

Passive sensitization of basophil leukocytes from a non-atopic adult by plasma from allergic children

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Basophil leukocytes from a non-atopic donor, who responded well to anti-IgE, were depleted of their native membrane-bound IgE by acid treatment and passively sensitized with plasma containing either *Phleum pratense*-, *Dermatophagoides pteronyssinus*- or dog dander- specific IgE obtained from 18 allergic children. Histamine release was then performed on the passively sensitized cells and the results were compared with those of bronchial provocation test (BPT), allergen-specific serum IgE (RAST), skin prick test (SPT), and conventional histamine release test (HR). A high coincidence rate was found between BPT, RAST and histamine release after passive sensitization (HR-PS), but compared to HR, it was lower. This could be because several of the patients had non-responding basophils (i.e. no release after challenge with anti-IgE) in the conventional histamine release assay. The lower rate was not related to a lack of antigen-specific IgE, since after passive sensitization of basophils, anti-IgE and allergen provocation could induce release. It is concluded that plasma from allergic children with non-responding basophil leukocytes contain antigen-specific IgE capable of binding to Fc-receptors on the basophils of a non-atopic donor. In addition it was found that the plasma could change the cell reactivity (maximal histamine release) of the donor cells, since the amount of histamine released varied according to the plasma used for passive sensitization. The lack of histamine release response in some patients could be because their own membrane-bound IgE is unable to induce mediator release or, more likely, activation of one or more of the subcellular steps involved in the release is impaired.

Key words: allergy; basophil cell reactivity; histamine release; passive sensitization.

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CLINICAL ASPECTS

The diagnostic value of histamine release tests in children is unclarified and the results vary. The present study and other reports in the literature indicate a high incidence of non-responding basophil leukocytes among children. When performing passive sensitization with plasma from allergic children on basophil leukocytes from a non-atopic healthy adult a high coincidence rate was found between BPT, RAST and histamine release after passive sensitization. This material, however, is selected, with all children responding with positive bronchial tests. Further studies should be carried out to ascertain whether false negative results in the histamine release test can be excluded by passive sensitization in children with non-responding basophil leukocytes.

Histamine release from human basophil leukocytes fulfils the demands of a specific and sensitive *in vitro* assay for diagnosing Type I allergy in adults (5, 11, 13, 16, 18), but in children the results vary and the clinical value of histamine release assays is still a subject of debate (5, 7, 13, 14, 15).

However, it has been reported that basophils from a few adult patients (4-6%) with a verified Type I allergy do not respond with histamine release upon challenge with specific antigen (9, 10). There are no reports in the literature about the frequency of non-re-

sponders among children, but our impression is that it is higher. Although non-IgE mechanisms could be responsible for these patients' symptoms, an impairment of allergen-induced basophil release due to low amounts of specific IgE bound to the basophils, defective IgE, or impaired basophil cell function, should also be taken into consideration and if possible ruled out (1, 2, 6, 8, 9).

To investigate this further we used frozen plasma from allergic children for passive sensitization, and to assess its clinical value we correlated the results of the usual diagnostic tests in allergy diagnosis to passive sensitization of basophils from a responding donor.

MATERIAL AND METHODS

Plasma from 18 children (8 girls and 10 boys), median age 10.9 years (range 6–14), were used. All had a well-documented history of extrinsic asthma responding to bronchodilating therapy and verified by a positive case history. Skin prick test (SPT), allergen specific serum-IgE (RAST), conventional histamine release from basophil leukocytes (HR) and bronchial provocation test (BPT) were performed.

At the time of examination and blood collection none of the children was receiving systemic

steroids, and bronchodilating drugs were suspended at least 6 h before the testing, and neither aminophylline nor antihistamines had been given for 24 h. None of the children had received immunotherapy.

Venous blood was sampled immediately prior to SPT and BPT. The plasma used for passive sensitization was separated and stored at -20°C for 2½ years prior to this study. Samples were frozen and thawed only once. The effect of storage on the plasma was not examined.

Basophil leukocytes responding well to anti-IgE from the same non-atopic healthy adult donor were used throughout the passive sensitization experiments. These experiments were carried out during a period of 1 month and five patients were tested in each assay.

SPT was carried out on the volar surface of the forearm with each extract in concentrations of 0.1, 1.0, and 5 HEP (Histamine Equivalent Prick) per ml. The wheals were recorded and calculated as described earlier (14). Results were scored as shown in Table 1.

BPT was carried out as described earlier (14) by inhalation of nebulized aqueous allergen extract in increasing concentrations of 0.01, 0.1, and 5 HEP. A positive reaction was defined as a decrease in FEV₁ by 20 % or more.

Results were scored as shown in Table 1.

RAST was determined and performed as previously (14) by ALK, Denmark and the results were expressed in allergy classes 0 to 4 and scored according to Table 1.

Histamine release from patients' basophil leukocytes was determined by a fluorometric method as described earlier by Diamant & Patkar (3). Histamine release was calculated in percent of the total histamine content, and the spontaneous release was deducted. The results from the histamine release were scored as shown in Table 1.

Passive sensitization of non-atopic basophil leukocytes with plasma from allergic children

Basophils from a non-atopic donor were depleted of their own membrane bound IgE by acid treatment and passively sensitized with plasma from 18 allergic children containing

Table 1
The score system

Score	Negative	Positive
BPT	Negative	> 20 % fall in FEV ₁ with the HEP extracts
SPT	< 75 % of histamine ref. with 5 HEP	> 75 % of histamine ref. with 5 HEP
RAST	Allergy class 0-1	Allergy class 2-4
HR	< 20 % release with 5 HEP extracts	> 20 % release with 5 HEP extracts
HR-FH	< 0.3 ng histamine with 5 HEP extracts	> 0.3 ng histamine with 5 HEP extracts

HEP = Histamine Equivalent Prick

either dog dander-, grass-, or house-dust mite-specific IgE. The donor's own plasma served as a negative control. The results of the histamine release after passive sensitization was then compared with those of previously performed BPT, SPT, RAST and conventional histamine release.

To dissociate and remove cell-bound IgE from the basophils of the non-atopic donor cells, washed blood samples were adjusted to pH 3.6 for 5 min at 4°C with NaH₂PO₄

buffer, and the cells washed free of IgE and resuspended in Pipes-AMC buffer, pH 7.4 (4, 9). Fixation of plasma IgE by passive sensitization of basophils was performed by incubating an equal volume of IgE-deprived cells with an equal volume of serum for 120 min at 37°C. The cells were thereafter washed three times and resuspended in Pipes-ACM buffer, pH 7.4 (4, 9).

The histamine release was measured with the new glass microfibre-based histamine assay

Table 2
Relation between responses in the different tests

Pt. No.	BPT	SPT	RAST	HR		HR-PS		Allergen
				Allergen	A-IgE	Allergen	A-IgE	
1	+	+	-	-	-	+	+	mite
2	+	+	+	+	+	+	+	mite
3	+	+	+	+	+	+	+	dog
4	+	+	+	+	+	+	+	dog
5	+	-	+	-	-	+	+	dog
6	+	+	+	-	-	+	+	dog
7	+	+	+	+	+	+	+	dog
8	+	+	+	+	+	+	+	grass
9	+	-	+	+	+	+	+	grass
10	+	+	+	+	+	+	+	grass
11	+	+	+	-	-	+	+	dog
12	+	+	+	+	+	+	+	mite
13	+	+	+	+	+	+	+	mite
14	+	+	+	+	+	+	+	dog
15	+	+	+	+	+	+	+	dog
16	+	+	-	-	+	-	+	mite
17	+	-	-	-	-	-	+	mite
18	+	+	-	+	+	-	+	dog

Table 3
Comparison between histamine release after passive sensitization and conventional histamine release (HR) with the other tests

		BPT		RAST		HR		SPT	
		pos	neg	pos	neg	pos	neg	pos	neg
HR-PS	pos	15	0	14	1	11	4	13	1
	neg	3	0	0	3	1	2	3	1
Coincidence %		83		94		72		78	
HR	pos	12	6	11	1			11	1
	neg	6	0	3	3			4	2
Coincidence %		63		74				72	

(12). In this assay histamine release is performed in the glass microfibre-prepared microtitre plates by addition of 20 μ l antigen, 50 μ l buffer, and 50 μ l washed blood per well and incubated for 60 min at 37°C. During incubation released histamine is bound with high affinity to the glass microfibrils, 90% of the histamine in the solution being absorbed to the fibres. The microtitre plates are then washed to remove cells and interfering substances. Fibrebound histamine is detected by a fluorometric method (7, 12). Released histamine (net release) from the cells is expressed as nanogram histamine per sample. The sensitivity of the assay is 0.3 ng histamine per sample, and the histamine standard curve is linear up to at least 5 ng (12).

Allergens and anti-IgE

The allergens used, were grass mixture, house-dust mite and dog dander, these were delivered

as standardized allergen extracts by ALK, Denmark in stock solutions of 10 HEP/ml. Extracts were reconstituted and diluted on the day of testing and then discarded. For histamine release experiments the allergens were used in final concentrations of 0.05, 0.5, and 5 HEP/ml.

Anti-IgE (400,000 U/ml) from Behringwerke, Germany was used in final concentrations of 4, 40, and 400 U/ml in the reaction mixture.

Reagents

For histamine release experiments by the microfibre method the anti-IgE and allergens were diluted in Pipes-AMC, a buffered solution (pH 7.4) consisting of 10 mM piperazine-N, N (bis-ethane sulfonic acid), 140 mM sodium acetate, 5 mM potassium acetate, 0.6 mM CaCl_2 , 1.1 mM MgCl_2 , glucose 1 mg/ml, human serum albumin 0.3 mg/ml, and heparin, LEO, Denmark, 15 IE/ml.

This study was conducted in accordance with the Helsinki Deklaration II.

RESULTS

Experiments were designed to verify whether all the native IgE was removed from the donor cells after acid elution, since histamine release experiments were performed before and after removal of membrane bound IgE. Comparison of the histamine release patterns before and after IgE removal showed that after acid elution the donor cells lost all releasing activity when challenged with anti-IgE, and this activity was recovered after passive sensitization with plasma (results not shown).

Table 2 shows for each patient the relation between responses obtained by the different tests according to the suspected allergen. Note that all patients had a positive BPT. Complete concordance (positive or negative) between all four "diagnostic" procedures was only found in 10/18 (56%) of the patients. Table 3 shows that the best coincidence percent was found between RAST vs HR-PS 94% and in decreasing order BPT vs HR-PS 83%, SPT vs HR-PS 78%,

Table 4

Cell reactivity after HR-PS when challenging donor cells with allergen or anti-IgE

Pt. No.	Anti-IgE	Allergen
1	0.9	0.7
2	1.2	1.2
3	1.1	0.9
4	1.0	0.8
5	0.5	0.9
6	0.9	0.3
7	0.6	0.3
8	0.3	0.3
9	0.6	0.3
10	0.3	0.3
11	0.4	0.3
12	0.3	0.3
13	0.6	1.1
14	0.8	0.6
15	0.9	1.0
16	0.6	0.3
17	1.6	1.1
18	0.5	1.2
X_{donor}	0.7	-

Each value represents the mean net histamine release in nanograms based on three determinations ($\text{SD} = \pm 0.05$). Cell reactivity of the donor cells was estimated for each series of experiments. The mean X_{donor} $\text{SD} = 0.7 \pm 0.05$ was used for the statistical analysis ($P > 0.1$).

HR vs HR-PS 72 %, RAST vs HR 74 %, SPT vs HR 72 %, and BPT vs HR 63 %.

It was found that all patient plasma could sensitize the responding donor cells (verified by anti-IgE challenge after passive sensitization), while five patients (28 %) had non-responding basophils with the conventional histamine release test. Six patients did not react with histamine release upon allergen challenge in the conventional assay, but four of them released histamine upon allergen challenge after passive sensitization. When comparing the histamine release assays with BPT it is seen that only three patients were negative to the tested allergens in the HR-PS, whereas six patients were negative in the HR.

Table 4 shows the maximum release of histamine in nanograms after passive sensitization with plasma from 18 different patients, using the same non-atopic donor basophils and challenging the donor cells with anti-IgE and the relevant allergen. The maximal histamine release of the donor cells was estimated at each new set up and compared with the passive sensitization performed with the donor's own plasma and the allergic childrens' plasma. It is shown that sensitization with donors own plasma resulted in almost identical maximal release response in all experiments, but this varied according to the plasma used for passive sensitization from the different patients. (The variation is not statistically significant using Wilcoxon's one sample test.)

DISCUSSION

Allergen-mediated basophil histamine release has proven to be a reliable test for diagnosing Type I allergy in adult atopic patients, and a positive correlation has been shown between the severity of clinical symptoms, skin test, bronchial provocation, and histamine release (13, 18). The diagnostic value of histamine release in children is unclarified and the results vary (7, 14, 16). This could be because more child patients have non-responding/immature basophils (i.e. no histamine release is observed on challenge with anti-IgE). A high frequency has been found in newborns

where eight of 32 (25 %) cord blood samples did not respond with histamine release after challenge with anti-IgE (4). In the present study five of 18 allergic children (28 %) had non-responding basophils.

Recently, we have shown that the new microfibre-based histamine release test is a valuable diagnostic tool in diagnosing Type I allergy in children (7). The present study was undertaken to examine whether the lack of histamine release in some allergic children is due to a lack of/or defective allergen-specific IgE, or to impaired basophil cell function. This might explain the false negative results of histamine release in children.

To elucidate this further we used plasma from the 18 allergic children to perform passive sensitization on responding basophils from a non-atopic adult donor. Prior to passive sensitization the donor cells were deprived of their own membrane bound IgE, since we have found that more free IgE-Fc-receptors for binding antigen-specific IgE are obtained by the acidification procedure. This increases passive sensitization and causes an enhanced cell reactivity and sensitivity (9).

Table 2 shows that six of the 18 patients had non-responding basophils to both allergen and anti-IgE when challenged in the conventional histamine release test. Five patients (Nos. 1, 5, 6, 11, 17) were negative upon anti-IgE challenge in the HR. When plasma from the same 18 patients were used for HR-PS, only two plasma samples (Nos. 16, 18) were unable to sensitize the cells (sensitization was verified by histamine release after challenge of the cells to allergen). But all patients became positive anti-IgE responders. This indicates that patients 5, 6 and 11 are allergic since they have positive RAST and HR-PS test. It is doubtful whether patients 1 and 18 are allergic, whereas patient 17 seems to be non-allergic with non-responding basophils. Three patients (Nos. 16, 17, 18) were negative both in RAST and HR-PS test, which suggests they are not allergic. Non-IgE mechanisms might be responsible for their symptoms.

It can be concluded that some allergic children with non-responding basophils possess

membrane-bound IgE, since IgE from these patients will sensitize responding basophils. This indicates that the Fc-portion of the IgE-molecule possesses sufficient affinity for its receptor to sensitize responding basophils.

Concerning the hypothesis that the unresponsiveness of basophils in some allergic children is associated with impaired IgE receptor-complex activation or subcellular dysfunctioning (10), we have recently examined the influence of enhanced IgE receptor stimulation on histamine release in non-responding basophils (10). It was found that by stimulating protein kinase C activity with a phorbol diester (phorbol 12-myristate 13-acetate) non-responding basophils became responsive to both anti-IgE and specific antigen. These results showed that cells possessed antigen-specific IgE on the cell membrane and the defect was most likely due to an impaired cell function. From this study we cannot locate the defect more precisely, but it can be concluded that passive sensitization is a valuable experimental tool. We are aware that the material is selected and that further studies should include plasma samples from patients with negative BPT.

It seems less likely that acid-eluted and passively sensitized basophils react unspecifically, since basophils with non-allergic donor plasma did not respond to allergen challenge and no unspecific reactions were recorded.

Although not proved by this study, the clinical implication could be that false negative results in the histamine release test can be excluded by passive sensitization, thus increasing the diagnostic value of histamine release tests in children.

Finally, we also found that the cell reactivity of the same donor cells varied according to the plasma used for sensitization. Since there is no correlation between RAST value and cell reactivity (9) the variation in maximal histamine release could be due to unknown modulating factors in the plasma.

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REFERENCES

1. Conroy, M. C., Adkinson, N. & Lichtenstein, L. M.: Measurement of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. *J. Immunol.* **118**, 1317-1321, 1977.
2. Conroy, M. C., Adkinson, N. F. & Lichtenstein, L. M.: Passive sensitization of human basophils: evidence for heterogeneity in the IgE molecule. *Int. Arch. Allergy Appl. Immunol.* **60**, 106-109, 1979.
3. Diamant, B. & Patkar, S.: Histamine release from washed whole blood. A method suitable for routine diagnosis of type I allergy. *Int. Arch. Allergy Appl. Immunol.* **67**, 13-17, 1982.
4. Ebbesen, F., Skov, P. S., Jensen, C., Norn, S. & Søndergaard, I.: Basophil histamine release in cord blood. *Allergy* **41**, 562-567, 1986.
5. Malling, H.-J.: Histamine release from basophil granulocytes. A comparative investigation between bronchial provocation and histamine release in asthma patients. *Ugeskr. Læg.* **139**, 2312-2316, 1977.
6. Mendoza, G. R., Minagawa, K., Orner, F. B., Stiehm, E. R.: Basophil releasability in the newborn: Factors limiting immunoglobulin E-mediated histamine release. *Pediatrics* **69**, 188-192, 1982.
7. Nolte, H., Schiøtz, O. & Skov, P. S.: A new glass microfibre-based histamine analysis for allergy testing in children. Results compared with conventional leukocyte histamine release assay, skin prick test, bronchial provocation test and RAST. *Allergy* **42**, 366-373, 1987.
8. Siriganian, R. P.: Automated histamine analysis for in vitro allergy testing. II. Correlation of skin test results with in vitro whole blood histamine release in 82 patients. *J. Allergy Clin. Immunol.* **59**, 214-222, 1977.
9. Skov, P. S., Jensen, C., Nolte, N., Mosbech, H. & Norn, S.: Regulatory role of cell-bound IgE on histamine release. Drug receptors and dynamic processes in cells. In: Schou, J. S., Geisler, A. & Norn, S. (eds.): Alfred Benzon Symposium 22 in Copenhagen, Munksgaard, 1986.
10. Skov, P. S., Norn, S., Weeke, B. & Nolte, N.: Impaired basophil histamine release from allergic patients. *Agents Actions*, **20**, 303-306, 1987.
11. Skov, P. S., Mosbech, H., Norn, S. & Weeke, B.: Sensitive glass microfibre-based histamine analysis for allergy testing in washed blood cells. *Allergy*, **40**, 213-218, 1985.
12. Skov, P. S., Norn, S. & Weeke, B.: A new method for detecting histamine release. *Agents Actions*, **14**, 414-416, 1984.
13. Skov, P. S., Norn, S. & Weeke, B.: Basophil histamine release in patients with hay fever. Results compared with specific IgE and total IgE during immunotherapy. *Clin. Exp. Immunol.* **27**, 432-439, 1977.

14. Stafanger, G., Andersen, J. K., Schwartz, B. & Østerballe, O.: Specific diagnosis of exogenous bronchial asthma in children. *Allergy* 41, 110-117, 1986.
15. Vanto, T., Viander, M., Koivikko, A., Schwartz, B. & Løwenstein, H.: RAST in the diagnosis of dog dander allergy. *Allergy* 37, 75, 1982.
16. Wegner, F., Kockamp, R., Rutschke, A. Becker, B. & Reinhardt, D.: Superiority of the histamine release test over case history, prick test, and radio-allergosorbent test in predicting bronchial reactivity to the house dust mite in asthma children. *Klin. Wochenschr.* 61, 43-47, 1983.
17. Weyer, A., Ougen, P., Danden, J. P., Marchaud, F. & David, B.: Passive in vitro sensitization of human basophils with *Dermatophagoides farinae* specific IgE. *Agents Actions* 18, 178-181, 1986.
18. Østerballe, O., Malling, H.-J. & Weeke, B.: Specific diagnosis of exogenous bronchial asthma in adults. Comparison between case history, intracutaneous test, RAST, histamine release from basophil leukocytes and bronchial provocation. *Allergy* 34, 175-185, 1979.

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