

Original article

Organization of secondary lymphoid tissue and local IgE formation to *Staphylococcus aureus* enterotoxins in nasal polyp tissue

Background: Bilateral nasal polyposis (NP) is characterized by high concentrations of IgE in NP tissue, which show no relation to the atopic status. We aimed to study the relationship between systemic and local IgE formation, nasal carriage of *Staphylococcus aureus* and nasal polyposis.

Methods: In serum and nasal tissue homogenates from 24 NP patients and 12 controls, we determined concentrations of total IgE and IgE antibodies to inhalant allergens and *S. aureus* enterotoxins (SAEs; A,B,C,D,E,TSST) by ImmunoCAP. Tissue cryosections were stained for CD3, CD20, CD38, CD23, FcεR1, IgE and SEA/SEB.

Results: We demonstrated a higher incidence of *S. aureus* colonization (17/24) and IgE antibodies to SAEs in NP tissue (12/24) compared with controls (3/12 and 0/12, respectively). Total IgE and IgE antibodies in serum and NP tissue were dissociated because of local polyclonal IgE formation in NP tissue. Staining of NP tissue revealed follicular structures characterized by B and T cells, and lymphoid accumulations with diffuse plasma cell infiltration.

Conclusions: We demonstrated the organization of secondary lymphoid tissue in polyp tissue and a polyclonal hyper-immunoglobulinemia E associated with the presence of IgE antibodies to SAEs, colonization with *S. aureus*, and tissue eosinophilia in a relevant subgroup of polyp patients.

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A hallmark of bilateral nasal polyposis in western countries is the pronounced tissue eosinophilia that can be found in about 70–90% of cases as well as elevated local IgE levels in polyp fluid (1–4). Therefore, an allergic aetiology of nasal polyps has been presumed, but never firmly demonstrated (5). In a retrospective study by Settipane and Chafee (6), polyps were present in 2.8% of atopic patients, and in 5.2% of non-atopic subjects. Furthermore, nasal polyps (NP) occur more frequently in subgroups of patients with asthma and aspirin sensitivity (6). About 40–80% of patients with aspirin sensitivity suffer from polyposis, and about 15% of polyp patients are hypersensitive to aspirin (6). In studies involving large series of patients with nasal polyposis, asthma was found in 20–70% of patients, and non-allergic asthma is significantly more frequently linked to polyps compared with allergic asthma (6). In a recent study we demonstrated that atopy based on positive skin-prick tests to inhalant allergens was not related to total IgE or IgE antibodies in NP tissue and that there was no impact of atopy on the levels of IL-5, IL-4, eotaxin, LTC₄/D₄/E₄, sCD23, ECP and number of tissue eosinophils in NP tissue (2). Whether the high concentrations of IgE

antibodies in polyp tissue are the result of a local production or are preferentially derived from the serum remains to be elucidated. In about half of the polyp specimens, we demonstrated polyclonal IgE formation specific IgE to *Staphylococcus aureus* enterotoxins (SAE), high levels of local IgE antibodies, and a high prevalence of asthma (2). Similarly, we demonstrated that IgE antibodies to SAE were more often found in serum from patients with severe asthma than in those with mild asthma (7). Recent evidence suggests that SAE could modify airway disease by acting as superantigens (8, 9). Superantigens are microbial or viral protein toxins with potent immunostimulatory properties. These properties are attributable to their unique ability to crosslink major histocompatibility complex (MHC) class II molecules with T-cell receptors forming a trimolecular complex that activates a much larger number of resting T cells (as many as one in five T cells) than does a conventional antigen (one in 10⁵–10⁶ T cells) (10). Several bacterial superantigens have been described, of which the family of classical *S. aureus* enterotoxins (SEA, SEB, SEC, SED, SEE and TSST) are the most widely studied. In addition, staphylococcal protein A (SPA) (11) as well as the SAE

TSST-1 have also been demonstrated to possess B-cell superantigen moieties, inducing polyclonal IgE synthesis (12, 13). Because of the widespread carriage of *S. aureus* and the ubiquitous expression of their toxins it is clear that the nasal immune system is under constant challenge by these extremely powerful agents, which are therefore likely to influence the response to other challenges.

It was therefore tempting to study the relationship between nasal carriage of *S. aureus*, IgE formation to *S. aureus* enterotoxins and nasal polyposis. Furthermore, we aimed to determine whether IgE is produced locally in NPs or is a result of extravasation, by comparison of IgE specificities in tissue and serum and by analysis of B- and T-lymphocyte accumulations in tissue.

Methods

Patients

Nasal tissue and serum were obtained from 12 control and 24 bilateral NP patients at the Department of Otorhinolaryngology, Ghent University Hospital. All control subjects (median age 33.6 years, range 18–71 years; five females/seven males) were skin-prick test negative, in generally good health, and none had a history of nasal or sinus disease, allergic disease (asthma, rhinitis, dermatitis), upper respiratory tract infection in the previous month, use of any intranasal medications, decongestants, antihistamines or oral steroids during the last month. Bilateral polyposis was diagnosed based on history, clinical examination, nasal endoscopy and sinus computed tomography (CT) scan. The median age of NP patients was 46 years (range 22–78 years; seven females/17 males). A history of asthma was reported in eight nasal polyp patients. Twelve patients were atopic, as indicated by positive cutaneous skin-prick test responses to common aeroallergens. The panel of allergens comprised: grass pollen mix (*Dactylis glomerata*, *Festuca elatior*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*); mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Tyrophagus putrescentiae*, *Acarus siro*, *Lepidoglyphus destructor*); moulds (*Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Penicillium notatum*) and *Curvularia spicifera* (ALK-Abelló, Horsholm, Denmark). Negative and positive controls (10 mg/ml histamine solution) were also included with each skin-prick test. Patients treated with oral corticosteroids within the last 4 weeks prior to surgery were excluded. The ethical committee of the Ghent University Hospital approved the study, and informed consent was obtained from all subjects.

Measurement of total IgE, IgE antibodies and albumin in serum and tissue homogenates

All samples were immediately processed, separated and stored in aliquots at –80 °C until analysis. Approximately 0.5 cm³ tissue strips were collected from the surface of each inferior turbinate (turbintomy, 0.2–0.5 g of tissue) or bilateral NP (0.2–2.0 g of tissue), during routine septal and sinus surgery, respectively. All (*n* = 24) freshly obtained tissue specimens were weighed, and 1 ml of 0.9% NaCl solution was added per every 0.1 g tissue. The tissue was then homogenized with a mechanical homogenizer (B. Braun, Melsungen, Germany) at 1000 rpm for 5 min on ice as described previously (3). After homogenization, the suspensions were centrifuged at 1500 *g* for 10 minutes at 4 °C, and the supernatants separated. Blood samples were allowed to clot at room temperature for

20–30 min, centrifuged at 1500 *g* for 10 min at 4 °C, and serum was separated.

All supernatants and sera were assayed for total IgE and IgE antibodies by the UniCAP system (Pharmacia Diagnostics, Uppsala, Sweden) and for albumin by nephelometry. IgE antibodies were determined for *S. aureus* enterotoxins (SEA, SEB, SEC, SED, SEE and TSST-1); grass pollen mix (*D. glomerata*, *F. elatior*, *L. perenne*, *P. pratense*, *P. pratensis*); trees mix (*Alnus incana*, *Betula verrucosa*, *Corylus avellana*, *Quercus alba*, *Salix caprea*); house dust mix (*D. pteronyssinus*, *D. farinae*, *Blatella germanica*) and moulds mix (*A. alternata*, *Aspergillus fumigatus*, *C. herbarum*, *Penicillium notatum*, *Candida albicans*, *Helminthosporium halodes*). ImmunoCAP coated with human serum albumin or glycine were used to evaluate any non-specific binding of IgE.

Immunohistochemistry

Specimens were snap frozen in liquid nitrogen cooled methyl butane and stored at –80 °C. Cryostat sections were prepared (6 µm) and mounted on SuperFrost Plus glass slides (Menzel GmbH & Co. KG, Braunschweig, Germany), packed in aluminium paper and stored at –30 °C until staining.

Sections were stained for CD3, CD20, CD38, CD23, FcεRI and IgE. Specimens were fixed in acetone. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in tris buffered saline (TBS) containing 0.1% sodium azide for 20 min. Primary antibodies or negative controls, consisting of the corresponding isotype control, were incubated for 1 h and detected using the labelled streptavidin biotin (LSAB) technique conjugated with peroxidase according to the manufacturer’s instructions (labelled streptavidin-biotin; Dako, Copenhagen, Denmark). The peroxidase activity was detected using AEC Substrate chromogen (Dako), which results in a red-stained precipitate. Finally sections were counterstained with haematoxylin and mounted.

Biotinylated SAE staining and blocking experiments

Specimens were fixed in acetone and endogenous peroxidase was blocked. Biotinylated SEA (40 µg/ml) and a mixture of non-biotinylated SEA/biotinylated SEA (1000 µg/ml/40 µg/ml), to determine the specificity of the biotinylated SAE staining (Toxin Technology, Sarasota, FL, USA) were applied and incubated

Table 1. IgE concentrations and eosinophil counts in controls vs nasal polyps

	Controls (<i>n</i> = 12) [median (IQR)]	<i>P</i> -value	Nasal polyps (<i>n</i> = 24) [median (IQR)]
Nasal culture			
<i>S. aureus</i> + nasal tissue	3/12 (25%)	0.02	17/24 (71%)
Nasal Tissue			
IgE (kU/l)	17 (7–25)	<0.001	654 (156–1263)
Albumin (g/l)	15.6 (12.7–17.3)	0.002	19.7 (17.7–23.2)
IgE/albumin	1.28 (0.90–2.14)	<0.001	27.08 (10.2–59.8)
SAE IgE ab+ (%)	All negative (0)	0.009	12/24 (50)
Eosinophils (%)	0.3 (0.1–0.7)	<0.001	11.5 (2.9–24.3)
Serum			
IgE (kU/l)	31 (15–64)	0.004	160 (50–291)
Albumin (g/l)	48.1 (46.2–49.7)	NS	44.5 (40.9–54.3)
IgE/albumin	0.32 (0.67–1.19)	0.04	3.5 (1.12–6.93)
SAE IgE ab+ (%)	0/8 (0)	NS	3/24 (12.5)
Eosinophils (%)	0.9 (0.4–1.8)	<0.001	4.8 (2.6–7.1)

Table 2. IgE antibody expression in serum and nasal polyp tissue

Diagnosis	Skin-prick test	Serum					Nasal tissue															
		IgE (kU _A /l)					IgE ab to <i>S. aureus</i> enterotoxins (kU _A /l)					IgE (kU _A /l)						TSST1				
		Total IgE (kU/l)	gx1	hx2	mx2	tx9	SEA	SEB	SEC	SED	SEE	TSST1	gx1	hx2	mx2	tx9	SEA		SEB	SEC	SED	SEE
NP	Negative	13.3	-	-	-	-	-	-	-	-	-	6.1	-	-	-	-	-	-	-	-	-	-
NP	Negative	8.8	-	-	-	-	-	-	-	-	-	10.5	-	-	-	-	-	-	-	-	-	-
NP	Negative	78.5	-	-	-	-	-	-	-	-	-	97.1	-	-	-	-	-	-	-	-	-	-
NP + Asth	Negative	20.2	-	-	-	-	-	-	-	-	-	141.3	-	-	-	-	-	-	-	-	-	-
NP	Negative	277.0	-	-	-	-	-	-	-	-	-	145.3	-	-	-	-	-	-	-	-	-	-
NP	Negative	48.5	-	-	-	-	-	-	-	-	-	97.4	-	-	4.17	-	-	-	-	-	-	-
NP	Negative	50.2	-	-	-	-	-	-	-	-	-	250.7	-	-	5.78	5.01	-	-	-	-	-	-
NP	Negative	157.0	-	-	-	-	-	-	-	-	-	160.1	-	-	5.88	4.57	-	-	-	-	-	-
NP	Negative	278.0	-	-	-	-	-	-	-	-	-	1776.5	4.62	4.29	12.21	9.13	-	-	-	-	-	4.03
NP+Asth	Negative	114.0	-	-	-	-	-	-	-	-	-	957.4	6.66	6.22	11.68	9.39	4.59	-	-	-	-	5.94
NP	Negative	887.0	-	-	-	-	-	-	-	-	-	1455.0	3.91	4.02	6.95	5.21	4.85	-	-	-	-	4.91
NP	Negative	265.0	-	-	-	-	-	-	-	-	-	1233.7	7.13	5.92	13.71	9.65	4.61	11.19	12.17	6.03	18.97	7.46
NP	hdm	163.5	-	3.11	-	-	-	-	-	-	-	322.9	nd	18.85	4.30	4.30	-	-	-	-	-	-
NP	hdm	130.0	-	1.72	-	-	-	-	-	-	-	620.4	4.50	6.92	7.03	5.93	-	-	-	-	-	-
NP	gx, tx	273.0	1.51	-	0.92	3.39	-	-	-	-	-	718.1	5.25	nd	9.51	10.71	-	-	-	-	-	-
NP	hdm, gx	1310.0	0.59	34.70	-	-	-	-	-	-	-	688.3	4.56	111.81	4.34	3.91	-	-	-	-	-	-
APA	hdm	402.5	-	0.38	-	-	-	-	-	-	-	1073.7	4.69	4.14	6.43	4.25	-	-	-	-	-	-
NP	gx	64.8	4.71	-	-	-	-	-	-	-	-	514.7	37.10	6.59	14.71	11.19	5.38	-	-	-	-	6.80
NP	hdm, tx	815.0	-	1.17	-	26.60	0.39	0.40	-	-	0.84	1686.8	6.16	7.92	10.46	23.99	4.51	-	-	-	-	4.18
NP + Asth	gx	37.5	0.54	-	-	-	-	-	-	-	-	1348.2	9.84	6.34	nd	nd	4.05	-	-	3.94	-	-
NP + Asth	hdm	38.5	-	0.38	-	-	-	-	-	-	-	365.2	43.01	-	5.72	-	-	8.14	7.70	4.40	-	-
NP + Asth	hdm, gx	331.0	0.46	18.10	-	-	-	-	-	-	-	1066.5	5.78	45.91	12.54	10.47	7.96	4.69	-	-	6.22	14.18
APA	hdm, gx	615.0	5.20	3.06	-	-	-	-	-	-	-	2500.0	66.81	11.21	12.40	10.23	-	-	4.24	-	4.46	-
APA	hdm, gx	217.0	1.38	6.35	-	0.97	-	-	-	-	-	1577.7	7.65	15.75	nd	nd	4.77	-	-	8.43	6.65	-

NP: nasal polyps; NP + Asth: nasal polyps with concomitant asthma; APA: aspirin hypersensitivity, polyps and asthma syndrome; hdm: house dust mite; gx: grass mix; hx: house dust mite mix; mx: moulds mix; tx: trees mix; nd: not done; -: below detection level.

overnight at 4 °C. The sections were rinsed with TBS and incubated with streptavidine-conjugated peroxidase (Dako) for 30 min. After rinsing with TBS, sections were incubated with AEC chromogen (Dako) for 10 min rinsed, counterstained with haematoxylin and mounted.

Statistical analysis

Data are expressed as median and interquartile range (IQR). When comparisons were made between groups, significance of between-group variability was first established using the Kruskal–Wallis test. The Mann–Whitney *U*-test was then used for between-group comparison. Spearman rank correlation coefficient (*r*) was used to assess the relationship between the parameters. The chi-square test was used for comparison of frequencies. *P*-values <0.05 were considered to be statistically significant.

Results

Nasal culture from the middle meatus demonstrated staphylococcal colonization in 17/24 NP patients and in three of 12 controls (*P* = 0.02). Table 1 demonstrates a significant increase in concentrations of IgE, albumin, and eosinophil counts in nasal polyp tissue compared with control tissue. Total IgE and IgE antibody concentrations were in all cases higher in tissue compared with serum. The IgE/albumin ratios in polyp tissue and in serum were dissociated, indicating that tissue IgE is rather the result of a local IgE production than of extravasation. Furthermore, IgE antibodies in polyp tissue only showed a partial relation to IgE antibody in serum and to skin prick tests (Table 2). In a subgroup of 16 patients, a particular pattern of IgE expression was found in NP tissue. This polyclonal type of IgE expression is characterized by IgE antibodies to all measured common aeroallergens and is associated with the highest levels of IgE antibodies in NP tissue. IgE specific to SAE could be demonstrated in 12 of 24 NP tissue homogenates and in three of 24 serum samples of NP subjects, but in none of the controls. Eosinophil counts and IgE concentrations were significantly higher in those NP positive for IgE antibodies to SAEs than in SAE-negative samples (*P* < 0.001) (Fig. 1).

Follicular structures were found in six of 24 NP samples, whereas diffuse lymphoid accumulations were found in all NP samples (Fig. 2). Follicular structures stained positive for IgE, CD3, CD20, and CD23, whereas FcεRI was found only outside the follicle (Fig. 3). Plasma cells expressing CD38 were absent from the inner area of the follicular structures, but prominent in the diffuse lymphoid accumulations. The latter stained positive for IgE, CD3, CD20 and, FcεRI, but not for CD23 (Fig. 3). SAE binding was positive in follicular structures and in some areas with lymphoid accumulations. The specificity of the SAE binding was confirmed by staining with an excess of non-biotinylated SEA to biotinylated SEA, which completely blocked the signal. Follicular structures or SAE staining were not found in control tissue.

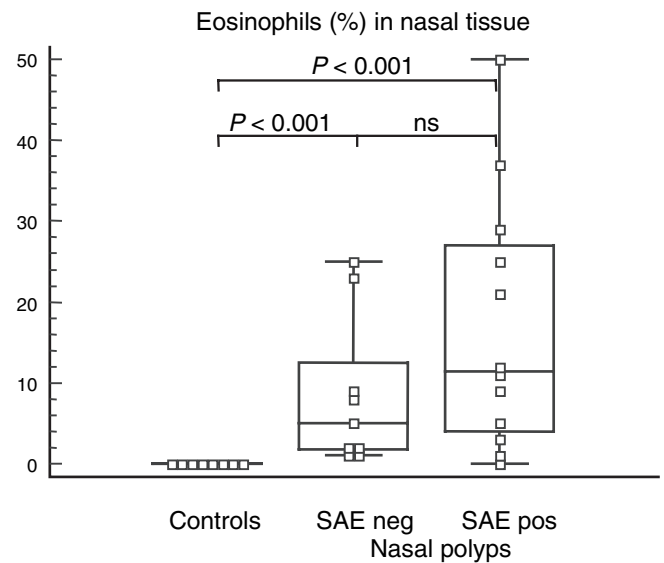
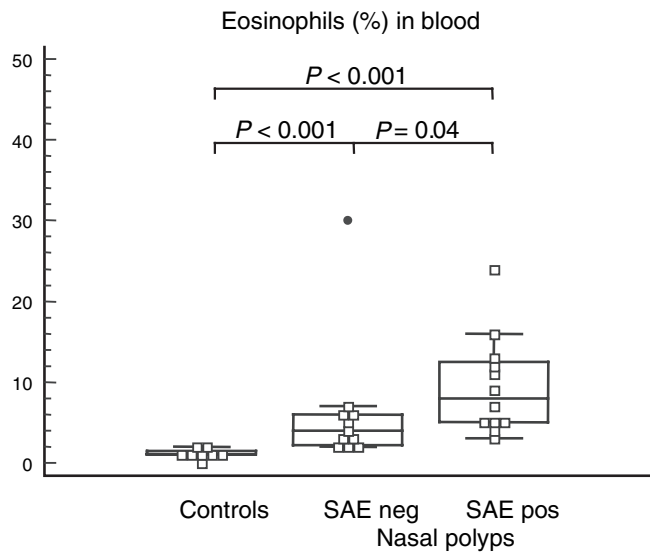
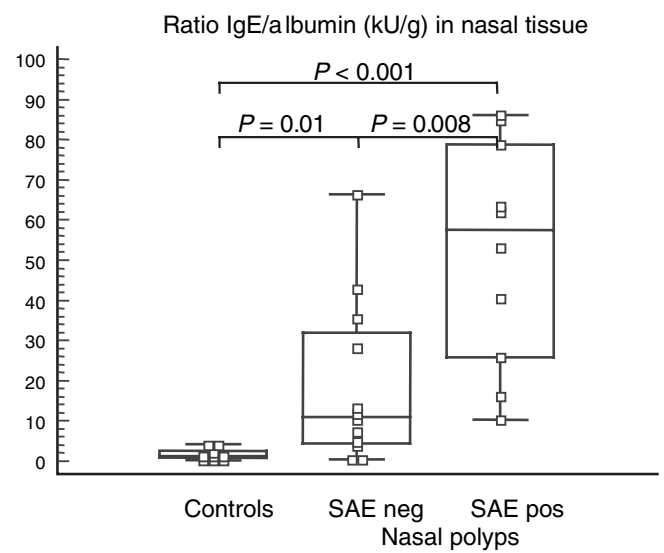
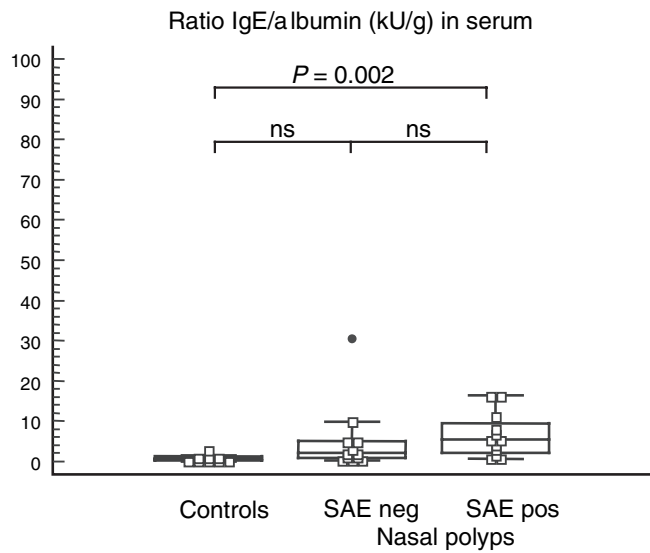
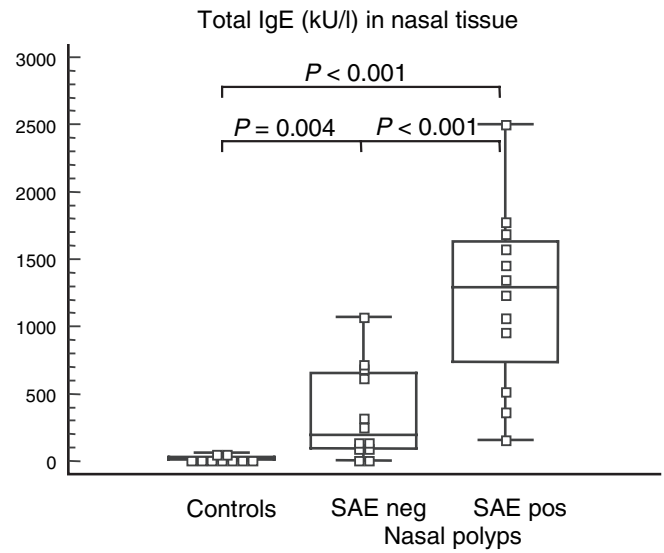
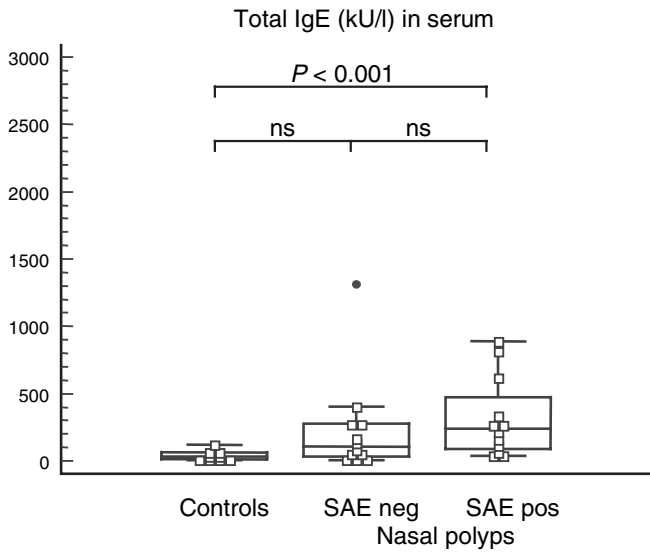
Discussion

We demonstrated the organization of secondary lymphoid tissue in NP tissue and a polyclonal hyper-immunoglobulinemia E associated with the presence of IgE specific to SAEs, colonization with *S. aureus*, and increased eosinophilic inflammation in a relevant subgroup of NP patients. Furthermore, the dissociation in IgE levels and IgE antibodies between NP tissue and serum suggests that IgE in NP are mainly because of a local production. We demonstrated binding of biotinylated SAE to follicular structures and in lymphoid accumulations in NP tissue. The follicular structures were mainly characterized by an accumulation of B cells and T cells, whereas lymphoid accumulations showed diffuse plasma cell infiltration. These data suggest an organization of secondary lymphoid tissue with polyclonal B-cell activation in nasal polyps because of chronic microbial colonization and release of enterotoxins, which is likely to be the cause of IgE switch and formation.

The nasal vestibule is the ecologic reservoir for *S. aureus*, with about 25% of the population being permanent carriers (14). A large proportion of the population (approximately 60%) harbours *S. aureus* intermittently, and the strains change with varying frequency (14). Notably, only a minority of people (approximately 20%) almost never carry *S. aureus* (14). The differences could be due to host factors and/or to competition between members of the nasal flora (e.g. *Corynebacterium* spp.) (15). Indeed, a higher incidence of *S. aureus* colonization of skin or mucous membranes may be due to injuries, abnormal leucocyte function and inflammation (e.g. atopic dermatitis), viral infections (e.g. influenza), metabolic abnormalities (e.g. diabetes mellitus and uraemia) and miscellaneous other conditions (e.g. malnutrition, old age, malignancies, etc). Whereas nearly all strains of *S. aureus* produce enzymes and haemolysins that contribute to their pathogenicity, it has been generally accepted that only some strains produce superantigens. However, recent data using multiplex polymerase chain reaction (PCR)-DNA enzyme immunoassays and considering both, the newly described SAE genes (*SEG*, *SEH*, *SEI*, and *SEJ*) and the classical toxins (*SEA*, *SEB*, *SEC*, *SED*, *SEE* and *TSST*), demonstrated that the overall rate of toxin gene-positive isolates reached 73.0%. More than half of the isolates (55.0%) harboured the combination of *SEG* and *SEI*, whereas other toxin genes amplified were *TSST* (20.3%), *SEA* (15.9%), and *SEC*

Figure 1. Comparison of total IgE concentrations, IgE/albumin ratio's and eosinophil percentages in serum and nasal tissue of controls, NP patients without or with IgE antibodies to SAE (NP + SE). The Box-and-whisker plot represents the median and the 10th, 25th, 75th, 90th percentile. Statistical analyses performed by the Mann–Whitney *U*-test (NS = not significant).

Local IgE formation in nasal polyp tissue



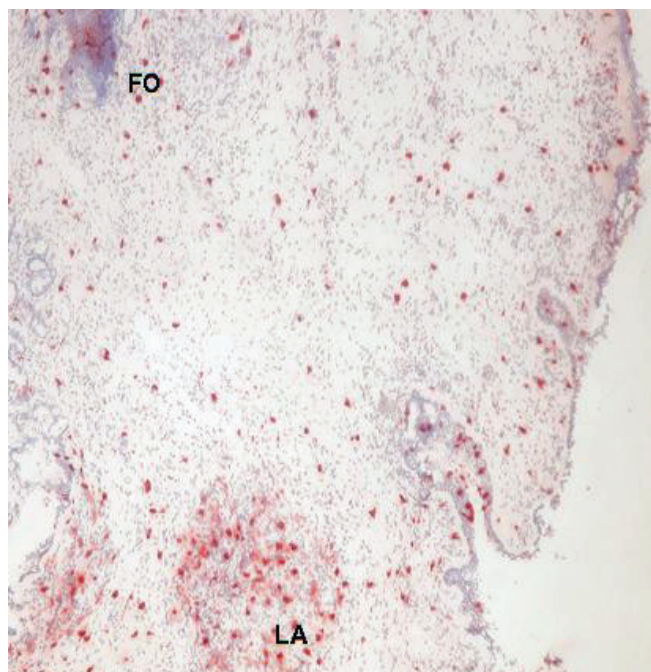


Figure 2. Nasal polyp tissue stained for IgE demonstrating a follicular structure (FO) and lymphoid accumulations (LA) ($\times 10$).

(11.2%) (15). In the present study, we demonstrate a significantly higher incidence of nasal colonization with *S. aureus* in NP patients (71%) than in controls (25%). However, only in 50% of polyp tissues, IgE antibodies to classical SAE were detectable. These data probably underestimate the impact of enterotoxins, as contact to only the classical, but not to the newly described enterotoxins was studied because of the current lack of validated tests for IgE antibodies to the latter.

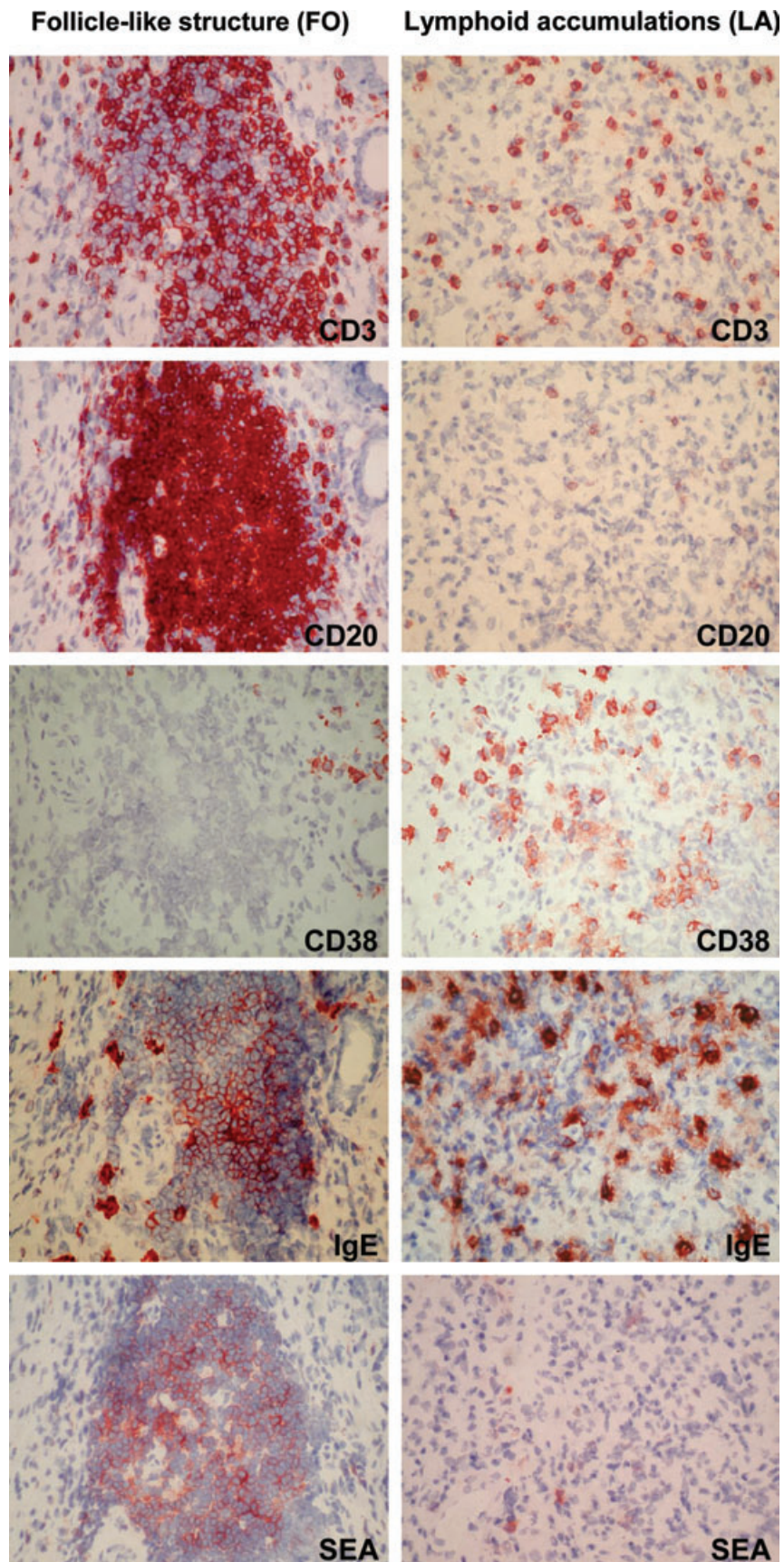
Detailed analysis of Table 2 reveals two patterns of IgE expression: 'the allergic type' and 'the polyclonal type' that can be found either isolated or combined. The 'allergic' type of IgE expression is characterized by increased concentrations of total IgE and IgE antibody specificities in nasal tissue that correspond those in serum and to the skin-prick test results. In clear 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 pattern is associated with a hyper-immunoglobulinemia and only a small fraction of the total can be explained by IgE antibodies. Polyclonal expression was described in 16 of 24 NP tissues and was associated with IgE antibodies to SAEs in 12 cases, indicating that other than the classic enterotoxins might have acted as superantigens (most probably from *S. aureus*, but possibly also from other micro-organisms). Furthermore, 12 of the 16 polyp patients showed a combined expression pattern,

with the polyclonal type of IgE expression in addition to an existing allergy.

The earliest quantitative studies on humoral responses to antigenic stimuli showed that the amount of measurable specific antibody produced often only represented a small fraction of the newly synthesized total (13). Parasites, viruses, fungi and bacteria can obliterate specific immune responses by triggering the machinery of polyclonal lymphocyte responses, thus resulting in a lack of specificity of antibodies or T-cell responses to the microbial agents (16). There is convincing evidence that cytokines released from activated T cells may substitute non-specifically for T-helper (TH) cells in stimulating B cells (17). Two types of polyclonal stimuli exist that can trigger B-lymphocyte proliferation and differentiation in the absence of antigen: (i) those derived from microbial products, such as lipopolysaccharide or unmethylated single-stranded DNA motifs (CpG oligonucleotides), which stimulate B cells; and (ii) T cells activated by a third-party antigen, which stimulate B cells in a non-cognate fashion via CD40 ligand (18) and cytokine production, referred to as bystander help. Recent data indicated that memory B cells have two response modes. In the antigen-dependent mode, they undergo a massive expansion and differentiation towards short-lived plasma cells (19, 20). In contrast, in the polyclonal mode, all memory B cells respond to microbial stimuli by undergoing continuous proliferation and differentiation. In this way, a constant level of plasma cells and immunoglobulin in serum could theoretically be maintained throughout a human life-span (21). Because this mechanism is non-specific, it would act indiscriminately to maintain the broad spectrum of antibody specificities generated during the antigen-driven immune response as well as non-antibody active immunoglobulin molecules. The protection is relative to a particular pathogen or toxin and may be mediated by preformed antibodies, by rapid secondary responses or by a combination of both (19, 20). However, in the case of powerful bacterial toxins such as superantigens, protection relies entirely on pre-existing neutralizing antibodies as there is no time to mount a secondary response to achieve protection.

The high IgE concentrations in polyp tissue could be preferentially derived from the serum, as an extravasation and deposition of serum proteins in polyp tissue has been shown (1). However, IgE has been found in NP tissue enriched over serum suggesting that IgE synthesis occurs locally in the mucosa. In mucosal tissues of allergic rhinitis and asthma patients, mRNA for the ϵ -chain of IgE was associated with a significant proportion of B cells by using *in situ* hybridization (22–24). This supports the hypothesis of local IgE synthesis as

Figure 3. Follicular structures and lymphoid accumulations in nasal polyp tissue are stained for CD3, CD20, CD38, IgE, and SAE binding ($\times 40$).



mRNA expression would be required for synthesis and secretion of IgE. Using immunohistochemical methods, different research groups have reported varying success in visualizing IgE protein associated with B cells in nasal mucosa. Recently, however, Kleinjan et al. (25) visualized IgE protein in association with both B-cells and plasma-cells in the nasal mucosa of patients with allergic rhinitis. In our present study we describe two prominent IgE positive areas: first, the follicular structures, which are characterized by an organization of T cells (CD3) and B cells (CD20), found in six of 24 NP samples; secondly, in all polyps we found diffuse lymphoid accumulations that contain T cells (CD3) and plasma cells (CD38). B-cell foci have been described before in nasal polyp tissue (26), associated with a reduced expression of activin A in those areas, which points to a reduced suppression of plasma cells development (26, 27). There is increasing evidence that SAEs can directly affect the frequency and activation of the B-cell repertoire. Studies with TSST-1 have shown that this staphylococcal superantigen may play an important role in the modulation of allergic disease, since it can augment isotype switching and synthesis of IgE, both *in vitro* (18) and *in vivo*, in a SCID mouse model (28). Although TSST-1-induced activation of B cells *in vitro* is indirect and dependent on increased expression of CD40 ligand on T cells, a more recent study has also provided evidence for a direct effect by demonstrating TSST-1-induced expression on B cells of B7.2 (12), a molecule that has been shown to enhance Th2 responses and to be involved in IgE regulation.

An augmented local synthesis of IgE may, under appropriate circumstances, increase allergic reactivity, but when it is excessive, it has been suggested to actually suppress specific reactivity by saturation of Fcε receptors on mast cells through polyclonal IgE and/or an inhibition of specific antibody synthesis to environmental allergens by the polyclonal response (29). These mechanisms could explain why nasal symptoms and markers of nasal mucosal inflammation did not increase in relation to natural seasonal allergen exposure in highly ragweed-allergic patients with polyps (30) and why nasal provocation in nasal polyps is largely unsuccessful although elevated IgE levels are present (31, 32). However, the backside of this local polyclonal IgE production may be the permanent triggering of the IgE-mast cell-FcεRI cascade that maintains polyp growth (33).

Conclusion

We demonstrate the organization of secondary lymphoid tissue in nasal polyposis with polyclonal B-cell activation and hyper-immunoglobulinemia E associated with the presence of locally formed IgE antibodies to staphylococcal enterotoxins, and increased eosinophilic inflammation in a relevant subgroup of nasal polyp patients. The increased prevalence of colonization with *Staphylococcus aureus* and subsequent chronic microbial stimulation in NP may be a causative or disease modulating factor, being dependent upon a former damage of the mucosa by unrelated pathomechanisms.

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