



# NEW HORIZONS — ALLERGY —

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## Staphylococcal superantigen effects, their impact and diagnosis

### Summary

Gram-positive *Staphylococcus aureus* germs constitutively have the possibility to release classical and egc-locus derived enterotoxins (SEs) which have superantigen activity, and effectively modify the functions of T- and B-cells, eosinophils, and other inflammatory and structural cells. The stimulation may lead to a TH2-polarized eosinophilic inflammation as well as a multiclonal IgE production, aggravating allergic disease in the skin and the upper and lower respiratory tract. Interestingly, IgE antibodies to enterotoxins have been found in atopic eczema, in allergic rhinitis and nasal polyp patients, in aspirin-sensitive patients, in severe asthma and COPD sufferers, and in early childhood wheezers. As a principle, the presence of those IgE antibodies indicated an amplification of the inflammatory process on the dermal and mucosal level, and thus a more severe course of disease, often linked to the clinical expression of aspirin sensitivity and/or steroid insensitivity. Consequently, the awareness of such impact of superantigens on a given chronic disease is of great value, and SE-specific IgE antibodies detecting ImmunoCAP are currently the mainstay for diagnosis.

We here summarize the current evidence for an active role of SEs in allergic rhinitis, nasal polyps, asthma, COPD and finally early childhood wheezing, and discuss the diagnostic steps to be taken to unravel superantigenic impact. New therapeutic approaches are evolving, but need further development to tackle this currently underestimated clinical challenge.

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For further information please refer to a recent review, on which this paper is based:  
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### Introduction

Gram-positive *Staphylococcus aureus* germs, to facilitate colonization and impair the hosts self-defense, constitutively have the possibility to release a bunch of enterotoxins which have superantigen activity, and effectively modify the functions of T- and B-cells, eosinophils, and other inflammatory and structural cells (1,2). The classical *S. aureus* enterotoxins (SE) comprise SEA-SEE and TSST-1 (Toxic shock syndrome toxin-1), however, further enterotoxins have been described recently, derived from the egc-gene locus (3); these seem to be of relevance, as they frequently are produced by nasal *S. aureus*, and partially are unrelated to the production of classical enterotoxins (T. van Zele, unpublished). Staphylococcal enterotoxins are able to activate T-cells via the T-cell receptor (TCR)-MHC class II-complex independent from the antigen-specific groove by binding to the variable beta-chain of the TCR, and therefore are called superantigens. The susceptibility of a T-cell to superantigens therefore is dependent on the usage of a specific beta-chain repertoire, possibly leading to the activation of abundant T-cells in a given tissue (normally, far

less than 1% of T-cells are activated by a specific antigen). Once activated, T-cells would produce interleukins including IL-4, IL-5, IL-13, eotaxin and many others, which would lead to a severe eosinophilic inflammation and local immunoglobulin E (IgE) production. Other direct actions of superantigens on B-cells, epithelial cells, eosinophils etc. have been described, adding to the enormous inflammatory potential of *S. aureus* derived superantigens.

### *S. aureus* enterotoxins in allergic rhinitis

About 25% of the population is permanent carrier of *S. aureus* in the nostrils, and approximately 20% of all human staphylococcal infections are autogenous (4). Shiomori *et al.* (5) examined the nasal carriage of *S. aureus* and its superantigen production, and found that the rate of nasal carriage of *S. aureus* was significantly higher in patients with perennial allergic rhinitis (44%) than that of control subjects (20%,  $p < 0.01$ ). Moreover, the nasal symptom scores of the *S. aureus*-

positive patients were significantly higher than those of the *S. aureus*-negative patients ( $p < 0.05$ ). Rossi *et al.* determined the prevalence of IgE specific to SEA, SEB, SEC, SED and TSST-1 in serum of patients suffering from persistent allergic rhinitis and/or allergic asthma and confirmed an increased concentration in serum of eosinophilic cationic protein, ECP, in patients with IgE antibodies to SE (6). Furthermore, these patients should significantly increased concentrations of “total IgE” and house dust mite specific IgE antibodies in serum compared to allergic rhinitis patients without IgE antibodies to SE.

These and other observations in allergic rhinitis subjects suggest that allergic rhinitis predisposes to Staphylococcal colonization, and confirm the role of SEs in amplifying the IgE-response and eosinophilic inflammation, resulting in an increase in symptoms.

### The role of SEs in nasal polyposis

The finding of IgE antibodies to *S. aureus* enterotoxins SEA and SEB in nasal polyp tissue homogenates (7) indicated that these superantigens could be involved in the pathogenesis of nasal polyposis. Nasal polyps (NP) are characterized by abundant eosinophils, Th2-cell activation, overproduction of IgE, and originally were thought to represent an allergic disease. The concentrations of IgE, IL-5, eotaxin, eosinophil cationic protein (ECP), and the cys-leukotrienes (LT) are significantly higher in polyp tissue compared to controls, and eosinophil survival is prolonged by IL-5 (8). Recent evidence accumulates, that SE, acting as superantigens, induce a substantial inflammatory reaction in a large subgroup of NP, and strongly modify the disease (7). An important subgroup of polyp patients demonstrates a multiclonal IgE formation, including IgE to *S. aureus* enterotoxins, a high IgE level, increased levels of ECP and IL-5, and a high prevalence of asthma. Further studies suggested that bacterial superantigens could induce IgE-synthesis in nasal polyps and impact the degree of eosinophilic inflammation (7,9).

There is increasing evidence that SEs can not only indirectly affect B-cells via T-cell derived cytokines, but also directly affect the activation of the B cell repertoire. Functional studies in B-cells have shown that *S. aureus* protein A induces proliferation of these cells (10). Studies with TSST-1 indicated that staphylococcal superantigens may play an important role in the modulation of allergic disease, since they may augment isotype switching and synthesis of IgE (10). In mucosal tissues of hay fever and asthma patients, mRNA for the e-chain of IgE was found in a significant proportion of B cells using in situ hybridisation (11-13), supporting the hypothesis of a truly local IgE synthesis in the airway mucosa. The IgE/albumin ratios in polyp tissue and in serum are dissociated, again indicating that tissue IgE is rather the result of a local IgE production than of extravasation. Furthermore, IgE antibodies in polyp tissue only show a partial relation to IgE antibodies in serum and to skin prick test results. In a substantial subgroup of patients, the following typical pattern of IgE expression in polyp tissue is found: a polyclonal type of IgE expression with IgE antibodies to common aeroallergens in low quantities and a high level of IgE. These findings resemble those in eczema, where colonization of the inflamed skin with *S. aureus* clearly contributes to the high IgE levels in serum and to the severity of the disease (14).

Detailed analysis of IgE expression in serum and tissue of NP patients reveals two patterns: “the allergic type” and “the polyclonal type” that can be found either isolated or combined (15). The “allergic” type of IgE expression is characterized by increased concentrations of IgE and IgE antibody specificities in nasal tissue that correspond to those in serum and to the skin prick test results. In contrast, the “polyclonal” IgE expression is a local process and IgE antibodies found in polyp tissue are only partially reflected in serum of the same patients and are independent of the skin prick results. Notably, this polyclonal expression is often associated with a hyper-immunoglobulinemia, and only a small fraction of the total can be explained by IgE antibodies of known specificity. Polyclonal expression was described in 16/24 NP tissues and was associated with IgE antibodies to SEs in 12 cases, indicating that other than the classic enterotoxins might have acted as superantigens (15). This local polyclonal IgE production may however lead to a permanent triggering of the IgE-mast cell-FcεRI cascade, evoked by a variety of allergens, and may thus contribute to the mucosal inflammation. This way, a patient could react to allergens via IgE antibodies, although she/he was not primarily atopic or allergic, and the patient’s skin prick tests are negative!

From the first study in patients with local IgE against staphylococcal enterotoxins (7) it appeared that the highest IgE concentrations were observed in polyp samples of aspirin sensitive, typically non-allergic subjects. Further studies revealed that indeed IgE-concentrations correlated with markers of eosinophilic inflammation, which are typically higher in aspirin-sensitive than in tolerant subjects (16, 17), and in subjects with or without IgE antibodies to SE. More recently, we extended our observations demonstrating that the production of cysteinyl-leukotrienes, leukotriene B<sub>4</sub>, and lipoxin A<sub>4</sub> is up-regulated in NP tissue of patients with an immune response to *S. aureus* enterotoxins versus SE IgE antibody negative NP patients (18). Today, we suggest an indirect relationship between SEs and aspirin sensitivity, with the severity of inflammation as link between both observations, as a direct link has not been shown. However, it is clinically interesting to realize the background for a likely coincidence of high IgE levels with aspirin sensitivity.

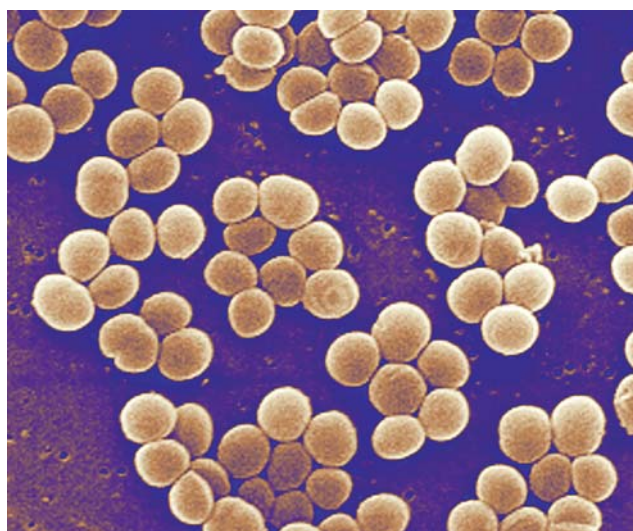


Figure 1. *Staphylococcus aureus*.

## ***S. aureus* also impacts the lower airways: asthma and COPD**

Until recently, there only was indirect evidence that SEs possibly could also impact lower airway disease, especially in poorly controlled asthma. By studying the TCR  $\gamma$ - $\delta$  repertoire of broncho-alveolar lavage (BAL) cells and peripheral blood mononuclear cells (PBMCs) from subjects with poorly controlled asthma ( $FEV_1 < 75\%$ ), subjects with well-controlled asthma and control subjects, D. Leung and co-workers found a significantly higher expression of  $\gamma\delta$  T cells in BAL fluid of poorly controlled asthmatics compared to the other groups. Increased  $\gamma\delta$  BAL T cells were present in the CD4(+) and CD8(+) subsets, suggesting activation by SEs (19).

We took advantage of a mouse model of allergic asthma to study the effects of nasal or bronchial applications of SEs on the development of allergic asthma in previously sensitized mice (20). Whereas nasal and bronchial application resulted in a significant, but modest increase in the total cell counts in bronchial alveolar lavage fluid, repeated exposure of OVA-sensitized mice to nasal and nebulized OVA clearly aggravated bronchial inflammation. Thus, in bronchial tissue of OVA-sensitized mice, the inflammatory response was aggravated compared to SEs or allergen alone by consecutive SEB contact via the nose and the bronchi.

Also in humans, evidence for a direct impact of enterotoxins on lower airway disease is growing. Based on our previous findings, we used a sensitive and highly specific screening tool, the SE ImmunoCAP<sup>®</sup>, to detect IgE antibodies to SEs in serum of mild and severe asthmatics, classified by lung function and need for drug treatment, versus controls. IgE antibodies to SEs were found significantly more frequent in severe asthmatics (62%) versus controls (13%,  $p=0.01$ ), and were associated with increased concentrations of total IgE antibodies in serum and severity of eosinophilic inflammation defined as high concentrations in serum of ECP (17).

This study has now been extended, investigating asthmatics from mild disease to chronic persistent disease requiring management at steps 4 or 5 of the BTS/SIGN guidelines (21).  $FEV_1$  progressively declined from BTS Step 1 ( $95.0 \pm 9.3$ ) to BTS Step 4/5 ( $59.8 \pm 19.9$ ), and 41% of the severe asthmatics had required a hospital admission, 96% had required oral steroids within the last year. Levels of IgE antibodies to SE were significantly higher in the mild and severe asthmatics than in the controls, and positive results were found significantly more often in serum from severe asthmatics than mild asthmatics and controls. These findings contrasted with the results of measures of IgE antibodies to indoor aeroallergens, with over 70% of mild and moderate asthmatics having specific IgE against these common aeroallergens, but no difference between the control subjects and severe asthmatics. Aspirin sensitive severe asthmatics and patients with a history of nasal polyps were significantly more likely to have IgE antibodies to SEs.

We also studied the expression of IgE and IgE antibodies to SEs in chronic obstructive pulmonary disease (COPD) patients, smokers without COPD, and healthy controls (22). IgE antibodies to SE were found rarely in controls and smokers, but often in patients with stable and exacerbated COPD (38.9%).

We therefore propose a crucial role for SEs in the pathophysiology of upper and lower airway disease, linked to severity of eosinophilic inflammation, IgE synthesis, but also clinical disease severity, to be confirmed in a larger population as well as in confirmatory treatment studies. Of interest, enterotoxins may impact sensitized and non-sensitized airways, and will lead to an increase of IgE synthesis in relation to the respective disease background. Such impact may take place early in life, as we recently demonstrated in pre-school children: Amongst pre-school wheeze phenotypes, persistent wheezers were most commonly sensitized to SE (23), and showed increased airway reactivity.

## **Clinical implications and perspectives**

In summary, there is accumulating evidence that superantigens, primarily derived from *S. aureus*, may have a major impact on upper and lower airway disease such as nasal polyposis and asthma, COPD and early wheezing. Superantigens at least appear to modify, if not cause, severe airway disease. Staphylococcal enterotoxins may furthermore affect treatment possibilities, as it was shown that these compounds may alter steroid sensitivity and expression of glucocorticoid receptor beta (24). Dexamethasone caused a 99% inhibition of phytohemagglutinine (PHA)-induced PBMC proliferation, but only a 19% inhibition of the SEB-induced, 26% inhibition of the TSST-1, and 29% inhibition of the SEE-induced PBMC proliferation, demonstrating that superantigens can induce steroid insensitivity. At the same time, stimulation of normal PBMCs with SEB induced a significant increase of glucocorticoid receptor beta compared with PHA and unstimulated cells, a possible mechanism to induce glucocorticoid insensitivity.

*S. aureus* frequently colonizes the nostrils in healthy subjects, and can be found in acute and chronic rhinosinusitis (5). We recently reported an increased colonization rate of *S. aureus* in nasal polyps, but not in other chronic rhinosinusitis (CRS) conditions (25). Colonization with *S. aureus* was present in 63.6% of subjects with polyps, with rates as high as 66.7% and 87.5% in the subgroups with asthma and aspirin sensitivity, which were significantly higher than in controls and subjects with CRS (33.3% and 27.3%, respectively). Furthermore, repeated swabbing of the middle meatus in 8 subjects with polyps suggested long-term colonization with *S. aureus*. IgE antibodies to *S. aureus* enterotoxins, using a combination of different enterotoxins, were present in 27.8% in polyp samples, with rates as high as 53.8% and 80% in the subgroups with asthma and aspirin sensitivity, compared to 15% in controls and 6% in subjects with CRS, respectively (25). The concentration of ECP, reflecting the eosinophilic inflammation, was significantly increased in polyp samples with the presence of IgE antibodies to enterotoxins versus samples without IgE, confirming a strong inflammatory effect of superantigens. In subjects with NPs and co-morbid asthma or aspirin sensitivity, rates of colonization and IgE response in nasal tissue homogenates were further increased, paralleled by increases of ECP and IgE. These figures indicate that there is a strong relation between staphylococcal colonization and tissue immune responses to enterotoxins in upper and lower airways (25).

For diagnostic purposes, *S. aureus* can be detected in the middle nasal meatus by swabs, but would only poorly predict production of and immune response to its enterotoxins. The potential production of enterotoxins by these germs, once cultured, can be shown by PCR or protein assays, but clinical studies to show the clinical relevance in an individual patient have not yet been performed. The ability to produce enterotoxins by a given germ may also vary due to varying conditions in the nasal environment or number of colonies present. In contrast, the presence of IgE antibodies to SEs indicates a former or present stimulation of the local immune system by the respective enterotoxin, and can be tested in tissue homogenates, and, depending on the involved organ (asthma rather than rhinosinusitis) and the severity of the inflammation, SE-specific IgE antibodies can also be found in the peripheral blood. A polyclonal IgE response pattern, high “total IgE” and increased ECP would indicate the activity of the superantigens in modifying the inflammation. This should especially help to differentiate from atopic diseases, which also might co- or preexist.

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