Relation between skin tests, inhalation tests, and histamine release from leucocytes and IgE in house-dust mite allergy

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Kerrebijn, K. F., Degenhart, H. J., and Hammers, A. (1976). *Archives of Disease in Childhood*, 51, 252. **Relation between skin tests, inhalation tests, and histamine release from leucocytes and IgE in house-dust mite allergy.** This study was concerned with the correlation between skin test, bronchial provocation test, and histamine release from leucocytes and the serum level of total and allergen-specific IgE in patients atopic to house-dust mite allergen (*Dermatophagoides pteronyssinus*) as measured by an intracutaneous skin test. It is concluded that, assuming a positive provocation test to be evidence of allergy, estimation of the allergen-specific IgE concentration in the serum will not offer any major advantages over the intradermal skin test in determining the clinical significance of house-dust mite allergy.

Of the factors which play a role in triggering an asthmatic response, hypersensitivity to inhaled allergens is important. This probably consists of both a nonspecific component in which complement plays a role (Berrens, 1974), and a specific component mainly based on a type 1 sensitivity reaction (Coombs, 1968), though a type 3-like reaction may also be important (Booij-Noord *et al.*, 1972). The reaction of allergen with antibody molecules present on the cell membranes of mast cells, basophils, and other leucocytes will produce a chain of intracellular enzyme reactions, resulting in the release of histamine and other vasoactive amines which cause obstruction of the bronchi due to swelling of the mucosa and spasm of smooth muscle tissues. However, histamine can be released in nonspecific reactions as well. The presence of specific IgE antibodies to allergens (reagins) suggests that inhalation of these allergens may result in bronchial obstruction.

There are various methods besides the skin test to detect the presence of reagins, qualitatively or quantitatively in serum (Wide, Bennich, and Johansson, 1967; Coombs *et al.*, 1968). Bronchial obstruction occurs after provocation with allergen when the allergen-specific IgE subfractions are biologically active. This can be investigated in-

directly by means of a test in which the allergen is inhaled through an aqueous aerosol. Three types of response can be differentiated (Van Lookeren Campagne, Knol, and de Vries, 1969). (1) Bronchial obstruction occurring during or immediately after exposure to the allergen. (2) Delayed bronchial obstruction occurring within 6–12 hours of exposure. (3) An immediate reaction followed by a delayed reaction. When allergen is added to a suspension of leucocytes from a patient with IgE antibodies to this allergen, histamine will be released (Ishizaka *et al.*, 1969; Lichenstein, Levy and Ishizaka, 1970). Approximately 50% of this histamine comes from basophils, the other 50% from eosinophils and neutrophils (Lichenstein and Norman, 1969).

The present study was concerned with the correlation between skin test, bronchial provocation test, and histamine release from leucocytes and the serum level of total and allergen-specific IgE in patients atopic to house-dust mite allergen (*Dermatophagoides pteronyssinus*) as measured by an intracutaneous skin test.

Methods

Twenty asthmatic children 8–12 years of age were studied. The children were not desensitized and did not take any drugs during the investigation.

Concentration of allergen. The water-soluble fraction of house-dust mite (*D. pteronyssinus*) allergen was prepared as follows. 500 mg mites were suspended in 250 ml 0.1 mmol/l phosphate buffer, pH 6.8, and stirred at room temperature for 2 hours. The insoluble material was removed by filtration (G-5 glass filter) and the resulting clear solution immediately lyophilized. This preparation was stored at -20°C . All allergen concentrations were freshly prepared from lyophilized material on the morning of the test and all tests were done with the same allergen solution in every subject. Blood samples, in which histamine release and IgE were determined, were taken on 3 consecutive days just before the provocation test.

Skin tests were performed with allergen concentrations of 1000, 100, 10, and 1 $\mu\text{g/ml}$. The initial concentration at *bronchial provocation* was 100 $\mu\text{g/ml}$. When this failed to induce an immediate response, the test was repeated 7 days later with 1000 $\mu\text{g/ml}$. A total of 29 provocation tests were performed in the 20 patients under investigation. **Histamine release** from leucocytes was measured with allergen concentrations of 1000 and 100 $\mu\text{g/ml}$.

Histamine release test. The amount of histamine released from 1 ml of a leucocyte suspension was determined after incubation with an allergen solution for 60 minutes using the method described by May, Lyman, and Alberto (1970). Total histamine content was not determined by direct butanol extraction, but by extraction after 30 minutes' incubation in a boiling waterbath. All tests were performed in triplicate and each result is the average of three tests. The basal histamine release due to the test procedure was also determined and a correction therefore was made. As there is a linear relation between the concentration of leucocytes and the absolute histamine release, and as the concentration of leucocytes cannot be standardized, histamine release is not stated in absolute amounts but in percentages of the maximum histamine release.

Allergen-specific IgE. It was essential that a similar allergen be used in all tests. Instead of using the commercially available house-dust Phadebas kit, the concentration of allergen-specific IgE in the serum

was therefore measured in the following way. The total amount of IgE was determined using the total IgE Phadebas kit. Allergen was then linked to glass pearls. After incubation of the allergen carrier complex with the patient's serum, the IgE bound to allergen was eluted with an acid buffer solution (pH 2.5). Thereafter the IgE in the eluate was estimated using the total-IgE Phadebas kit (Degenhart *et al.*, in preparation).

Bronchial provocation. On the morning of the day before testing the vital capacity (VC) and the forced expiratory volume in one second (FEV_1) were determined before and after inhalation of the solvent for 10 minutes. Pulmonary function was recorded with a Lode spirometer No. D 53. The nebulizer used was the Doppel-inhalator (made by Blümel, Wiesbaden) with an airflow of 8 l/min. Pulmonary function was determined before, immediately after and 1, 8, and 24 hours after inhalation of allergen. All provocation tests were performed during a clinically optimal period in hospital. The initial FEV_1/VC value was 55% in 2 inhalation tests, 60–70% in 4, and 71% or more in 23. The provocation test was regarded as positive when the VC or FEV_1 were reduced by more than 15% of the initial level, and doubtfully positive when the reduction varied from 10–15%. Bronchial hyper-reactivity (de Vries, 1970) was not measured but the histamine threshold was 8 mg/ml or more in at least 2 tests in every case.

Skin tests. These were performed intradermally on the volar surface of the forearm using four concentrations of allergen.

Results

Histamine release. The accuracy of the histamine concentration measurements was checked by carrying out 10 determinations in duplicate at each of the concentrations of 20, 30, 40, 50, and 60 $\mu\text{g/l}$. The standard deviation of the single determination was 4.5% in the 20–60 $\mu\text{g/l}$ range. Table I shows the SD and the standard error of the mean in the range of a low to a marked histamine release. The reproducibility on various days was examined in 23 asthmatic children with a

TABLE I
Accuracy of estimations of histamine release (HR)

Histamine release in % of maximum HR	No. of estimations	Mean histamine release in % of maximum HR	SD	SEM
0–9	30	5	3	0.6
10–19	26	15	5	1.0
20–29	20	26	4	0.8
30–39	22	36	5	1.0
40–49	36	45	5	0.8
50–59	46	55	7	1.0
60–69	40	65	6	1.0
≥ 70	12	81	7	2.2

positive skin test to house-dust allergen (Fig. 1.) Though the histamine release varies on different days, it is in the same order of magnitude in the majority of cases, i.e. it shows either a marked increase ($\geq 40\%$), a moderate increase (20–39%), or a slight increase ($< 20\%$). In 4 healthy controls provocation did not result in histamine release.

FIG. 1.—Histamine release (highest value in % of maximal histamine release) on different days, house mite allergen (*Dermatophagoides pteronyssinus*). Broken lines indicate marked, moderate, or slight release.

IgE. The accuracy and reproducibility of IgE measurements can be assessed from the calibration line in Fig. 2. This was composed of triplicate estimations at eight different IgE concentrations. The SD of the single determination of IgE was calculated from 36 duplicate determinations and is approximately 2 units/ml in the 1–200 units/ml range. In this range the SD is independent of the IgE concentration. The linkage of IgE to allergen may be affected by factors in the

FIG. 2.—Standard curve of the IgE determination with the Phadebas-kit.

serum, the nature of which is largely unknown (Aalberse, Reerink-Brongers, and Vermeulen, 1973).

The significance of these factors for the determination of allergen-specific IgE currently cannot be quantitated. It appears to be negligible, however, in the range of the IgE levels measured in the present study (Degenhart *et al.*, in preparation).

Results of comparative studies. Tables II and III show the results obtained in 12 children with strongly positive skin tests ('skin titre' 10 or 1 $\mu\text{g/ml}$), in 2 with moderately positive skin tests ('skin titre' 100 $\mu\text{g/ml}$) and in 6 with negative skin tests to house mite allergen. The following is apparent from these two tables.

- (1) A strongly positive intradermal skin test (10 or 1 $\mu\text{g/ml}$) may be accompanied by marked or by moderate histamine release on leucocyte provocation.
- (2) The three types of response to provocation may be associated with strongly positive skin tests.
- (3) Histamine release is low and a positive response to provocation is absent when skin tests are negative.
- (4) A good correlation exists between a positive provocation test and high histamine release ($\geq 40\%$) and vice versa. When the provocation test is negative, histamine release will be low.*
- (5) The result of the provocation test (immediate with or without delayed response; not immediate but a delayed response) varies with the concentration of allergen used.
- (6) An obvious relation exists between the mite-specific and total serum IgE concentration on the one hand and the skin titre, response to provocation, and magnitude of the histamine release on the other.

As the IgE level increases the skin titre will be more strongly positive, the provocation test will be positive more often, and histamine release will be higher. A definite relation exists between the concentration of specific IgE in the serum and the skin titre. The relation between the concentration of specific IgE in the serum and histamine release is less marked but present. The multiple regression coefficient is 0.88. The average concentration of allergen-specific IgE in the serum was 1 unit/ml in normal controls.

*Statistical analysis (χ^2 test) shows—(a) Provocation dose 100 $\mu\text{g/ml}$: immediate response, $\chi^2 = 1.56$, NS; delayed response, $\chi^2 = 7.21$, $0.10 > P > 0.05$. (b) Provocation dose 1000 $\mu\text{g/ml}$: immediate response, $\chi^2 = 7.91$, $0.05 > P > 0.025$; delayed response, $\chi^2 = 13.16$, $0.005 > P > 0.001$.

TABLE II

Correlation of skin titre, leucocyte histamine release, provocation test, and serum IgE with house-dust mite extract (*D. pteronyssinus*)

Case no.	Lowest concentration at which skin test was positive (μg/ml)	Histamine* release with 100 or 1000 μg/ml in % of maximum HR	Provocation test						IgE		
			100 μg/ml			1000 μg/ml.			Total (units/ml)	Mite-specific†	
			Response			Response				(units/ml)	% of total IgE
			Imme- diate	After 8 h	After 24 h	Imme- diate	After 8 h	After 24 h			
1	1	75	+	+	+				573	15	2.6
2	10	73	—	+	+	+	+	+	1781	24	1.3
3	1	69	—	+	+	+	+	+	1061	13	1.2
4	1	60	+	+	+				3990	36	0.9
5	1	59	—	+	+	+	+	+	775	14	1.8
6	10	59	—	+	+	+	+	+	260	9	3.5
7	10	37	—	—	—	—	—	—	3752	28	0.7
8	1	31	+	+	+				688	15	2.2
9	10	27	—	+	+	±	—	—	922	14	1.5
10	1	25	—	—	—	±	—	—	156	6	3.9
11	10	25	—	—	—	—	—	—	3976	33	0.8
12	10	17	—	+	+	+	+	+	2145	18	0.8
13	100	51	—	—	—	—	+	+	213	0	0
14	100	19	—	—	—	—	—	—	210	5	2.3
15	Neg	14	—	—	—	—	—	—	210	0	0
16	Neg	3	—	—	—	—	—	—	130	0	0
17	Neg	25	—	—	—	—	—	—	49	6	12.2
18	Neg	18	—	—	—	—	—	—	180	0	0
19	Neg	3	—	—	—	—	—	—	42	0	0
20	Neg	0	—	—	—	—	—	—	76	0	0

*Highest of 3 estimations.

†Average of 3 estimations on 3 consecutive days.

1 unit = approximately 2 ng.

Discussion

In the present study the relation of the results of the skin test, provocation test, and histamine release from leucocytes and serum IgE concentration (total and allergen-specific) was examined. As house dust is believed to be among the most important inhalant allergens in the Netherlands and as the house-dust mite (*D. pteronyssinus*) was found to contribute to a considerable extent to the allergens in house dust (Voorhorst *et al.*, 1967), the study was primarily concerned with mite allergen, though other investigators report the correlation of diagnostic procedures with regard to house dust allergy to be inferior to those of tests for other allergens (e.g. animal danders and pollen) (Berg, Bennich, and Johansson, 1971; Aas and Johansson, 1971).

The method used here to estimate specific IgE offers several advantages over the classic radio-allergosorbent test (RAST) (Johansson, Bennich, and Wide, 1968), which is at present universally available using the Phadebas house-dust kit. However, in this study the comparative studies have been done with allergen of the same batch. Moreover, coated glass pearls used as carriers are

more stable and their linkage to allergen is more rapid and satisfactory than that of normal carriers. An additional advantage of our method is that the concentration of specific IgE is determined in units of weight rather than in reference serum units. In comparing various tests, the following requirements should be met. (1) A potent allergen showing the highest possible degree of purity and originating from the same batch should be used in all tests. (2) All tests should be performed at the same time. (3) The tests should be performed during optimum clinical and physiological conditions. Drugs which may affect the results, such as corticosteroids, disodium cromoglycate, antihistamines, or bronchodilators, should not be administered during the tests. It should be stated whether patients have been desensitized.

All requirements were met in the present study. The results show that a high concentration of reagins in the skin (i.e. a positive skin test with a low allergen concentration) is invariably associated with an increased serum level of specific IgE. However, testing for IgE does not appear to be more sensitive than skin testing, as observed by Hogarth Scott in a number of cases (Hogarth-

TABLE III

Correlation of provocation test, skin titre, serum IgE, and histamine release (HR) with house-dust mite extract (D. pteronyssinus)

Lowest concentration at which skin test was positive	No.	Total IgE (units/ml)		Specific IgE (units/ml)			Provocation test			Histamine release (% maximum HR)		
		≥ 500	< 500	≥ 10	1-9	0	Positive	Doubtful*	Negative	≥ 40	20-39	< 20
1 or 10	12	10	2	10	2	0	8	2	2	6	5	1
100 or 1000	2	0	2	0	1	1	0	1	1	1	0	1
Negative	6	0	6	0	1	5	0	0	6	0	1	5
Provocation test	No.	Total IgE (units/ml)		Specific IgE (units/ml)			Lowest concentration at which skin test was positive (μg/ml)			Histamine release (% maximum HR)		
		≥ 500	< 500	≥ 10	1-9	0	1 or 10	100 or 1000	Negative	≥ 40	20-39	< 20
Positive	8	7	1	7	1	0	8	0	0	6	1	1
Doubtful*	3	1	2	1	1	1	2	1	0	1	2	0
Negative	9	2	7	2	2	5	2	1	6	0	3	6
Specific IgE (units/ml)	No.	Total IgE (units/ml)		Lowest concentration at which skin test was positive (μg/ml)			Provocation test			Histamine release (% maximum HR)		
		≥ 500	< 500	1 or 10	100 or 1000	Negative	Positive	Doubtful*	Negative	≥ 40	20-39	< 20
≥ 10	10	10	0	10	0	0	7	1	2	5	4	1
1-9	4	0	4	2	1	1	1	1	2	1	2	1
0	6	0	6	0	1	5	0	1	5	1	0	5
Histamine release (% maximum HR)	No.	Total IgE (units/ml)		Specific IgE (units/ml)			Lowest concentration at which skin test was positive (μg/ml)			Provocation test		
		≥ 500	< 500	≥ 10	1-9	0	1 or 10	100 or 1000	Negative	Positive	Doubtful*	Negative
≥ 40	7	5	2	5	1	0	6	1	0	6	1	0
20-39	6	4	2	4	2	0	5	0	1	2	1	3
< 20	7	1	6	1	1	5	1	1	5	1	0	6

*Doubtful, i.e. provocation with 1000 μg/ml is followed by a reduction of the VC and/or FEV₁ by 10-15% within 60 minutes or by > 15% after 8 or 24 hours.
1 unit = approximately 2 ng.

Scott *et al.*, 1973). A positive skin test or an increased serum concentration of allergen-specific IgE does not imply, however, that inhalation of allergen will result in marked histamine release or in bronchial obstruction. This agrees with the findings reported by Van Lookeren Campagne *et al.* (1969) and Aas (1970).

Bronchial obstruction following provocation usually occurs in the event of marked histamine release. Provocation tests may also be positive when provocation results in moderate or low histamine release. Histamine release following provocation is found to decrease as nonspecific histamine release increases (Fig. 3). If the effect of provocation is expressed by the total amount of histamine released spontaneously and after provocation, the

relation between the two tests is maintained and provocation may result in bronchial obstruction while total histamine release is low.

De Vries (1970) noted that the initial pulmonary function and the histamine threshold (which may be regarded as a parameter of the degree to which the bronchi are susceptible to aspecific stimuli) are dependent upon one another. With a diminished pulmonary function the result of provocation may therefore be positive due to nonspecific factors. For this reason every provocation test was preceded by inhalation with the solvent and provocation was only performed when the result of inhalation with solvent was negative. All patients were tested during a clinically quiet period in which their pulmonary function was optimal. In all provoca-

FIG. 3.—Relation between allergen-induced and spontaneous histamine release (HR) in % of maximal histamine release.

tion tests but one in which the initial FEV₁/VC value was <70%, there was no immediate response with 100 µg/ml. A positive provocation test can therefore be considered to be due to an allergic reaction.

Aas and Johansson (1971) observed a correlation between the RAST and the provocation test with house-dust in 59% of the cases. Berg *et al.*, (1971) observed identical results of RAST and provocation tests with various allergens in 74% of the cases. In these studies RAST and provocation tests were not performed at the same time and allergens of various batches were used. A study by Wide *et al.* (1971), which satisfied most of the criteria mentioned, showed that the results of 220 RAST and provocation tests with pollens, animal danders, and house-dust were similar in 95% of the cases. Foucard (1973) observed a correlation between a strongly positive prick test and a positive RAST in 86% of the cases. Miyamoto *et al.* (1974) studied the correlation between a high skin titre and the RAST and between the provocation test and the RAST with house-dust mite extract. Positive results correlated in 85% and negative results in 76.5% of the cases.

Our findings broadly agree with these results. When the skin test is negative, little if any specific IgE will be present in the serum and bronchial obstruction or histamine release after provocation will not be detectable to any appreciable extent.

Conclusion

Assuming a positive provocation test, performed during a period when the clinical and pulmonary

physiological conditions are suitable, to be evidence of allergy, estimation of the allergen-specific IgE concentration in the serum will not offer any major advantages over the intradermal skin test (in which a 'skin titre' is determined by allergen dilutions) in determining the clinical significance of house-dust mite allergy. The skin test has the advantages that it can be rapidly and readily performed and that it is cheap. Estimation of the IgE concentration has the advantage of causing less discomfort to the patient and of being easier to perform in longitudinal studies or mass screening. When the skin titre is 10 or 1 µg/ml, or the serum specific IgE concentration is >10 units/ml (>20 ng), the provocation test will very likely be positive in approximately 80% of patients. The histamine release test is not a suitable routine procedure as it is time consuming and technically difficult, but it is a useful reference test.

The present study was undertaken with the financial support of the Health Organization TNO and the Netherlands Asthma Fund. The authors are indebted to Dr. R. Voorhorst for making available the house-dust mite allergen.

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