

Specific IgE antibodies in nasal secretion from patients with allergic rhinitis and with negative or weakly positive RAST on the serum

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Summary

Nasal secretions from eighteen patients with allergic rhinitis with a positive case history, intradermal test and nasal provocation test, but with negative or only weakly positive RAST (radioallergosorbent test) on the serum against a total of thirty-five allergens, were studied. In the RAST an immunosorbent-purified anti-IgE with D ϵ 2 specificity was used, which raised the detection limit. Nasal secretion was collected by washing the nasal mucosa with 0.9% and 18% NaCl solution respectively, and the latter secretion was also lyophilized and concentrated. In ten cases RAST was slightly positive on the nasal secretion, and in three of the concentrated secretions the RAST value was higher than on the serum. In none of the serum or nasal secretion samples was RAST positive according to the cut off value for a positive result defined by the reference system used in Phadebas RAST*. From these results it is concluded that RAST analyses of nasal secretion from patients with allergic rhinitis is of no appreciable value in routine clinical allergological diagnosis. However, the increased sensitivity of RAST obtained with isotope-labelled anti-D ϵ 2 may be useful in the serological diagnosis of patients with low grade allergy having low levels of IgE antibodies in serum.

Introduction

By means of the radioallergosorbent test (RAST) (Wide, Bennich & Johansson, 1967), specific IgE antibodies (reagins) can be demonstrated in serum from patients with allergic rhinitis. Usually there is good agreement between the occurrence of IgE antibodies in the serum and a positive case history, intradermal test and nasal provocation test (Johansson, Bennich & Berg, 1972; Wide, 1973). IgE antibodies have also been found in nasal secretion from patients with allergic rhinitis who showed a positive serum RAST for the same allergens (Johansson *et al.*, 1973; Okuda, 1975; Merrett *et al.*, 1976; Deuschl, 1976). Calculations of the concentrations of reagins in nasal secretion have indicated that IgE antibodies are produced locally in the nasal mucosa (Deuschl, 1976).

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Sometimes patients present with allergic rhinitis and have a positive case history, and a positive nasal provocation test against one allergen, but corresponding IgE antibodies cannot be demonstrated in the serum with the RAST technique (Berg & Johansson, 1974). Such patients might have such a limited production of reagins in the vicinity of the nasal mucosa that the antibody levels are only measurable in the local secretion, and not in the serum. This hypothesis is supported by some recently published investigations (Merrett, 1975; Huggins & Brostoff, 1975; Merrett *et al.*, 1976).

The aim of the present work was to study the occurrence of IgE antibodies in nasal secretion from a group of patients with clinically established allergic rhinitis but with undetectable or very low levels of IgE antibodies in the serum.

Material and methods

Eighteen patients (mean age 26 years, range 21–41 years) with allergic rhinitis were studied at a time when no obvious symptoms of rhinitis were present. The criteria for selection were a positive history of allergy, a positive intradermal test and a positive nasal provocation test for a particular allergen, while the RAST result for the same allergen in the serum was negative or weakly positive. Most of these patients also had allergic rhinitis with a positive case history, intradermal test and nasal provocation test, and at the same time a clearly positive RAST in the serum for one or more other allergens. Some patients also had mild allergic asthma.

Allergic rhinitis with low or non-demonstrable serum levels of IgE antibodies occurred for twelve different allergens, and the total number of such allergen reactions was thirty-five, comprising animal epithelium, pollens, mites and house dust (sixteen, eleven, three and five reactions, respectively). At the time of the present investigation two patients had been receiving parenteral hyposensitization against two of these allergens for the previous 7 and 9 months, respectively.

In order to calculate the albumin concentration in native, undiluted nasal secretion, a separate study was conducted on a further eight patients with allergic rhinitis with a positive case history, intradermal test and nasal provocation test and with a clearly positive RAST. These patients had no obvious symptoms of rhinitis at the time of sampling.

The intradermal tests were read after 10–15 min and were considered positive if the sum of the largest perpendicular diameters of the weal was 20 mm or more. Positive nasal provocation tests were characterized by sneezing, itching and a running nose and/or obstruction of the nasal air passage. For a detailed description of the evaluation of skin and provocation tests, see Fagerberg, Nilzén & Wiholm (1972). The results of the intradermal tests and nasal provocations are given in Table 1. Different batches of allergens were used *in vivo* and in the RAST.

A sample of nasal secretion (secretion A) was taken from all eighteen patients in the main study by the nasal spray washing (NSW) method described previously (Johansson & Deuschl, 1976), using 0.9% NaCl solution. At the same time a sample of venous blood was taken. From thirteen of the eighteen patients a further sample of nasal secretion (secretion B) was collected about 1 week later. On this occasion the nasal mucosa was washed with 1 ml of 18% NaCl solution in each nasal cavity, by the method described by Merrett *et al.* (1976), but with a modification in that the solution was sprayed on to the mucosa. The samples of serum and nasal secretion were stored at -20°C pending analysis. Secretion B, which amounted to 5–8 ml from each patient,

Table 1. Results of skin tests and nasal provocations expressed as the highest allergen dilution giving a positive reaction

	Concentration (w/v)				
	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
Intradermal test					
All allergens	6	21	8		
Allergens with RAST on serum <0.2 PRU†	3	7	3		
Nasal provocation					
All allergens			3	22	10*
Allergens with RAST on serum <0.2 PRU†			1	7	5

* In five cases the positive reaction was obtained at a dilution of 1/20, which was the strongest available extract.

† PRU = Phadebas RAST units.

was divided into two parts, one of which (1–2 ml) was kept unchanged for analyses. The remainder was dialysed in Visking 23/100 dialysis tubing against three changes of 100 vol. each of 0.9% NaCl solution and then freeze-dried. At the time of analysis it was reconstituted with the smallest possible amount of 0.9% NaCl solution to get all of the dry substance in solution (secretion C).

In the separate study of the eight patients mentioned above, in whom the albumin concentration in native, undiluted nasal secretion was calculated, secretion was collected by the method of Lorin, Gaerlan & Mandel (1972), in which a dry strip of filter paper of known weight was applied against the nasal mucosa of one nasal cavity for a few minutes. The paper was then weighed again, whereafter albumin and other soluble substances were extracted in a known volume of isotonic saline solution. The secretion solutions were stored at -20°C until analysed for their albumin concentration. As, with this method, the degree of dilution of the secretion sample was known, the absolute albumin concentration in the original undiluted nasal secretion could be calculated.

On samples of serum and secretion, quantitative determinations of albumin were performed, using single radial immunodiffusion, and of IgE antibodies by RAST, as described previously (Johansson, Högman & Killander, 1968; Johansson, Bennich & Berg, 1971). In the present study the Phadebas RAST® (Pharmacia Diagnostics, Uppsala) reference system and allergen discs were used. In a few cases of less common allergens, we prepared our own allergen discs. Radiolabelled anti-IgE from Phadebas RAST®, which has both D_e1 and D_e2 specificity, was only used in a few experiments for checking RAST results in secretion samples. In the majority of cases ^{125}I -labelled, immunosorbent-purified anti-IgE with D_e2 specificity (Bennich & Johansson, 1970) was used in the RAST. The RAST result was considered to be negative when the concentration of IgE antibodies was <0.2 PRU (Phadebas RAST units), which corresponds to a five-fold dilution of Phadebas RAST® reference D. The count rate of 0.2 PRU is about twice the radioactivity obtained when testing serum or nasal secretion

samples from healthy persons. By a weakly positive RAST level is meant a value ≥ 0.2 but < 1.0 PRU.

Results

The mean absolute albumin concentration in the undiluted nasal secretion collected with filter paper strips was calculated to be 1.04 g/l (range 0.38–2.73 g/l), which corresponded on the average to 3.3% (range 1.2–8.7%) of the albumin concentration in the serum. The mean albumin concentration in secretion A was 0.84 g/l (range 0.18–2.88 g/l) (Fig. 1) and in secretion B 0.58 g/l (range 0.11–1.25 g/l), whereas in secretion C, which consisted of concentrated secretion B, the mean value was 2.49 g/l (range 0.42–5.36 g/l), corresponding to a mean increase in concentration of 3.4 times.

In twenty-two cases the serum level of specific IgE antibodies was weakly positive i.e. ≥ 0.2 but < 1.0 PRU (Fig. 2). No corresponding IgE antibodies were found

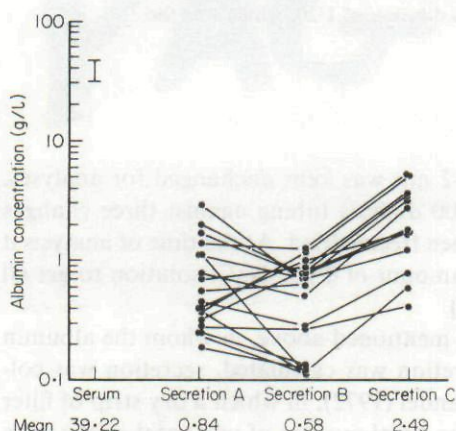


Fig. 1. Albumin concentrations in serum and different nasal secretions. The vertical line (I) near the ordinate shows the range for serum levels. Secretion A, nasal washing with isotonic saline solution; secretion B, nasal washing with 18% saline solution; secretion C, secretion B after additional dialysis, freeze-drying and reconstitution. The arithmetic mean for each type of sample is given.

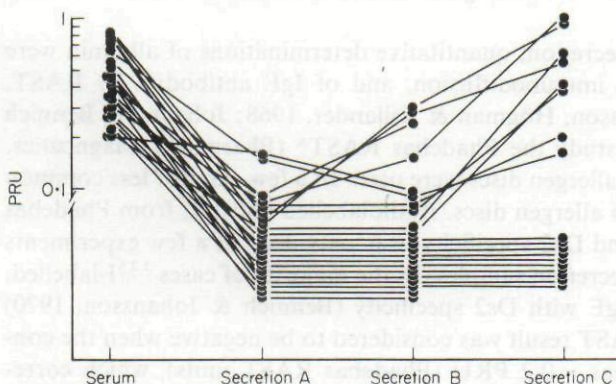


Fig. 2. Concentrations of specific IgE antibodies in serum and different nasal secretions from patients with serum levels ≥ 0.2 < 1.0 PRU (Phadebas RAST units). For notations see legend to Fig. 1.

(<0.2 PRU) in any secretion A samples. In secretion B, RAST was weakly positive in three cases, but in all cases the antibody level was lower than in the serum. In secretion C, RAST was weakly positive in seven of eighteen cases. Of these seven values, three were higher than the corresponding serum values, but in no cases was the concentration ≥ 1.0 PRU.

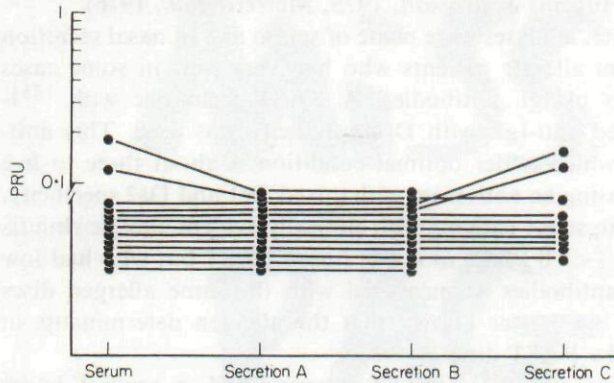


Fig. 3. Concentrations of specific IgE antibodies in serum, and different nasal secretions from patients with serum levels <0.2 PRU (Phadebas RAST units). For notations see legend to Fig. 1.

In thirteen cases the serum content of specific IgE antibodies was lower than 0.2 PRU (Fig. 3). In none of the different types of nasal secretion samples were corresponding IgE antibodies found. Among these thirteen cases with negative serum RAST, animal epithelium allergens were more common (eight of thirteen cases, 62%) than in the group with weakly positive serum RAST (eight of twenty-two cases, 36%).

In order to test whether the use of a hypertonic NaCl solution as a diluent gave an increased count rate in RAST, two RAST-positive sera were diluted 1:2 with 18% NaCl. The final 1.5 mol/l NaCl had very little influence on the RAST values and for none of the five tested allergens was RAST more positive with this method than when isotonic NaCl was used as the diluent.

To compare the results of the RAST method used in the present study and Phadebas RAST®, nine samples of secretion B, all of which were negative in our RAST method to seven allergens (totally thirteen tests)—mainly mites and house dust—were analysed with Phadebas RAST® isotope-labelled anti-IgE, where the sensitivity was increased to detect as little as 0.2–0.5 PRU. In no case was a positive reaction (≥ 0.2 PRU) obtained, which precludes the possibility of significant levels of antibody-active $F(ab')_2$ -fragments in our secretion samples.

As controls, serum and secretion A from thirteen of the eighteen patients were tested against thirty-two allergens, for which the history of allergy and intradermal tests were negative. For all these allergens RAST was negative (<0.2 PRU).

Discussion

In previous investigations of the clinical importance of circulating reagins a small number of patients have been reported in whom IgE antibodies have not been demonstrated in the serum with the RAST technique despite a positive history of allergy and positive skin and provocation tests (Berg & Johansson, 1974). Although these discrepancies may be partly due to the use of different batches of allergen extracts *in vivo* and *in vitro*, most of these patients are presumed to have a low grade allergy with

little production of reagins. Earlier studies have shown that IgE antibodies are probably locally produced in or in the vicinity of the nasal mucosa and that these are then transported selectively to the secretion (Deuschl, 1976). It should thus be possible in certain cases of low grade allergic rhinitis to detect IgE antibodies more easily in nasal secretion than in the serum. This hypothesis has in fact been supported by some recent investigations (Merrett, 1975; Huggins & Brostoff, 1975; Merrett *et al.*, 1976).

To test this hypothesis further, analyses were made of serum and of nasal secretion collected in different ways from allergic patients who had very low, in some cases non-demonstrable serum levels of IgE antibodies. A RAST technique with ^{125}I -labelled immunosorbent-purified anti-IgE with D ϵ 2 specificity was used. This anti-serum gives a RAST method which under optimal condition is about three to five times more sensitive than that using an antiserum with mixed D ϵ 1 and D ϵ 2 specificity. By this method it was possible to select patients with clinically evident allergic rhinitis who were classified as negative (<1.0 PRU) in the ordinary RAST but who had low serum concentrations of IgE antibodies as measured with the same allergen discs but with an anti-IgE (D ϵ 2). Thus it was known that the allergen determinants in question were represented on the RAST discs.

The patients with a negative (<0.2 PRU) RAST in serum had, in general, lower provocation titres than those with a positive RAST. However, it is probably more significant that among the RAST-negative patients an overrepresentation of animal dander allergies occurred. It is well known that animal dander allergens are more heterogeneous than pollen allergens (Fagerberg & Wide, 1970). Thus, the most likely explanation for the discrepancy between RAST and *in vivo* tests in this group of patients is differences in the allergen composition of the preparations used *in vivo* and in RAST. A limited reagin production is likely to be the reason for some of the cases of discrepancy, although a certain number of non-specific skin and provocation tests cannot be ruled out.

Nasal secretion collected by the NSW method, using isotonic NaCl solution, did not show a higher concentration of IgE antibodies than did the serum in any of the patients. As the collection procedure in the NSW method implies dilution of the secretion of unknown magnitude, our results do not preclude the possibility that the reagin levels might be higher in the native nasal secretion than in the corresponding serum. To obtain an idea of the degree of dilution, the albumin concentration in samples of undiluted nasal secretion collected on dry strips of filter paper inserted in the nose was compared with that in NSW samples. Undiluted nasal secretion from patients with allergic rhinitis contained, on the average, 1.04 g/l albumin, corresponding to 3.3% of the serum concentration, and the mean albumin concentration in NSW samples was 0.84 g/l. Mygind, Weeke & Ullman (1975) reported an albumin concentration of 3.9% of the serum albumin level in nasal secretion collected by the filter paper method from patients with allergic rhinitis.

The similarity in albumin concentration between undiluted secretion and secretion after NSW indicates that the latter method yields a relatively undiluted sample. The fact that no IgE antibodies were demonstrated in NSW despite a sensitive RAST technique cannot therefore, it seems, be attributed to a dilution effect.

With Phadebas RAST[®], Merrett *et al.* (1976) obtained a positive reaction (≥ 1.0 PRU) in nasal secretion from five of nineteen patients with allergic rhinitis despite the fact that corresponding serum samples were negative (<1.0 PRU). It is conceivable that their collection technique, with 18% NaCl solution, might have given a concen-

trated secretion sample. In our study, however, the albumin concentration in secretion samples obtained by nasal washing with 18% NaCl (secretion B) was, as a rule, distinctly lower than in samples obtained by NSW with 0.9% NaCl. This shows that 18% NaCl does not generally give a more concentrated nasal secretion sample. Neither does the high NaCl concentration seem to enhance the sensitivity of RAST. It is possible, on the other hand, that washing with strongly hypertonic NaCl might give a selective increase of IgE antibodies in the nasal secretion and this is supported by our finding of IgE antibodies in secretion B in three cases. In no patient, however, have we found a higher content of IgE antibodies in the nasal secretion than in the corresponding serum sample. The lack of agreement between our results and those of Merrett *et al.* may be due to the selection of patients. It has been suggested by Merrett *et al.* (1976) that some patients with recently acquired allergic rhinitis may have a higher concentration of reagins in the nasal secretion than in the serum.

Another conceivable explanation for the negative RAST results in the nasal secretion is that the IgE antibodies in the samples were partly in the form of $F(ab')_2$ fragments. The isotope-labelled anti-IgE present in Phadebas RAST® includes specificity for the D ϵ 1 antigen, which is localized to the $F(ab')_2$ fragment of IgE, in contrast to the D ϵ 2 antigen. The result of our comparison between the RAST used in the present study and Phadebas RAST®, however, speaks against this hypothesis.

In most previous investigations of IgE antibodies in nasal secretion, measured by RAST, the secretion has been collected after washing the nasal mucosa with a saline solution (Johansson *et al.*, 1973; Merrett *et al.*, 1976; Deuschl, 1976). Such a method gives a dilution of unknown degree, making a comparison between reagin levels in native, undiluted nasal secretion and serum impossible. Okuda (1975), using a micro-method for collection of nasal secretion that did not involve dilution, found a concentration of IgE antibodies in the serum, measured by RAST, that was, on average, about twice that in the nasal secretion. Huggins & Brostoff (1975) collected nasal secretion with a dry strip of filter paper from patients with low-grade allergy to mites. After extraction in physiological saline solution, the extract was concentrated by freeze-drying and reconstituted. In the majority of cases RAST was positive in the secretion but negative in the serum. Unfortunately the degree of any possible increase in concentration was not given.

In the present study secretion B was concentrated correspondingly by freeze-drying and reconstituted in a minimal amount of saline solution (secretion C). The mean albumin concentration in this concentrated secretion was 2.49 g/l, corresponding to three to four times the concentration of secretion B, which was clearly higher than in secretion collected both by the filter strip method and by NSW with isotonic saline solution. Despite the fact that these secretion samples were probably more concentrated than native, undiluted nasal secretion, the level of specific IgE antibodies was higher than in the serum in only three cases, and in no case was it ≥ 1.0 PRU.

The authors cannot explain the difference between their results and those of Huggins & Brostoff (1975). It is remarkable, however, that Huggins & Brostoff found three times higher values, expressed in ct/min, for serum than for secretion samples from non-atopic subjects. This is not the case in the RAST system used in the present study.

Thus in the present study no evidence was found that IgE antibodies are present in a higher concentration in the nasal secretion than in the serum in patients with allergic rhinitis. Even if it is possible that in rare cases reagins may occur in a higher concentration in the nasal secretion, we do not consider that *in vitro* diagnosis by

RAST should be performed routinely on nasal secretions samples. For scientific studies of allergic rhinitis, on the other hand, secretion analyses are of great interest. It seems possible, however, that with a more sensitive RAST method in serum analysis, e.g. with the use of isotope-labelled anti-De2, a number of patients with low grade allergy may be discovered.

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