

## Original article

# Allergen-reactive antibodies are found in nasal fluids from patients with birch pollen-induced intermittent allergic rhinitis, but not in healthy controls

**Background:** Increased levels of allergen-reactive immunoglobulins (Igs) have been reported in nasal fluids from patients with intermittent allergic rhinitis (IAR) sensitive to ragweed and grass. The aims of this study were to make a detailed characterization of nasal fluid Igs in birch pollen-induced IAR.

**Methods:** Nasal fluids were obtained from 23 patients with birch pollen-induced IAR during and after the birch pollen season, and from 20 healthy controls. Nasal fluid total and Bet v 1-reactive (IgA), IgE and IgG as well as albumin were analyzed by immunoassays. The integrity of IgA and IgG, and the molecular form of IgA were assessed by Western blotting and column fractionation, respectively.

**Results:** Nasal fluid total IgE and IgG, but not IgA, were higher in patients compared with controls. Western blotting indicated no significant degradation of IgA (including S-IgA) and IgG. Most of the IgA, including Bet v 1-reactive antibodies, was of the secretory form and of the IgA1 subclass. Bet v 1-reactive IgA and IgG were present in all patients, but was mostly nondetectable in controls. No significant differences in the levels of Bet v 1-reactive IgA and IgG were found in patients during the birch pollen season compared with off season. Both Bet v 1 and Bet v 2-reactive IgE were nondetectable in most samples.

**Conclusions:** Nasal fluid Bet v 1-reactive IgA and IgG were found in all patients with birch pollen-induced IAR, but not in controls. However, no significant differences were found between patients during and after the birch pollen season.

**M. Benson<sup>1,2</sup>, J. Reinholdt<sup>3</sup>,  
L. O. Cardell<sup>1</sup>**

<sup>1</sup>Allergy Laboratory, Department of Oto-Rhino-Laryngology, Malmö University Hospital, Malmö, Sweden; <sup>2</sup>Department of Pediatrics, Queen Silvia Children's Hospital, Gothenburg, Sweden; <sup>3</sup>Department of Oral Biology, Royal Dental College, Aarhus, Denmark

Key words: Bet v 1; degranulation; immunoglobulins; intermittent allergic rhinitis.

Mikael Benson, MD, PhD  
Department of Pediatrics  
Queen Silvia Children's Hospital  
SE-416 85 Gothenburg  
Sweden

Accepted for publication 17 January 2003

Allergen-specific immunoglobulin A (IgA), IgE and IgG have been demonstrated in nasal secretions from patients with intermittent allergic rhinitis (IAR) sensitive to grass and ragweed, but are usually not found in healthy controls (1–5). Moreover, seasonal increases have been reported in patients with ragweed-induced IAR (4, 5). These observations suggest that allergen-reactive Igs in nasal fluids could be of interest as local markers of inflammation in IAR. The allergen reactivities of nasal fluid Igs in birch pollen-induced IAR are not well defined. Bet v 1 is the main allergen, based on IgE reactivity and skin prick tests (6). This was substantiated by a recent study of tears from patients with allergic conjunctivitis, where IgE was found to be directed against Bet v 1. By contrast, IgA was directed against other, nonallergenic moieties (7). However, in a pilot study we found Bet v 1-reactive IgA in nasal fluids from patients with birch pollen-induced IAR, but not in healthy controls (8). A possible explanation for these contradictory findings could be that analysis of nasal fluid IgA and IgG may be confounded by degradation of these Igs in the nasal cavity (9–11). Thus, assessment of the structural integrity

of IgA and IgG is important in studies of nasal fluid IgA and IgG. It should be noted that IgA and IgG have different molecular forms, which complicate interpretation of nasal fluid Ig levels. Immunoglobulin A has two subclasses and IgG four subclasses. While nasal fluid IgG is mainly derived from plasma, IgA is mostly present as a locally produced dimer, secretory IgA (S-IgA), which is bound together by the secretory component (SC) (reviewed in 3).

The aims of this study were to make a detailed characterization of total and allergen-reactive IgA, IgE, and IgG in nasal fluids from patients with birch pollen-induced IAR during and after the pollen season, and from healthy controls.

## Materials and methods

### Study design

Twenty-three patients with birch pollen-induced IAR and 20 healthy controls were prospectively enrolled in the year 2000, as previously described (12). The patients were diagnosed after start of

symptoms during the birch pollen season. To avoid cross-reactivity against hazel and alder or other pollens off-season samples were taken during the late fall of the same year. The controls were diagnosed during the birch pollen season. The median age of the patients and the controls were 42 (18–69) and 31 (24–66) years, respectively. Fourteen patients and 15 controls were women. The diagnosis of birch pollen-induced IAR was based on a positive history of IAR for at least 2 years, and positive skin prick tests against birch pollen. Patients were excluded if they had a history of persistent rhinitis, had positive skin prick tests against perennial allergens, had been given immunotherapy, local or systemic glucocorticoid (GC) treatment less than 4 weeks before the study, had contraindications for GC, or were pregnant. The controls were all symptom-free, had no history of allergic disease, and had negative skin prick tests against a panel of allergens, including birch pollen. The study was approved by the Ethics Committee of the Medical Faculty, Lund University.

### Nasal fluid collection and processing

Nasal lavage fluids were obtained as previously described (13). Briefly, after clearing excess mucous by forceful exsufflation, sterile isotonic saline solution at room temperature was aerosolized into each nostril, while alternately clearing the other. The nasal fluids were allowed to return passively and collected in a graded test tube, until 5 ml was recovered. The median volumes of introduced fluid in the patients were 7 ml (5–8) off season, 7 ml (5.5–9) during season, and 7 ml (6–8) in the controls. The total number of cells per millilitre was counted in a Bürker chamber. The fluids were then centrifuged at 1334 *g* for 10 min at 4°C. The supernatant was separated from the pellet. The pellet was used to prepare slides that were stained according to the May-Grünwald and Giemsa method for morphological assessment of cells in the fluid by two independent microscopists. The percentages of eosinophils out of a total of 100 cells/slide were computed. All fluid samples were processed within 30 min after the lavage.

### Preparations of birch allergens

Three different preparations of birch allergen were employed for the assessment of antibody responses: (i) Pure Bet v 1 major allergen produced by recombinant technique (rBet v 1) (14); (ii) Pure Bet v 1 in the form of a native preparation representing more than 24 different isoforms, (native Bet v 1) (15); (iii) A crude aqueous extract of birch pollen. The three preparations were the products and generous gifts of ALK-ABELLÖ, Hørsholm, Denmark.

### Quantitation of immunoglobulins and albumin in nasal lavage fluid

Levels of IgA and IgG, Ig isotypes and antibodies to birch allergens were quantitated by enzyme-linked immunosorbent assay (ELISA) protocols sharing several essential points. Nunc polystyrene microplates (code, 269620) were used as solid phase throughout and proteins for coating were incubated at 5 µg/ml in 10 mM Tris buffered saline, pH 7.5 containing 0.1% sodium azide (TBS) in volumes of 100 µl at room temperature overnight. The TBS supplied with 0.15% Tween 20 (TBST) served as combined washing and blocking solution and as diluent for unknowns, standards, antibody reagents, and alkaline phosphatase conjugates, all of which were incubated at room temperature for 4 h. Pilot experiments showed that TBST used in this way, with the type of

plates employed, prevented background reactions that may arise from nonspecific binding of test sample Igs and other postcoating reagents to the plate material (16). Optimal dilutions of commercial antibodies (from DAKO, Glostrup, Denmark, unless otherwise stated) were determined in pilot experiments. Unknowns, appropriately prediluted, were analyzed at six serial threefold dilutions in duplicate wells, and a similar dilution series (eight steps) of the relevant standard was included in each plate. After development of plates with chromogenic substrate (p-nitrophenyl phosphate in diethanolamine buffer, pH 9.8) optical densities (OD) at 405 nm were read using a Multiscan RC ELISA reader (Lab-systems, Helsinki, Finland). Titration curves for standard and unknowns were fitted from a four-parameter logistic model using the Genesis Lite software package (Lab-systems) and concentrations of unknowns were calculated as the mean of three, occasionally two, determinations based on OD values corresponding to the log-linear part of the standard curve. Concerning the individual assay protocols, total IgA and IgG were quantitated by sandwich ELISA using isotype-specific rabbit antibodies for coating and enzyme-conjugated versions of the same antibodies for detection (third layer). Secretory-IgA purified from human colostrum (16) served as standard in the IgA assay whereas isotype standards for the IgG assay were obtained from DAKO. Birch allergen-reactive IgA and IgG antibodies in nasal wash were quantitated in gravimetric units (ng/ml) as described (17). Briefly, for the quantitation of IgG antibodies, wells with test samples were coated with the allergen (Bet v 1 or crude pollen extract) whereas other wells of the plate with dilutions of a standard of the relevant Ig isotype were coated with isotype-specific rabbit antibodies. After incubation of the wells with sample, standard, or buffer (background control), all wells were developed by sequential incubation with biotinylated isotype-specific rabbit antibodies, enzyme-conjugated streptavidin, and chromogenic substrate. The OD values for dilutions of samples and standard were recorded after subtraction of the corresponding background controls. In the case of IgG, titration curves for most test samples were largely parallel to the standard curve, testifying to the reliability of the assay. A similar, calibrated ELISA quantitated IgA antibodies, except that the standard curve was generated by titration of purified S-IgA2 (11) in wells coated with an A2 subclass-specific monoclonal antibody (Nordic, Tilburg, The Netherlands). Using an A2 subclass standard for quantitation of IgA antibodies of mixed subclass composition was acceptable because the biotinylated IgA-specific rabbit antibody subsequently used for development reacted equally with the A1 and A2 subclasses (11). This method of calibration, involving a catching antibody of relatively low affinity ( $K_a = 3.7 \text{ H}/10^7$ ) (18) for the standard, generated a standard curve parallel to the titration curve for Bet v 1-reactive IgA antibodies in most test samples. Total IgE was determined by a competitive radioimmunoassay, whereas Bet v 1-reactive and Bet v 2-reactive IgE antibodies were determined with fluoroenzyme immunoassays (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Levels of albumin in nasal fluid were determined using a competitive radioimmunoassay (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

### Analysis of integrity of immunoglobulin molecules

Immunoglobulin A, S-IgA, and IgG in nasal wash were analyzed with respect to integrity of  $\alpha$  chains, SC, and  $\gamma$  chains by Western blotting essentially as described (11). Briefly, samples of nasal wash were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions and electroblotted onto polyvinylidene difluoride membranes. To examine  $\alpha$  chains or SC,

blots were incubated with  $\alpha$  chain- or SC-specific rabbit antibodies followed by alkaline phosphatase conjugated swine antibodies to rabbit IgG. To examine  $\gamma$  chains, blots were incubated with biotinylated  $\gamma$  chain-specific antibodies followed by alkaline phosphatase conjugated streptavidin. Blots were developed with a chromogenic substrate of 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium. Lanes of  $M_r$  standards were stained with amido black.

#### Molecular characterization of total IgA and IgA antibodies to Bet v 1

The molecular form of total IgA in nasal secretions was examined as previously described (11). Briefly, nasal wash samples were fractionated on a calibrated Superose 6 size-exclusion chromatography column (Pharmacia, Uppsala, Sweden) whereafter the separated S-IgA (400 kD) and serum IgA (160 kD) forms were quantitated in eluent fractions by sandwich ELISA, using IgA standards of the homologous molecular form. After plotting of IgA concentration against eluent fraction number, the molecular distribution of IgA in the sample was calculated by planimetry (10). Bet v 1-reactive IgA antibodies in eluent fractions were quantitated by ELISA against IgA standards of homologous form as described above. Similar to total IgA, the molecular distribution of IgA antibodies was calculated by planimetry.

#### Subclass analysis of Bet v 1-specific IgA antibodies

The subclass distribution of Bet v 1-specific S-IgA and serum IgA antibodies was determined by analysis of the corresponding Superose 6 eluent fractions of nasal wash, essentially as described by Hocini et al. (19). The assay analyzes that IgA1 (including S-IgA1), but not IgA2, molecules are cleavable in the hinge region by bacterial IgA1 proteases and that such cleavage prevents the detection of IgA1 antibodies in an appropriately constructed ELISA (20). Briefly, all IgA1 in a sample of the test fraction was cleaved by incubation overnight at 37°C with an excess of meningococcal IgA1 protease (own product) (21). A control sample was incubated with buffer. The digested sample and the control were then titrated in parallel in ELISA wells coated with recombinant Bet v 1, the wells being subsequently developed with Fc $\alpha$ -specific mouse monoclonal antibody (reacting with both subclasses) followed by enzyme-labeled rabbit antibody to mouse IgG. By relating the titration curve for the digested sample (only IgA2 antibodies detectable) to that of the control (both subclasses detectable), the subclass distribution of Bet v 1-specific antibodies was obtained.

#### Nomenclature

The revised nomenclature for allergy according to recent EAACI recommendations is used (22).

#### Statistics

The levels of nasal fluid cells and mediators were compared between patients and healthy controls using the Mann-Whitney test. Wilcoxon's signed rank test was used to make paired comparisons of the same variables during and after season and between patients. Spearman rank correlation test was used for correlation analyses. *P* values < 0.05 were considered significant. The results are stated as median and range.

## Results

#### Nasal fluid eosinophil counts and albumin concentrations

Eosinophil counts in nasal fluid were higher in the patients during the birch pollen season compared with patients during off season, 1.1 cells  $\times 10^4$ /ml (0.1–8.3) [median (range)] vs 0 (0–0.34), *P* < 0.001, and compared with the controls, 0 (0–0.30, *P* < 0.001). Albumin concentrations were higher in the patients during the season than after, 100 (10.4–763) vs 35.8 (4.3–332) mg/l, *P* < 0.01, and compared with the controls, 23.6 (5.2–450), *P* < 0.01.

#### Integrity of IgA and IgG in nasal fluid

Fourteen samples of nasal wash from patients (in season) and five from controls were analyzed by immunoblotting. Dominant and distinct bands identified as  $\gamma$  chains,  $\alpha$  chains, and SC by their reactivity with corresponding antibodies were of similar mobility in allergics compared with controls. In some samples from both allergics and controls, probing with anti- $\gamma$  or anti- $\alpha$  chain antibodies revealed an additional faint band of approximately 45 kD, corresponding to heavy chains depleted of one constant domain. Notably,  $\alpha$  chain analysis did not reveal Fc $\alpha$  and Fd $\alpha$  fragments characteristic of bacterial IgA1 protease activity in any samples.

#### Molecular forms of total IgA and allergen-reactive IgA antibodies in nasal wash

Different molecular forms of IgA perform differently in quantitative solid-phase immunoassays (23, 24). To select appropriate calibration standards for quantitative ELISA, we first analyzed the balance of secretory and serum forms in total IgA and allergen-reactive IgA antibodies. Analysis of size-exclusion column eluent fractions of five randomly selected nasal wash samples from patients in season and seven samples from controls showed that S-IgA constituted from 62% (31–88) of total IgA in the allergics to 92 (61–96)% in the controls. Quantitation of Bet v 1-reactive S-IgA and serum IgA antibodies in the eluent fractions from patients revealed that the S-IgA form predominated, 81% (62–91), irrespective of the molecular distribution of total IgA. Fractions of samples from controls were without antibodies. Based on these results standards selected for the quantitation of total and Bet v 1-reactive IgA in unfractionated samples were in the form of S-IgA.

#### Levels of nasal fluid immunoglobulins and antibodies to birch allergens

Total IgA levels in the patients during the season did not differ significantly compared with off season, nor with controls (Table 1). In contrast, total IgE and total IgG in

Table 1. Nasal fluid concentrations of total IgA, IgE, and IgG

|            | Total IgA [ $\mu$ g/ml] | Total IgE [kU/ml]  | Total IgG [ $\mu$ g/ml] |
|------------|-------------------------|--------------------|-------------------------|
| Controls   |                         |                    |                         |
| Median     | 38.5                    | 2.5                | 3.8                     |
| Range      | 16.3–115.4              | 1.1–3.0            | 0.7–51.3                |
| N          | 20                      | 19                 | 20                      |
| Patients   |                         |                    |                         |
| Off season |                         |                    |                         |
| Median     | 40.5                    | 2.5                | 5.2                     |
| Range      | 10.6–122.6              | 2.1–13.4           | 0.6–67.9                |
| N          | 20                      | 20                 | 20                      |
| Season     |                         |                    |                         |
| Median     | 54.5                    | 2.8** <sup>1</sup> | 8.7**** <sup>1,2</sup>  |
| Range      | 18.1–199.6              | 2.3–11.3           | 2.5–108.6               |
| N          | 21                      | 23                 | 21                      |

\*\*  $P < 0.01$ , \*\*\*\*  $P < 0.0001$ .

<sup>1</sup> Compared with healthy controls [Mann–Whitney].

<sup>2</sup> Compared with patients after season [Wilcoxon signed rank test].

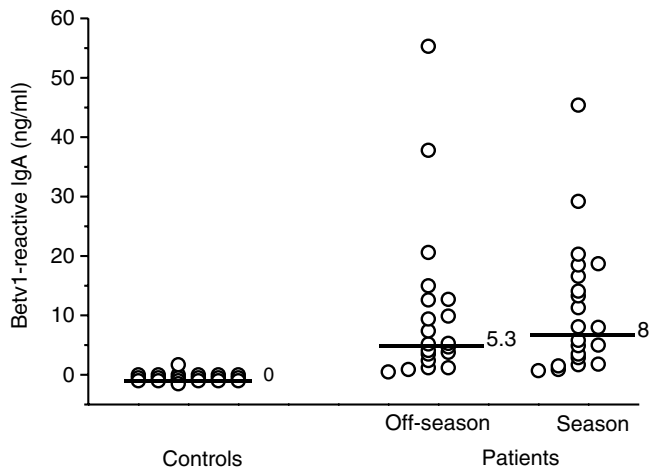


Figure 1. Bet v 1-reactive IgA in nasal fluids from patients during ( $n = 21$ ) and off season ( $n = 20$ ) and controls ( $n = 20$ ). Horizontal bars indicate median values.

the patients during season were significantly higher than the levels after season and in controls (Table 1).

Immunoglobulin A as well as IgG antibodies reacting with rBet v 1 were present in nasal fluid from all patients in the season (IgA; 8.0 (0.7–45.4) ng/ml; IgG: 15.8 (1.1–165) ng/ml) and were not significantly higher compared with off season (IgA; 5.3 (0.5–55.3) ng/ml; IgG: 14.4 (0–180) ng/ml). In the controls rBet v 1-reactive IgA and IgG were mostly nondetectable (IgA: 0 (0–1.7) ng/ml; IgG: 0 (0–9.0) ng/ml) and lower than in the patients, both before and after season ( $P < 0.0001$ ) (Fig. 1).

The subclass distribution of rBet v 1-reactive IgA antibodies was assessed for a subset of five patient samples, the S-IgA and serum IgA molecular form being analyzed separately by the IgA1 protease-based assay. In all five samples IgA1 was the predominant subclass of

S-IgA (median 94 (95–55)%) as well as of serum IgA (86 (95–52)%) antibodies.

The IgE antibodies to rBet v 1 were detected in four samples from the patients during the birth pollen season, in one of the patients off season, and in none of the controls. The IgE antibodies reacting with Bet v 2 were not detected in any of the groups.

Native Bet v 1 exists in many isoforms with variable antigenic properties (28). To examine if reactivity to rBet v 1 reliably reflected IgA responses to the allergen, IgA reactivity to native Bet v 1 was analyzed as well. Levels of native Bet v 1-reactive IgA correlated with rBet v 1-reactive IgA in nasal fluid from the patients (during as well as after season) as well as from the controls ( $r = 0.95$ ,  $P < 0.0001$ ). Furthermore, similar to rBet v 1, levels of native Bet v 1-reactive IgA in the patients during season (4.5 (1.5–25.8) ng/ml) and after season (6.2 (1.1–33.6) ng/ml) were significantly above levels in the controls (0.2 (0–0.6) ng/ml) ( $P < 0.0001$ ).

Birch pollen contains potential allergens other than Bet v 1. To examine if Bet v 1-reactive IgA reflected the overall IgA reactivity to birch pollen, we measured IgA antibodies reacting with a crude extract containing all pollen constituents in nasal fluids from patients during the season. These IgA antibody levels (4.5 (2.4–28.8) ng/ml) were significantly correlated to those against native ( $r = 0.78$ ,  $P < 0.01$ ) and recombinant ( $r = 0.7$ ,  $P < 0.01$ ) Bet v 1, indicating that Bet v 1 was a major target of naturally induced nasal IgA antibodies to birch pollen.

## Discussion

Total and allergen-reactive Igs can be measured in nasal fluids from patients with IAR, but usually not in healthy controls (1–5). In two studies of IAR sensitive to ragweed allergen-reactive IgA was found to increase during the pollen season (4, 5). This suggests that allergen-reactive Igs could be relevant as local markers of inflammation.

In this study nasal fluid levels of total IgE and IgG, but not IgA, were higher in patients than in controls. Increased IgE agrees with previous studies (1, 2, 13), whereas IgA and IgG have varied (1–5). It should be noted that IgA and IgG may be degraded by enzymes of microbial and/or host origin (9–11). Nasal bacteria may produce IgA1 proteases, which specifically cleave IgA1, including S-IgA1, molecules in the hinge region, leaving the molecules as Fab $_{\alpha}$  (monovalent) and Fc $_{\alpha}$  (or Fc $_{\alpha}$ : SC) fragments (10, 11). In addition, the SC of S-IgA molecules may be degraded by enzymes in secretions (25). Immunoglobulin A1 is the main subclass in the nasal mucosa and secretions (3, 16). Moreover, in this study, analysis of five patient samples indicated that also Bet v 1-reactive IgA antibodies, whether of secretory or serum form, were predominantly IgA1 and hence susceptible to potential IgA1 protease activity. However, examination by Western blotting revealed only very limited, if any,

degradation of  $\alpha$  chains and SC, suggesting that most Bet v 1-reactive IgA antibodies were intact and functional. Likewise, no degradation of IgG was found.

Comparisons of absolute concentrations of antibodies in nasal secretions may be confounded by variable dilution by the lavage fluid (26). To compensate for this antibody responses are usually recorded relative to total isotype. However, as allergen-reactive antibodies in the nasal mucosa are largely produced locally (2), they would be confounded by transudated serum Ig if so recorded. In this study, transudation was evident from the high albumin levels in patient samples. Hence, and because we have previously found little variation of dilution in repetitive lavage samples (11), antibodies were measured directly in nasal lavage.

Bet v 1 is considered to be the main allergen in birch pollen-induced IAR, based on IgE-reactivity and skin prick tests (6). Analysis of IgA and IgE in tears from patients with birch pollen-induced allergic conjunctivitis has shown that while IgE was Bet v 1-reactive, IgA was mainly reactive to other, nonallergenic moieties. It was suggested that the protective effect of IgA, i.e. by competitively blocking binding of IgE to Bet v 1, thus would be lost (7). However, in this study Bet v 1-reactive IgA and IgG were found in almost all samples from the patients, and in none from the controls. By contrast, Bet v 1-reactive IgE was only detected in a small number of patients during the season, and Bet v 2-reactive IgE was nondetectable. Previous studies of grass pollen-induced IAR have, however, shown nasal fluid allergen-reactive IgE (2), suggesting that the concentrations of Bet v 1- and v 2-reactive IgE may be below the detection limits of the assays used in this study. Allergen-specific IgA and IgG in nasal fluids from patients but not controls agree with other studies of grass pollen- and ragweed-sensitive IAR (2, 4). However, in contrast to two studies of IAR sensitive to ragweed, there were no significant differences in allergen-reactive IgA levels between our patients during and season. This could depend on antigenic heterogeneity causing variable antibody reactivities. Native Bet v 1 consists of many different isoforms (15). It is thus possible that IgA reactivity against recombinant Bet v 1 may not be representative of reactivity against native Bet v 1. However, nasal fluid levels of native and recombinant Bet v 1-reactive IgA were highly correlated. To assess IgA reactivity against moieties other than Bet v 1, IgA reactive to native birch extract was analyzed. The levels of native birch-reactive IgA were similar to those for native Bet v 1-reactive IgA. This indicates that allergen-reactive IgA mainly reacted with Bet v 1. Further studies of IgA reactive to other moieties, e.g. Bet v 2, are needed to clarify

this. To our knowledge there are no previous reports comparing allergen-reactive IgG during and off season. However, similar to Bet v 1-reactive IgA, there were no differences in Bet v 1 reactive IgG during and off season. These findings do not indicate that Bet v 1-reactive IgA or IgG are suitable for assessing the degree of local inflammation in IAR. However, it is noteworthy that allergen-specific IgA and IgG antibodies were found in nasal fluids from patients both during and off season, but not in controls. This raises the question if these antibodies could be used for specific allergy diagnosis. In contrast to skin prick tests nasal lavage requires little training and is associated with little or no discomfort. This could be of interest in pediatric practice. Indeed, the method of obtaining nasal fluids in this report has previously been used in large pediatric studies (13). Another question is the functions of allergen-reactive antibodies. Whereas the pivotal role of allergen-reactive IgE in IAR is well documented, the significance of non-IgE antibodies is less clear. Immunoglobulin A antibodies have been considered protective because they may exclude antigens from mucosal tissues (27), their assumed function as blocking antibodies (28) and repeated, although not unanimous, observation of increased prevalence of atopic diseases in selectively IgA-deficient individuals (27, 28). Likewise, allergen-reactive IgG, or IgG subclasses, could have protective properties by blocking or regulatory mechanisms (29). However, high levels of allergen-reactive IgA and IgG in nasal fluids from patients with IAR, but not in controls, contradict the protective role of these antibodies (2). It should be noted that experimental studies indicate that IgA, IgE, and IgG may induce degranulation of eosinophils and neutrophils (30–35). In clinical studies positive correlations between IgA and eosinophil granule proteins have been found (4, 8).

In summary, nasal fluid Bet v 1-reactive IgA and IgG are found in patients with birch pollen-induced allergic rhinitis, but not in healthy controls. Further studies are needed to examine the functional role of these antibodies in allergic disease.

## Acknowledgments

We thank Henrik H. Jacobi for discussions and supply of birch allergen preparations, Ingemo Harrysson, R.N., for expert help with obtaining nasal fluids, Helena Gunnlaugsdottir, Ann Reuterborg, Bodil Persson, for skilful laboratory work. This study was supported by grants from the Swedish Medical Research Council, the Swedish Heart and Lung Foundation, the Tegger Foundation, and the Swedish Society against Asthma and Allergy.

## References

- JOHANSSON SG, DEUSCHL H. Immunoglobulins in nasal secretions with special reference to IgE. I. Methodological studies. *Int Arch Allergy Appl Immunol* 1976;**52**:364–365.
- PLATTS-MILLS TAE. Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. *J Immunol* 1979;**122**:2218–2225.
- BRANDTZAEG P. The Immune system of the nose and nasopharynx. In: MYGIND N, PIPKORN U, editors. *Allergic and vasomotor rhinitis: pathophysiological aspects*. Copenhagen: Munksgaard, 1987:91–120.
- REED CE, BUBAK M, DUNNETTE S, BLOMGREN J, PFENNING M, WENTZ-MURTHA P, WALLEN N, KEATING M, GLEICH GJ. Ragweed-specific IgA in nasal lavage fluid of ragweed-sensitive allergic rhinitis patients: increase during the pollen season. *Int Arch Allergy Appl Immunol* 1991;**94**:275–277.
- KITA H, JORGENSEN RK, REED CE, DUNNETTE SL, SWANSON MC, BARTEMES KR, BLOMGREN J, BACHMAN K, GLEICH GJ. Mechanism of topical glucocorticoid treatment of hay fever: IL-5 and eosinophil activation during natural allergen exposure are suppressed, but IL-4, IL-6, and IgE antibody production are unaffected. *J Allergy Clin Immunol* 2000;**106**:521–529.
- PAULI G, OSTER JP, DEVILLER P, HEISS S, BESSOT JC, SUSANI M, FERREIRA F, KRAFT D, VALENTA R. Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: diagnostic value for birch pollen and associated allergies. *J Allergy Clin Immunol* 1996;**97**:1100–1109.
- AGHAYAN-UGURLUOGLU R, BALL T, VRTALA S, SCHWEIGER C, KRAFT D, VALENTA R. Dissociation of allergen-specific IgE and IgA responses in sera and tears of pollen-allergic patients: a study performed with purified recombinant pollen allergens. *J Allergy Clin Immunol* 2000;**105**:803–813.
- BENSON M, SVENSSON PA, CARLSSON B, JERNÅS M, REINHOLDT J, CARDELL LO, CARLSSON L. DNA microarrays to study gene expression in allergic airways. *Clin Exp Allergy* 2002;**32**:1–8.
- SØRENSEN CH, KILIAN M. Bacterium-induced cleavage of IgA in nasopharyngeal secretions from atopic children. *Acta Pathol Microbiol Scand Sect* 1984;**C92**:85–87.
- KILIAN M, REINHOLDT J, LOMHOLT H, POULSEN K, FRANSEN EV. Biological significance of IgA1 proteases in bacterial colonization and pathogenesis: critical evaluation of experimental evidence. *APMIS* 1996;**104**:321–338.
- KIRKEBY L, RASMUSSEN TT, REINHOLDT J, KILIAN M. Immunoglobulins in nasal secretions of healthy humans: predominance of secretory immunoglobulin A1 [S-IgA1] and occurrence of neutralizing antibodies to IgA1 proteases of nasal bacteria. *Clin Diagn Lab Immunol* 2000;**7**:31–39.
- BENSON M, UDDMAN R, CARDELL LO. Epithelial cells in nasal fluids from patients with allergic rhinitis; how do they relate to the epidermal growth factor, eosinophils and eosinophil cationic protein? *Acta Otolaryngol* 2002;**122**:202–205.
- BENSON M, STRANNEGARD I-L, STRANNEGARD O, WENNERGREN G. Topical steroid treatment of allergic rhinitis decreases nasal fluid TH2 cytokines, eosinophils, eosinophil cationic protein, and IgE but has no significant effect on IFN-gamma, IL-1beta, TNF-alpha, or neutrophils. *J Allergy Clin Immunol* 2000;**106**:307–312.
- GAJHEDE M, OSMARK P, POULSEN FM, IPSEN H, LARSEN JN, VAN NEERVEN RJ, SCHOU C, LOWENSTEIN H, SPANGFORT MD. X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. *Nat Struct Biol* 1996;**3**:1040–1045.
- LOWENSTEIN H, SPARHOLT SH, KLYSNER SS, IPSEN H, LARSEN JN. The significance of isoallergenic variations in present and future specific immunotherapy. *Int Arch Allergy Immunol* 1995;**107**:285–289.
- AHL T, REINHOLDT J. Subclass distribution of salivary secretory immunoglobulin A antibodies to oral streptococci. *Infect Immun* 1991;**59**:3619–3625.
- ZOLLINGER WD, BOSLEGO JW. A general approach to standardization of the solid-phase radioimmunoassay for quantitation of class-specific antibodies. *J Immunol Methods* 1981;**46**:129–140.
- REIMER CB, PHILLIPS DJ, ALOISIO CH, BLACK CM, WELLS TW. Specificity and association constants of 33 monoclonal antibodies to human IgA epitopes. *Immunol Lett* 1989;**21**:209–215.
- HOCINI H, ISCAKI S, BOUVET JP, KAZATCHKINE MD, BELEC L. AN ELISA method to measure total and specific human secretory IgA subclasses based on selective degradation by IgA1-protease. *J Immunol Methods* 2000;**235**:53–60.
- REINHOLDT J. A method for titration of inhibiting antibodies to bacterial immunoglobulin A1 proteases in human serum and secretions. *J Immunol Methods* 1996;**191**:39–48.
- REINHOLDT J, KILIAN M. Comparative analysis of immunoglobulin A1 protease activity among bacteria representing different genera, species, and strains. *Infect Immun* 1997;**65**:4452–4459.
- JOHANSSON SG, HOURIHANE JO, BOUSQUET J, BRUIJNZEEL-KOOMEN C, DREBORG S, HAAHTELA T, KOWALSKI ML, MYGIND N, RING J, VAN CAUWENBERGE P, VAN HAGE-HAMSTEN M, WUTHRICH B. EAACI (the European Academy of Allergology and Clinical Immunology nomenclature task force). A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;**56**:813–824.
- DELACROIX DL, DEHENNIN JP, VAERMAN JP. Influence of molecular size of IgA on its immunoassay by various techniques. II. Solid-phase radioimmunoassays. *J Immunol Methods* 1982;**48**:327–337.
- DE FIJTER JW, VAN DENWALL BAKE AW, BRAAM CA, VAN ES LA, DAHA MR. Immunoglobulin A subclass measurement in serum and saliva: sensitivity of detection of dimeric IgA2 in ELISA depends on the antibody used. *J Immunol Methods* 1995;**187**:221–240.
- CROTTET P, CORTHESY B. Secretory component delays the conversion of secretory IgA into antigen-binding competent F[ab']<sub>2</sub>: a possible implication for mucosal defense. *J Immunol* 1998;**161**:5445–5453.
- NACLERIO R, TOGIAS R, FLOWERS B, PROUD D, KAGEY-SOBOTKA A, NORMAN P, LICHTENSTEIN L. Nasal lavage: a technique for elucidating the pathophysiology of allergic rhinitis. In: MYGIND N, PIPKORN U, DAHL R, editors. *Rhinitis and asthma*. Copenhagen: Munksgaard, 1990:213–221.

27. RUSSELL MW, KILIAN M, LAMM ME. Biological activities of IgA. In: OGRA PL, MESTECKY J, LAMM ME, STROBER W, BIENENSTOCK J, MCGHEE JR, editors. *Mucosal immunology*, 2nd edn. San Diego: Academic Press, **1999**:225–252.
28. STOKES CR, TAYLOR B, TURNER MW. Association of house-dust and grass-pollen allergies with specific IgA antibody deficiency. *Lancet* 1974;**31**: 485–488.
29. POULSEN LK. Regulation of the IgE allergic immune response by humoral and cellular factors. *APMIS* 2000;**98**(Suppl):1–43.
30. ABU-GHAZALEH RI, FUJISAWA T, MESTECKY J, KYLE RA, GLEICH GJ. IgA-induced eosinophil degranulation. *J Immunol* 1989;**142**:2393–2400.
31. GOUNNI AS, LAMKHIUED B, OCHIAI K, TANAKA Y, et al. High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* 1994;**367**:183–186.
32. GOUNNI AS, LAMKHIUED B, KOUSSIH L, RA C, RENZI PM, HAMID Q. Human neutrophils express the high-affinity receptor for immunoglobulin E [Fc epsilon RI]: role in asthma. *FASEB J* 2001;**15**:940–949.
33. RAJAKULASINGAM K, DURHAM SR, O'BRIEN F, HUMBERT M, et al. Enhanced expression of high-affinity IgE receptor [Fc epsilon RI ] alpha chain in human allergen-induced rhinitis with co-localization to mast cells, macrophages, eosinophils, and dendritic cells. *J Allergy Clin Immunol* 1997;**100**:78–86.
34. KANEKO M, SWANSON MC, GLEICH GJ, KITA H. Allergen-specific IgG1 and IgG3 through Fc gamma RII induce eosinophil degranulation. *J Clin Invest* 1995;**95**:2813–2821.
35. MOTEGI Y, KITA H, KATO M, MORIKAWA A. Role of secretory IgA, secretory component, and eosinophils in mucosal inflammation. *Int Arch Allergy Immunol* 2000;**122**(Suppl 1):25–27.