

Determination of IgE-specificities in nasal secretions and sera of allergic subjects by crossed radio-immunoelectrophoresis

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Summary

Allergen-specific IgE antibodies have not only been demonstrated in the serum, but also in the nasal secretions of allergic patients by means of the radio-allergosorbent test (RAST). In this preliminary study we adapted the technique of crossed radio-immunoelectrophoresis (CRIE) to nasal secretions in order to obtain further insight into the specificity of locally collected antibodies. Based on RAST results we compared CRIE patterns in serum, nasal secretions in 13 out of 23 grass pollen allergic subjects and two out of three house dust mite allergic subjects. In the nasal secretions the same IgE specificities were found as in the corresponding serum of the individual subject and as in a reference serum pool. These data indicate that the same specificity of IgE antibody can be found in the serum and the target organ. From these data there is no evidence for a local mucosal immune response that differs in specificity from the serum response.

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Introduction

Allergic subjects build specific IgE antibodies (IgE-specificities, IgE-SP) against epitopes located on 'allergenic proteins' of inhalant allergens, e.g. pollens or house dust mites. Some of these IgE-SP could be found in the majority of subjects allergic to the respective allergen, and some could only be found in a minority of the subjects [1–3]. Based on the results of these investigations it was concluded that allergen extracts contain major, intermediate and minor allergens [3]. Each individual forms a special composition of IgE-SP against the different 'allergenic proteins' ('fingerprints'), which can be demonstrated by crossed radio-immunoelectrophoresis (CRIE) [4,5].

CRIE has only been performed with sera of allergic patients. There is no direct information on IgE-SP found in secretions of the target organ, i.e. in the nasal secretions (NS) of allergic patients suffering from rhinitis. RAST

measurements performed with NS were compared to the one performed with sera [6–9]. No good correlation could be found. The results of these studies, the discrepancies between skin-prick test and nasal challenge test [10,11] as well as immunohistochemical investigations demonstrating IgE-synthesizing plasma cells [12,13] in the nasal mucosa, suggested that up to 80% of the IgE detected in NS would be produced locally [14]. Recently, a local lymphoid tissue in the mouse nasal mucosa was found: nasal mucosa-associated lymphoid tissue ('NALT'). However, this could not be directly transferred to the human nasal mucosa. Nevertheless, it is unclear whether there is a local mucosal immune response in man independent from the serum response. Investigation of IgE-SP in NS compared to IgE-SP in sera of individual allergic patients may be important to elucidate a suggested local IgE-synthesis [10,15,16] in the nasal mucosa or alterations of the composition of allergen-specific immunoglobulins in the target organ produced by immunotherapy [9].

In this preliminary study we applied CRIE for the comparative investigation of sera (S) and nasal secretions

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(NS) of individual allergic subjects. Our aim was to demonstrate the applicability of the technique for the investigation of NS. Furthermore, we wanted to show whether there is a difference in IgE-SP between S and NS.

Materials and methods

Timothy pollen allergic subjects

Clinical data. Allergy to timothy pollen was demonstrated in 23 subjects by history, skin-prick test, serum RAST and nasal challenge test. Nasal challenge was performed as recently described [17]. In brief they were done with freshly prepared extracts from lyophilized timothy pollen allergen preparations (Allergopharma, Reinbek, FRG). Alterations of the nasal flow were monitored by rhinomanometry. Allergic reactions were evaluated by a symptom score. NS were collected by blowing into parafilm. NS and S of the subjects were frozen at -20°C .

RAST. Specific IgE in S and NS was determined by RAST (Pharmacia, Freiburg, FRG) using timothy pollen allergen discs [18,19].

CRIE. Preliminary investigations showed very weak allergen bands using the diluted NS for CRIE. For further investigations NS was concentrated. Concentration was done by lyophilization of NS and reconstitution with a small volume of twice-distilled water.

CRIE [20] was performed with S and concentrated NS of five individual allergic subjects. In parallel, CRIE was performed using pooled NS ($n=6$) and an in-house reference grass pollen allergic pool serum ($n=41$, RAST-class 4). Ten micrograms protein [21] of the timothy pollen extract were separated in the first dimension and then precipitated in the second dimension in an agarose gel containing rabbit anti-timothy pollen serum ($60\text{ }\mu\text{l/ml}$, ALK, Copenhagen, Denmark). The discs were incubated overnight with S and NS (diluted 1:24) of the subjects. After incubation the discs were washed and incubated with $700\text{ }000\text{ c.p.m./12 ml}$ of [^{125}I]anti-human IgE (Pharmacia) at -20°C for 3, 7 and 14 days. Autoradiographs were obtained using Kodak X-Omat AR films (Kodak, Rochester, U.S.A.).

House dust mite (*Dermatoglyphoides pteronyssinus*) allergic subjects

S and NS of two *D. pteronyssinus* allergic patients who showed a positive clinical history, positive skin-prick test and nasal provocation test, were investigated. In these two cases the nasal lavage was obtained by a lavage technique (NL). Three millilitres of a salt solution were installed into the nose for 1 min and collected by bending the head forward.

For CRIE, a rabbit anti-*D. pteronyssinus* serum and $10\text{ }\mu\text{g}$ protein of a partly purified *D. pteronyssinus* whole mite body extract [22] were used. The sera were diluted 1:24. According to low specific IgE bindings detected by RAST, the concentrated NL of the subject was diluted 1:14. Because of lack of material to perform the CRIE, NL of the second subject was diluted 1:40.

Results

Timothy pollen allergic subjects

RAST. RAST-classes 2–4 (9.5–39.5% binding) were measured with the sera of the 23 timothy pollen allergic subjects. In 19 out of the 23 subjects (82%), timothy pollen-specific IgE could be detected in NS, expressed in RAST-classes 1–4 (1.8–34.3% binding). In 16 out of 23 (69%) subjects, the RAST-classes were lower in NS as compared to S. In the sera of four subjects RAST-classes

Table 1. RAST-measurements in sera and nasal secretion of 23 grass pollen allergic subjects. The samples of the subjects 1, 2, 4, 7 and 16 were used for the parallel investigation of IgE-SP and NS of individual patients

Subject number	Serum		Nasal secretion	
	RAST-class	Binding (%)	RAST-class	Binding (%)
1	4	35.5	3	16.6
2	4	39.5	3	24.2
3	4	39.9	1	3.1
4	4	37.7	4	28.3
5	3	21.8	2	3.8
6	3	17.3	2	9.4
7	3	26.1	4	33.9
8	3	24.0	2	6.5
9	3	23.6	3	14.5
10	3	21.9	1	2.5
11	3	21.5	3	20.4
12	3	26.7	1	3.4
13	3	17.5	0	1.4
14	2	9.5	2	4.5
15	4	38.9	3	13.1
16	4	33.9	4	33.7
17	3	27.4	4	34.3
18	4	37.5	3	15.0
19	4	35.8	0	1.3
20	4	33.7	1	1.8
21	4	37.4	3	14.1
22	3	25.7	0	1.0
23	4	31.2	0	1.5

Fig. 1. Parallel investigation of serum (S) and nasal secretion (NS) of a timothy pollen allergic subject (number 7) by CRIE. (†) Indicates the allergens (1) Ag 19 (*Phl p* VI), (2) allergen X¹, (3) Ag 25 (*Phl p* V), (4) Ag 27, (5) Ag 41. Time of autoradiography: 14 days.

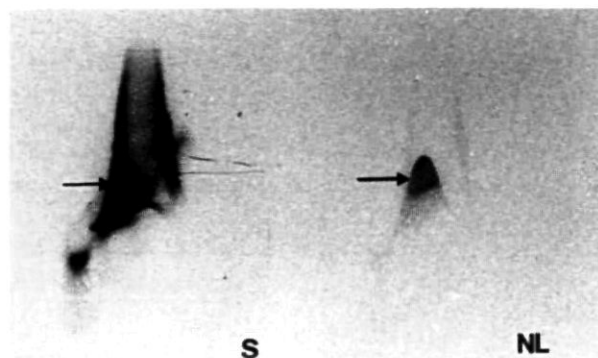
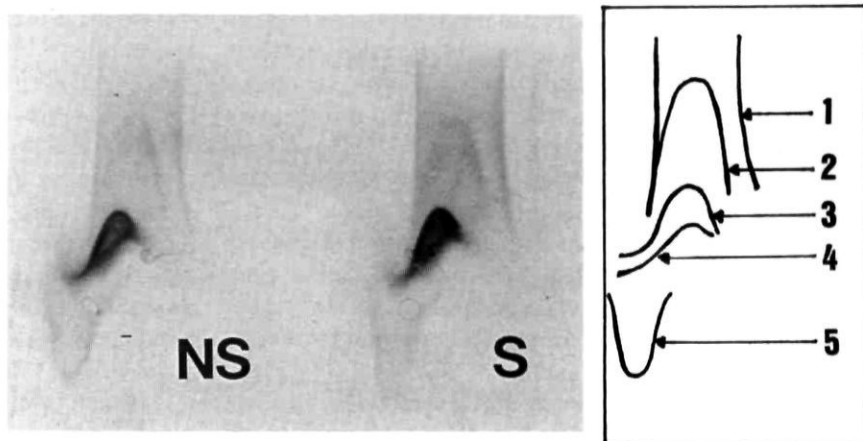


Fig. 2. CRIE of the serum (S) and the nasal lavage (NL) of a *D. pteronyssinus* allergic subject (number 2). (†) Indicates the major allergen *Der p* I. Time of autoradiography: 14 days.

3 and 4 were measured. No specific IgE could be detected in NS. Only in two subjects were RAST-classes higher in NS than in S (Table 1).

CRIE. In S and NS of five subjects (numbers 1, 2, 4, 7, 16) comparable IgE-SP patterns could be detected (Fig. 1, subject 7). The allergen pattern between S and NS were comparable. The pattern obtained in NS demonstrated the same radiostaining intensity for the individual allergen bands as in S. The IgE-SP detected with the pooled NS of six subjects (numbers 5, 6, 8, 9, 11, 15) corresponded well to the one of the in-house reference serum pool. Differences could only be seen in the radiostaining intensity of some allergen bands. IgE against Ag 19 (*Phl p* VI), Ag 25 (*Phl p* V) which are major allergens and against Ag 27 and Ag 41 (2–4, 23, 24) could be detected as well in NS and S of the individual subjects and in the pooled serum. In NS and S the recently described 'allergen X' could be detected [5]. The major allergens were specified according to literature [2,25].

House dust mite allergic subjects

RAST. With the sera, RAST-class 4 and with NL, RAST-classes 1 and 2 were measured.

CRIE. The allergen pattern was more complex and the radiostaining intensity of IgE specificities was more pronounced in S than in the respective NL. According to our results with NS obtained with the challenge method these differences may just reflect lower concentrations of specific antibodies due to dilution by the lavage technique. IgE-antibodies against the major allergen *Der p* I [1] as well as against two further allergen bands could be detected in S and NL of each subject (Fig. 2, subject 2).

Discussion

CRIE is a suitable technique to detect IgE antibodies formed by the individual patient against 'allergenic proteins' from inhalant allergens. According to our knowledge, allergen-specific IgE-antibodies in the secretions of the target organ—the nasal secretions (NS) in allergic rhinitis—were only measured by RAST. By RAST, quantitative differences of specific IgE between NS and sera of individual subjects were detected [7,8]. From these studies it has been suggested that this is a local production of IgE antibodies in the nasal mucosa which is considered to be as high as 80% in some cases [14]. Clinical studies showing positive nasal challenge tests and negative skin-prick tests in the same patients as well as immunohistochemical findings of IgE-synthesizing plasma cells in the nasal mucosa [12,13] seem to confirm the possibility of a local IgE production. In contrast we examined in which organ the immunological response, or the production of IgE in the case of allergic rhinitis, occurs [15,26]. The question of whether the local IgE-response in the nose is independent from, or part of, the serum response, cannot therefore be answered yet. The

transport mechanism of IgE into the nasal secretion is not well understood, but a simple transudation does not seem to be likely. This preliminary study therefore represents an initial investigation into the comparison of IgE-SP of the allergic organ with those of the serum. Differences in the composition of IgE-SP between S and NS would be of importance for the estimation of major/intermediate/minor allergens as well as for standardization of allergen extracts which are used for immunotherapy. An extension of these studies on other allergen-specific immunoglobulin isotypes, especially before and after immunotherapy, is being prepared.

By RAST we could demonstrate timothy pollen-specific IgE antibodies in the sera and nasal secretions of 19 out of 23 timothy pollen allergic subjects. In four subjects no specific IgE could be found in NS in contrast to high RAST-classes in S. This may be due to the method of challenge and collection of the secretions. Except for two cases RAST-classes were higher in S compared to NS of the individual subjects. As in previous studies [7,9,14] it may be suggested that in these two cases a local IgE production contributes to the high RAST in secretion. However, in these cases new IgE-specificities were not found.

It is difficult to collect high amounts of nasal secretion in a standardized way. The method of collecting the secretion may additionally alter the constituents of NS, as was shown by Brofeldt *et al.* [27]. The allergen challenge method resembles a natural situation in allergic rhinitis and enabled us to obtain sufficient quantities of secretion with reasonable amounts of antibodies in most subjects. We preferred, therefore, the technique described to the others reviewed by Mygind [28]. As we intended to investigate the IgE-SP pattern completely we performed CRIE with concentrated nasal secretions as described by Platts-Mills [9], and Deutschl and Johansson [29] in their RAST studies. Being careful not to disregard weakly stained bands, we choose NS with high IgE concentrations.

The parallel performance of CRIE in the sera and the secretions of five individual subjects with high RAST-classes (subject no. 7 had a higher RAST-class in NS than S) showed corresponding autoradiographic results. The IgE-SP identified in NS could also be identified in S of the respective subject showing only quantitative differences in the radiostaining intensity. No IgE-SP could be detected in S which were not determined in NS. The same result was obtained with the pooled NS of six subjects compared to an in-house reference serum pool. Because of lower percentage bindings the radiostaining intensity was reduced in the secretions. IgE to *Phl p* V, *Phl p* VI and to further allergens could be detected in S and NS. These findings indicate the qualitative conformity of timothy

pollen-specific IgE-SP [2,4,23,25] in serum and nasal secretion.

The RAST-classes of two *D. pteronyssinus* allergic subjects were lower in nasal lavage than in the sera of the same subjects. By CRIE, the pattern of IgE-SP was more pronounced in S than in NL. This may reflect the low concentration of IgE-antibodies due to the technique of collection (nasal lavage). IgE-SP against the major allergen *Der p* I [1,25] as well as against two other allergen bands could be detected in all sera and secretions. This indicates again the comparability of IgE-specificities in the different fluids.

In conclusion, we were able to show that IgE-SP cannot only be demonstrated in sera, but also in the nasal secretions of allergic rhinitis subjects by means of CRIE. According to these preliminary results, the composition of IgE-SP in serum corresponds to the composition of the nasal secretion. No evidence for a local IgE production altering the composition of IgE-SP in the target organ from that in the serum was obtained.

For further studies a better standardization of NS should be achieved by calibrating to total IgE levels or albumin content. This should then enable a precise comparison of the concentrations of IgE-SP in NS and S in order to exclude concentration effects on the CRIE results.

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