

Original article

Nasal carriage of *Staphylococcus aureus* in house dust mite allergic patients and healthy controls

Background: Staphylococcal colonization may influence the course of allergic diseases such as atopic dermatitis or allergic rhinitis. The frequency of *Staphylococcus aureus* (SA) nasal carriage and its possible influence on persistent allergic rhinitis was investigated.

Methods: In nasal lavages from 22 patients with house dust mite allergy and 18 healthy controls, the number of SA colony forming units per ml were assessed and related to nasal symptom scores, the concentrations of three inflammatory cell activation markers, nasal total IgE and 17 cytokines in nasal secretions.

Results: SA was found in 15/22 allergic patients and 4/18 controls ($P < 0.01$). Comparing allergic SA carriers with allergic noncarriers, nasal symptom scores tended to be higher ($P < 0.1$), and the cell activation markers ECP ($10^{2.23 \pm 0.33}$ vs $10^{1.45 \pm 0.50}$ ng/ml; $P < 0.05$) and elastase ($10^{2.70 \pm 0.21}$ vs $10^{2.12 \pm 0.34}$ ng/ml; $P < 0.01$), and nasal total IgE-levels ($10^{1.66 \pm 0.38}$ vs $10^{1.2 \pm 0.28}$ kU/ml; $P < 0.05$) were significantly higher in allergic SA carriers. Nasal SA carriers had a higher nasal IL-13/IFN- γ ratio ($P < 0.01$), and this was correlated with higher nasal total IgE in allergic patients ($r = 0.6$, $P < 0.05$).

Conclusion: Nasal SA carriage is frequent in patients with persistent allergic rhinitis. The data of this study suggest that they are not only secondary bystanders, but actively modulate the disease by promoting local IgE production.

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Staphylococcal infection causes a variety of diseases ranging from superficial skin infections to severe toxic shock syndrome (1). Moreover, staphylococcal colonization may modulate allergic disease. Patients with atopic dermatitis suffer from relapses of their disease following dermal overgrowth of this organism and most patients with atopic dermatitis develop IgE antibodies to staphylococcal antigens (2).

Staphylococcus aureus (SA) is a relevant source of several superantigens, which exert immunomodulatory and proinflammatory effects (1). Superantigens can bind to MHC class II of APC, and cross-link them to specific motifs of the TcR- β chain of CD4⁺ T-cells outside the antigen-binding groove. They may thus activate large amounts of T cells in an unspecific manner (3). On B cells, staphylococcal enterotoxins bind Fab and Fc γ , structures involved in antigen recognition and effector functions, and are thus able to stimulate a large B-cell fraction (4). In addition to professional antigen presenting cells, such as dendritic cells or macrophages, superantigen presentation on MHC-class II molecules of neutrophils, basophils and eosinophils has also been reported (3). Moreover, bacterial superantigens have been demonstrated to directly activate inflammatory effector cells including mast cells and macrophages (5). The cytokine

response to bacterial superantigens may differ in atopic and nonatopic subjects. In symptomatic atopics, staphylococcal enterotoxin B has been found to selectively stimulate IL-4 and IL-5, and to reduce IFN- γ production of PBMC, whereas no such effect was demonstrated in nonsymptomatic atopic or nonatopic subjects (6).

In recent years, researchers have focused on the potential role of staphylococcal superantigens in airway diseases (7, 8) such as allergic and nonallergic asthma (9, 10), nasal polyps (11, 12) and allergic rhinitis (13). In this study, the frequency of nasal SA carriage and its possible influence on symptoms and nasal biomarker concentrations in patients with persistent allergic rhinitis was investigated.

Methods

Study population

Forty nonsmoking subjects participated in this study, 17 were men and 23 were women. Their age ranged from 19 to 36 years. Patients and controls were students of the University of Ulm. An allergic history was obtained, and anterior and posterior rhinoscopy was performed. Participants were skin prick tested with inhalant allergens common in central Europe including various pollen, mites,

molds and animal dander. Not included were pregnant or lactating women, patients with chronic rhinosinusitis, acute rhinosinusitis within the last 6 weeks, clinically relevant nasal septal deviation, regular use of nasal decongestants, previous sinus surgery, bronchial asthma, lung emphysema, atopic dermatitis, previous immunotherapy, systemic therapy with corticosteroids or anti-inflammatory drugs, or nasal corticosteroid therapy within the last 6 weeks. A positive vote of the ethics committee of the University of Ulm was obtained (No. 57/2003).

The house dust mite (HDM) allergic patients had to meet all of the following additional criteria: a positive skin prick test to *Dermatophagoides pteronyssinus* (D. pter.) and/or *Dermatophagoides farinae* (D. far.), defined as a mean wheal diameter of at least 3 mm (14), positive D. pter. and/or D. far. serum specific IgE (ssIgE), defined as at least class III in the Pharmacia CAP-assay (15), and a positive conjunctival provocation test to D. pter. and/or D. far. as previously described (16). Briefly, 50 µl 1 : 10 diluted allergen extract (1000 AU/ml), and if negative, 50 µl undiluted extract (10 000 AU/ml, HAL, Germany) were applied to the lower conjunctival sac. The response was assessed with clinical symptom scores. The participants in the control group were negative in all of these tests. The study was performed from November 2003 to January 2004 outside the pollen season.

Nasal SA carriage and ssIgE to staphylococcal enterotoxin A and B

Nasal lavages were obtained in order to assess the number of SA colony forming units. Nasal lavages reach all relevant nasal areas including the nasal vestibule and the main mucus transport routes within the nasal cavity. In contrast to the qualitative nasal swab investigation, nasal lavages allow semi quantitative assessment of nasal SA colonization. After mucosal decongestion, patients were asked to recline their head some 45°, and to breathe in and hold. Then both nostrils were flushed with 5 ml of isotonic saline solution using a sterile disposable syringe. After 10 s the patients vigorously blew the solution into a sterile glass container. Nasal washes for bacterial culture were promptly transported to the laboratory and processed quantitatively. Therefore, a 1 : 100 dilution was prepared from each specimen using 0.9% (w/v) sterile NaCl solution. Samples of 0.01 ml from the undiluted and the 1 : 100 diluted specimen were plated onto sheep blood agar (Heipha-Biotest, Germany) and incubated at 36°C in the presence of 5% CO₂ for a minimum of 24 h. The detection level was 100 Colony forming units (CFU) of SA per ml original specimen. Colonies of SA were identified by typical colonial appearance, Gram stain, positive catalase reaction and the Slidex Staph plus, a commercial latex agglutination kit (bioMérieux, France) detecting clumping factor, protein A and a further antigen specific for SA. Equivocal results were cleared by detection of aurease using the RAPIDEC Staph (bioMérieux) (17). The specimen was considered positive, if at least 100 SA CFU/ml were grown.

In addition, the IgE-dependent immune reaction to staphylococcal superantigens was assessed in blood serum. Therefore, ssIgE to *Staphylococcus* enterotoxin A and B was determined using a fluorescence-immunoassay (UniCAP 100 Diagnostic System, Pharmacia, Freiburg, Germany) according to the manufacturer's recommendations. The limit of detection for specific IgE to *Staphylococcus* enterotoxin A and B was 0.35 kU/l.

Nasal symptom scores

Symptom scores for nasal obstruction, hypersecretion, and irritation were assessed on 10 cm long horizontal visual analog scales. The left scale ending of the line was marked as 'symptom not present' and the right end as 'maximum symptom intensity.' Each participant

marked the line at a position, which should reflect his/her personal estimate of the symptom severity. The distance from the left end of the line to this mark in cm was used as the symptom severity score.

Sampling of nasal secretions for biomarker determinations

Nasal secretions for biomarker determinations were obtained as previously described (18). Briefly, open cell flexible polyurethane foam samplers of 28 × 18 × 6 mm were placed into each nasal cavity posterior to the mucocutaneous junction under direct visualization and left in place for 10 min. Following removal, undiluted nasal fluid was extracted from the sampler by centrifugation.

Cell activation markers and total IgE in nasal secretions

Eosinophil cationic protein (ECP), tryptase and total IgE levels in nasal secretions were measured using a fluorescence-immunoassay (UniCAP 100 Diagnostic System) according to the manufacturer's recommendations. Sample dilutions were fivefold for ECP and tryptase, nasal total IgE was measured undiluted. The detection limit was 2 ng/ml for ECP, 1 ng/ml for tryptase and 2 kU/l for total IgE. Human PMN elastase was measured using a quantitative enzyme-linked immunosorbent assay (Bender MedSystems, Vienna, Austria). Samples were diluted 100-fold. The limit of detection for PMN elastase was 3 ng/ml.

Cytokine determination in nasal secretions

Nasal secretions were diluted 1 : 10 and cytokine concentrations were analyzed on a Bio-Plex™ Suspension Array System (Bio-Rad Laboratories, Munich, Germany). Employing the Multiplex Cytokine Assay for 17 cytokines (Bio-Rad Laboratories), nasal secretion concentrations of interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70), IL-13, IL-17, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1β (MIP-1β) were determined. Data were evaluated with the Bio-Plex Manager™ Software 3.0 (Bio-Rad Laboratories). The limit of detection was less than 0.5 pg/ml.

Statistical analysis

Frequency tables were evaluated with Fisher's exact test. Symptom scores in SA carriers and noncarriers were compared with the Kruskal Wallis test. Biomarker concentrations in nasal secretions were log-transformed to the base 10 and means ± SD are provided for data description. The influence of SA colonization on nasal biomarkers in allergic patients was compared with *t*-tests. Due to the exploratory character of the study, no corrections for multiple tests were performed. A two-sided α-level of 0.05 was set as the significance level in all tests performed.

Results

Nasal SA carriage and ssIgE to staphylococcal enterotoxin A and B

In 19 of 40 participants, nasal lavages were positive for SA. In two participants grew 10² CFU/ml, in three grew 10³, in six grew 10⁴, in seven grew 10⁵, and in one grew

Table 1. Frequencies of SA carriage in 18 nonallergic study participants and 22 patients with HDM persistent allergic rhinitis

	Nonallergic	Allergic	Total
SA negative	14	7	21
SA positive	4	15	19
Total	18	22	40

10⁶ CFU/ml. In 21 participants, no SA could be cultured. Nasal SA carriage was significantly more frequent in patients with HDM allergy than in nonallergic controls (*P* = 0.005, Table 1). In all participants, ssIgE levels for staphylococcal enterotoxin A or B were below 0.8 kU/ml, thus no relevant IgE-dependent systemic immunereaction to staphylococcal enterotoxins A and B was observed.

Polyvalent sensitization and nasal symptom scores

Nasal SA colonization was not associated with polyvalent sensitization in skin prick tests. In allergic patients with nasal SA, the number of positive prick tests (median: 6; interquartile range: 3–12) did not differ relevantly from those without nasal SA (8, 5–9, *P* > 0.5). Symptom scores were not significantly influenced by the nasal SA carrier state (*P* = 0.1, Fig. 1).

Cell activation markers in nasal secretions

Nasal secretion levels were higher in allergic SA carriers for elastase ($10^{2.70 \pm 0.21}$ vs $10^{2.12 \pm 0.34}$ ng/ml; *P* < 0.01), and ECP ($10^{2.23 \pm 0.33}$ vs $10^{1.45 \pm 0.50}$ ng/ml; *P* < 0.05)

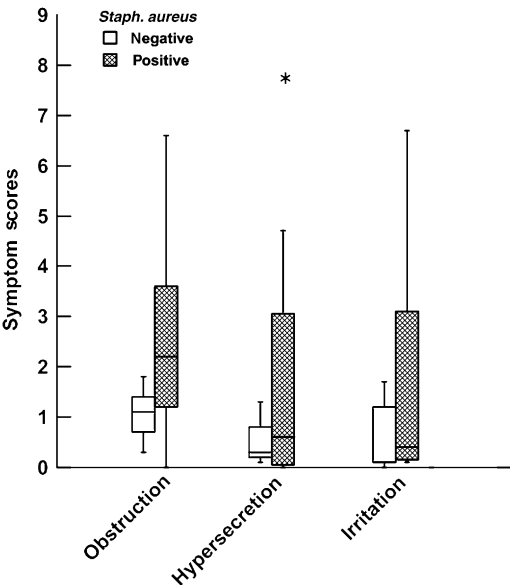


Figure 1. Symptom severity assessed on 10 cm visual analog scales in HDM-allergic patients with and without nasal staphylococcal colonization (*P* = 0.1, *n* = 22; asterisk: outlier).

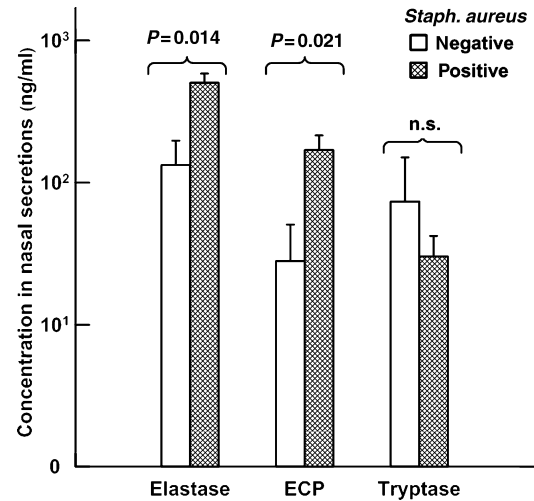


Figure 2. Inflammatory cell activation markers in nasal secretion in HDM-allergic patients with and without nasal staphylococcal colonization (*n* = 22).

when compared with SA negative HDM-allergic individuals. Levels did not differ significantly for tryptase (*P* = 0.25, Fig. 2). In nonallergic controls, nasal secretion levels for neutrophil elastase, ECP and tryptase did not differ significantly depending on nasal SA carrier state.

Nasal total IgE, serum total IgE, and serum HDM-specific IgE

In nasal secretions, allergic SA carriers had higher total IgE levels ($10^{1.62 \pm 0.37}$ kU/l) than allergic noncarriers ($10^{1.21 \pm 0.28}$ kU/l; *P* < 0.05, Fig. 3). In blood serum, total IgE levels in HDM-allergic patients with nasal SA ($10^{2.3 \pm 0.5}$ kU/l; mean $\pm$ SD) were also higher than in allergic noncarriers ($10^{1.9 \pm 0.3}$ kU/l), but this difference

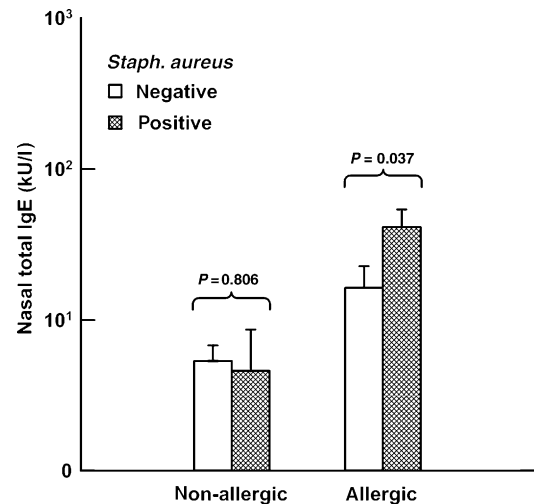


Figure 3. Total IgE levels in nasal secretions and nasal staphylococcal carriage in 18 healthy controls and 22 HDM allergic patients.

did not reach statistical significance ($P = 0.09$). Moreover, HDM-specific serum IgE levels tended to be higher in HDM-allergic SA carriers, when compared with HDM-allergic noncarriers. Levels for ssIgE to D. pter. were $10^{1.28 \pm 0.5}$ kU/l in allergic SA carriers and $10^{0.82 \pm 0.51}$ kU/l in allergic noncarriers ($P = 0.055$).

Nasal SA and cytokine levels in nasal secretions

Nasal SA carriage was associated with high IL-8 and IL-13, and low IFN- γ levels ($P < 0.01$). Moreover, high IL-8 and IL-13, and low IFN- γ levels were positively correlated with nasal total IgE in HDM-allergic patients ($r = 0.6$, $P = 0.015$), but not in nonallergic patients ($r = -0.18$; $P = 0.51$).

Discussion

The nose is regarded as the major site of SA carriage from where the organisms can spread to other parts of the body. The SA preferentially grows within the nasal vestibule, close to the mucocutaneous junction (19). Staphylococcal colonization has been identified as a relevant pathogenic factor in atopic dermatitis (2) and a significant role in the physiopathology of inflammatory airway disease has been proposed (8). Approximately 70% of nasal SA strains in Central Europe were found to produce staphylococcal superantigens (20), which may modulate the course of allergic rhinitis. In this study, the frequency of nasal SA carriage in HDM-allergic patients and controls without nasal allergy has been investigated. Clinically relevant HDM allergy was confirmed by medical history, skin prick tests, determination of HDM ssIgE, and positive conjunctival provocation tests. Patients and controls were recruited in the outpatient allergy unit. To achieve a comparatively homogenous population concerning risk factors for nasal SA carriage (19), only students of the University of Ulm were recruited. Patients with additional risk factors for nasal SA carriage such as nasal structural abnormalities (21), concomitant upper airway disease (22), atopic dermatitis (19), previous or concurrent immunotherapy (23, 24) or other conditions associated with increased frequency of skin punctures were excluded. The resulting small size of the study group and the large number of parameters investigated underline the exploratory character of this study.

Frequency of nasal SA carriage and severity of allergic rhinitis

Consistent with the results of a previous study (13), nasal SA carriage was significantly more frequent in patients with allergic rhinitis to HDM than in nonallergic controls ($P < 0.01$). Though not significant, symptom scores for nasal obstruction, hypersecretion and irritation were higher in allergic nasal SA carriers when compared with allergic noncarriers (Fig. 1). Moreover, allergic nasal SA

carriers had significantly higher nasal ECP and elastase levels (Fig. 2), which are considered sensitive indicators of disease severity (25).

Nasal SA carriage and IgE

In this study, superantigen production of cultured SA was not explicitly confirmed, however, assessment of the ever-growing number of SA superantigens is laborious and reliable information on the frequency of superantigen producing SA strains in Central Europe is available (20, 26). Nasal carriage of superantigen producing SA is probably more frequent than detected by the immune reaction elicited by SA superantigens (27). No ssIgE to SA superantigen was detected in this study, which might reflect that SA superantigen promoted IgE production in AR is mainly confined to the nasal cavity. However, because limited amounts of nasal secretions were available for biomarker detection, IgE to SA superantigens could only be assessed in blood serum.

In several studies, exposure to staphylococcal superantigens was assessed by the presence of ssIgE to SA-superantigens. Consistently, ssIgE to SA-superantigens was associated with high serum total IgE levels (28–30). However, this could be a mere epiphenomenon, reflecting that patients with high serum IgE levels are frequently polysensitized and produce specific IgE also to staphylococcal antigens. Conversely, a number of observations indicate a causative relation between SA superantigen exposure and high IgE levels. An increased IgE production from peripheral blood mononuclear cells in response to staphylococcal superantigen has been observed in patients with atopic dermatitis (31) and allergic rhinitis (32). Increased IgE-production is thought to depend on immunoglobulin class switching to IgE, controlled by signals delivered to B cells by T cells (5, 33). In addition to CD40 mediated B-cell activation, IL-4 or IL-13 are essential stimuli, whereas IFN- γ promotes switching to IgG. Increased IL-4 and decreased IFN- γ release from mononuclear cells of SA carriers in response to staphylococcal superantigen has been observed in patients with atopic dermatitis (6) and allergic rhinitis (13) suggesting that staphylococcal superantigens augment the T_{H2}- and IgE-bias of the immune response in atopic individuals.

Consistent with these results, serum total and HDM-specific IgE levels were higher in allergic SA carriers in this study, although in blood serum the differences just missed statistical significance. However, in nasal secretions of allergic SA carriers, total IgE levels were significantly higher when compared with allergic noncarriers (Fig. 3). High serum and nasal IgE levels were not associated with polyvalent sensitization. It is therefore not regarded as an unspecific epiphenomenon, secondary to a preexisting IgE-skewed immune response, but rather supports the concept of an IgE modulating activity of nasal SA in patients with allergic rhinitis. Nasal SA colonization and the association with local IgE production

have also been thoroughly investigated in nasal polyps. Gevaert et al. recently reported the organization of secondary lymphoid tissue and local IgE-formation to SA enterotoxins in nasal polyp tissue (34).

Nasal SA and cytokine levels in nasal secretions

In allergic rhinitis, immunoglobulin class switching is likely to occur within the nasal mucosa (35). To further investigate possible mechanisms; how SA superantigens may modulate nasal IgE-levels, the effects of nasal SA carriage on cytokine levels in nasal secretions were assessed. The nasal cytokine milieu of nasal SA carriers was characterized by higher IL-8 and IL-13, and lower IFN- γ levels ($P < 0.01$). Moreover, this set of cytokines was closely correlated with nasal IgE-levels in allergic ($r = 0.6$, $P = 0.015$), but not in nonallergic participants ($r = -0.17$, $P = 0.5$). Hence, in agreement with previous studies in PBMC (6, 13), nasal SA carriage in HDM-allergic individuals was associated with a nasal cytokine milieu, which is known to shift the T-cell/B-cell interaction towards an IgE response.

The role of IL-4 and IL-8 in these measurements appeared of particular interest. Consistent with a previous report (36), IL-4 levels in nasal secretions were not substantially different in participants with or without

allergic rhinitis and also not in SA carriers and noncarriers. Due to cytokine redundancy concerning B-cell effects, it is likely that IL-13 suffices to elicit an immunoglobulin class switching also in the absence of IL-4 (37). The association of IL-8 with IL-13 and IFN- γ points at immunomodulatory functions of airway epithelial cells (38). In nasal mucosa, IL-8 mainly derives from airway epithelial cells. It is a chemoattractant not only for PMN, but also for T cells (39), what may explain the association of IL-8 with the mainly T-cell derived cytokines IL-13 and IFN- γ .

In conclusion, the results of this study support a relevant role of SA in allergic rhinitis. Staphylococcal colonization was frequent in patients with persistent allergic rhinitis. It was associated with higher nasal IgE-levels. Moreover, staphylococcal colonization was related with high nasal IL-13 and low IFN- γ levels indicating immunomodulatory effects. The results of this study are consistent with the concept that staphylococcal superantigens enhance the T_{H2}- and IgE-bias of the immune response in individuals with allergic rhinitis.

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