novo CA, Kowalski ML, Kuna P, Ptasińska A, Holtappels G, van Cauwenberge P, Gevaert P, Johansson S, Bachert C: Aspirin sensitivity and IgE antibodies to *Staphylococcus aureus* enterotoxins in nasal polyposis: studies on the relationship. *Int Arch Allergy Immunol* 2004;133:255–260.

- ▶66 Hellings PW, Hens G, Meyts I, Bullens D, Vanoirbeek J, Gevaert P, Jorissen M, Ceuppens JL, Bachert C: Aggravation of bronchial eosinophilia in mice by nasal and bronchial exposure to *Staphylococcus aureus* enterotoxin B. Clin Exp Allergy 2006;36:1063–1071.
- ▶67 Barnes PJ: Intrinsic asthma: not so different from allergic asthma but driven by superantigens? Clin Exp Allergy 2009;39:1145–1151.
- ▶68 Herz U, Ruckert R, Wollenhaupt K, Tschernig T, Neuhaus-Steinmetz U, Pabst R, Renz H: Airway exposure to bacterial superantigen (SEB) induces lymphocyte-dependent airway inflammation associated with increased airway responsiveness – a model for non-allergic asthma. Eur J Immunol 1999;29:1021–1031.
- ▶69 Bachert C, van Steen K, Zhang N, Holtappels G, Cattaert T, Maus B, Buhl R, Taube C, Korn S, Kowalski M, Bousquet J, Howarth P: Specific IgE against *Staphylococcus aureus* enterotoxins: an independent risk factor for asthma. J Allergy Clin Immunol 2012;130:376–381 e378.
- ▶70 Bachert C, Zhang N: Chronic rhinosinusitis and asthma: novel understanding of the role of IgE ‘above atopy’. J Intern Med 2012;272:133–143.
- ▶71 Semic-Jusufagic A, Bachert C, Gevaert P, Holtappels G, Lowe L, Woodcock A, Simpson A, Custovic A: *Staphylococcus aureus* sensitization and allergic disease in early childhood: population-based birth cohort study. J Allergy Clin Immunol 2007;119:930–936.
- ▶72 Hollams E, Hales B, Bachert C, Huvenne W, Parsons F, de Klerk N, Serralha M, Holt B, Ahlstedt S, Thomas W, Sly P, Holt PG: Th2-associated immunity to bacteria in asthma in teenagers and susceptibility to asthma. Eur Respir J 2010;36:509–516.
- ▶73 Holt PG, Strickland DH, Wikstrom ME, Jahnsen FL: Regulation of immunological homeostasis in the respiratory tract. Nat Rev Immunol 2008;8:142–152.
- ▶74 Rohde G, Gevaert P, Holtappels G, Borg I, Wiethage A, Arinir U, Schultze-Werninghaus G, Bachert C: Increased IgE-antibodies to *Staphylococcus aureus* enterotoxins in patients with COPD. Respir Med 2004;98:858–864.