

Bet v 1-specific IgA increases during the pollen season but not after a single allergen challenge in children with birch pollen-induced intermittent allergic rhinitis

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Allergen-specific immunoglobulins of the Immunoglobulin A (IgA) type have been found in the nasal fluid of patients with allergic rhinitis. IgA may play a protective role, but there are also data which show that allergen-specific IgA can induce eosinophil degranulation. The aim of this study was to quantitate Bet v 1-specific IgA in relation to total IgA in the nasal fluid of children with birch pollen-induced intermittent allergic rhinitis and healthy controls, after allergen challenge and during the natural pollen season. Eosinophil cationic protein (ECP), Bet v 1-specific IgA and total IgA were analyzed in nasal fluids from 30 children with birch pollen-induced intermittent allergic rhinitis and 30 healthy controls. Samples were taken before the pollen season, after challenge with birch pollen and during the pollen season, before and after treatment with nasal steroids. During the pollen season, but not after nasal allergen challenge, Bet v 1-specific IgA increased in relation to total IgA in children with allergic rhinitis. No change was found in the healthy controls. The ratio of Bet v 1-specific IgA to total IgA increased from 0.1×10^{-3} (median) to 0.5×10^{-3} in the allergic children, $p < 0.001$. No change was seen after treatment with nasal steroids, although symptoms, ECP and eosinophils were reduced. In conclusion, allergen-specific IgA in relation to total IgA increases in nasal fluids during the pollen season in allergic children but not in healthy controls. These findings are compatible with the hypothesis that allergen-specific IgA plays a role in the allergic inflammation and further studies are needed to clarify the functional role of these allergen-specific antibodies.

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Mucosal surfaces represent a major interface between the host and the environment. Antibodies of the immunoglobulin A (IgA) isotype dominate the humoral immune defence at mucosal interfaces. IgA occurs in many molecular forms. In serum, IgA mainly occurs in a monomer form and, in secretions, IgA is mainly found in the form of secretory IgA, which consists of a polymeric IgA linked to a secretory component. Mucosal IgA has been studied comprehensively with respect to its protective effect

against various bacteria and viruses (1). Less is known about its significance in allergic airway disease. Not only allergen-specific IgE but also allergen-specific IgA and immunoglobulin G (IgG) have been demonstrated in the nasal secretions of patients with intermittent allergic rhinitis (IAR) who are sensitive to grass and ragweed (2, 3) and to birch pollen (4). Allergen-specific IgA has been reported to increase during the pollen season (3) but others saw no change in allergen-specific IgA during and after the pollen

season (4). IgA in nasal secretions mainly takes the form of secretory IgA, produced locally in the respiratory mucosa (5).

The role of allergen-specific IgA in allergy is not clear. IgA may be a competitive antibody binding to the allergen, thereby preventing immunoglobulin E (IgE) from binding to the allergen (6, 7). Other studies suggest that IgA, secretory IgA and IgG can induce eosinophil degranulation *in vitro* and that secretory IgA is the most effective (8–10). There are *in vivo* studies that support this theory (11).

The aim of this study was to quantitate the amounts of IgA antibodies reactive to Bet v 1, which is the main allergen in birch pollen (12), in the nasal secretions of children with birch pollen-induced IAR in comparison to healthy controls, before the pollen season, after challenge with birch pollen, during the pollen season and after treatment with topical steroids. We also wanted to study whether there was a correlation between Bet v 1-specific IgA and ECP.

Material and methods

Patients

The study was performed between January and May 2003 in Göteborg, Sweden. Thirty children with a history of birch pollen-induced IAR (13) and 30 healthy controls from the same school were included. The median age of the allergic children was 12.5 yr (range 11–16), while that of the controls was 13.4 yr (range 12–16). The allergic children had a positive history of IAR and a positive skin prick test (SPT) against birch. The SPT was performed on the volar side of the arm according to 'the Nordic guidelines' (14). Soluprick 10 HEP (ALK, Hörsholm, Denmark) was used; saline was used as a negative control and histamine dihydrochloride served as a positive control. The test was considered positive if the weal was at least 50% of the area of the weal induced by histamine dihydrochloride and had a minimum diameter of 3 mm. The SPT was performed when the children were first seen in

January. The controls were healthy children with no history of allergic rhinitis and a negative SPT for birch.

The children were first seen in January–February. Samples were taken before, and after challenge with birch pollen. See Fig. 1 for study protocol and time points for sampling. All the children were seen again during the birch pollen season when the allergic children had symptoms of allergic rhinitis. The median duration of symptoms before sampling during the pollen season was 7 days (range 2–20). Treatment with budesonide nasal spray 64 µg/dose (two doses in each nostril daily for 3 days and then one dose in each nostril) was initiated in the allergic group and, if needed, sodium cromoglycate eye drops (40 mg/ml, two drops in each eye, twice daily). The allergic children were seen again after 7–10 days. The exclusion criteria included a history of persistent symptoms, asthma, common cold, smoking or any kind of medication acting on the upper airways during the last 2 months before entering the study.

Pollen counts

Local air pollen counts were determined daily by the Department of Botany at Göteborg University (Fig. 2).

Allergen challenges

Allergen challenges were performed with birch pollen extract (0.1 ml of 5000 SBU/ml of Provocation test, 108 Birch, Allergopharma, Reinbeck, Germany) administered with a mechanical spray pump into each nostril. All children were challenged with the same dose. Ten of the controls were also challenged with the solvent (sodium chloride) as a control.

Nasal symptom scores

Nasal symptoms were recorded before nasal lavages. Nasal secretion, itching and blockage were scored from 0 to 3 (0 = no, 1 = mild, 2 =

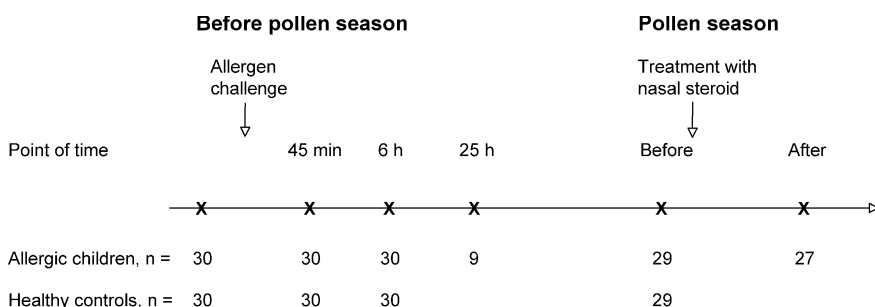


Fig. 1. Study protocol. X: Nasal lavages performed: Before pollen season at baseline, 45 min, 6 h and 25 h after allergen challenge. During the pollen season before and after treatment with a nasal steroid.

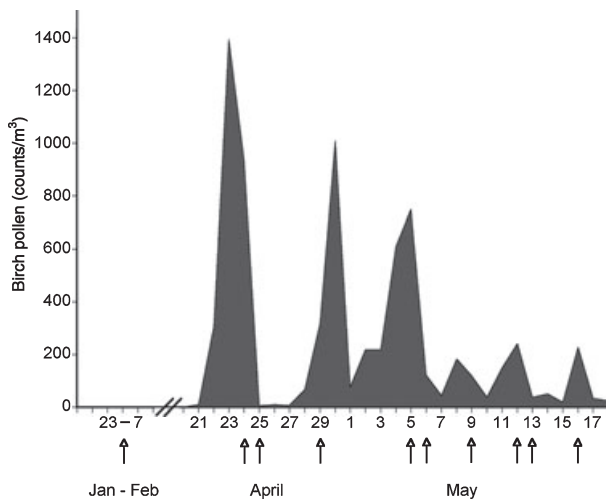


Fig. 2. Birch pollen counts/m³ January–May 2003 in Göteborg, Sweden. Median count during pollen season 119/m³. Arrows indicate dates for nasal lavage sampling before pollen season (January–February) and during the pollen season (April–May).

moderate, 3 = severe symptoms). The number of sneezes was counted during the last hour and transformed into a score (0, 0 sneezes; 1, 1–4 sneezes; 2, 5–9 sneezes; and 3, 10 or more sneezes) (15). The total symptom score was calculated by adding the 4 scores. The maximum score was 12.

Recovery of nasal lavage fluids

Nasal lavage fluids were obtained as previously described (16, 17). After clearing excess mucus by forceful exsufflation, 5–8 ml of sterile saline solution was delivered by aerosol into each nostril, while alternately clearing the other. The nasal fluid was allowed to return passively and was collected in a test tube, until 5 ml had been recovered. The collected fluid was kept on ice until centrifuged, 10 min at 500 *g* at 4°C. The supernatant was separated from the pellet. Before centrifugation, slides were prepared, dried and then stained according to the May-Grünwald-Giemsa method for the morphologic assessment of the cells in the fluid. Mean counts from duplicate (two independent investigators counting in a blinded fashion) slides were obtained. The numbers were expressed as the percentage of eosinophils, neutrophils and epithelial cells from 200 cells.

Nasal fluid mediator assays

Albumin was determined using competitive radioimmunoassays from Pharmacia Diagnostics AB (Uppsala, Sweden). ECP was determined

using an ELISA kit from MBL (Nagoya, Japan), according to the manufacturer's instructions.

Assays for total IgA and Bet v 1-specific IgA

Total IgA and Bet v 1-specific IgA were measured by ELISA as previously described (4) and presented in µg/ml and ng/ml respectively. Briefly, total IgA was measured by sandwich ELISA using isotype-specific rabbit antibodies for coating and enzyme-conjugated versions of the same antibodies for detection (third layer). Because IgA in nasal secretion mainly takes the form of secretory IgA (4), test samples were analysed against a standard of secretory IgA purified from human colostrums. The samples and standard were applied to the test plate in serial three-fold dilutions. After the development and reading of plates, titration curves for standard and unknowns were fitted using a four-parameter logistic model. Concentrations of unknowns were calculated as the mean of three, occasionally two, determinations based on optical density (OD) values corresponding to the log-linear part of the standard curve.

In the ELISA for Bet v 1-reactive IgA antibodies, test samples were titrated in wells coated with the allergen, recombinant Bet v 1 (gift from ALK). A standard curve was generated by titrating purified colostrum secretory IgA2 in wells of the same plate coated with a mouse monoclonal antibody specific for human IgA2 (Nordic, Tilburg, The Netherlands). After incubating the wells with sample, standard or buffer (background control), all the wells were developed by sequential incubation with biotinylated IgA-specific rabbit antibodies, enzyme-conjugated streptavidin and chromogenic substrate. OD values for dilutions of samples and standard were recorded after subtracting the corresponding background controls. Using an IgA2-subclass standard for the quantitation of IgA antibodies of mixed subclass, the composition was acceptable because the biotinylated IgA-specific rabbit antibody subsequently used for development reacted equally with the IgA1 and IgA2 subclasses (18). This method of calibration, involving a catching antibody of relatively low affinity ($K_a = 3.7 \times 10^{-7}$) (19) for the standard, generated a standard curve parallel to the titration curve for Bet v 1-reactive IgA antibodies in most test samples.

Statistical analysis

The results are described by median and range, while, for the IgA data, the inter-quartile range

(IQR), defined by the first and third quartiles, was used. Non-parametric tests were used as the data are non-normally distributed. Wilcoxon's signed-rank test was used to analyze changes between paired observations within individuals. Comparisons between the group of allergic children and healthy controls were performed using the Wilcoxon–Mann–Whitney *U*-test. Possible relationships between different variables were evaluated using Spearman rank-order correlation coefficients.

The Bonferroni–Holm method was used to adjust for multiple tests.

All statistical analyses were performed on a personal computer using the SPSS software package, version 11 (Chicago, IL, USA).

Ethics

The Ethics Committee at the Medical Faculty, Göteborg University, approved the study. All subjects provided written informed consent from their parents and themselves before entering the study.

Results

Symptoms

Two children (one from each group) were lost to follow-up as a result of change of school. Two children in the allergic group were not seen after treatment because of illness (upper airway infection).

The median and range values for symptom scores are presented in Table 1. All children in the allergic group had symptoms and signs of IAR 1 h after the allergen challenge and during the pollen season. Treatment with a nasal steroid reduced symptoms. All but two of the healthy controls were free of symptoms at all visits. The same investigator (C.K.) saw all the children during all the visits.

Eosinophils, ECP and albumin

The median and range values for eosinophils, ECP and albumin are presented in Table 1.

The percentage of eosinophils in nasal secretions increased 6 h after allergen challenge and during the pollen season in the allergic children ($p < 0.001$) and the allergic children had significantly higher levels of ECP 6 h after allergen challenge ($p = 0.01$) and during the pollen season ($p = 0.002$) than the healthy controls. No increase in eosinophils was seen in the controls. After treatment with budesonide, the eosinophils decreased. Albumin levels increased after allergen challenge in both groups, but no increase was seen during the pollen season and there was no difference between the groups at any time.

Levels of Bet v 1-specific IgA antibodies

Bet v 1-specific IgA increased from 4.4 to 6.3 ng/ml ($p = 0.047$) during the pollen season in the allergic children (Table 2). No increase was seen after the allergen challenge. There was no

Table 1. Symptom score, eosinophils, neutrophils, ECP and albumin

	Before season		45 min after provocation		6 h after provocation		25 h after provocation	Pollen season		After treatment
	Allergic (n = 30)	Control (n = 30)	Allergic (n = 30)	Control (n = 30)	Allergic (n = 30)	Control (n = 30)	Allergic (n = 9)	Allergic (n = 29)	Control (n = 29)	Allergic (n = 27)
<i>Symptom score</i>										
Median	0	0	6	0	0	0	0	8	0	3
Range	0–1	0	1–11	0–2	0–2	0–0	0–0	1–11	0–0	0–11
<i>Eosinophils (%)</i>										
Median	0	0	1	0	2	0	2	2	0	0
Range	0–1	0–6	0–8	0–7	0–8	0–15	0–12	0–47	0–2	0–40
<i>Neutrophils (%)</i>										
Median	28	23	26	19	51	40	42	29	20	15
Range	3–70	4–80	8–72	2–87	14–78	10–86	25–58	5–64	2–49	0–72
<i>ECP (ng/ml)</i>										
Median	24.6	11.2	10.3	3.5	34.9	7.4	24.2	34.8	7	9.9
Range	0.1–287.2	0.8–481.6	0–87.7	0.7–192.2	0–492.2	0–203.6	0–225.9	0.1–644.2	0–87.2	0–332.1
<i>Albumin (mg/ml)</i>										
Median	41	34	93	70	88	123	99	42	36	22
Range	4.2–278	7.6–233	3.5–508	7.4–608	6.4–662	16–489	4.6–372	4.6–112	6.9–298	4.6–249

Table 2. Bet v 1-specific IgA, total IgA and ratio of Bet v 1-specific IgA to total IgA

	Before season		45 min after provocation		6 h after provocation		25 h after provocation	Pollen season		After treatment
	Allergic (n = 30)	Control (n = 30)	Allergic (n = 30)	Control (n = 30)	Allergic (n = 30)	Control (n = 30)	Allergic (n = 9)	Allergic (n = 29)	Control (n = 29)	Allergic (n = 27)
<i>Spec IgA ng/ml</i>										
Median	4.4	3.8	1.7†††	2.3††	3.1	3.2	3.1	6.3***†	3.8	6.1
Range	0–125.3	0–47.6	0–17.6	0–15.8	0–162.5	0–22.2	0–59.9	3.3–38	0–18.1	0–69.6
<i>IQR</i>	1.5–12.5	1.4–7.6	1.2–5.4	0–5.5	1.7–9.5	0–8.7	0.8–10.8	5.1–16.3	2.6–7.7	2.0–11.1
Subjects below detection	2	5	4	9	2	9	1	0	1	1
<i>Total IgA µ/ml</i>										
Median	33.6	33.5	19***†††	31.6	26.1	38.6	28.5	13.7***††	34.1	16.2
Range	7–86	6–208	4–56	7.3–80.6	9.9–89.5	12.3–126.4	3.8–50.1	1.7–49.9	2–68.2	0.5–52.8
<i>IQR</i>	19.4–62.0	24.8–52.6	11.2–27	19.2–43.0	19.6–47.1	22.0–64.9	20.3–39.1	6.7–24.0	13.4–45.0	8.6–27.5
<i>Ratio $\frac{\text{Spec IgA ng/ml}}{\text{Total IgA µ/ml}}$</i>										
Median	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.5***†††	0.1	0.3
Range	0–1.6	0–0.9	0–4.7	0–0.3	0–2.0	0–0.4	0–9.9	0.2–9.6	0–1.0	0–5.5
<i>IQR</i>	0–0.3	0–0.2	0–0.3	0–0.2	0–0.3	0–0.2	0–1.1	0.3–1.6	0.1–0.2	0.1–0.8

Significant difference in relation to healthy controls * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Significant difference in relation to before allergen challenge † = $p < 0.05$, †† = $p < 0.01$, ††† = $p < 0.001$

Multiplicity adjusted for by using the Bonferroni-Holm method.

increase in the healthy controls during the pollen season or after allergen challenge.

Total IgA decreased in the allergic children 45 min after the allergen challenge and during the pollen season. No change was seen in the healthy controls.

We calculated Bet v 1-specific IgA in relation to total IgA in order to control the dilution factor.

The ratio of allergen-specific IgA to total IgA did not differ between the allergic children, 0.1×10^{-3} (0–1.6), and the healthy controls, 0.1×10^{-3} (0–0.9), before challenge (Table 2). The ratios are presented in Fig. 3. No change was seen in either group 45 min or 6 h after challenge. During the pollen season, the ratio increased significantly in the allergic children, median 0.5×10^{-3} (range 0.2–9.6), $p < 0.001$,

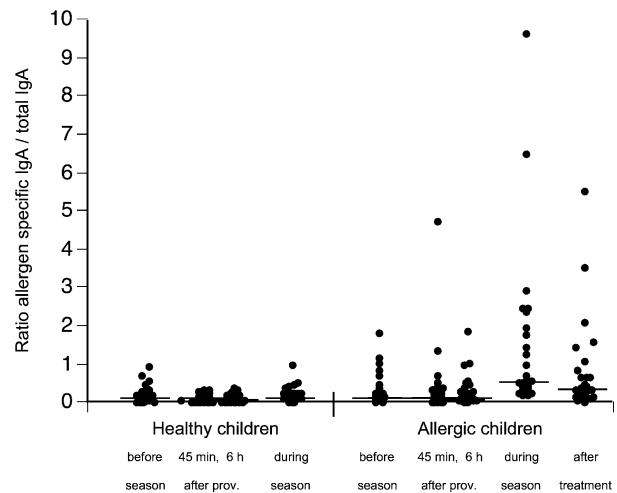
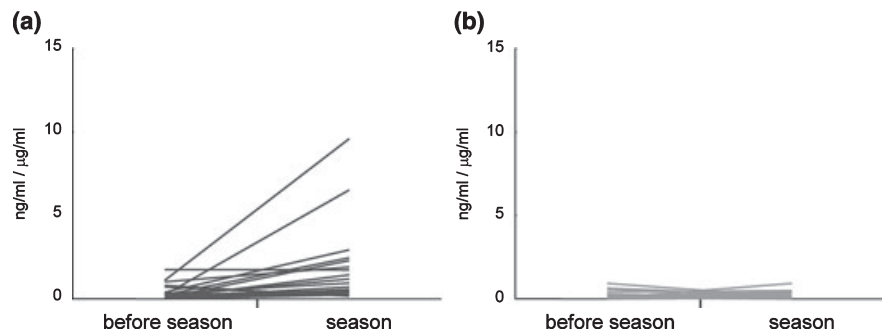


Fig. 3. Ratio of Bet v 1-specific IgA to total IgA. Median for each group is indicated.

Fig. 4. The change in the ratio of allergen-specific IgA to total IgA from before to during the pollen season for each individual. Allergic children (a) and healthy controls (b).



but no change was seen in the healthy controls, 0.1×10^{-3} (0–1.0) (Table 2, Fig. 4).

No significant decrease in the ratio of allergen-specific IgA to total IgA was seen after steroid treatment (Fig. 3).

We found no correlation between Bet v 1-specific IgA and ECP in the nasal secretions of allergic children during the pollen season ($r = 0.025$, $p = 0.90$) or after the pollen challenge ($r = 0.052$, $p = 0.79$).

Discussion

The allergic children in this study displayed a marked increase in the ratio of allergen-specific IgA to total IgA in nasal fluids during the pollen season, whereas the healthy controls showed no increase. This difference in ratio between the two groups could indicate that allergen-specific IgA plays a role in the allergic inflammation. No increase was seen in either group after allergen challenge, but this might be due to too little time for IgA production between the challenge and the nasal lavage.

When analyzing mediators in nasal fluids, it is important to take account of the possibility of dilution with the lavage fluids, the transudation of mediators and fluid from plasma, indicated by the increase in albumin, and secretion and absorption from the mucosa. Several lavages with short intervals in between might also interfere with the results. In order to control some of these problems, we chose to study the ratio of Bet v 1-specific IgA to total IgA.

In this study we saw a decrease in total IgA in the allergic children 45 min after allergen challenge and during the pollen season, which is when they had symptoms of allergic rhinitis. This is contradictory to other studies (4). In accordance with our findings others have found considerable variations in levels of IgA in repetitive samples (18, 20, 21). Previous studies on nasal secretions have indicated that such differences may be explained by variations in secretion rate in combination with the existence of a reverse relationship between IgA concentration and the rate of secretion. These parameters undergo a certain diurnal variation (22), which is why we took most of the samples in the mornings. Earlier studies have shown that S-IgA of the IgA1 subclass is the predominant immunoglobulin in nasal secretions (18) and this subclass is susceptible to bacterial IgA1 proteases. Bacteria producing these proteases, for example *Streptococcus pneumoniae* and *Haemophilus influenzae* are very common amongst children which also could explain large variations in IgA levels in nasal

secretions. Symptomatic children with nasal secretions might loose more mediators when they blow their nose before sampling than children without symptoms. This could explain the decrease we saw in all mediators (ECP, Bet v 1-specific IgA and total IgA) 45 min after allergen challenge in the allergic children. Studying the ratio of Bet v 1-specific IgA to total IgA in each sample compensates for these intra-individual variations and the varying dilutions of the different samples.

Secretory IgA is the dominant form of IgA in nasal secretions and it is mainly produced locally in the nose. IgA from serum can reach the nasal secretions by transudation. An increase in transudation from serum, indicated by increased levels of albumin, could therefore increase the serum IgA in nasal secretions. In our study, we found no increase in total IgA when albumin was increased and IgA from serum is therefore unlikely to confound the results. Albumin levels were increased after allergen challenge in both allergic children and healthy controls, indicating transudation from serum. This was probably due to unspecific inflammation caused by the actual proceedings of nasal lavages because similar results were obtained when we performed nasal lavages at the same time points but without the allergen challenge (data not shown).

The pathophysiology of allergic rhinitis is divided into two different phases, an early phase, which occurs soon after allergen challenge and is dominated by the IgE-induced release of mediators, and a later phase, 6–8 h after challenge, which is dominated by eosinophil inflammation (23). The ongoing allergen exposure during the pollen season is characterized by an inflammation in which the two mechanisms are intertwined. The allergic children displayed clear symptoms 45 min after the challenge (early phase), but the majority had no symptoms after 6 h (the later phase). However, there was a clear picture of ongoing allergic inflammation, with a significant rise in eosinophils and significantly higher ECP levels in the allergic children compared to the healthy controls 6 h after allergen challenge. There was no significant increase in allergen-specific IgA 6 h after allergen challenge, indicating that the increase in allergen-specific IgA in the allergic children during the pollen season is not because of influx from serum but from increased local production in the nasal mucosa. Immunoglobulin production is not instant and this could be the reason why we saw no increase in allergen-specific IgA 6–25 h after allergen challenge. The allergic children reported symptoms during the pollen season and they had a more

pronounced inflammatory response, as measured by eosinophils and ECP, in nasal secretions than they had before the season. No changes in ECP or eosinophils were seen in the healthy controls during the pollen season.

Treatment with a local steroid reduced symptoms, eosinophils and ECP in most of the allergic children. No decrease in allergen-specific IgA was seen after treatment. This agrees with a previous study of adults (24). In contrast to this study of children, the study of adults revealed that allergen-specific IgA is found in the nasal secretions of patients with IAR but not in healthy controls (4) and the allergic patients had the same levels of allergen-specific IgA during and after the pollen season. They studied the absolute concentrations of Bet v 1-reactive IgA and not Bet v-reactive IgA in relation to total IgA. However, ragweed-specific IgA has been studied in the nasal fluid of rhinitis patients sensitive to ragweed and it has been shown to increase during the pollen season (3). In the bronchoalveolar (BAL) fluids of patients with allergic asthma and healthy controls, ragweed-specific IgA was found in both groups and no difference was seen between the two groups before allergen challenge, but afterwards the levels were significantly higher in the sensitized group (25).

It has been suggested that IgA is the most prominent class of immunoglobulins in the nasal mucosa and allergen-specific IgA is produced locally in the nasal mucosa (2), but little is known of its role in the allergic inflammation.

IgA plays a protective role as a binding antibody to inhibit the binding of IgE to the allergen (7, 26). However, allergen-specific IgA, induces eosinophil degranulation (8, 10) and studies have demonstrated a correlation between allergen-specific IgA and ECP in the BAL fluid (25) and in the induced sputum (11) of patients with allergic asthma. In this study Bet v 1-specific IgA increased in relation to total IgA in the allergic children during the pollen season indicating a pathophysiologic role for Bet v 1-specific IgA. However, in this study, we found no correlation between ECP and Bet v 1-specific IgA in nasal fluids after allergen challenge or during the pollen season. Woerly et al. (27) have reported that eosinophils can secrete Th2 cytokines after activation with IgA immune complexes, indicating yet another role of allergen-specific IgA.

In conclusion, Bet v 1-specific IgA increases in nasal secretions during the pollen season in allergic children with birch pollen-induced rhinitis but not in healthy controls. Furthermore, there is a marked increase in the ratio of allergen-specific IgA to total IgA in the nasal secretions of

allergic children during the pollen season. These findings are compatible with the hypothesis that allergen-specific IgA plays a role in the pathogenesis of allergic inflammation. Further studies are needed to identify the mechanism.

Acknowledgments

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