

Ragweed IgE and IgG₄ antibody in nasal secretions during immunotherapy

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Summary

We have developed sensitive amplified immunoassays for measurement of IgE and IgG₄ ragweed (RW) antibodies in unconcentrated nasal washes. IgE to *Amb a* I (formerly antigen E) can be assayed to <0.1 ng/ml using IgE capture by anti-IgE on microtitre plates and an alkaline phosphatase-conjugated *Amb a* I with an amplification substrate technique. IgG₄ to whole RW extract was assayed to <0.01 ng/ml by amplification ELISA using monoclonal anti-IgG₄. Nasal washes (NW) (10 ml) and serum were obtained in December from 22 RW-sensitive patients before and after 1 and 2 yr of RW immunotherapy (IT), and assayed for *Amb a* I IgE or RW IgE and RW IgG₄ antibodies. *Amb a* I IgE could be measured in the NW of 15/22 pre IT, 19/22 at 1 yr IT, but only 3/10 at 2 yr IT (compared with pre-IT, $P < 0.05$). Mean *Amb a* I IgE in NW was 0.66, 0.36 and 0.21 ng/ml at pre, 1 yr and 2 yr IT (P -values > 0.05). Mean serum RW IgE, was 76, 55 and 27 ng/ml at pre, 1 yr and 2 yr IT (P -values > 0.05). *Amb a* I IgE in nasal washes was correlated with RW IgE in serum ($r = 0.56$, $P < 0.001$, $n = 44$). RW IgG₄ was detectable in NW of 15/22 pre-IT, 18/22 at 1 yr IT and 9/10 at 2 yr IT (P -values > 0.05). Mean RW IgG₄ in NW did not change with IT (0.12, 0.16 and 0.20 ng/ml at pre, 1 yr and 2 yr IT, P -values > 0.05), whereas serum RW IgG₄ rose substantially from a mean 23 ng/ml pre IT to 197 ng/ml at 1 yr IT and 1324 ng/ml at 2 yr IT (P -values < 0.0001). Nevertheless, RW IgG₄ in NW and serum was significantly correlated ($r = 0.59$, $P < 0.001$, $n = 44$). We conclude that using sensitive immunoassays IgE and IgG₄ RW antibodies can be measured in unconcentrated NW of about two-thirds of ragweed-sensitive patients. Both isotypes in NW have strong correlation with corresponding serum antibodies, but the significant rise of serum RW IgG₄ after IT is not reflected in NW of asymptomatic subjects out of season. Whether allergic inflammation can increase IgG₄ in NW remains to be determined.

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Introduction

Allergic reactions of the respiratory tract involve the respiratory mucous membrane, an organ of external secretion. Inhaled allergen initially encounters the external secretions and the antibody content of these secretions

could potentially influence allergic reactions. Some reports have shown that the allergen-specific IgE in nasal secretions or in nasal mucosa is much more related to clinical symptoms than serum antibody levels [1–6]. Furthermore, in one study the results of the RAST on nasal secretions were in good agreement with bronchial provocation testing [7]. The increase of timothy-specific IgE during the pollination season was significantly more pronounced in nasal secretions than in serum [8]. The local synthesis of IgE antibody has also been suggested by (1) the finding of IgE-forming cells [9,10] and specific IgE antibody [1] in the mucosa of the respiratory tract, and

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(2) the analysis of the antibodies in nasal secretions and in sera [5,8,11–15]. Therefore there is reason to believe that for the study of respiratory allergy measurement of nasal secretions may be of greater significance than measurements in sera.

Allergen-specific IgE or reaginic antibody [2–7,15–23] and specific IgG [15,18,23], IgA [15,18,23,24] and IgM antibodies [23] have been found in nasal secretions using a variety of immunochemical techniques. In previous studies specimens had to be concentrated prior to assay because of low antibody concentrations in nasal washes. Some have tried to collect concentrated nasal secretions by (1) placing a strip of filter paper in the nose [2,4,25], (2) irritating the nasal passages with 18% saline to stimulate nasal secretions [5,7], (3) spray washing [26] or (4) suction prior to tissue removal [14]. None of these techniques have allowed routine measurement of IgE antibodies in unconcentrated nasal fluids or washes. To overcome this difficulty, we have developed sensitive enzyme-linked immunosorbent assays (ELISA) for measurement of IgE and IgG₄ ragweed (RW) antibodies in unconcentrated nasal washes. Using these assays, we have now compared the levels of RW antibodies in nasal secretions and in serum in hay fever patients during 2 years RW immunotherapy.

Materials and methods

Subject selection and treatment

Twenty-two adult patients with a history consistent with ragweed hay fever were recruited prior to the 1985 ragweed season in Baltimore, Maryland. To be entered into the study, patients had to have a positive intradermal skin test (10 mm weal) to a concentration of 10 PNU/ml or less of ragweed extract. Beginning in February 1986, patients received RW (Greer Labs., Lenoir, U.S.A.) immunotherapy rising to a maintenance dose of 2 µg of *Amb a I* over 3 months. In February 1987, 12 patients dropped out, the remaining 10 patients had the maintenance level treatment gradually increased to 20 µg/ml.

Blood for RW antibody assays was obtained 1 month after the end of pollen season (November) pre IT (1985), after 1 yr of IT (1986) and after 2 yr IT (1987). Sera were stored at –70°C until assays were performed. Unfortunately, 10 serum samples from pre IT (1985) were accidentally lost. Nasal washes were obtained each year in December, 1 month later than blood samples.

Ten non-atopic normal adult subjects without personal or family allergic history were selected as normal controls for RW antibody measurements.

Nasal washes

Specimens were collected while the subjects were sitting. They held their breath and extended their neck 45° from the horizontal. Next, 5 ml of normal saline were instilled into each nostril. After 5–10 sec, the subject expelled the wash fluid into a plastic receptacle. We have previously shown that in the unobstructed nose 8 ± 1 ml of fluid is returned using this method [27]. The wash fluids were centrifuged at $5000 \times g$ for 15 min. The aqueous phase was removed from the mucous gel and stored at –70°C until the assays were performed.

Nasal Amb a I IgE assay

Conjugation of alkaline phosphatase to Amb a I. The conjugate was prepared according to a method modified from that of Engvall and Perlman [28]. Glycerol was removed from the *Amb a I* solution (NIH research reference, NIH, Bethesda, U.S.A.) by dialysis against 0.01 M, pH 7.2 phosphate-buffered saline (PBS) overnight. Alkaline phosphatase suspension (0.6 ml of a 8.3 mg protein/ml, 1000 units activity/ml; Sigma type VII-S, Sigma Chemical Co., St Louis, U.S.A.) was centrifuged in the cold for 10 min at $2000 \times g$. The supernatant was discarded and the pellet mixed with 2 mg *Amb a I* in 1 ml PBS. After overnight dialysis against PBS, 25% glutaraldehyde (w/v) (Sigma Chemical Co.) was added to the mixture to make a final concentration of 0.2% (w/v) and vortexed. After 2 hr at room temperature the free glutaraldehyde was removed by dialysis against PBS overnight and then against 0.05 M, pH 8.0 Tris-HCl buffer overnight. The enzyme-conjugated *Amb a I* was diluted to 4 ml with Tris-HCl buffer containing 1% bovine serum albumin (BSA), 0.001 M MgCl₂ and 0.02% Na₂S₂O₃, and stored at 4°C.

Amb a I IgE ELISA. An ELISA with enzyme-conjugated *Amb a I* and an amplification substrate was used for measurement of *Amb a I* IgE in nasal washes. Polystyrene microtitre plates (PGC Scientific Lab., Gaithersburg, U.S.A.) were coated with 0.12 µg/well goat anti-human IgE (affinity purified with PS myeloma protein in our laboratory) in 100 µl of 0.05 M carbonate buffer (pH 9.6) overnight at 4°C, and then washed three times with buffer [0.05 M Tris-HCl (pH 7.5), 0.15 M NaCl, 0.05% Tween] (TBS-T20). After washing, 150 µl/well of 0.2% BSA-TBS-T20 was added and incubated for 1 hr at 37°C and then discarded. One hundred microliters of reference serum or unknown nasal wash sample were then added, each diluted in undiluted negative nasal wash pool from non-atopic subjects. Reference serum No. 5089 was used for a standard curve, beginning with 1:400 dilution. Its value of RW IgE (2782 ng/ml) has previously been

Table 1. Comparison of the three *Amb a I* IgE assays

	Labelled antigen	Labelled antibody	
		Three steps	Four steps
Coating 4°C overnight ($\mu\text{g}/\text{well}$)	Goat anti-IgE 0.12	<i>Amb a I</i> 0.05	<i>Amb a I</i> 0.05
Serum 4°C overnight	No. 5089	No. 5089	No. 5089
Monoclonal antibody 37°C 2 hr	—	—	Anti-IgE, 0.12 $\mu\text{g}/\text{well}$
Enzyme conjugate 37°C 2 hr	<i>Amb a I</i> 1:1000	Goat anti-IgE 1:1000	Goat anti-mouse IgG 1:1000

All three assays employed the enzyme amplification system.

estimated in our laboratory [29]. After overnight incubation and washing three times with TBS-T20, 100 μl of alkaline phosphatase-conjugated *Amb a I* were added to the wells at a 1:1000 dilution in 0.2% BSA-TBS-T20 and incubated for 2 hr at 37°C. After a final washing, 50 μl of the substrate [nicotinamide adenine dinucleotide phosphate (NADP)] from an ELISA Amplification System kit (Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, U.S.A.) were added and incubated for 15 min at room temperature. Then, 50 μl of amplifier (alcohol dehydrogenase and diaphorase) from the same kit were added and incubated for another 15 min at room temperature. The reaction was stopped by addition of 50 μl of 0.3 M H_2SO_4 . Absorbance was read at 492 nm on an automated MR700 microplate reader (Dynatech Laboratories, Alexandria, U.S.A.) The level of *Amb a I* IgE in unknown nasal washes was calculated by interpolation from the dilution curve of reference serum which was mathematically fitted by a three-parameter spline function [30].

Comparison among three *Amb a I* IgE assays. In order to choose the most sensitive assay for measurement of nasal IgE, two other indirect ELISAs (using enzyme-conjugated antibody) were developed for comparison. In these two assays, monoclonal anti-human IgE (ascites fluid, clone 4.15, kindly provided by Dr Andrew Saxon), alkaline phosphatase-conjugated goat anti-human IgE (Sigma Chemical Co.), and affinity-purified alkaline phosphatase-conjugated goat anti-mouse IgG (Tago Inc., Burlingame, U.S.A.) were used. These three assay procedures are briefly compared in Table 1.

Nasal RW IgG₄ assay

An indirect binding ELISA with monoclonal anti-human IgG₄ and amplification substrate was used in the measurement of nasal RW IgG₄. Polystyrene microtitre plates were coated with RW extract (Research Resources

Branch, National Institute of Allergy and Infectious Diseases, Bethesda, U.S.A.) 0.02 $\mu\text{g}/\text{well}$ in 100 μl of 0.1 M acetate buffer (containing 1.0 M NaCl, pH 6.5) overnight at 4°C and then washed three times with TBS-T20. After washing, 150 $\mu\text{l}/\text{well}$ of 0.2% BSA-TBS-T20 were added to the wells with incubation for 1 hr at 37°C and then discarded. Reference serum or nasal wash sample was added exactly as in the *Amb a I* IgE assay except that the reference sera were obtained from a starting 1:20 000 dilution of reference serum No. 6630 in which the absolute value of RW IgG₄ (7 $\mu\text{g}/\text{ml}$) has been estimated in our laboratory [31]. Monoclonal anti-human IgG₄ (clone HP 6025, kindly provided by Dr R. G. Hamilton and then purified from ascites by a DEAE column in our laboratory) was added to the wells at a 1:2000 dilution in 0.2% BSA-TBS-T20 and incubated for 2 hr at 37°C. After washing, 100 μl of affinity-purified alkaline phosphatase-conjugated goat anti-mouse IgG (Tago Inc.) were added to the wells at a 1:1000 dilution in 0.2% BSA-TBS-T20 and incubated for 2 hr at 37°C. After another washing, amplification substrate was added and the remaining steps were the same as those for the *Amb a I* IgE assay.

The precision and reproducibility in RW-IgG₄ assay have been described elsewhere [32]. The sensitivity of the present assay is 0.01 ng/ml.

Serum RW IgG₄ assay

An indirect ELISA with conventional substrate was used to assay IgG₄ antibody in serum. The procedure in this assay was the same as in the measurement of nasal RW IgG₄ except for the following: (1) phosphate-buffered saline (0.05 M Na_2HPO_4 , pH 7.4, 0.15 M NaCl) (PBS) was used instead of TBS; (2) after coating with RW in microtitre plates, the block step with 0.2% BSA-PBS-T20 was omitted; (3) twofold dilutions of reference serum No. 6630 and unknown serum started from 1:25 and were diluted in 0.2% BSA-PBS-T20; (4) monoclonal anti-human IgG₄ and goat anti-mouse conjugate were diluted 1:3000; and (5) conventional substrate, *p*-nitrophenyl-

phosphate (NPP) 1 mg/ml in diethanolamine buffer (pH 9.8), was used instead of the ELISA Amplification System kit. The precision and reproducibility in this assay is the same as nasal RW IgG₄ assay; its sensitivity is 0.1 ng/ml.

Serum RW IgE assay

Serum RW IgE was measured using a standard agarose-based RAST [33]. The precision and reproducibility in this assay has been described previously [29]; its sensitivity was 0.3 ng/ml.

Nasal albumin and protein assays

The albumin in nasal washes were measured by radio-immunoassay as previously described [34]. The protein in nasal washes was measured by the Bio-Rad protein assay (Bio-Rad Labs, New York, U.S.A.).

Statistical analysis

Analysis of data was performed with CLINFO software provided by The Johns Hopkins Clinical Research Center. The data obtained were log transformed. Paired or unpaired *t*-tests were used for group comparison. Linear regression analysis was employed to examine correlations between antibody levels and albumin levels. Chi-square analysis was used to compare frequency of antibody response. For performance assessment, the ELISA precision profiles were calculated according to Ekins [35].

Results

Choice of ELISA for nasal Amb a I IgE assay

Use of the amplification substrate system conveyed approximately 10-fold more sensitivity than the conventional substrate, *p*-nitrophenylphosphate (NPP), in the Amb a I IgE assay (Fig. 1). Among the three amplification ELISAs for Amb a I IgE, the labelled antigen (enzyme-conjugated Amb a I) ELISA had the lowest background, best parallelism and the highest sensitivity, as shown in Fig. 2; its sensitivity was 0.1 ng/ml. Therefore we chose enzyme-conjugated Amb a I ELISA with amplification substrate to measure nasal IgE. The results obtained in this assay were reproducible among replicates (mean CV $\pm$ s.e.m. = 0.05 ± 0.006 ; $n = 76$), between dilutions (0.17 ± 0.03 ; $n = 20$), and between assays (0.18 ± 0.02 ; $n = 62$).

Nasal and serum RW antibody levels

IgE and IgG₄ RW antibody levels both in nasal washes and in serum were below the threshold for detection in all 10 non-atopic subjects.

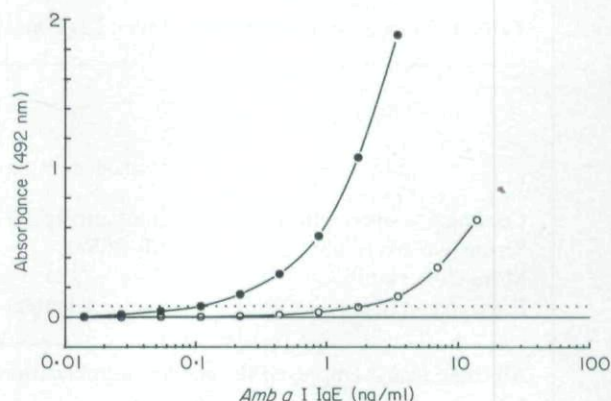


Fig. 1. Comparison of amplification ELISA (●) and conventional ELISA (○) for a Amb a I IgE assay using alkaline phosphatase-conjugated Amb a I (see text). The sensitivity of the amplified ELISA is 0.1 ng/ml or about 10-fold greater than the conventional ELISA.

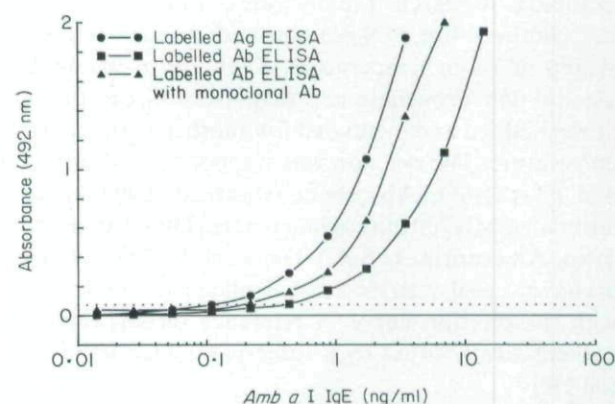


Fig. 2. Comparison of enzyme-conjugated antigen ELISAs and enzyme-conjugated antibody ELISAs. The procedure of labelled antigen assay involved sequential coating/incubation with goat anti-IgE, serum IgE, and finally labelled Amb a I (see text). The sequence for labelled antibody assays was (1) Amb a I, serum IgE, and labelled goat anti-IgE; or (2) Amb a I, serum IgE, monoclonal anti-IgE, labelled goat anti-mouse IgG. The sensitivities are 0.1 ng/ml for labelled Amb a I assay, and 1.74 ng/ml and 0.44 ng/ml for labelled antibody assays, respectively.

Amb a I IgE could be measured in the nasal washes of 15/22 pre IT, 19/22 at 1 yr IT, but only 3/10 at 2 yr IT (compared with pre IT, $P < 0.05$) (Table 2). Mean Amb a I IgE in nasal washes was 0.66, 0.36 and 0.21 ng/ml at pre, 1 yr and 2 yr IT (P -values > 0.05). Mean serum RW IgE was 76, 55 and 27 ng/ml at pre, 1 yr and 2 yr IT (P -values > 0.05) (Table 2). Thus, both mean nasal Amb a I IgE levels and mean serum RW IgE levels decreased during IT, but the changes did not reach statistical

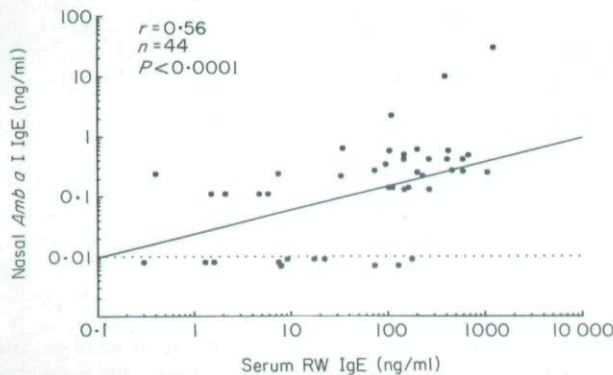
Table 2. Nasal and serum IgE and IgG₄ ragweed antibodies in atopic patients during 2 yr of immunotherapy

Antibody	Before IT		1 yr M IT		2 yr H IT	
	Percentage of specimens measurable	Mean†	Percentage of specimens measurable	Mean†	Percentage of specimens measurable	Mean†
Nasal <i>Amb a I</i> IgE	68 (15/22)	0.66	86 (19/22)	0.36	30 (3/10)*	0.21
Serum RW IgE	100 (12/12)	76	100 (22/22)	55	100 (10/10)	27
Nasal RW IgG ₄	68 (15/22)	0.12	82 (18/22)	0.16	90 (9/10)	0.20
Serum RW IgG ₄	67 (8/12)	23	100 (22/22)	197†	100 (10/10)	1324**

† Geometric mean of measurable samples (ng/ml).

* Compared with the percentage measurable before IT, $P < 0.05$ (chi-square analysis). ** Compared with the antibody level before IT, $P < 0.0001$ (non-paired *t*-test).

1 yr M IT, 1 yr immunotherapy with moderate dose of ragweed; 2 yr H IT, 1 yr immunotherapy with moderate dose of ragweed, followed by another year IT with high dose of RW.

**Fig. 3.** Relationship between nasal *Amb a I* IgE and serum RW IgE.

significance. However, *Amb a I* IgE in nasal washes was correlated with RW IgE in serum ($r = 0.56$; $P < 0.0001$) (Table 3, Fig. 3).

In contrast with IgE, RW IgG₄ increased markedly in serum but non-significantly in nasal washes during IT. RW IgG₄ was detectable in nasal washes of 15/22 pre IT, 18/22 at 1 yr IT and 9/10 at 2 yr IT (P values > 0.05). Mean RW IgG₄ in nasal washes did not change significantly with IT (0.12, 0.16 and 0.20 ng/ml at pre, 1 yr and 2 yr IT; P -values > 0.05), whereas serum RW IgG₄ rose almost 10-fold from a mean 23 ng/ml pre IT to 197 ng/ml at 1 yr IT and 1324 ng/ml at 2 yr IT (P -values < 0.0001) (Table 2). Nevertheless, RW IgG₄ in nasal washes and serum were significantly correlated ($r = 0.59$; $P < 0.001$) (Table 3, Fig. 4).

Figures 5 and 6 show the mean percentage of initial RW antibody levels in nasal wash and in serum from the study group. The nasal *Amb a I* IgE and serum RW IgE appeared to decrease in parallel after IT, although there

was no statistically significant fall from initial levels (Fig. 5). However, for RW IgG₄ there was not a parallel change between nasal and serum levels during IT. Nasal RW IgG₄ levels did not change much ($P > 0.05$), while serum RW IgG₄ rose substantially ($P < 0.0001$) (Fig. 6).

Nasal albumin and protein concentrations

Nasal secretions were diluted by the instilled 10 ml of normal saline in nasal washes. In order to compare the diluted antibodies in nasal washes with those in undiluted serum, we evaluated albumin content as an intravascular marker in nasal secretions. In nasal washes, albumin levels were significantly correlated with protein levels $r = 0.91$; $P < 0.0001$; $n = 64$). Geometric mean albumin levels were significantly higher in atopic patients (16.18 μ g/ml) than non-atopic subjects (1.71 μ g/ml; $P < 0.0001$). After 2 yr IT, mean albumin level tended to decrease (18.5 μ g/ml pre IT, 18.21 μ g/ml at 1 yr IT and 9.3 μ g/ml at 2 yr IT), but no statistically significant differences were observed ($P > 0.05$).

Intercorrelations

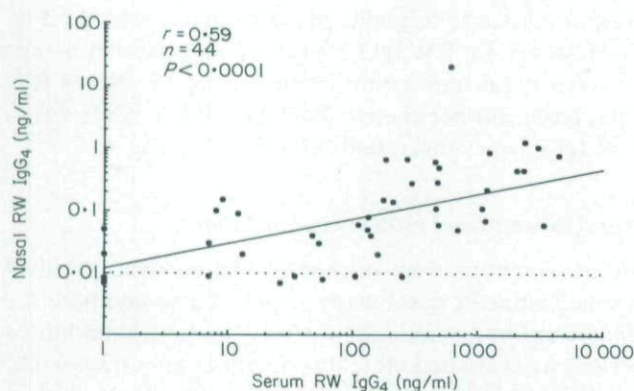
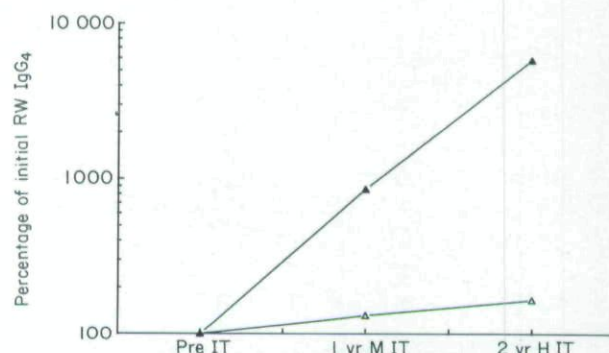
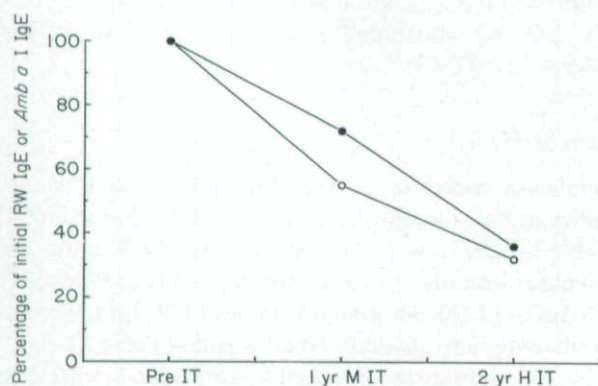
Significant correlations were found between RW antibodies in nasal washes and in serum (P -values < 0.0001) (Table 3, Figs 3 and 4), and between nasal RW antibodies and nasal albumin ($P < 0.005$ for *Amb a I* IgE; $P < 0.03$ for RW IgG₄) (Table 3). *Amb a I* IgE and RW IgG₄ were not significantly correlated in nasal washes (Table 3).

Nasal albumin is supposedly transudated into nasal secretions from plasma. The range of nasal albumin levels in patients was from 1.0 to 628 μ g/ml. In order to see if the nasal antibodies came from blood, we calculated nasal RW antibodies levels per 10 μ g of nasal albumin, and then compared albumin-normalized nasal antibody levels to

Table 3. Correlation between nasal albumin, nasal and serum RW antibody levels in atopic patients during immunotherapy

Correlations	n	Correlation coefficients	P-value
Nasal <i>Amb a</i> I IgE and serum RW IgE	44	0.56 (0.34)	0.0001 (0.02)
Nasal RW IgG ₄ and serum RW IgG ₄	44	0.59 (0.61)	0.0001 (0.0001)
Nasal <i>Amb a</i> I IgE and nasal albumin	54	0.50	0.005
Nasal RW IgG ₄ and nasal albumin	54	0.30	0.03
Nasal <i>Amb a</i> I IgE and nasal RW IgG ₄	54	0.26	0.06

Values in parentheses are correlation coefficients between nasal antibodies normalized for albumin content and serum antibody levels.

**Fig. 4.** Relationship between nasal RW IgG₄ and serum RW IgG₄.**Fig. 6.** The mean percentages of initial values for nasal (Δ) and serum RW IgG₄ ($\bullet$) levels during IT. Nasal IgG₄ did not change significantly ($P > 0.05$) during IT, while serum IgG₄ rose substantially ($P < 0.0001$). See Table 2 for abbreviations.**Fig. 5.** The mean percentages of initial values for nasal *Amb a* I IgE ($\circ$) and serum RW IgE ($\bullet$) levels during IT. Both nasal IgE and serum IgE tended to decrease during IT, but there were no statistically significant changes from initial values (P -values > 0.2). See Table 2 for abbreviations.**Table 4.** The ratios of mean RW IgE/IgG₄ in nasal washes and in serum

	Before IT	1 yr M IT	2 yr H IT
Nasal RW IgE/IgG ₄	5.50	2.25	1.05
Serum RW IgE/IgG ₄	3.30	0.28	0.02

See Table 2 for abbreviations.

serum antibody levels. As shown in Table 3, the coefficient of correlation between nasal *Amb a* I IgE and serum RW IgE was lower ($r = 0.34$, $P < 0.02$) after normalization for albumin than the unnormalized coefficient ($r = 0.56$; $P < 0.0001$). In contrast the coefficient of correlation

between nasal and serum RW IgG₄ levels was changed insignificantly by normalization ($r=0.59$, 0.61). This suggests that most nasal RW IgG₄ may come from serum. The mean ratios of RW IgE/IgG₄ in nasal washes were consistently and significantly higher than the same ratio in serum (Table 4). This observation is compatible with the notion that all nasal IgE does not come from serum.

Discussion

The measurement of small quantities of antibodies in unconcentrated nasal secretions or washings is a technical achievement for those interested in seasonal allergy and immunity. Innovative techniques are required and only recently have enhanced ELISAs with amplification systems able to measure down to 10 pg immunoglobulin been developed [36]. Another problem for measurement of specific IgE is the competitive interference by specific antibodies of other classes, mainly IgG antibody, in patients on allergen immunotherapy [37–39]. Strategies to avoid this interference include an antibody-capture ELISA employing an enzyme-conjugated antigen. Such assays have been reported for measurements of dust-mite IgE [40], crude rye grass IgE [41] and *Amb a* I IgE [42].

In the present report, we combined an enzyme amplification system for increased sensitivity and an antibody-capture ELISA (using enzyme-conjugate *Amb a* I) to measure *Amb a* I IgE in nasal washes. This assay allowed us to measure *Amb a* I IgE to less than 0.1 ng/ml without interference by other isotypes. This assay was shown to detect IgE antibody in 68% of unconcentrated nasal washes from ragweed-allergic subjects (Table 2). This assay had lower non-specific binding, better parallelism and higher sensitivity than the traditional indirect ELISA using an enzyme conjugate to anti-IgE (Fig. 2). Another amplification ELISA was found useful in measuring RW IgG₄ in nasal secretions, with even greater sensitivity (<0.01 ng/ml).

Using these assays, we measured IgE and IgG₄ RW antibodies levels in nasal secretions and in serum during immunotherapy. A dramatic rise in serum RW IgG₄ was found after immunotherapy, but this rise was not reflected in the nasal washes (Fig. 6, Table 2). This finding is in keeping with the results of RW IgG reported by Tse *et al.* [19], and is similar to the results of Platts-Mills *et al.* [24]. Platts-Mills *et al.* reported the increase of RW IgG and RW IgA in nasal washes after immunotherapy [24], but later he confirmed that the increase of serum IgG after immunotherapy was much greater than the increase of IgG in nasal secretions [15]. This suggests that regional injections of allergen mainly stimulate the subcutaneous lymph nodes or spleen to produce IgG₄ which then is translocated into the serum. The small amount of RW IgG₄ in nasal secretions both before and after IT could

come from plasma via vascular permeability or be derived from local synthesis. The possibility of transudation from the vascular compartment was supported by quite similar correlations between nasal RW IgG₄ and serum RW IgG₄ before or after normalization for albumin content (Table 3). Albumin is frequently measured in external secretions to estimate the magnitude of plasma contamination [43]. The nasal washes we evaluated were collected in December from asymptomatic patients out of season. At that time vascular permeability was likely to have been minimal. That some IgG₄ may be produced locally is supported by reports of IgG₄-forming cells in respiratory mucosa [44,45], higher fractions of allergen-specific IgG and IgA in nasal secretions than in serum [15], and evidence for a barrier preventing diffusion of antibody to the mucosal surface [46].

After immunotherapy, mean RW IgE levels remained steady or decreased both in serum ($P>0.05$) and in nasal secretions ($P<0.05$ by chi-square analysis) (Table 2, Fig. 5). Changes in serum IgE antibody after immunotherapy have been well investigated. Usually there is an initial rise of serum IgE and followed by a failure of the customary rise during the ragweed season and ultimately by a decline to pre-treatment levels, usually achieved by 1–2 yr [47]. Our findings are in good agreement with this paradigm.

A significant correlation was found between *Amb a* I IgE in nasal washes and RW IgE in serum ($r=0.56$, $P<0.0001$) (Table 3, Fig. 3), which is in agreement with the results from previous studies [2,12]. After normalization of IgE in nasal washes for albumin content, this correlation was reduced (from $r=0.66$ to $r=0.34$) (Table 3). This weaker correlation suggests that IgE in nasal washes is derived, at least partly, from a non-serum source, most likely local production. Furthermore, the fact that the ratios of mean RW IgE/IgG₄ in nasal washes were higher than those in the corresponding serum specimens (Table 4) provides more evidence for local production of IgE. Our suggestion corresponds well with findings by other investigators who have reported that IgE:IgG ratios and allergen-specific IgE:total IgE ratios were higher in nasal secretions than in serum [11,15]. We were unable to measure total IgE in nasal washes, thereby precluding examination of fractional IgE content in nasal washes versus serum. Furthermore, the number of IgE-containing cells was found to be increased in respiratory mucosa as compared to lymph nodes and spleen [9]. These results taken together with those presented here suggest that there is a local synthesis of IgE in respiratory mucosa. The antibody capture reversed-amplification ELISA for specific IgE we have presented here provides a useful tool to further our understanding of the clinical importance of this discrepancy between local and circulating levels of IgE and IgG antibodies.

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