Superantigens and Nasal Polyps

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Nasal polyps represent an often severe T-cell–orchestrated eosinophilic upper airway disease with currently unknown pathogenesis, often associated with lower airway disease, such as asthma. Superantigens, predominantly derived from *Staphylococcus aureus*, are potent activators of T cells, induce the synthesis of IgE in B cells, and have direct effects on pro-inflammatory cells, such as eosinophils. IgE antibodies to *S. aureus* enterotoxins have been described in polyp tissue, linked to a local polyclonal IgE production and an aggravation of eosinophilic inflammation. Furthermore, such IgE antibodies have also been described in the sera of patients with asthma, and linked to severity of disease and steroid insensitivity. This review summarizes our current understanding of the possible role of *S. aureus* enterotoxins in chronic severe airway disease, such as nasal polyposis.

Introduction

Nasal polyposis, a disease involving the nose and sinuses, represents an excellent example of a mostly severe, chronic, eosinophilic inflammation of the airways and often is linked to comorbidities, such as asthma and aspirin sensitivity. Edematous, semitranslucent masses develop from the mucosal linings of the middle nasal meatus, the middle turbinate, and the sinuses, and prolapse into the nasal cavities [1]. Nasal polyps cause long-term symptoms, such as prominent nasal obstruction, postnasal drip, loss of smell, and a feeling of a "full head," and can have a severe impact on the quality of life of the patient. Furthermore, patients might suffer from headache and facial pain, and can develop severe bony, orbital, or cerebral complications caused by secondary infections.

The prevalence of nasal polyps in the general population is considered to be low. A postal questionnaire survey of a population-based random sample of 4300 adult women and men aged 18 to 65 years recently performed in Finland [2] demonstrated a prevalence of nasal polyposis of 4.3%. This figure, however, might be an underestimation, because a significantly higher prevalence was reported in autopsy studies [3]. The incidence is higher in men than in women and significantly increases after the age of 40 years. However, nasal polyps occur more frequently in subgroups of patients with asthma and aspirin sensitivity [4]. Approximately 40% to 80% of patients with aspirin sensitivity suffer from polyposis, and approximately 15% of polyp patients are hypersensitive to aspirin. In studies involving large series of patients with nasal polyposis, asthma was found in 20% to 70% [5], and nonallergic asthma was significantly more frequently linked to polyps compared with allergic asthma.

A hallmark of bilateral nasal polyposis in adults, which is the focus of this review, is the abundant number of eosinophils within the tissue, which can be found in approximately 70% to 90% of cases in western countries [6]. Consequently, oral and topical corticosteroids represent the major treatment strategies, followed by surgical interventions; however, recurrences are frequent, regardless of treatment, especially in a subgroup of polyp patients with systemic disease manifestations, making a combination of repeated surgical interventions and a long-term drug treatment necessary.

Recently, an increasing body of knowledge on the role of cytokines, chemokines, and adhesion receptors emerged from studies in different models of eosinophilic airway inflammation [7]. However, although our knowledge on the pathomechanisms involved in the regulation of eosinophilic inflammation is increasing tremendously, the etiology of nasal polyposis is currently very obscure. Recent findings point to bacterial organisms and their products to be involved in the initiation or modification of the disease [8•,9]; more precisely, superantigens from germs such as *Staphylococcus aureus* have been suspected to amplify the inflammation in terms of severity and chronicity.

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. It is understood that approximately 25% of the population are permanent carriers of *S. aureus*, and although 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* [10]. These include injury to skin or mucous membranes, abnormal leukocyte function, and viral infections (*eg*, influenza), but also metabolic abnormalities (*eg*, diabetes mellitus, uremia) and miscellaneous

conditions (*eg*, malnutrition, age, malignancies). Under appropriate conditions, the organism leads to a variety of clinical conditions, affecting the skin, lungs, heart, central nervous system, bones and joints, gastrointestinal tract, and the blood system [10]. 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 related to the production of coagulase enzymes, and many conditions result from invasion by this bacterium, these organisms also contain a number of cellular antigens and produce a variety of toxins with superantigenic properties [11,12]. These enterotoxins have been shown to modulate atopic skin disease, such as atopic dermatitis (atopic eczema/dermatitis syndrome [AEDS]) by acting as allergens and superantigens [13]. *S. aureus* enterotoxins (SAEs) can influence the activity of both immunomodulatory and pro-inflammatory effector cell types, and, therefore, have a potentially important role in the pathogenesis of chronic inflammatory disease, including eosinophil-related airway diseases. This review summarizes our recent knowledge on the potential role of SAEs in nasal polyposis.

Pathomechanism of Nasal Polyps

Nasal polyps are characterized by their edematous nature, showing pseudocyst formations centrally, and a subepithelial accumulation of inflammatory cells, among which EG2+ (activated) eosinophils are a prominent feature in approximately 80% of polyps [6,14]. Albumin and other plasma proteins are deposited within the pseudocysts, adjacent to the eosinophilic infiltration. Histomorphologic characterization of polyp tissue reveals frequent epithelial damage, a thickened basement membrane, and edematous to sometimes fibrotic stromal tissue, with a reduced number of vessels and glands, but virtually no neural structure [4,15]. In small polyps, not larger than 5 mm, growing on normal-appearing mucosa of the middle turbinate in patients with bilateral polyposis, numerous subepithelial EG2+ eosinophils were present in the luminal compartment of the early stage polyp, forming a cap over the central pseudocyst area [14]. These observations suggest a central deposition of plasma proteins, regulated by the subepithelial, mainly eosinophilic inflammation, a pathogenetic principle of polyp formation and growth.

Several studies have been focused on the recruitment and survival of eosinophils in nasal polyp tissue, and the cytokines and chemokines mediating these processes [7,16]. There is evidence today that interleukin (IL)-5 plays a major role in the recruitment, activation, and inhibition of the apoptosis of eosinophils [17], but other related cytokines might also contribute to a network of factors [18–22]. IL-5, a key cytokine for the maturation and activation of mature eosinophils, was found to be significantly increased in nasal polyps, compared with healthy controls and other forms of sinusitis, independent of the atopic status of the patient [1,17,23]. Among eosinophil-related cytokines, IL-5 correlates best with eosinophil cationic protein, indicating its close relationship to the degree of eosinophilic inflammation. High concentrations of IL-5 were found in subjects with nonallergic asthma, and aspirin sensitivity, conditions linked to severe tissue eosinophilia, and eosinophils themselves could possibly contribute to IL-5 release, as documented by immunohistochemistry. The key role of IL-5 was supported by the finding that treatment of eosinophil-infiltrated polyp tissue with neutralizing anti–IL-5 monoclonal antibodies (mAbs), but not anti–IL-3 or anti–granulocyte-macrophage colony-stimulating factor (GM-CSF) mAbs in vitro, resulted in eosinophil apoptosis and decreased tissue eosinophilia in vitro [24]. Transforming growth factor (TGF)-β1, a cytokine with IL-5–counteracting activities, is only produced in low quantities and bound to the extracellular matrix in its latent, inactive form [14]. TGF-β1 is a potent fibrogenic cytokine that stimulates extracellular matrix (ECM) formation, and, thus, is involved in fibrosis, acts as a chemoattractant for fibroblasts, but inhibits the synthesis of IL-5, and abrogates the survival-prolonging effect of hematopoietins (IL-5 and GM-CSF) on eosinophils [25].

The eosinophilic inflammation in polyps is orchestrated by T cells that have been characterized as activated [26]. They represent a mixed population, consisting of CD4+ and CD8+ cells, and show a mixed Th1/Th2 profile. However, inflammatory cells such as eosinophils, macrophages, or mast cells also might contribute to the release of cytokines, as was especially shown for eosinophils, contributing IL-4 and IL-5 in an autocrine manner [17,27].

Recent studies have shown that nasal polyps also express high levels of regulated upon activation, normal T-cell expressed and secreted (RANTES) and eotaxin, the predominantly recognized eosinophil chemoattractants [14,28]. According to our data [14], it appears that eotaxin, rather than RANTES, in cooperation with IL-5, plays a key role in chemoattraction and activation of eosinophils in polyp tissue. However, RANTES might be involved in the localization of the eosinophils near the epithelium. Employing three-color immunofluorescence staining [29], it was demonstrated that both the number of eosinophils and the proportion of vessels positive for vascular cell adhesion molecule (VCAM)-1 were significantly increased in nasal polyps compared with the turbinate mucosa of the same patients. Moreover, treatment with topical glucocorticosteroids decreases the density of eosinophils by the inhibition of VCAM-1 and chemokine expression in polyps [30].

Although elevated total IgE was found in polyp fluid, there was no difference between polyps from allergic and nonallergic subjects [31]. However, it was noted that total IgE was higher in polyp fluid than the corresponding sera in both allergic and nonallergic polyp subjects. Local IgE production could also be demonstrated in nasal polyps

associated with negative skin tests and the serum radioallergosorbent test (RASTs) [32]. However, the specificity of IgE antibodies in polyp tissue has not been fully analyzed. According to recent findings, high total IgE concentrations are most likely due to local production of *S. aureus* enterotoxins, acting as superantigens and inducing a polyclonal IgE formation [8•].

Staphylococcus aureus–derived Enterotoxins

Superantigens differ in several aspects from conventional peptide antigens. Similar to peptide antigens, superantigens are presented by major histocompatibility complex (MHC) class II molecules. However, they are not digested within the antigen-presenting cells, and they are not presented in the MHC peptide-antigen binding groove. The unprocessed superantigen binds directly to conserved amino acid residues that are outside the peptide-antigen binding groove. Whereas recognition of peptide antigens by the T-cell receptor (TCR) is restricted by MHC alleles, recognition of superantigens generally is not MHC restricted. Conventional peptide antigens require recognition by all five variable elements (Vβ, Dβ, Jβ, Vα, Jα) of the TCR. In contrast, the recognition of superantigens by TCR almost exclusively depends on the TCR Vβ chain. Therefore, the frequency of T cells responding to superantigens exceeds that of conventional peptide antigens, activating up to 30% of circulating T cells. As a result, superantigens elicit a strong primary response, which is not seen with conventional peptide antigens [33•].

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 [34], B cells, macrophages, antigen presenting cells, eosinophils, and epithelial cells. SAEs are a family of structurally related heat-stable proteins of approximately 27 kDa molecular mass. Several staphylococcal superantigens have been described, of which the *S. aureus*–derived enterotoxins (SAEs) are the most widely studied, comprising several major serologic types: five prototypic "classic" SAEs (types A to E) [11,35], and the newly characterized SAEs (types G to Q) [36,37], of which the group of enterotoxins originating from the *egc*-gene cluster appear to be of special interest [38•]. Furthermore, superantigens can also be formed by other germs, such as *Streptococcus pneumoniae*, *Mycoplasma*, or viruses.

Toxic shock syndrome toxin (TSST-1), a causative agent of TSS, 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 cytokines and to act as a nominal antigen to induce proliferation and immunoglobulin secretion in human B cells [39,40]. In addition, in patients with atopic dermatitis, TSST-1 induces high amounts of IgE synthesis in the presence of low concentrations of interferon (IFN)-γ [41,42]. Similar to the SAEs, studies have demonstrated that there are naturally occurring variants of this superantigen also, which might or might not be as fully effective as the wild-type TSST-1 [43,44].

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 [45]. Although there have been conflicting reports on the superantigenic nature of these agents, a recent study has demonstrated that highly purified ETA and ETB induce selective polyclonal expansion of human T cells [46]. Studies of staphylococcal virulence factor, protein A, have suggested that this toxin directly influences the activity of a subset of B cells and leads to a T-cell–independent depletion of these cells in vivo [47,48]. Staphylococcal protein A (SPA) is known to bind preferentially to the VH3 family of Ig heavy-chain variable gene products. Like T-cell superantigens, SPA probably interacts with residues in the exposed regions of the heavy chain molecule outside the classic Ag-binding site and provides a signal that can lead to biased Ig production by human B cells expressing VH3 [49].

Superantigenic Effects on T Lymphocytes

Studies in patients who develop glomerulonephritis following methicillin-resistant *S. aureus* infection have demonstrated that a marked increase in CD4+, CD8+, and TCR Vβ + T cells, and a variety of cytokines (including IL-1β, IL-2, IL-6, IL-8, IL-10) and tumor necrosis factor (TNF)-α are released in these patients, compared with healthy subjects [50].

Stimulation of peripheral blood mononuclear cells (PBMCs) from atopic dermatitis (AD) patients with SAE A or B displayed significantly stronger proliferative responses than cells from controls. After culture of SAE-stimulated cells, no difference was observed in the expression of TCR Vβ segments in the controls compared with pre-stimulation, but particular Vβ segments were intensely expressed in AD patients, displaying distinct patterns. Many of these Vβ segments corresponded with those known to be induced by SAEs. These results suggest a polyclonal proliferation of T cells in the peripheral blood of AD patients [51]. SAE B selectively stimulated the production of IL-5 in peripheral blood mononuclear cells of AEDS sufferers and allergic asthmatics, but not in asymptomatic atopics or nonatopics [52].

Application of SAE B on the skin of normal individuals and patients with AD resulted in an inflammatory reaction at the application site [53], and it could be derived from a mouse model that this inflammation was T-cell dependent [54,55]. The cutaneous inflammation is exacerbated by the superantigens primarily by two pathways: 1) polyclonal activation of T cells and 2) induction of antigen-specific T cells that promote the generation of antigen-specific IgE

antibodies, which subsequently play a role in "conventional" allergen-mediated reactions.

Superantigenic Effects on B Lymphocytes

There is increasing evidence that the SAEs can also directly affect the frequency and activation of B cells, and subsequently influence the expression of the B-cell repertoire. Studies, predominantly with SPA, have demonstrated that this superantigen influences the activity of B cells by binding the fragment, antigen-binding (Fab) region of human Igs, whose heavy chains are encoded by V (H) cluster III genes [47,56,57]. SAE A fails to induce B-cell proliferation and differentiation in the absence of T cells. However, it induces survival of B cells uniquely expressing VH3-containing IgM, independent of light-chain use [58,59]. Although SAE B does not induce T-cell–independent proliferation nor differentiation of VH3-expressing cells, it enhances the survival of B cells. In contrast, SAE D induces a 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 of these SAEs might function as unique B-cell superantigens by rescuing them from apoptosis. TSST-1 augments isotype switching and synthesis of IgE, both in vitro [41,42] and in vivo [60] in a mouse model of severe combined immunodeficiency disease (SCID). 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 [61], a molecule that has been shown to enhance Th2 responses and to be involved in IgE regulation.

Superantigenic Effects on Pro-inflammatory Cells

There are fewer studies investigating the effects of superantigens on 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 SAEs via the MHC class II molecules expressed on their cell surface. Incubation of human eosinophils in the presence of GM-CSF, however, leads to an increased expression of human leukocyte antigen (HLA)-DR and a subsequent proliferation of resting CD4+ T cells, in response to SAEs A, B, and E [62]. 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 SAEs [62]. A preliminary study has recently suggested that classic SAEs and TSST-1 might also directly affect eosinophil activity by inhibiting apoptosis and modulating important cell-surface antigens, including the upregulation of CD11b, CD45, and CD69, and downregulation of CD34 and CD54 expression [63].

Macrophages also act as accessory cells for a T-cell activation/proliferation response to SAEs [62,64,65], 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 SAE A leads to a concentration-dependent increase in synthesis and secretion of IL-8 and messenger RNA encoding IL-8 [66]. Incubation of a monocyte-cell line with this enterotoxin, however, led to the release of lower concentrations of 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 [66]. SAE A might activate macrophages via both MHC class I and MHC class II molecules [67], and SAE B induces IL-12 production in macrophages [68].

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. SPA is capable of cross-linking IgE molecules on mast cells [69]. Genovese *et al.* [70] have investigated the effect of SPA on the release of several mediators from mast cells isolated from human heart tissue, and demonstrated that SPA led to an increased release of histamine, tryptase, and leukotriene C4 (LTC4).

Staphylococcal Superantigens in Nasal Polyposis

If we match the possible effects of SAEs on lymphocytes and inflammatory cells with the current pathophysiologic understanding of the inflammatory reaction in nasal polyps, the parallels are striking. The severe eosinophilic inflammation driven by a mixed pattern of T cells, the local IgE synthesis present in polyp tissue, and the sometimes poor response to glucocorticosteroids could be induced by superantigens derived from *S. aureus*, a major colonizer of the nasal cavity. However, what is the evidence, and what are the deficits in knowledge so far?

In a study taking swabs from the middle meatus of patients with chronic rhinosinusitis and nasal polyps, we found strikingly more frequent colonization with coagulase-positive *S. aureus* strains in polyp patients compared with controls (subjects with chronic rhinosinusitis or without sinus disease) (Unpublished data). Approximately 70% of these strains would be able to produce classic enterotoxins under appropriate conditions, and, furthermore, are likely to synthesize other enterotoxins from the *egc*-gene cluster that have not yet been studied in airway disease, but have been described in AEDS subjects [38•,71]. There was a partial overlap only in terms of pro-

duction of classic and *egc*-gene cluster SAEs by strains sampled from the skin, indicating that also for the nose, studying reactions to merely classic enterotoxins might underestimate the prevalence of SAE impact. Studies on the enterotoxin production of strains harvested from nasal polyp patients have shown that classic enterotoxins are released, and an extention of these studies to other superantigens is currently ongoing. We were able to detect enterotoxins at the protein level in polyp tissue from patients with polyposis, but sensitivity of these tests is insufficient so far (Unpublished data). Furthermore, techniques to demonstrate enterotoxin production in human fluids are needed to screen for the presence of these superantigens in a given organ.

The colonization of the nasal cavity is likely to be dependent on a disturbance of the local immunity or mucosal barrier function. For example, the rate of nasal carriage of *S. aureus* in patients with perennial allergic rhinitis (PAR) was significantly higher than that of control subjects, and the rate of nasal carriage of classic superantigen-producing *S. aureus* in these patients was significantly higher than that of control subjects [72]. In vitro evaluation of the response of peripheral blood lymphocytes to SAE B 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 larger amounts of IL-4 and IL-5 in a dose-dependent manner. In contrast, the control lymphocytes were seen to produce significantly larger amounts of IFN-γ, compared with lymphocytes derived from PAR subjects. Triggers such as viral infections or other sources of damage to the mucosal barrier are likely to have a comparable impact. Animal models should help to determine such "start-off" mechanisms.

S. aureus strains express a distinct array of receptors that recognize human ECM proteins. This family of microbial cell surface proteins has been shown to specifically bind ECM proteins, such as fibronectin, fibrinogen, and collagen [73]. In patients with atopic dermatitis, binding of *S. aureus* was localized primarily to the stratum corneum, with immunocytochemical staining showing a redistribution of fibronectin, an observation not seen in normal skin [74]. Immunohistochemical staining of nasal polyps also showed an increased expression of fibronectin in the epithelium and subepithelium compared with control mucosa [14]. Therefore, specific inflammatory processes in nasal polyps could facilitate colonization of the epithelium with *S. aureus*.

Currently, we use specific IgE antibodies to SAEs in tissue (and sera) as a marker for current or former immune reactions to SAEs. We [8•] demonstrated that in approximately 50% of the polyp homogenates, and more than 60% of tissues derived from polyp patients with concomitant asthma, specific IgE antibodies to SAE A and/or B were present, and this finding was linked to high total IgE and polyclonal IgE formation against inhalant allergens and fungi. Similar findings have recently been made in serum samples from patients with asthma, as discussed later. Although elevated total IgE and IgE antibodies to SAEs are linked in most cases, there are different patterns of IgE antibodies in polyp samples:

- No IgE to SAEs, low total IgE and IgE/no IgE to inhalant allergens, indicating no impact of enterotoxins (~30%)
- IgE to SAEs, high total IgE and IgE to inhalant allergens, most likely based on superantigen activity in an allergic subject (~50%)
- IgE to SAEs, moderate to high total IgE, but no IgE to inhalant allergens, indicating superantigen activity in a nonallergic subject (~ 10%)
- High total IgE and IgE to inhalant allergens, but no IgE to SAEs, indicating that other than the classic enterotoxins might have acted as superantigens (~10%)

There is evidence that IgE antibodies in polyp tissue are formed locally, as has been shown for allergic rhinitis [75]. IgE specificities found in polyp tissue are only partially reflected in serum of the same patient, and are independent of skin prick test results. Although total IgE in tissue is elevated, total IgE in serum is normal in many patients with polyps. Analysis of dispersed tissue cells by the EliSpot technique demonstrated a significant increase in the number of IgE-producing cells in polyp tissue versus normal mucosa (M. Wagenmann, personal communication). Studies using immunohistochemistry recently allowed us to demonstrate lymphocyte aggregates, which stained positive for SAEs, and consisted of B and T cells, surrounded by IgE-producing plasma cells (Unpublished data). Further investigations on the switch of immunoglobulin production to IgE in polyp tissue are ongoing.

Of interest, in SAE A/SAE B–specific IgE-positive polyp samples compared with controls, the eosinophilic inflammation was significantly more severe in terms of synthesis of IL-5, eotaxin, and cysteinyl leukotrienes, and these patients more often had asthma and/or aspirin sensitivity [8•]. Again, these findings in polyps were supported by data from asthma patients. In contrast, patients with chronic rhinosinusitis without polyp formation did not show increased total or specific IgE-antibody formation compared with controls, nor did they elicit a comparably strong eosinophilic inflammation.

Results of a recent study in patients with aspirin sensitivity and nasal polyposis (ASNP) showed significantly increased specific IgE antibodies to SAEs in the group of ASNP compared with ATNP (aspirin tolerant nasal polyposis) and in both groups, compared with controls. Interestingly, seven out of 13 patients with ASNP were positive for specific IgE to SAEs (54%) in contrast with seven out of 27 in the ATNP group (26%) and none out of 12 subjects in the control group (Unpublished data). Concentrations of

IL-5, ECP, and total IgE were significantly increased in ASNP compared with ATNP patients and controls, as was shown before in polyp tissue unrelated to aspirin sensitivity. Therefore, SAEs might be related to aspirin sensitivity, and might have an impact on the severity of eosinophilic inflammation. Further studies are needed to clarify the link between enterotoxins and aspirin sensitivity.

Because asthma is frequently associated with polyposis, and SAE-IgE–positive polyp patients more frequently have asthma compared with SAE-negative polyp subjects, we recently extended our investigations to the lower airways. Studies in mice have shown that SAE B triggers airway recruitment of several pro-inflammatory cell types (including TCR Vβ(+) and TCR Vβ(-) T cells, eosinophils, neutrophils, and TNF-α(+) macrophages) and release of cytokines (including IL-4 and TNF-α, but not IFN-γ), which was associated with an increased airway responsiveness in these animals that resembled asthma [76•,77]. Importantly, the increase in airway responsiveness was particularly CD4+ T-cell–dependent. In humans, it seems that the expression of TCR-Vβ8(+) T cells obtained in the bronchoalveolar lavage (BAL) of patients with poorly controlled asthma is significantly increased, compared with cells in the BAL of patients with well-controlled asthma and normal control subjects, suggesting that SAEs potentially trigger T-cell activation in poorly controlled asthma [78]. Comparable studies on T cells from polyp tissue are lacking so far.

To allow screening of sera from asthmatic patients, we characterized the patterns of IgE antibody responses specific to SAEs in nasal polyps and established a mix, consisting of three SAEs (SAE A, SAE C, TSST-1), that was shown to possess high specificity and sensitivity [79•]. IgE antibodies to the SAE mix were found significantly more often in sera from patients with asthma, compared with controls (49% vs 13%). Furthermore, within the group of asthmatic patients, defined by their decreased lung function in spite of the use of regular, high-dose, inhaled or systemic corticosteroid therapy, IgE antibodies to SAEs were found more often in severe compared with mild asthmatics (62% vs 41%). This increase was paralleled by an increase in serum ECP. Although IgE to SAEs was related to total IgE, some patients demonstrated high IgE levels without specific antibodies, indicating the possible impact of superantigens other than the classic enterotoxins; and some patients had IgE to SAEs, but a normal total IgE, indicating that the immune response to SAEs was independent of an atopic status. These data again suggest a close relation between the presence of IgE antibody to SAEs and the severity of asthma in terms of lung function and eosinophilic inflammation, as previously was described in nasal polyposis.

Although ill-defined corticosteroid insensitivity is a well-known phenomenon in severe polyposis and asthma, recent studies have suggested that SAEs might lead to poor disease control by corticosteroids, because it was shown that they can induce steroid insensitivity in PBMCs [80]. Compared with the level of dexamethasone-induced inhibition of proliferation of PBMCs in response to phytohemagglutinin stimulation, the level of dexamethasone-induced inhibition of proliferation in response to stimulation with SAE B, SAE E, and TSST-1 was reduced threefold to fivefold. Furthermore, SAE B also significantly increased the expression of glucocorticoid receptor beta, compared with PHA and control cells. Similarly, a comparison of control B cells versus B cells from cystic fibrosis patients 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 of cystic fibrosis B cells, was inhibited by treatment with dexamethasone [81].

Therapeutic Perspectives

It has been shown that antibiotic treatment to reduce the impact of colonizing *S. aureus* in subjects with AEDS leads to a sustained reduction of skin symptoms [82•]. As yet, no study has documented the therapeutic impact of antibiotics on nasal polyposis, but such a study is currently ongoing. However, although treatment with antibiotics results in a significant reduction in colony counts of *S. aureus* in AEDS patients, subjects are quickly recolonized when the antibiotic is discontinued [83], and this might also be expected for the nasal cavity, representing a reservoir for *S. aureus*. Furthermore, disease severity and bacterial counts have been shown to be reduced by immunosuppressive treatment, such as steroids [84] or cyclosporine A [85]. This suggests that antibiotic therapy can be combined with effective anti-inflammatory agents to achieve long-term control of disease, and to overcome steroid insensitivity.

Peptide antagonists have been developed to prevent T-cell activation and lethal toxic shock induced by staphylococcal superantigens in mice [86]. Similar approaches are not yet developed for humans and might be inadequate for long-term therapy.

Conclusions

There is now increasing evidence that staphylococcal toxins act as both superantigens and conventional allergens, and, therefore, play a role in modulating chronic inflammatory disease. Studies of patients suffering from AEDS have convincingly demonstrated the association between colonization with *S. aureus* and production of staphylococcal superantigens and severity of disease, thus proposing a putative modulatory role for *S. aureus* enterotoxins in the pathogenesis of AEDS. Similarly, there is increasing evidence that *S. aureus* superantigens might also have the potential to influence the pathogenesis of upper and lower airway disease, including nasal polyposis and bronchial asthma. The evidence for the role of staphylococcal superantigens in the etiology of airways disease, however, is preliminary and, at best, circumstantial. Consequently, more basic studies and properly controlled clinical trials are

clearly required. Additionally, understanding the impact of superantigens might also elucidate novel therapeutic strategies for these disorders.

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This paper is the first to describe IgE antibodies to *Staphylococcus aureus* enterotoxins in nasal polyp tissue and to show the link to severity of eosinophilic inflammation. Please note that incidences are based on IgE to only two classic enterotoxins, and many more have been described recently.

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