

# Mucosal tissue polyclonal IgE is functional in response to allergen and SEB

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## Keywords

IgE; mast cells; nasal polyps; polyclonal; superantigens.

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## Abstract

**Background:** *Staphylococcus aureus* may modify airway disease by inducing local formation of polyclonal IgE antibodies (abs), the role of which is unknown.

**Methods:** Nasal mucosal tissue and serum was obtained from 12 allergic rhinitis (AR) and 14 nasal polyp (NP) subjects. Skin prick tests were performed, and total and specific IgE abs to inhalant allergens and enterotoxin B were determined in serum and tissue. Tissue fragments were stimulated with anti-IgE, enterotoxin B, or grass and house dust mite allergens in different concentrations for 30 min. RBL SX38 cells were sensitized with NP homogenates containing IgE and stimulated with grass pollen extracts.

**Results:** In AR patients, degranulation of tissue mast cells upon allergen exposure and presence of specific IgE to inhalant allergens corresponded in almost all cases. Total IgE concentrations in serum and mucosal tissue homogenates highly correlated. In contrast, in NP patients, reactivity of tissue mast cells upon allergen exposure and presence of specific IgE to inhalant allergens or *Staphylococcus aureus* enterotoxin B corresponded for tissue, but not for serum. Total IgE was significantly higher in tissue compared to serum and failed to show correlation. Tissue IgE to grass pollen was functional to degranulate RBL cells.

**Conclusion:** We here demonstrate that mucosal IgE abs in NP tissue are functional and able to activate mast cells; specific IgE abs in NP tissue can be found independently of their presence in serum. We postulate that superantigen-induced polyclonal IgE in airway disease contributes to chronic inflammation by continuously activating mast cells.

Evidence is accumulating that *Staphylococcus aureus* (SA) plays an important role as disease modifiers in upper and lower airway disease, esp. nasal polyps (NP) and asthma, by releasing enterotoxins (*Staphylococcus aureus* enterotoxins (SAEs)) (1–5). *Staphylococcus aureus* enterotoxins have been shown to polyclonally activate T-cells, releasing preferentially

Th2 cytokines (6) and amplifying eosinophilic inflammation, and B-cells to induce polyclonal IgE and IgG/IgG4 production (7). The presence of SAE-IgE antibodies (abs) in the tissue is associated with high total IgE and eosinophil cationic protein (ECP) values; high IgE titers of up to 5000 kU/l can be found in SAE-IgE+ polyp tissue, as demonstrated earlier (1). Apart from an effect on T- and B-cells, other impact of SAEs on the local inflammation has been shown, such as the inhibition of T regulatory cells (8), the reduction of eosinophil apoptosis (9) and the induction of chemokines from epithelial cells (10). We recently could demonstrate that the impact of SAEs on the local immune reaction in NP tissue increases the risk of co-morbid asthma (11). Indirect evidence from the comparison of IgE levels in serum and

## Abbreviations

abs, antibodies; AR, allergic rhinitis; GP, grass pollen allergens; HDM, house dust mite allergens; IT, inferior turbinate; NIP, 4-hydroxy-3-iodo-5-nitrophenylacetate; NP, nasal polyps; PGD2, prostaglandin D2; SA, *Staphylococcus aureus*; SAE, *Staphylococcus aureus* enterotoxins; SEB, *Staphylococcus aureus* enterotoxin B; SPT, skin prick test; TCM, tissue culture medium.

tissue points to a local IgE formation; however, a truly local switch to IgE production in NP still needs to be established. Furthermore, the functionality of local IgE in NP has been discussed and has been questioned based on the failure of demonstrating seasonal symptoms and seasonal mediator release in patients with ragweed allergy and NP (12). However, *Staphylococcus aureus* enterotoxin B (SEB)-induced IgE abs may be functional, as elegantly demonstrated in human basophils by transfer experiments (13), SEB itself may act as an allergen, and polyclonal IgE directed to various inhalant allergens may be functional as continuous mast cell activators.

We therefore investigated the *ex vivo* degranulation of tissue mast cells upon allergen exposure in NP tissue from polyp subjects in comparison with inferior turbinates (IT) from allergic rhinitis (AR) patients to study the functionality of IgE abs. Total IgE, specific IgE abs to SAEs including SEB, and to grass pollen and house dust mite allergens (HDM) in serum and mucosal tissue of 12 AR patients and 14 NP subjects were measured to describe the status as either 'systemic atopic' or 'local polyclonal', based on the ratio of specific to total IgE and the tissue vs serum IgE concentrations. The mucosal tissues were stimulated with anti-IgE, and the allergens HDM, grass pollen allergens (GP) and SEB; mast cell activation was measured as prostaglandin D2 (PGD2) release, as described before (14). We furthermore tested the transferability of this IgE reactivity using RBL SX38 cells.

## Material and methods

### Patients

Nasal mucosal tissue and serum was obtained from 26 adult patients (12 AR patients and 14 NP subjects (mean age 46 years 17 men, nine women)). Nasal polyp tissue samples were obtained during routine endonasal sinus surgery, and IT tissue samples were obtained from allergic patients who were undergoing septoplasty because of anatomic variations at the ENT department, University Hospital Ghent, Belgium, scheduled for surgery unrelated to the study. The diagnosis of AR was based on the history (more than 2 years of typical symptoms) and on a positive skin prick test (SPT) to grass pollen and/or house dust mite; the diagnosis of NP was based on history, clinical examination, nasal endoscopy and CT scan of the sinuses. All patients fulfilled the criteria of bilateral NP (chronic rhinosinusitis with NP) according to the EP<sup>3</sup>OS guidelines (15); however, symptoms of AR and NP were not clearly distinguishable. All patients gave their written informed consent and the ethics committee of the Ghent University Hospital approved the study. All patients were asked not to use topical or oral corticosteroids 4 weeks prior to inclusion, and oral antihistamines for 48 h prior to SPT. No patient was currently on allergen immunotherapy or omalizumab treatment.

Total IgE and specific IgE abs to SAEs including SEB, and specific IgE abs to *Phleum pratense* (GP) and *Dermatophagoides pteronyssinus* (HDM) in serum and mucosal tissue [IT and polyp tissue (NP) homogenates] were mea-

sured using the CAP system (Phadiatop; Phadia, Uppsala, Sweden).

### Measurement of mediators in supernatants of SEB and allergen-stimulated tissue fragments

Mechanical disruption of human nasal tissue was performed as described before (6). The tissue fragments were stimulated with either tissue culture medium (TCM) (negative control), 10 µg/ml  $\epsilon$ -chain specific anti-human IgE antibody (Dako Belgium N.V., Heverlee, Belgium), 10 µM ionomycin (Calbiochem, VWR International, Leuven, Belgium), 0.5 µg/ml SEB (Sigma-Aldrich NV/SA, Bornem, Belgium) or the allergen extracts grass pollen [*Phleum pratense* (ALK Abello, Horsholm, Denmark)] and house dust mite [*Dermatophagoides pteronyssinus* (ALK Abello)] in different concentrations for 30 min. Concentrations of PGD2 were measured in tissue supernatants by ELISA (Cayman Chemicals, Ann Arbor, MI, USA) following the instructions of the manufacturer. The amount of endotoxin contamination in all solutions (allergen extracts, SEB, anti-IgE) was measured by the *Limulus ameobocyte* lysate assay (GenScript, Aachen, Germany) and was found to be below 0.04 EU/ml.

### Degranulation assay with IgE from NP tissue homogenates

The rat basophilic leukemia cells line RBL SX38 transfected to express the human high-affinity IgE receptor FcεRI was used as an *in vitro* model (16). Cells were seeded onto 96-well plates at a density of  $4 \times 10^5$  cells/well and cultured for 24 h. Cells were sensitized with NP tissue homogenates from patients or control antibody (NIP-IgE) at 200 ng/ml overnight (17). Sensitized cells were stimulated with grass pollen extracts (1000SQ-U/ml, *Phleum pratense*; ALK Abello) or control antigens (NIP-BSA). Tissue homogenates of three different groups of NP patients was used in this assay: (i) tissue homogenates of patients without tissue IgE to grass pollen and with negative SPT to grass pollen; (ii) tissue homogenates of patients with increased tissue IgE to grass pollen and with negative SPT to grass pollen; and (iii) tissue homogenates of patients with tissue IgE to grass pollen and with positive SPT to grass pollen.

Cell degranulation was detected by measuring beta-hexosaminidase release. This was assayed for by following the breakdown of the fluorogenic substrate, 4-methylumbelliferyl-*N*-acetyl-beta-D-glucosaminide. Fluorescence was measured by exciting the enzymatic product at a wavelength of 360 nm and detecting emission at 450 nm. Results were expressed as a percentage of degranulation compared to exposure to anti-IgE (20 µg/ml).

### Statistical analysis

Further nonparametric statistical analysis was performed using the Mann-Whitney *U* two-tailed test for unpaired comparisons when comparing the median of concentrations between two groups. Association between a categorical variable and groups was investigated via Fishers' exact test. The significance level was specified as  $\alpha = 0.05$ .

**Table 1** The results for 12 allergic rhinitis patients. House dust mite (HDM) and grass pollen (GP) specific IgE antibodies (abs) and total IgE values are given for serum and corresponding homogenized inferior turbinate tissue samples; no patient was SAE-IgE positive in serum or tissue. Furthermore, skin prick test (SPT) results and allergens that caused tissue mast cell degranulation, defined as an increase of prostaglandin D2 release of at least 100% upon allergen exposure vs controls, are listed. The percentage of specific IgE abs for GP and HDM from total IgE abs in serum and tissue has been calculated. Detection levels are 0.1 kU/l for serum, 0.35 kU/l for tissue homogenates

Inferior turbinate sample numbers	Mast cell degranulation upon exposure to	SPT	Serum (kU/l)				Homogenates (kU/l)				
			IgE	d1-sIgE	g6-sIgE	% sIgE of tIgE	IgE	SAE-IgE	d1-sIgE	g6-sIgE	% sIgE of tIgE
1	HDM	HDM	85	5.6	BDL	6.6	43	BDL	7.4	BDL	17.1
2	HDM	HDM	46	4.5	BDL	9.7	155	BDL	22.8	BDL	14.7
3	HDM and grass	HDM and grass	1400	34.7	96.1	9.3	1111	BDL	28.3	74.3	9.2
4	HDM	HDM	181	3.2	BDL	1.8	448	BDL	11.8	BDL	2.6
5	Grass	Grass	61	BDL	4.4	7.2	44	BDL	BDL	4.1	9.3
6	Grass	Grass	166	2.0	56.1	35	474	BDL	BDL	199.5	42
7	Grass	Grass	20	BDL	8.3	41.9	147	BDL	BDL	70.0	47.6
8	Grass	Grass	91	BDL	11.2	12.3	30	BDL	BDL	4.5	14.8
9	Grass	Grass	72	BDL	3.0	4.2	32	BDL	1.5	3.2	14.7
10	Grass	Grass	200	BDL	1.9	1	218	BDL	BDL	5.3	2.4
11	HDM and grass	HDM and grass	111	2.1	BDL	1.9	160	BDL	37.1	23.3	37.8
12	HDM	HDM	120	8.3	0.3	7.2	45	BDL	5.4	BDL	11.9

SAE, *Staphylococcus aureus* enterotoxins.

## Results

### AR patients

In Table 1, the results for HDM- and GP-specific IgE abs and total IgE values are given for serum and corresponding homogenized IT tissue samples of 12 AR patients; no patient was SAE-IgE positive in serum or tissue. Furthermore, allergens that caused tissue mast cell degranulation, defined as an increase of PGD2 release of at least 100% upon allergen exposure vs control, are listed. Reactivity of tissue mast cells upon allergen exposure and presence of specific IgE abs to the allergens *Phleum pratense* and/or *Dermatophagoides pteronyssinus* corresponded in 23/24 cases for tissue specific IgE abs (in one case, HDM-specific IgE abs in the tissue did not induce mast cell degranulation upon allergen exposure) and in 21/24 cases for serum IgE abs (in one case, no allergen-specific IgE abs could be detected in the serum, although it was present in the tissue and resulted in mast cell reactivity; in two cases, specific IgE abs in the serum were not mirrored in the tissue, and no mast cell reactivity was found).

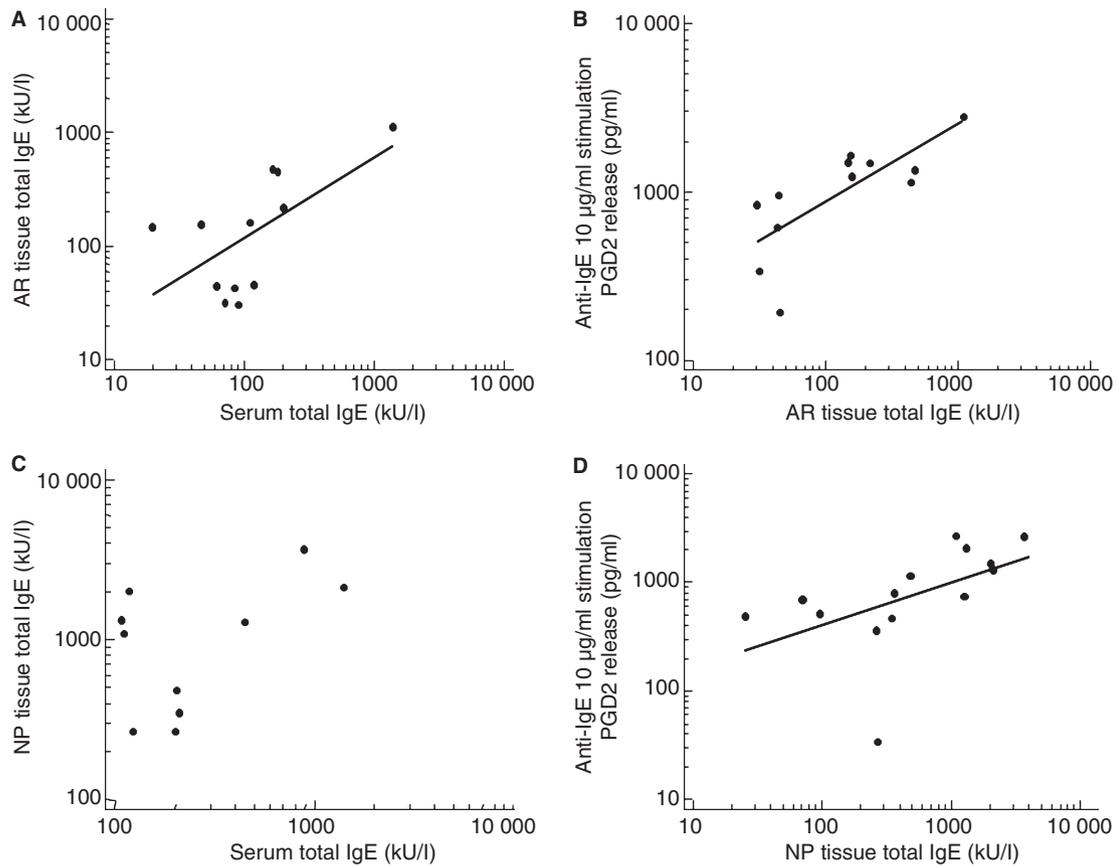
In AR patients, total IgE in serum [101 (66.2–173.5)] and IT tissue homogenates [151 (43.7–332.8)] highly correlated (Fig. 1A;  $r = 0.91$ ,  $P < 0.0001$ ). Tissue total IgE also significantly correlated to the release of PGD2 upon anti-IgE exposure ( $r = 0.82$ ,  $P < 0.001$ ); a tissue total IgE of 200 kU/l corresponded to a PGD2-release of 1095 pg/ml ( $y = 736.1734 + 1.7942 x$ ) upon challenge with 10 µg/ml anti-IgE (Fig. 1B). Of the total IgE, 14.7% (9.3–27.5%) in tissue and 7.2% (3.1–11%) in serum could be explained by specific IgE abs to the two tested inhalant allergens, GP and HDM.

Allergen stimulation with different concentrations of grass pollens led to the release of PGD2 in a Gaussian distribution, with a magnitude of release upon 1000 SQ-U/ml app. corresponding to that upon anti-IgE at a concentration of 10 µg/ml (Fig. 2A).

### NP patients

Table 2 lists HDM, GP- and SAE-specific IgE abs and total IgE values for serum and corresponding polyp tissue samples of 14 NP patients. Allergens causing tissue mast cell degranulation, defined as earlier, are also listed. Tissue mast cell reactivity in six patients (nr 13–18) was found to SEB, GP and HDM, corresponding to the specific IgE abs in the NP tissue; however, specific IgE abs could only be found in a third of cases in serum in those patients. Total IgE concentrations were significantly higher in tissue compared to serum in all these patients [141 (111–210) vs 421 (264–1311) kU/l,  $P = 0.0004$ ]. A group of four patients demonstrated specific IgE abs in both, serum and tissue (nr 19–22); tissue mast cell reactivity in these patients corresponded in 12/12 cases to the specific IgE abs in the NP tissue and in 10/12 cases to the specific IgE abs in serum, resembling the situation in AR patients. A last group of four patients (nr 23–26) did not have any specific IgE abs in serum or tissue, and total IgE was only moderately increased in serum and tissue.

In the relevant NP patients with at least one specific IgE ab ( $n = 10$ ), total IgE in serum [141 (111–210)] and tissue [420.8 (264–1311) kU/l] did not show a correlation (Fig. 1C;  $r = 0.4948$ ,  $P = 0.1460$ ); specific IgE abs to *Phleum pratense*



**Figure 1** (A) In allergic rhinitis patients, total IgE in serum and inferior turbinate tissue homogenates highly correlated ( $r = 0.91$ ,  $P < 0.0001$ ); (B) Tissue total IgE also significantly correlated to the release of prostaglandin D2 (PGD2) upon anti-IgE exposure ( $r = 0.82$ ,  $P < 0.001$ ). A tissue total IgE value of 200 kU/l corresponded to a PGD2-release of 1095 pg/ml ( $y = 736.1734 + 1.7942x$ ) upon challenge with 10 g/ml anti-IgE; (C) In the relevant nasal polyp

patients with at least one specific IgE ab ( $n = 10$ ), total IgE in serum [141 (111–210)] and tissue [420.8 (264–1311)] did not show a correlation ( $r = 0.4948$ ,  $P = 0.1460$ ); (D) Tissue total IgE significantly correlated to the release of PGD2 upon anti-IgE exposure ( $r = 0.74$ ,  $P < 0.005$ ); a tissue total IgE of 200 kU/l corresponded to a PGD2-release of 656 pg/ml ( $y = 537.5736 + 0.5920x$ ) upon challenge with 10 g/ml anti-IgE.

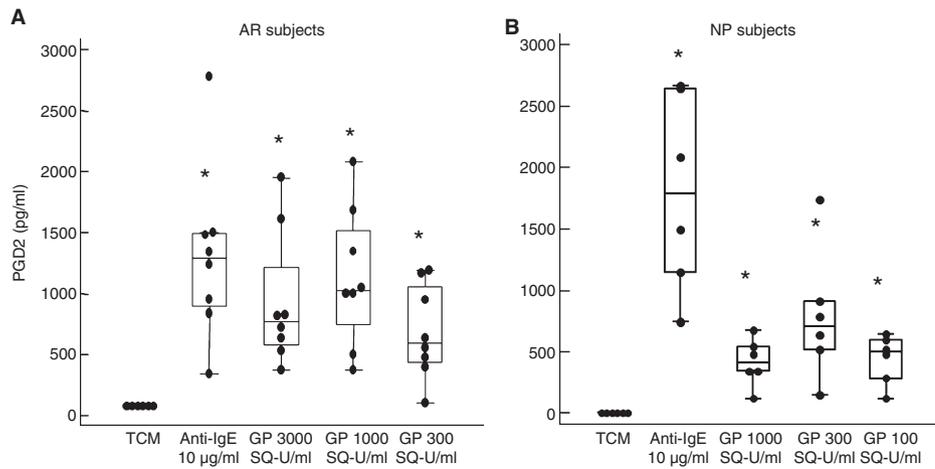
and *Dermatophagoideis pteronyssinus* in tissue and serum did not correlate either (data not shown). We observed a significant difference in the contribution of specific IgE abs to the total IgE concentrations (Fig. 3); compared to AR subjects, the percentage of specific IgE abs to GP and HDM out of the total IgE was significantly lower in NP tissue with 2.8% (0.5–6.4%;  $P < 0.005$ ) (Fig. 3A) and in serum with 0.5% (0.1–8.1%,  $P < 0.03$ ) (Fig. 3B).

Tissue total IgE significantly correlated to the release of PGD2 upon anti-IgE exposure ( $r = 0.74$ ,  $P < 0.005$ ); a tissue total IgE of 200 kU/l corresponded to a PGD2-release of 656 pg/ml ( $y = 537.5736 + 0.5920x$ ) upon challenge with 10 µg/ml anti-IgE (Fig. 1D), without statistical difference to the release in AR patients. Again, stimulation with different allergen concentrations led to the release of PGD2 in a Gaussian distribution for GP. The range of PGD2 release in patients ( $n = 6$ ) with IgE abs to grass pollen in tissue, but not in serum, peaked at 300 SQ-U/ml, and only represented

app. a third of the PGD2 release upon anti-IgE (Fig. 2B). The stimulation with SEB ( $n = 8$ ) also resulted in a relevant PGD2 release in patients with SEB-IgE abs in the polyp tissue [median interquartile range (IQR) 536.3 (185.1–888.8)].

#### IgE from NP tissue homogenates mediates basophil degranulation

Tissue homogenates containing specific IgE to grass pollen-mediated RBL cell degranulation after stimulation with grass pollen irrespective of the patient's SPT negativity or positivity. Thus, local tissue IgE to grass pollen is functional, with a magnitude of response of app. 10% compared to anti-IgE stimulation (set as 100% degranulation) or 12% compared to NIP-IgE + NIP-BSA stimulation. Tissue homogenates with increased IgE but with no IgE to grass pollen could not induce any RBL cell degranulation after stimulation with grass pollen (Fig. 4).



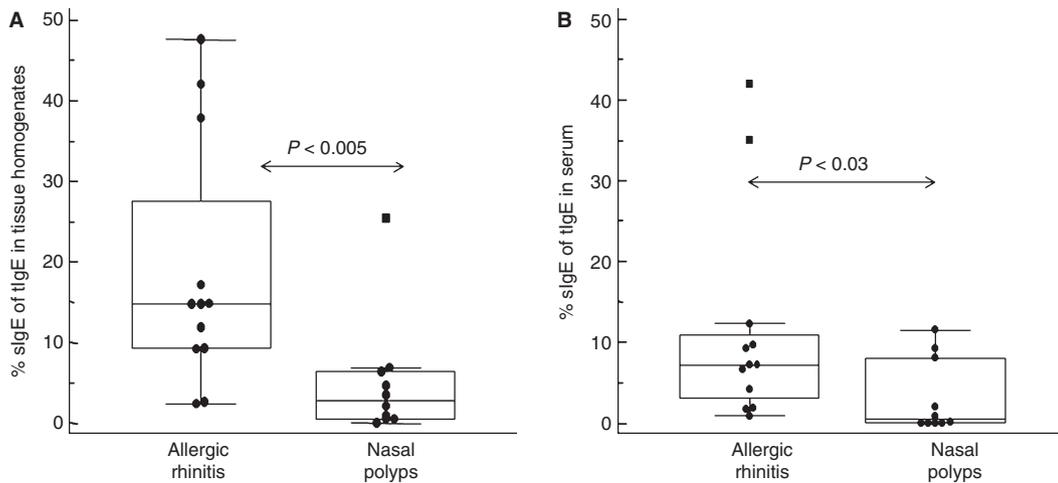
**Figure 2** (A) Allergen stimulation of allergic rhinitis tissue samples ( $n = 8$ ) with different concentrations of grass pollen (GP) led to the release of prostaglandin D2 (PGD2) in a Gaussian distribution, with a magnitude of release upon 1000 SQ-U/ml app. corresponding to that upon anti-IgE at a concentration of 10 g/ml.  $*P < 0.01$

vs tissue culture medium (TCM); (B) The range of PGD2 release in nasal polyp patients ( $n = 6$ ) with IgE-antibodies to GP in tissue, but not in serum, peaked at 300 SQ-U/ml, and only represented app. a third of the PGD2 release upon anti-IgE.  $*P < 0.01$  vs TCM.

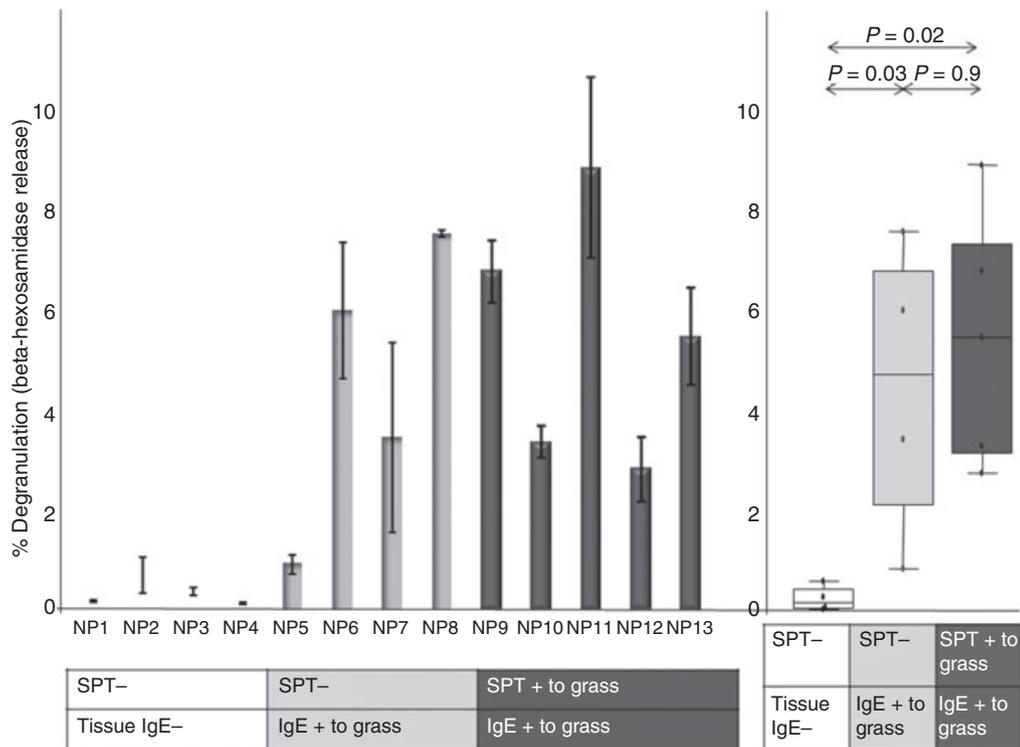
**Table 2** The results for 16 nasal polyp (NP) patients. House dust mite allergens (HDM), grass pollen allergens (GP) and SEB specific IgE antibodies (abs) and total IgE values are given for serum and corresponding homogenized polyp tissue samples. Furthermore, skin prick test (SPT) results and allergens that caused tissue mast cell degranulation, defined as a minimum increase of prostaglandin D2 release of at least 100% upon allergen exposure vs controls, are listed. The percentage of specific IgE abs for GP and HDM from total IgE abs in serum and tissue has been calculated. Patients nr 13–18 demonstrate a local polyclonal pattern; nr 19–22 a systemic atopic pattern, and nr 23–26 do not show IgE specificities

NP sample numbers	Mast cell degranulation upon exposure to	SPT	Serum (kU/l)					Homogenates (kU/l)				
			IgE	SAE-IgE	d1-sIgE	g6-sIgE	% sIgE of tIgE	IgE	SAE-IgE	d1-sIgE	g6-sIgE	% sIgE of tIgE
13	HDM and grass and SEB	Grass and HDM	111	BDL	2.1	BDL	1.9	1090	3.9	200	76.5	25.4
14	HDM and grass and SEB	None	203	BDL	BDL	BDL	0	481	6.38	11.8	4.8	3.8
15	HDM and grass and SEB	HDM	108	BDL	8.7	BDL	8.1	1311	2.4	23	4	2.1
16	HDM and grass and SEB	HDM	447	0.7	0.6	BDL	0.1	1275	7.2	5.4	5.8	0.9
17	HDM and grass and SEB	None	117	BDL	BDL	BDL	0	2003	6.9	4.6	4.5	0.5
18	HDM and grass and SEB	HDM	891	3.96	0.7	BDL	0.1	3663	21.5	9	8.1	0.5
19	Grass and SEB	Grass	200	0.5	BDL	1.9	0.9	264	5.2	BDL	16.8	6.4
20	SEB	None	210	3.6	BDL	BDL	0	349	31.7	BDL	BDL	0
21	HDM and grass	HDM and grass	1400	0.4	34.7	96.1	9.3	2107	BDL	31.1	66	4.6
22	HDM	HDM	123	0.6	14.2	BDL	11.6	268	BDL	18.3	BDL	6.0
23	HDM	None	142	BDL	BDL	BDL	0	361	BDL	BDL	BDL	0
24	None	None	19	BDL	BDL	BDL	0	25	BDL	BDL	BDL	0
25	None	None	154	BDL	BDL	BDL	0	96	BDL	BDL	BDL	0
26	None	None	14	BDL	BDL	BDL	0	71	BDL	BDL	BDL	0

SAE, *Staphylococcus aureus* enterotoxins; SEB, *Staphylococcus aureus* enterotoxin B.



**Figure 3** Compared to allergic rhinitis subjects, the percentage of specific IgE antibodies to GP and house dust mite allergens out of the total IgE values was significantly lower (A) in nasal polyp tissue with 2.8% (0.5–6.4%;  $P < 0.005$ ) and (B) in serum with 0.5% (0.1–8.1%,  $P < 0.03$ ) vs 14.7% (9.3–27.5%) and 7.2% (3.1–11%), respectively.



**Figure 4** Tissue supernatants from nasal polyp patients containing IgE antibodies (abs) to grass pollen could mediate RBL SX38 cell degranulation after stimulation with grass allergen independent of the presence or absence of skin prick test (SPT) reaction to grass pollen. Supernatants were derived from patients with negative SPT and no GP-IgE abs in the tissue (white), with negative SPT and GP-IgE abs in the tissue (light gray), and from patients who were SPT positive and had GP-IgE abs in the tissue (dark gray).

**Discussion**

We here for the first time demonstrate that mucosal tissue polyclonal IgE abs, here from NP, are functional and able to activate mast cells *ex vivo* upon allergen challenge; apart

from typical inhalant allergens, also SEB may serve as an allergen, and also serves as an indicator for superantigen impact on the mucosal inflammation. Specific IgE abs in NP tissue constitute a significantly smaller fraction of the total IgE than in IT of AR subjects, reflecting the polyclonal

character, and may be found in significantly higher concentrations compared to, and independently of, their presence in the serum. Mucosal polyclonal IgE abs may induce mast cell degranulation to numerous inhalant allergens, and we therefore postulate that polyclonal IgE abs in airway disease contribute to the persistent inflammation by continuously activating mast cells.

Whereas in AR, allergen in an optimal concentration leads to a release of PGD2 comparable to anti-IgE, the relative release of PGD2 in NP upon allergen exposure is smaller and reaches its maximum at a lower allergen concentration. These differences may be because of the distribution of specific IgE abs on the mast cell surfaces in the atopic vs the polyclonal situation. We may expect that the chance for IgE receptor bridging on the mast cell membrane is diminished with the polyclonal IgE, although not blocked. Other effects such as the activation status of the mast cells may also impact the releasability, as discussed before (14); however, these findings need further study.

Whereas total and allergen-specific IgE concentrations in IT of AR patients correspond to those in serum, and predict equally well reactivity of tissue mast cells to inhalant allergen exposure, total and allergen-specific IgE in NP tissue may be unrelated to that in the serum. We here identified a group of patients with NP with a total IgE greater 100 kU/l, but without detectable IgE abs to grass pollen in the serum; those patients were likely to demonstrate polyclonal IgE formation within the polyp tissue. In fact, total IgE concentrations in SAE-IgE positive polyp samples reached significantly higher total IgE levels compared to the serum of the same patient, which was different from the situation in AR patients. Furthermore, specific IgE abs in polyp patients only constituted a fraction of the total mucosal tissue IgE compared to AR subjects (14.7 vs 2.8%,  $P < 0.005$ ), which also was reflected in the serum of those patients (7.2 vs 0.6%,  $P = 0.03$ ). In a former publication (18), we detailed specific IgE abs to four allergen mixtures (grass pollen, house dust mites, molds and trees, comprising 20 allergens) and additionally to six SAE allergens in a group of 16 NP patients; we here recalculated the specific IgE fractions to 26 allergens of the total IgE and found values of 4.15% (IQR 2.35–9.3) in tissue and 1.9% (IQR 0.48–3.45) in serum. This allows assuming that, to explain 100% of total IgE abs, one would need several hundred allergen-specific IgE abs. We therefore suggest that SA-derived superantigens induce a polyclonal IgE formation to multiple inhalant and noninhalant allergens, driven by the abundant activation of T- and B-cells. SAEs such as SEB themselves also may serve as allergens in this case and may thus contribute to the continuous mast cell degranulation in NP.

It has been speculated that such polyclonal IgE may be nonfunctional or may even prevent bridging of IgE molecules by the fact that so many different IgE specificities on the mast cell surface compete for the IgE-binding sites of an allergen (19). However, passive sensitization experiments with basophils required ratios of polyclonal to antigen-specific IgE of greater than 500 : 1 to suppress basophil histamine release. Furthermore, polyclonal human IgE abs in sera of atopic dermatitis patients, via the high-affinity IgE receptor,

enhanced survival and cytokine production in mast cells and may amplify the allergic response (20). Freshly isolated basophils from atopic dermatitis patients released up to 59% of total histamine in response to staphylococcal enterotoxins but only with toxins to which patients had specific IgE (13). Stripped basophils sensitized with sera of atopic dermatitis patients containing IgE to toxin released histamine from basophils only when exposed to the relevant toxin. Sensitization of basophils with sera lacking IgE antitoxin did not result in release of histamine to any of the toxins tested.

Here, we show that allergen stimulation of NP tissue with different concentrations led to the release of PGD2 from mast cells following a Gaussian distribution; SEB, in tissue SAE-IgE positive samples, also was able to degranulate the mast cells effectively. These findings suggest that not only SEB can act as an allergen itself in NP, but that SA superantigens can induce the polyclonal formation of IgE specificities, which are directed against multiple inhalant allergens, and thus may maintain a continuous activation of mast cells as part of the pathomechanism by which SAEs impact on mucosal inflammation. As the release of mediators upon exposure may be lower and the pattern of mast cell activation may be chronic rather than acute, seasonal peaks of inhalant allergens may not be discriminated by the patients (12). The reduction in magnitude of response to the allergens of polyp tissue mast cells vs AR tissue mast cells may have different, so far unraveled reasons, including IgE polyclonality, presence of blocking abs including IgG4-abs (7), or high concentrations of soluble low affinity IgE receptors (1). Further work needs to be carried out to answer this question.

The results in the tissue explants could be reproduced in a totally different experiment where tissue homogenates containing IgE to grass pollen could mediate RBL SX38 cell degranulation after stimulation with grass allergen independent of the presence or absence of SPT reaction to grass pollen. The magnitude of the response was significant but reduced, pointing to the possibility of a soluble factor leading to reduction, which is transferable in tissue homogenates. These findings underline the functionality of mucosal tissue IgE and further show the limitations of classical allergy tests such as the SPT to diagnose mucosal disease.

*Staphylococcus aureus* products have been demonstrated to heavily activate T- and B-lymphocytes as well as other inflammatory cells and to bias the T-cell pattern toward Th2 and against T regulatory cells (6, 7); we have recently shown that SEB and staphylococcus aureus protein A (SPA) contribute to T-cell activation and mast cell degranulation in upper airway disease, respectively (6). However, we here demonstrate an additional mechanism by which probably locally formed SEB-specific and polyclonal IgE abs directed to various inhalant allergens may contribute to the persistence of inflammation. In fact, we have recently shown that the presence of SAE-IgE abs and high tissue total IgE concentrations greater than 1400 kU/l in NP tissues were associated with severe inflammation and served as predictors for co-morbid asthma (11). We hypothesize that similar mechanisms may be relevant in severe lower airway disease with or without upper airway disease (2, 11).

A proof-of-concept study on the role of polyclonal IgE in NP disease has been undertaken to support our concept; the results of this anti-IgE (omalizumab) treatment vs placebo will unravel the usefulness of this concept in the near future (21).

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### Conflict of interest

None.

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