

Relationship between Specific Circulating IgE, Basophil Cell-Bound IgE and Histamine Release Induced by Purified Allergens of *Dermatophagoides Farinae*

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Abstract. In this study we analysed the presence and proportions of IgE specific for Df 6 and Df 11, two purified allergens of *Dermatophagoides farinae*. The characterisation of serum IgE and cell-bound IgE for 3 *D. farinae*-sensitive patients were performed by CRIE, RAST and PRIST assays. Furthermore the basophils from these same patients were studied by histamine release assays in the presence of Df 6 and Df 11. All the individual patient's cell and serum IgE samples displayed the presence of IgE antibodies specific for Df 6 and Df 11, but the relative quantities of the IgE for these two specificities were characteristic of each patient. The ratios (Specific IgE for Df 11) : (Specific IgE for Df 6) (ratio 11 : 6) were similar in the serum and on the cells for an individual patient. As judged by histamine release assays, the basophil sensitivities towards Df 6 and Df 11 were very different from one atopic patient to another. Moreover cell sensitivities reflected the proportion of Df 6- and Df 11-specific IgE antibodies found in the serum and in the cell eluate.

Introduction

Immunoglobulins E are now recognized as the major antibodies responsible for immediate-type hypersensitivity reactions in humans. These antibodies can be either free in the serum or bound with high affinity to specific IgE receptors on mast cells or basophil granulocytes [1]. The combination of the cell-bound IgE antibodies with the corresponding allergenic molecules may lead to the degranulation of these cells. The concomittant release of mediators present in those cells, such as histamine, can be quantified.

The quantitative relationship between serum IgE and cell-bound IgE has been studied in animal models as well as in human patients. Comparing the total IgE in the serum and on the cells, Conroy et al. [2] and Stahl Skov et al. [3] found a correlation between free circulating IgE and the number of IgE molecules on the basophil leucocytes of atopic subjects. The quantities of specific IgE in the serum and on the cells have also been shown to be correlated in pollen-sensitive patients [3].

The relationship between the quantity of basophil-bound IgE and the characteristics of the histamine-release curves has been studied by several investigators.

Most of the previous studies on the cellular response from IgE-sensitized basophils or mastocytes were performed in the presence of anti-IgE [4, 5], or crude allergen extracts [6]. The latter situation is not far from natural conditions of allergen exposure but the histamine-release patterns are difficult to interpret since they result from simultaneous triggerings of the IgE of different specificities by their corresponding allergens. Similar studies with semi-purified pollen extracts were performed by Stahl Skov et al. [3] and Aalberse et al. [7], and a correlation between the quantity of cell-bound IgE specific for the pollen allergen and the cellular sensitivity towards this allergen was shown.

The existence of multiple allergenic molecules in crude allergen extracts of various origin has now been established [8-10]. The cross immunoelectrophoretic methods simultaneously allow the identification of these allergenic molecules and the individual IgE responses to those antigens in different patient's sera. Thus, studying the IgE response towards *D. farinae* extract in sera of patients sensitive to this mite, it has been shown that two allergens, Df 6 and Df 11, can be characterized as major allergens in *Dermatophagoides farinae* mite [11]. Df 6 and Df 11 have been purified

and they are available to investigate the IgE response towards these allergens in *D. farinae*-sensitive patients.

We here report on the proportions of cell-bound IgE and serum IgE antibodies specific for Df 6 and Df 11 in 3 mite-sensitive patients. To analyse the serum IgE and cell-eluted IgE, CRIE and RAST techniques have been used. The basophil sensitivities towards Df 6 and Df 11 were determined considering the histamine release curves obtained from the blood leucocytes of these patients.

Materials and Methods

Allergens

Partially Purified Extract. The preparation of the partially purified extract from *D. farinae* mite cultures was done as previously described in detail [12]. The extract, named Df 80d, constituted the experimental material.

Purification of Allergens. The purified allergens Df 6 and Df 11 were prepared according to the procedure previously described [13] and summarized in figure 1.

Antibodies

The immunization procedure was as follows. 3 rabbits of 2.5 kg received 100 mg of the partially purified extract Df 80d, in 9 ml of physiological saline solution, mixed with the same volume of in-

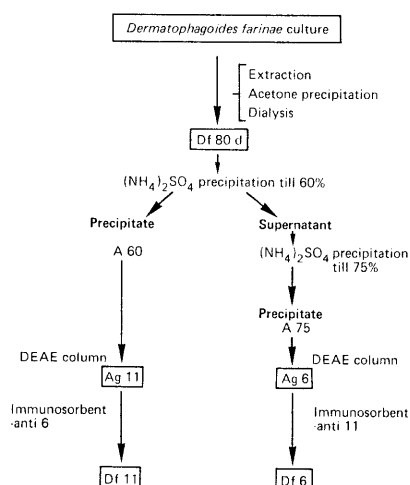


Fig. 1. Preparation of Df 6 and Df 11 from *D. farinae* mite culture.

complete Freund's adjuvant. The resulting emulsion was inoculated subcutaneously at 10 points along the spine for one part and a 5-ml inoculation of the same emulsion was done by the intraperitoneal route. After 1 month the rabbits were boosted with 10 mg of Df 80d in 1 ml of physiological saline solution, by intravenous injection. This was repeated 1 month later. 1 week after the last booster they were bled. Sera were harvested, pooled and concentrated four times.

Crossed Immunoelectrophoretic Methods

Crossed immunoelectrophoresis (CIE) was performed as described by Weeke [14] with some slight modifications. CIE was carried out on 7 × 5 cm plates (gel dimensions: 2 × 5 cm and 5 × 5 cm for the first dimension and the antibody-containing gels, respectively). A 10- to 20-μl volume of each fraction was applied in the well. First- and second-dimension electrophoreses were performed under 20 V/cm for 30 min and 2 V/cm for 18 h, respectively. The temperature of the cooling water was 15°C. Specified amounts of rabbit antiserum were included in the second dimension electrophoresis gel. Agarose gels were prepared at 1% solution in Tris-Veronal buffer, pH 8.7 (75 mM Tris, 24.5 mM Veronal, 0.63 mM calcium lactate, 0.2 mM sodium azide). After electrophoresis the gels were pressed, washed, pressed again, dried at room temperature.

Crossed radioimmuno-electrophoresis (CRIE) was performed as described by Weeke et al. [15] with several modifications. After CIE (on 5 × 7 cm plates) the unstained gels were incubated overnight at room temperature with the 17-fold diluted individual sera or the cell eluates from mite-sensitive patients. After washing, ¹²⁵I-labeled rabbit antiserum against IgE (kindly supplied by Pharmacia, France) was added after dilution in 8 ml of incubation buffer to give 8 × 10⁴ cpm/ml. This buffer is a 50-mM phosphate buffer pH 7.5 containing 0.3% bovine serum albumin, 150 mM sodium chloride, 15 mM sodium azide, 2.7 mM Na₂EDTA, 1% Tween 20. After an overnight incubation, the gels were washed, dried and placed in an X-ray cassette covered with a Kodak X-omat film (Eastman Kodak Co. Rochester N.Y.) and an intensifier screen. Autoradiography was performed at -70°C. The specificity of the radiostaining was checked by using controls such as serum incubation buffer or serum or cell eluate from a non-allergic subject. In our experiments, an exposure time of 5 days for the sera and cell samples was chosen since it gives a specific radiostaining. Finally each glass gel was stained by Coomassie brilliant blue R 250 in order to visualize the corresponding pattern.

Radioimmunoassays

Radioallergen sorbent Test (RAST). The purified allergens Df 6 or Df 11 were coupled to CNBr-activated paper discs as described by Ceska et al. [16]. RAST was performed according to the technique described by Wide et al. [17]. A 100-μl volume of serum or cell eluate was incubated with an allergen-coated paper disc for 3 h at room temperature. The disc was washed and 50 μl of ¹²⁵I-anti-IgE (Pharmacia, France) were added. After an overnight incubation the disc was washed and counted in a gamma counter for bound radioactivity. Cell eluates were concentrated 4 times by lyophilisation before IgE determination. The experimental results were corrected for control values obtained with the serum of a non-sensitive subject. The results were expressed as the ratio of the values obtained with Df 11- to those obtained with Df 6-coated discs. This ratio gives an estimation of the proportion existing between IgE of two

different specificities. Measures on serum and cell eluates were performed in the same experiment.

PRIST. The Phadebas test (Pharmacia, Uppsala, Sweden) was used for the determination of total IgE in the studied sera and in the cell eluates. The levels of total IgE were quantified by the method of Ceska and Lundkvist [18]. The results are given in international units per milliliter.

Source of Human Sera and Leucocytes

Leucocytes and the corresponding sera were obtained from 3 mite-sensitive patients with their informed consent. Patients A, B and C were selected by the following criteria. Allergic asthma and/or year-round rhinitis as clinical symptoms, positive skin tests to mite extracts and specific IgE antibodies for *D. farinae* as determined by RAST with Df 80d-coated discs. All the patients were treated with mite extracts at the moment of this study. A non-allergic control, having negative skin test and RAST towards *D. farinae*, was included in the study.

Isolation of Leucocytes

Leucocytes were isolated from 40 ml of venous blood as described by May et al. [19]. The cells were washed 3 times with Tris-albumin-EDTA and the last washing fluid was kept for IgE determination. The percentage of basophils was determined on an aliquot of the cells by the method of Moore and Watson James [20]. 20% of this leucocyte suspension was used for histamine release experiments, the remaining 80% was centrifuged and the cell-bound IgE was eluted.

Acid Treatment of the Cells for IgE Elution

IgE elution from the basophils was performed as described by Ishizaka and Ishizaka [21]. In short, the leucocytes were incubated for 5 min at 0°C in 0.75 ml glycine-HCl buffer pH 2.5, then the suspension was centrifuged for 5 min at 4°C. The supernatant was immediately neutralised with an equal volume of 0.1 M Na₂HPO₄. The cell pellet was treated again for acid elution with 0.75 ml buffer and the second supernatant neutralised and added to the first one. These eluates and the sera were stored at -20°C.

Histamine Release from Human Leucocytes

Non-treated aliquots of cells were washed with Tris-albumin buffer and incubated with dilutions of purified allergens from *D. farinae* Df 6 and Df 11. A volume of 0.1 ml of each dilution was mixed with 0.9 ml cell suspension containing 1-2 × 10⁶ cells/ml. Incubations were performed in duplicate with constant agitation at 37°C for 40 min. The tubes were centrifuged (300 g, 8 min, 4°C) except for the incubation intended for the determination of total histamine content. 0.85 ml of supernatant (or cell suspension) was mixed with 0.1 ml 4 N perchloric acid and, after centrifugation, the histamine content was quantified by an automatized fluorometric method [22].

The histamine values refer to histamine base. The percentage histamine release was calculated by the formula 100 (e-b)/(c-b), where e = mean of fluorometric readings for the experimental supernatant, b = mean of blanks and c = mean of total histamine.

Antigen concentrations are expressed as grams of protein per millilitre, as measured by the method of Lowry et al. [23]. The antigen concentration inducing half of the maximal percentage of histamine released is designed as D 50.

Results

Total IgE

Table 1 summarises the data on the total IgE amount in the serum and in the cell eluates, as well as the number of total IgE molecules per basophil. No IgE was found in the control serum and in the last washing liquid before the acid treatment of the cells. Patients A and B had a serum IgE concentration of 300 IU/ml, whereas patient C presented a serum level of 1,600 IU/ml. Percentage of basophils for patients A, B and C were 0.61, 1.25 and 1.9, respectively. The quantity of cell-bound IgE per basophil was calculated from the number of basophils treated, the quantity of IgE eluted and the molecular weight of IgE (1.9 × 10⁵ daltons).

Specific IgE

Presence of Specific IgE Antibodies for Df 6 and Df 11. The presence of IgE for Df 6 and Df 11 in sera and cell eluates from the 3 mite-sensitive patients was investigated by CRIE technique. Control experiments were carried out to determine the exposure time giving a specific radiostaining.

Figure 2 presents the CIE pattern of Df 80d. The antigens contained in this extract giving rise to rabbit IgG antibodies were immobilized in the gel and revealed by Coomassie blue staining. Among those antigens, Df 6 and Df 11, two major allergens of the *D. farinae* mite, are indicated. A series of identical unstained plates was prepared for the CRIE experiments.

Figure 3a displays the CRIE results obtained after binding of the specific IgE contained in the mite-sensitive patients sera. For those 3 serum samples, positive radiostaining was observed with Df 6 and Df 11.

Table 1. Total IgE: sera and cell eluates from 3 mite-sensitive patients and 1 non-allergic subject

Patient	IgE, IU/ml		Molecules IgE/basophil
	serum	cell eluate	
A	300	10	56,000
B	310	13	44,000
C	1,600	24	88,000
Control	<2	3.6	23,000



Fig. 2. Crossed immunoelectrophoresis of *D. farinae* extract. Antigen: 102 µg of Df 80d; Antibodies: 3 µl cm⁻² of rabbit serum pool against Df 80d.

The sera give rise to different intensities of radiostaining for Df 6 and Df 11. The CRIE results with the corresponding cell eluates (fig. 3b) showed only visible radiostaining with Df 11, while with Df 6 the X-ray film gave very faint images.

Proportion of Specific IgE for Df 6 and for Df 11. The ratios (Specific IgE for Df 11) : (Specific IgE for Df 6) as determined in the serum or in the cell eluate by the RAST technique are presented on table IIa. They are similar for the free IgE in the serum or for the cell-bound IgE in the same patient but differ from 1 patient to another. In patients B and C, the ratio is in favour of the specific IgE for Df 11 while it is the contrary in patient A. In this technique, serum dilution curves may present plateauing values for low and high IgE concentrations. For this reason the ratios 11/6 may be influenced by the dilution of the serum at which the RAST determinations are performed. In figure 4 an example of such a serum dilution curve is presented with the serum of patient C. As seen on this figure the ratio 11/6 was independent of the dilution.

As shown by the results in table III, the treatment of an IgE-containing sample at pH 2.5 for 20 min at 4°C did not alter the activity of the human IgE molecule. The decrease observed for the total and specific IgE values was only due to the dilution of the sample, as the same values were obtained after treatment at low pH and after a 20-min stay at pH 7.4 in the control experiment.

Table II. Specific IgE for Df 11
Specific IgE for Df 6

in sera and cell eluate from 3 mite sensitive patients

Patient	a		b
	ratio IgE $\frac{\text{Df 11}}{\text{Df 6}}$		ratio $\frac{\text{CS 11}}{\text{CS 6}}$
	serum	cell eluate	cellular sensitivities (CS)
A	0.1	0.1	0.1
B	3.00	2.77	450.0
C	1.10	1.54	12.5

Table III. Total IgE and specific IgE for Df 11 in serum of patient C before and after treatment by acid (pH 2.5) or neutral (pH 7.4) buffer

	Before treatment	After treatment with	
		acid buffer	neutral buffer
Total IgE, IU/ml	1,600	230	230
Specific IgE (Ratio $\frac{\text{cpm serum C}}{\text{cpm control}}$ serum)	32.4	12.6	12.2

Histamine Release and Cellular Sensitivities for Df 11 and Df 6

The results of the histamine release experiments on basophil cells from patients A, B and C to a challenge by Df 6 and Df 11 are presented in figure 5. The maximal quantity of histamine released for both allergens was not significantly different but the aspect and relative position of the curves differed from 1 patient to another. For patient C the D₅₀ histamine release by Df 11 corresponded to a concentration 10 times lower than that of Df 6 and for patient B to a concentration 450 times lower. This is completely reversed for patient A since D₅₀ histamine release occurred with a concentration of Df 11 ten times higher than that of Df 6.

The ratio of the cellular sensitivity towards Df 11 to the sensitivity towards Df 6 are reported in table IIb. These ratios were determined in comparing the values of the allergen concentrations inducing 50% of the maximal histamine release when experiments were performed with washed cells (fig. 5). Considering pa-

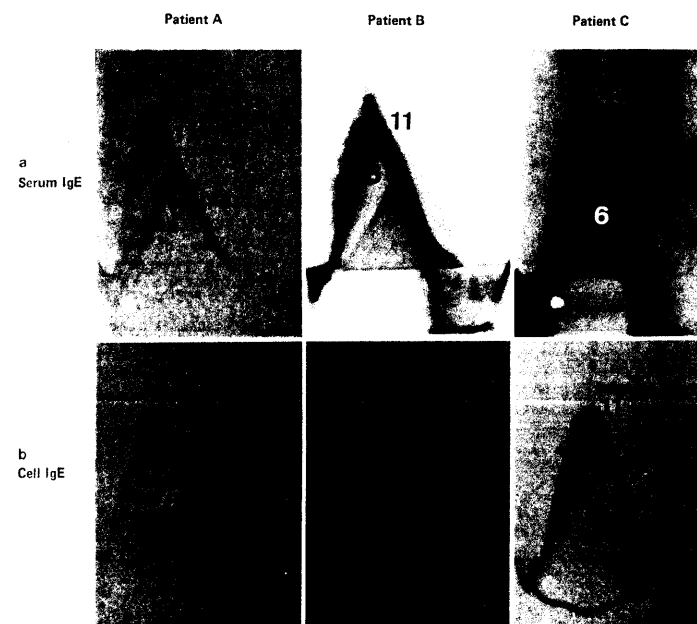


Fig. 3. X-ray of the CRIE of *D. farinae* extract (Df 80d) with sera (a) and cell eluates (b) from mite-sensitive patients.

tients B and C, the higher the ratio 11/6 was in the cell eluate (table IIa), the greater were the differences between the cell sensitivities for each of these allergens (table IIb). For patient A, the ratio 11/6 for the cell-bound IgE lower than 1 corresponds to an inversion in the proportion of the cellular sensitivities to these antigens when compared to the two other patients.

Discussion

Earlier studies concerned with the relationship between serum IgE, cell-bound IgE and basophil sensitivity were limited to the analysis of total IgE [3, 24] or IgE specific of one particular allergen [3, 25]. Until now, to our knowledge, no study has been undertaken to determine the ratio between levels of serum IgE with different specificities. On the basophil sensitivities towards different allergens, King et al. [26] published results on Ag E and Ag K from ragweed. As the

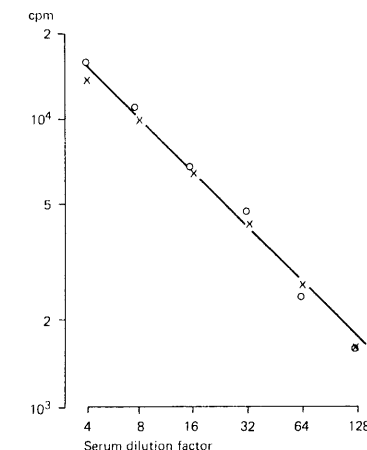


Fig. 4. Dilution effect on specific IgE determination by RAST experiments, o = Df 6-specific IgE; x = Df 11-specific IgE.

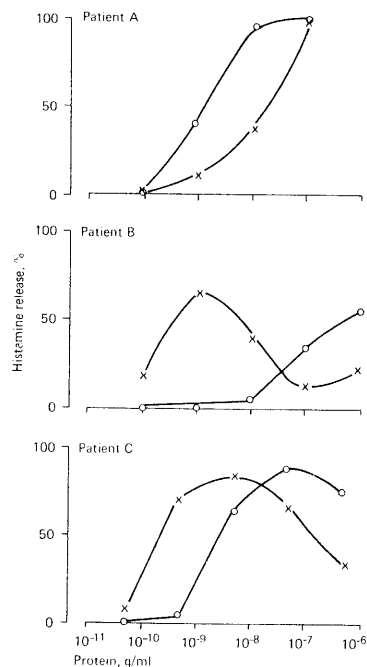


Fig. 5. Histamine release response towards Df 6 and Df 11. o = Df 6; x = Df 11.

latter allergens were cross-reacting, no clear-cut conclusion could be drawn from the ratio of the cellular sensitivities towards Ag E and Ag K.

In the particular case of *D. farinae*, previous results [11] have shown that mite-sensitive patients presented an individual pattern of IgE specificities in their serum. The frequent occurrence of IgE versus 2 major allergens, Df 6 and Df 11, was also noted. The availability of these non-cross-reacting allergens in a highly purified form prompted us to analyse the relative quantities of the IgE of these two specificities in the serum and on the basophils from *D. farinae*-sensitive patients.

CRIE experiments revealed the presence of specific IgE for Df 6 and Df 11 in all three sera. The identification of the individual IgE specificities in cell eluates has been more difficult due to the weak concentration of IgE antibodies. Indeed, it is known that the degree

of radiostaining is proportional to the amount of IgE antibody [27].

The number of total IgE molecules per basophil reported here was close to the number reported in literature [3]. This amount of cell-bound IgE may be overestimated as IgE can be present on other cells, though, the cell-washing procedure can remove IgE of low cell affinity.

As far as specific IgE is concerned, earlier studies [28] evaluated the specific IgE levels in different experimental samples, comparing the results so obtained with a standard dilution curve. In this study we directly compared values of specific IgE for two different allergens. The ratio was calculated between the cpm values obtained for each allergen after subtraction of the background value.

Ratio 11/6 may differ according to the method used for its determination, because compared to RAST, CRIE and histamine release determination are dependent on some particular parameters. In CRIE, Df 6 and Df 11 are immobilized by anti-Df 6 and anti-Df 11 IgG antibodies of the rabbit sera raised against Df 80d. So, the antigenic determinants of Df 6 and Df 11 which remained available may be differently recognized by the human IgE molecules according to the genetic background of the patient. In the histamine measurements of basophil sensitivity, the configuration and the valency of Df 6 and Df 11 could be important factors [29]. For these reasons, the same absolute value cannot be expected for the ratio 11/6 determined by the three different techniques in the same patient. Moreover in all these techniques effects of the quantity as well as of the affinity of specific IgE for Df 6 and Df 11 on the experimental values cannot be dissociated.

The ratio of specific IgE for Df 6 and Df 11 in the serum and on the cell samples of 3 mite-sensitive patients was studied by different techniques. The fact that within each patient the same ratio 11/6 values were found for the serum and the basophil-eluted IgE is in favor of equal affinity of these immunoglobulins for the cell IgE receptors. Great differences were observed for the ratio 11/6 values between the different patients, denoting the occurrence of individual sensitivity patterns towards the different mite components. It would be worthwhile to study whether hyposensitization efficiency varies following the ratio 11/6 of the patients. Moreover the possibility for new hyposensitization schedules with different combinations of purified allergens such as Df 6 and Df 11 does now exist.

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