

Clinical significance of specific IgE determination on nasal secretion

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Summary

The clinical use of RAST on the nasal secretions was investigated in seventeen atopic patients, with asthma or rhinitis, who had shown at a first diagnostic screening, some difficulties in the identification of the responsible allergen(s). The results of the skin tests, of the RAST on the serum and on the nasal secretions and of the specific provocation test (bronchial or nasal) were compared. In some cases the basophil degranulation test was performed. The results of the RAST on the nasal secretions were in perfect agreement with the provocation test. The skin tests and the RAST on the serum showed many discrepancies, particularly for *Dermatophagoides*, epidermal derivatives of cat and dog and moulds, and less frequently for *Graminaceae* and other pollens. It is concluded that RAST analysis on nasal secretions is useful in clinical diagnosis of allergy especially for *Dermatophagoides*, epidermal derivatives and moulds. Most false positive results were observed with the RAST on serum; in fifteen cases it was positive, while all the other tests, basophil degranulation test included, were negative. The data suggest that IgE may have a low affinity for basophil receptors.

Introduction

Identification of the allergic component of bronchial asthma and rhinitis is based upon the clinical examination, the skin tests, the RAST and the provocation test. All these tests are sometimes falsely positive or falsely negative and the provocation test is complicated and feasible only in specialized centres and not practicable as a routine.

The production of antibodies IgE, IgG or IgA, against inhaled substances takes place mainly at the mucosal level (Deuschl, 1976; Platts-Mills, 1979). We have compared the results of all these tests with the RAST performed on the nasal secretions, to evaluate its potential for clinical use.

Other authors have used the RAST on the nasal secretions (Merret *et al.*, 1976;

Huggins & Brostoff, 1975; Deuschl & Johansson, 1977) with varying results, mainly due to the selection of the subjects and to the use of different techniques to collect nasal secretions. This investigation was performed on subjects who, on a routine screening, failed to identify the allergen responsible for their symptoms.

Materials and methods

Subjects

We selected seventeen patients, whose age range was 16 to 53 years. In all of them the results of the skin tests and of the RAST on the serum were not in agreement with the clinical history and had shown multiple positive results (except for two cases, Nos 11 and 16). Thirteen subjects were affected by bronchial asthma (seven had asthma and rhinitis) and four had only rhinitis.

Skin tests

Skin tests were performed in the form of prick tests, with extracts by Hollister-Stier (Bayropharm, Italy), using the method of Norman (1978). We used pollen mix of *Graminaceae*, trees and *Compositae*, *Parietaria officinalis*, *Dermatophagoides pteronyssinus*, epidermal derivatives of dog and cat, *Alternaria tenuis*, *Cladosporium herbarum*, *Mucor racemosus*, *Penicillium notatum* and *Aspergillus fumigatus*.

Nasal secretion

The nasal secretion samples were collected according to the method by Merret *et al.* (1976). Briefly, we instilled in each nostril, by means of spray, 1 ml NaCl 18% solution. After removing the instilled solution, the nasal secretions were collected in plastic containers and at once frozen at -20°C . We added 50 $\mu\text{l/ml}$ Tween 20 to each sample.

Total IgE

The concentration of total IgE in the serum and the nasal secretions was carried out using PRIST (Pharmacia, Sweden).

Specific IgE determination

The determination of the specific IgE in the serum and the nasal secretions was carried out by the RAST, (Pharmacia, Sweden). Results are expressed in specific counts bound (%SCB), computed by subtracting from the number of counts of the examined sample, those of a pool of negative sera. We accepted as a positive value SCB greater than 2%.

The concentration of IgA, IgG and albumin both in the serum and the nasal secretions was obtained using the technique of Mancini, Carbonara and Heremans (1965) (ICL Scientific, U.S.A.).

Specific bronchial provocation test

The test was carried out only in subjects with a $\text{FEV}_1:\text{VC}$ ratio $> 70\%$, and who had not taken antihistamines, corticosteroids or β_2 mimetic drugs for at least 72 hr.

The aqueous allergenic extracts were prepared by Hollister-Stier. We used an increasing dose of allergen (diluting the extract in a physiological solution), starting from 5 PNU for the pollens of *Graminaceae*, *Parietaria* and Wormwood and from 25 PNU for the others, administered by an ultrasonic micronizer de Vilbiss 65.

If the subject did not respond to the initial concentration, he was exposed, after 20

min, to a double concentration, up to the maximum dose of 1000 PNU. Measurements made immediately after the exposure and respectively at 10, 20, 60 min and then after 6 hr. We evaluated the VC, the FEV₁, the MEF₂₅₋₇₅. The test was considered positive if the FEV₁ and the MEF₂₅₋₇₅ fell > 20% (Vitalograph).

Specific nasal provocation test

It was performed by means of a posterior rhinorheomanometer (Mercury NR2). The test was considered positive after an increase of the nasal resistances of 100% and/or the appearance of rhinorrhoea, sneezes, itching and nasal obstruction. The measurements were made as for the bronchial provocation test.

Basophil degranulation test

The basophil degranulation test was performed according to the technique of Benveniste *et al.* (1977). We considered positive degranulation percentages greater than 40%.

Results

The results of the prick test, of the RAST on the serum and on the nasal secretions and of the provocation test in the studied subjects are in Table 1. We considered the provocation test as the reference parameter and in our study it agreed with the patients' clinical state. We observed many discrepancies among the various tests (Table 2) and particularly: (1) agreement of the four tests in 20/50; (2) agreement only of the prick test and of the RAST on the serum in 3/50; (3) agreement only of the RAST on the secretion and of the provocation test in 3/50; (4) agreement only of the prick test in 8/50; (5) agreement only of the RAST on the serum in 15/50; (6) agreement of the RAST on the serum, RAST on the secretion and of the provocation test in 1/50.

Analysing the results for single allergens, the four tests gave good agreement for the Graminaceae pollens (Table 3), only in one subject (No. 7) the RAST on the serum was positive while all the other tests were negative. Good agreement was also observed with Wormwood and *Parietaria* pollens (Table 4). False positives occurred in two cases (Nos 5 and 9) with the prick test and in three cases (Nos 1, 4 and 14) with the RAST on the serum. Furthermore, in one subject (No. 4) the RAST on the serum and the prick tests were positive, while the RAST on the nasal secretion was negative. A provocation test was not carried out on this subject, but the clinical story seemed to exclude an allergy for this pollen.

The RAST on the nasal secretions always agreed with the provocation test ($r = 1$, computed considering the data as either positive or negative.). The correlation coefficient was significative for the prick test ($r = 0.6$), but not for RAST on the serum ($r = 0.4$).

With *Dermatophagoides pteronyssinus*, (Table 5), the prick test alone was positive in three cases (Nos 1, 2 and 8), the RAST on the serum in two (nos 7 and 15), the RAST on the secretion only in one (No. 6), in agreement with the provocation test.

The correlation coefficient was significative only for the RAST on the secretion ($r = 1$).

The results for the epidermal derivatives of dog and cat are combined in Table 6. All four tests were simultaneously positive in four cases (Nos 2, 7, 8 and 13), while the prick

Table 1. Results of the prick tests, of the RASTs on the serum and on the nasal secretions and of the provocation test. In the table we compare only the allergens that were positive

Patients and symptoms	Serum	Prick test	RAST (% SCB)		Provocation test
			Serum	Secretion	
(1) F.M. asthma	<i>Dermatoph.</i>	+	1.5	1.1	—
	Dog ep.	+	1.1	0.9	—
	Mucor	—	3.7	2.1	+
	<i>Alternaria*</i>	—	3.6	0.9	—
	Gramin.	++	20.0	3.6	+
	Wormwood	—	2.0	0.2	—
(2) L.S. asthma	<i>Dermatoph.</i>	+	0.8	1.3	—
	Cat ep.	++	41.9	10.3	+
	Gramin.	++	10.5	4.9	+
	Dog ep.	—	2.5	0.4	—
(3) O.B. asthma rhinitis	<i>Dermatoph.*</i>	+	2.9	1.3	—
	Cat ep.	—	6.2	0.9	—
	<i>Penicillium</i>	—	2.2	1.1	—
	Gramin.	++	9.6	2.8	+
	Wormwood	+++	32.0	6.2	+
(4) D.M. asthma rhinitis	Gramin.	++	44.1	4.0	+
	<i>Parietaria</i>	—	2.6	1.1	—
	Wormwood*	++	15.0	0.6	ND
(5) M.S. asthma rhinitis	Gramin.	+++	30.7	15.4	+
	<i>Parietaria</i>	+	0.4	1.4	—
	Wormwood	+++	22.1	3.5	+
(6) G.M. rhinitis	<i>Dermatoph.</i>	—	0.4	2.6	+
	<i>Parietaria</i>	+++	24.0	4.5	+
	Wormwood	+++	18.0	2.9	+
(7) R.G. asthma rhinitis	<i>Dermatoph.</i>	—	2.0	1.2	—
	Cat ep.	++	14.5	3.9	+
	Gramin.*	—	9.7	0.8	—
(8) N.M. asthma	<i>Dermatoph.</i>	+	0.5	0.1	—
	Dog ep.	++	33.0	2.8	+
(9) V.B. rhinitis	Gramin.	+++	16.0	5.0	+
	<i>Parietaria</i>	+	0.5	0.5	—
(10) F.C. rhinitis	Cat ep.	+	0.5	0.2	—
	<i>Penicillium</i>	+	0.9	0.1	—
(11) A.C. asthma	<i>Cladosp.</i>	—	0.5	2.1	+
(12) C.D. rhinitis	Gramin.	++	29.4	4.8	+
	Wormwood	+	2.8	2.0	+
(13) F.D. asthma rhinitis	<i>Dermatoph.*</i>	++	13.7	0.9	—
	Cat ep.	++	34.3	5.1	+

Table 1 continued

Patients and symptoms	Serum	Prick test	RAST (% SCB)		Provocation test
			Serum	Secretion	
(14) A.R. asthma	Gramin.	+++	25.0	8.2	+
	Wormwood	—	2.2	0.2	—
(15) P.G. asthma	Parietaria	+++	45.0	8.4	+
	<i>Dermatoph.*</i>	—	6.8	0.4	—
(16) P.P. asthma	<i>Alternaria</i>	—	1.2	3.2	+
(17) E.B. asthma	Dog ep.*	—	29.0	0.9	—
	Cat ep.	—	11.0	0.7	—
	<i>Parietaria</i>	++	11.0	3.6	+
	Gramin.	+++	39.4	8.4	+
	<i>Mucor</i>	—	2.7	1.8	—
	<i>Penicillium</i>	—	3.2	1.3	—
	<i>Cladosp.</i>	—	2.4	0.8	—

* Allergens for which the basophils degranulation test was performed

test and the RAST on the serum by themselves were positive respectively in two (Nos 1 and 10) and in three cases (Nos 3 and 17 positivity for cat and dog epithelium).

Complete agreement between the RAST on the nasal secretion and the provocation test was observed ($r=1$) also for these allergens. The correlation coefficient for the prick test ($r=0.4$) and for the RAST on the serum ($r=0.4$) were not significant.

For the moulds the prick test by itself was positive in one case (No. 10) and the RAST on the serum in five (Nos 1, 3 and 17 positivity for three moulds); furthermore in one case the RAST on the serum and on the secretions and the provocation test were positive, while the prick test was negative. In two cases (Nos 1 and 11) the RAST on the secretions was positive in association with the provocation test. The correlation

Table 2. Frequency of the positive results combinations occurring in the studied subjects (PT as reference parametre)

Tests	Positive results (n)	Percentage
Prick test, RASTs,* RASTsc,† PT‡	20	40
RASTs only	15	30
Prick test only	8	16
RASTsc and PT only	3	6
Prick test and RASTs only	3	6
RASTs, RASTsc and PT only	1	2

* RASTs = RAST on the serum

† RASTsc = RAST on the secretion

‡ PT = provocation test

Table 3. *Graminaceae* pollens. Comparison among the positive results of the performed tests

Patients	Prick test	RAST (% SCB)		Provocation test
		Serum	Secretion	
(1) F.M.	++	20.0	3.6	+
(2) L.S.	++	10.5	4.9	+
(3) O.B.	++	9.6	2.8	+
(4) D.M.	++	44.1	4.0	+
(5) M.S.	+++	30.7	15.4	+
(7) R.G.	-	9.7	0.8	-
(9) V.B.	+++	16.0	5.0	+
(12) C.D.	++	29.4	4.8	+
(14) A.R.	+++	25.0	8.2	+
(17) E.B.	+++	39.4	8.4	+
Total positive results	9	10	9	9

Table 4. Wormwood and *Parietaria*. Comparison among the positive results of the performed tests

		RAST (% SCB)		
	Prick test	Serum	Secretion	Provocation test
Wormwood				
(1) F.M.	—	2.0	0.2	—
(3) O.B.	+++	32.0	6.2	+
(4) D.M.	++	15.0	0.6	ND
(5) M.S.	+++	22.1	3.5	+
(6) G.M.	+++	18.0	2.9	+
(12) C.D.	+	2.8	2.0	+
(14) A.R.	—	2.2	0.2	—
Total positive results	5	7	4	4
Parietaria				
(4) D.M.	—	2.6	1.1	—
(5) M.S.	+	0.4	1.4	—
(6) G.M.	+++	24.0	4.5	+
(9) V.B.	+	0.5	0.5	—
(15) P.G.	+++	45.0	8.4	+
(17) E.B.	++	11.0	3.6	+
Total positive results	5	4	3	3

coefficient was not significant for the prick test ($r = -0.2$) or for the RAST on the serum ($r = -0.5$), contrasting with the RAST on the nasal secretions ($r = 1$).

Basophil degranulation test

We performed the *in vitro* basophil degranulation test, in presence of the specific antigen, in four subjects who had specific IgE in the serum, but not in the nasal

Table 5. *Dermatophagoides pteronyssinus*. Comparison among the positive results of the performed tests

Patients	Prick test	RAST (% SCB)		Provocation test
		Serum	Secretion	
(1) F.M.	+	1.5	1.1	—
(2) L.S.	+	0.8	1.3	—
(3) O.B.	+	2.9	1.3	—
(6) G.M.	—	0.4	2.6	+
(7) R.G.	—	2.0	1.2	—
(8) N.M.	+	0.5	0.1	—
(13) F.D.	++	13.7	0.9	—
(15) P.G.	—	6.8	0.4	—
Total positive results	5	4	1	1

Table 6. Epidermic derivatives (dog and cat). Comparison among the positive results of the performed tests

		RAST (% SCB)		
	Prick test	Serum	Secretion	Provocation test
Dog epithelium				
(1) F.M.	+	1.1	0.9	—
(2) L.S.	—	2.5	0.4	—
(8) N.M.	++	33.0	2.8	+
(17) E.B.	—	29.0	0.9	—
Total positive results	2	3	1	1
Cat epithelium				
(2) L.S.	++	41.9	10.3	+
(3) O.B.	—	6.2	0.9	—
(7) R.G.	++	14.5	3.9	+
(10) F.C.	+	0.5	0.2	—
(13) F.D.	++	34.3	5.1	+
(17) E.B.	—	11.0	0.7	—
Total positive results	4	5	3	3

secretions, and with negative prick tests. In all subjects it was completely negative, while it was positive in the controls. In three patient (Nos. 3, 4 and 13), who had specific IgE in the serum, but not in the nasal secretions and had a positive prick test, the *in vitro* basophils degranulation test was positive.

IgA concentration in the serum and in the nasal secretions

The levels of IgA in the serum were between 1.56 g/l and 3.5 g/l (mean 2.8 ± 0.56); in the nasal secretions the levels were between 0.10 g/l and 0.35 g/l (mean 0.16 ± 0.07).

Albumin concentration in the serum and in the nasal secretions

The levels of albumin in the serum were between 31.6 g/l and 60 g/l (mean 45.1 ± 7.6); in the nasal secretions the levels were between 0.10 g/l and 2.5 g/l (mean 0.75 ± 0.8).

Total IgE concentration in the serum and in the nasal secretions

We recorded values between 50 u/ml and 1000 u/ml in the serum with a mean of 402 ± 331 , while in the nasal secretions they ranged from 0.5 u/ml to 40 u/ml with a mean of 13.9 ± 11.9 .

Discussion

The use of this set of tests enabled us to identify the offending allergen in all the subjects studied, who (except for Nos 11 and 16) had positive skin tests and/or RAST on the serum for several allergens. An excellent agreement was observed between the RAST on the nasal secretions and the provocation test. This was regarded by us as a reference for the evaluation of the results and agreed with the clinical assessment of these patients. The prick test and the RAST on the serum showed many discrepancies, especially with the *Dermatophagoides*, the epidermal derivatives and the moulds. Whereas the RAST on the nasal secretion seems useful for *Dermatophagoides*, the moulds and the epidermal derivatives; and could be widely used in diagnosis even as an alternative to the provocation test.

The RAST on the serum was positive in fifteen cases, in whom all the other tests and the clinical history were negative. To clarify these results we performed the basophil degranulation test in four of these subjects, who had high percentages of SCB. In all the test was negative. This result suggests that specific IgE has a low affinity for the basophils receptors and therefore cannot induce a specific degranulation. Other reports (Godfrey *et al.*, 1978; Conroy, Adkinson & Lichtenstein, 1979; Miadonna *et al.*, 1980) support this suggestion of a low affinity of IgE for basophil receptors. Merret (1976) explains the presence of these falsely positive IgE tests (i.e. IgE with no reaginic activity) by a mechanism that would prevent basophils and mast-cell degranulation by interfering with the attachment of the IgE to the specific receptors or to the allergens.

In the cases with only the prick test positive, these were mild reactions (1+) and might have been caused by a non-specific irritant action of the extracts on the skin. We do not think that these responses are due to IgG4 or IgD since the provocation test was negative.

In three subjects with a positive provocation test, we found the IgE only in the nasal secretions. A similar result has been reported by Huggins and Brostoff (1975). The production of the antibodies for the inhaled substances (Butcher, Salvaggio & Leslie, 1975; Deuschl, 1976; Platts-Mills, 1979) takes place mainly in the lymphoid tissue of

respiratory tract and is found in nasal secretions and in the serum. The response to a parenterally administered allergen takes place in the central organs of the immune system (e.g. spleen or draining lymph nodes). After a specific immunotherapy, IgE and IgG levels vary only slightly in the nasal secretions. Further, it has been observed that after tetanus toxoid immunization the specific IgE is found only in the serum, and not in nasal secretions (Miadonna, unpubl. data). We cannot explain why in these three subjects IgE is not present in the circulation, unless the IgE is produced by lymphoid cells of the mucosa placed in an extremely superficial position.

Three subjects had positive prick test and positive RAST on the serum, the RAST on the nasal secretions and the provocation test being negative. The specific basophil degranulation test was positive in these three, thus confirming the reaginic activity of the circulating IgE. It is unexplained why this IgE with affinity for the basophil receptors is not present in the nasal secretions. One possibility is that these subjects have been immunized parenterally and not through the mucosae, although this appears unlikely since we are dealing with inhalants.

As a control on our collection of nasal secretions, we measured the concentration of total IgE, IgA and of albumin. These results are in agreement with published data.

Finally, the perennial allergens (*Dermatophagoides*, cat and dog epithelium and moulds) used in the prick test and in the RAST on the serum were less satisfactory in comparison with the seasonal allergens. This might be due to poor purification and specificity of these extracts and different antigenic characteristics or to distribution in the environment affecting immunization.

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