

Passive sensitization and histamine release of basophils

IgE and cellular factors regulating histamine release

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This study had two purposes. First, to examine a possible functional heterogeneity of IgE regulating basophil histamine release and the effect of using two different donor cells for passive sensitization experiments. Second, to investigate basophils not releasing histamine to anti-IgE by stimulating protein kinase C with the addition of the phorbol-ester, TPA. In consecutive experiments responding donor basophils were passively sensitized with plasma from non-responding subjects. Thus, the first set of experiments included passive sensitization of acid treated donor basophils from one atopic and one non-atopic patient with plasma from 29 children with exogenous asthma to grass pollen, cat dander, or dust mites. Different secretagogues (anti-IgE, Concanavalin A, and N-formyl-methionyl-leucyl-phenylalanine) induced different histamine release responses due to a cellular property of the basophils not related to the type of IgE bound to the cell membrane. It was demonstrated that the allergen-induced histamine release did not depend on the extract or type of IgE when the biological activity of each extract and serum-specific IgE levels were similar. However, the atopic donor cells released significantly ($P < 0.05$) more histamine than non-atopic donor cells. Thus, histamine release depends on the type of secretagogues and a cellular property which is maybe influenced by the presence of serum factors and a certain type of IgE in the serum of atopics. The second set of experiments included 10 patients (6 atopics and 4 non-atopics) with non-histamine releasing basophils. In the presence of 10 ng/ml TPA, however, seven of 10 patients released histamine at anti-IgE challenge. Three months later two additional patients became responsive in the presence of TPA. By passive sensitization of responding donor basophils the non-responding patients were shown to possess functionally intact IgE. Thus, the discrepancies sometimes observed between clinical symptoms, serological IgE-antibody measurements and histamine release testing in allergic patients may be related to a cellular property of basophils.

Key words: allergy; basophil; histamine; IgE; non-responders; passive sensitization; TPA.

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The biological sensitivity to allergens may depend more on the cell response of mast cells and basophils than on circulating IgE. Hence, in some patients, discrepancies between clinical symptoms and serological IgE antibody measurements may be explained by differences in the cellular properties of allergic patients (1, 3, 19). Thus, it is of importance to delineate the

factors controlling the cell response in allergic patients.

Allergen-specific histamine release from basophil leukocytes may be used as a diagnostic tool in allergy diagnosis (10, 16). However, basophils from a few allergic patients (6-12%) will not release histamine at antigen challenge, and will give rise to false negative test results (7,

10, 19). By elucidating the mechanisms responsible for this phenomenon one may enhance the sensitivity of the histamine release assay. This study had two purposes. First, to elucidate the regulatory role of the type of allergen-specific IgE bound to the basophil cell-membrane of either atopic or non-atopic donor cells. Second, to further examine basophils from allergic and non-allergic patients which do not release histamine at antigen challenge *in vitro*.

MATERIAL AND METHODS

All subjects were selected from a group of 124 children referred to us with suspected exogenous asthma (10). Medical histories and the results of prior skin prick tests, allergen-specific serum-IgE (Phadebas RAST), and basophil histamine release were reviewed as previously outlined (10). Two series of experiments were performed. In the first series plasma from 29 children with exogenous asthma (13 girls and 16 boys, median age 9.6 years, range 4–14) were used. Allergic asthma was confirmed by allergen bronchial provocation tests. The atopic patients suffered from exogenous asthma to grass pollen ($n = 13$), house dust-mite ($n = 10$) or cat dander ($n = 6$). The patients were also selected on the basis of the RAST result (in duplicate) i.e. they all expressed RAST values between 17–24 protein rating units (PRU).

The second series comprised 10 patients with bronchial asthma (6 atopics: 4 boys and 2 girls, median age 7.4 years, range 4.1–8.3, and 4 non-atopics with intrinsic asthma: 2 boys and 2 girls, median age 9.2 years, range 7.1–12.4). However, these patients did not demonstrate histamine release (i.e. basophils releasing < 0.2 ng histamine per sample) to either anti-IgE or allergen. In non-responding patients only the basophil histamine release test was performed twice within 24 h and again 90 days later.

None of the children received concomitant medication or had ever been hyposensitized. In all patients informed consent was obtained from parents or guardians.

Donor cells for passive sensitization were obtained from venous blood samples from one non-atopic healthy adult donor and one atopic

adult who was clinically allergic to house dust mites and showed positive skin prick test, histamine release test, and a RAST value of 17 PRU to *Dermatophagoides pteronyssinus*. Passive sensitization experiments were performed within 1 month and passive sensitization with plasma from 10 patients were performed and analysed in one run.

To dissociate and remove cell-bound IgE from the donor basophils we used the acid elution method of Skov et al. (7, 18). Briefly, the blood samples were washed three times in physiological saline and once in buffer, and then resuspended in NaH_2PO_4 buffer (pH 3.6, 5 min, 4°C). Thereafter, the acid-treated cells were washed free of IgE and resuspended in Pipes-AMC buffer (pH 7.4).

The plasma samples used to passively sensitize the acid-treated donor basophils were obtained from the above-mentioned children. Plasma from the adult donors served as controls. Acid-treated cells were incubated with an equal volume of EDTA-plasma from each patient for 120 min at 37°C . To remove plasma the cells were then washed three times and resuspended in Pipes-AMC (pH 7.4). All serum samples were stored at -20°C for < 3 months. Samples were only frozen and thawed once.

Histamine release was performed as previously described (6). Briefly, histamine release was performed in microtitre plates coated with a glass fibre-matrix which selectively binds histamine (17). Prior to histamine release experiments the basophil cell number was adjusted in each experiment. The basophils were enumerated by the procedure of Gilbert & Ornstein (4). Triplicate 50 μl aliquots (approximately 3.5 to 4×10^4 basophils ml^{-1}) were incubated in the microtitre wells and challenged with antigen for 1 h at 37°C . Released histamine (net release) from the cells was expressed as nanogram histamine per sample and calculated on the basis of a histamine standard curve included in all experiments. A net histamine release ≥ 0.2 ng histamine per sample was defined as a positive response (10). Fibrebound histamine was detected by the OPT-fluorometric method (17).

Reagents: Phorbol 12-myristate-13-acetate (TPA) from Sigma was used at a final concen-

tration of 10 ng/ml. Anti-IgE (461, 620 U/ml) from Behringwerke (West Germany) was used at final concentrations of 0.9, 900, 9000 U/ml. Concanavalin A (Pharmacia, Sweden) was used at final concentrations of 12.5, 25.0, and 50.0 µg/ml. N-formyl-methionyl-leucyl-phenylalanine (FMLP) from Sigma was used at final concentrations of 10⁻⁶, 10⁻⁵, and 10⁻⁴ M. Glass microfibre-prepared microtitre-plates were provided by Lundbeck Diagnostics (Copenhagen, Denmark). The following Pharmalgen allergens were used: *Phleum pratense*, *Dermatophagoides pteronyssinus* and cat dander at final concentrations of 1, 100, and 1000 BU/ml. Extracts were reconstituted and diluted at the day of testing and then discarded. The histamine releasing agents were diluted in Pipes-AMC buffer (6).

Statistics

The nonparametric Wilcoxon signed rank test for paired data or the Wilcoxon non-paired rank-sum test for non-paired data were used. The test of significances, mean, and standard deviation (SD) reported were calculated from the mean ± SD for triplicate determinations. The maximal histamine release represented the

highest observed histamine value at any of the above-mentioned concentrations.

RESULTS

Passive sensitization of non-atopic donor basophils

Challenge experiments were performed before (untreated cells) and after removal (acid-treated cells) of membrane bound IgE. Table 1a reports that after acid treatment the donor cells lost all releasing capability when challenged with anti-IgE. However, this activity was restored when the acid-treated cells were passively sensitized with donor's own plasma or plasma from atopic children.

The acid treatment could not effectively abolish the Concanavalin A-mediated histamine release, although a decrease in the release was observed. Passive sensitization of these cells restored the histamine release to Concanavalin A. In contrast, the FMLP-mediated histamine release was not significantly (*P* > 0.05) altered by acid treatment or passive sensitization.

The histamine release response (in absolute values) induced by the individual secretagogues (Concanavalin A, anti-IgE, and FMLP) dif-

Table 1a
Secretagogue-mediated histamine release from non-atopic donor basophils

Secretagogue		Untreated donor cells (<i>n</i> = 6)	Acid-treated donor cells (<i>n</i> = 6)	Donor cells passively sensitized with:	
				own plasma (<i>n</i> = 6)	plasma from atopic children (<i>n</i> = 29)
Anti-IgE (U/ml)	0.9	0.4±0.1	0.1±0.01	0.3±0.1	0.2±0.2
	900	0.4±0.1	0.1±0.02	0.3±0.1	0.7±0.3
	9000	0.2±0.1	0.1±0.01	0.3±0.2	0.5±0.3
Concanavalin A (µg/ml)	12.5	1.4±0.3	0.4±0.2	1.4±0.2	0.7±0.1
	25.0	1.4±0.3	0.4±0.1	1.2±0.3	1.1±0.3
	50.0	1.1±0.3	0.3±0.1	1.0±0.2	1.0±0.3
FMLP (M)	10 ⁻⁶	0.3±0.1	0.5±0.1	0.6±0.2	0.4±0.2
	10 ⁻⁵	1.0±0.4	0.8±0.2	1.2±0.3	0.5±0.2
	10 ⁻⁴	0.8±0.3	0.8±0.2	1.1±0.2	0.5±0.3

Each value represents the mean ± SD histamine in ng/sample, (*n*) indicates number of experiments performed

Table 1b
Allergen-mediated histamine release from non-atopic donor basophils

Allergens		Untreated donor cells	Donor cells passively sensitized with:		RAST [PRU]
			own plasma	plasma from atopic children	
Grass (<i>n</i> = 13) (BU/ml)	1	0.0	0.0	0.2±0.1	20
	100	0.0	0.0	0.7±0.2	(17-23)
	1000	0.0	0.0	0.9±0.2	
Mite (<i>n</i> = 10) (BU/ml)	1	0.0	0.0	0.8±0.3	23
	100	0.0	0.0	0.9±0.2	(19-24)
	1000	0.0	0.0	0.7±0.3	
Cat (<i>n</i> = 6) (BU/ml)	1	0.0	0.0	0.3±0.1	18
	100	0.0	0.0	0.8±0.2	(17-22)
	1000	0.0	0.0	0.7±0.2	

Each value represents the mean ± SD histamine in ng/sample, (*n*) indicates number of passive sensitization experiments with atopic plasma. RAST values in protein rating units (PRU) are median and range in parentheses

fered significantly (*P* < 0.05). For example, Concanavalin A and FMLP induced more histamine release from the same donor cells than anti-IgE. The plasmas from atopic children, however, did not change the amount of hista-

mine released from the donor cells after passive sensitization since the histamine release response induced by the secretagogues was not significantly different (*P* > 0.05) from the response of untreated cells.

Table 2
Allergen-mediated histamine release from the mite allergic donor cells

Allergen		Untreated donor cells	Acid-treated donor cells	Donor cells passively sensitized with:	
				own plasma	plasma from atopic children
Grass (<i>n</i> = 13) BU/ml	1	0.0	0.0	0.0	0.4±0.2
	100	0.0	0.0	0.0	1.1±0.2
	1000	0.0	0.0	0.0	1.3±0.3
Mite (<i>n</i> = 10) BU/ml	1	0.6±0.1	0.0±0.1	0.5±0.1	0.7±0.2
	100	0.6±0.1	0.1±0.1	0.6±0.2	1.4±0.3
	1000	0.5±0.1	0.0±0.1	0.6±0.2	0.9±0.2
Cat (<i>n</i> = 6) (BU/ml)	1	0.0	0.0	0.0	0.4±0.1
	100	0.0	0.0	0.0	1.2±0.2
	1000	0.0	0.0	0.0	1.1±0.2

Each value represents the mean ± SD histamine in ng/sample, (*n*) indicates number of passive sensitization experiments with atopic plasmas

Table 1b shows the data when non-atopic donor cells were passively sensitized with plasma from atopics and challenged with the appropriate allergens. Untreated donor cells did not release histamine at allergen challenge; however, acid-treated donor cells passively sensitized with atopic plasma responded to allergen challenge. The histamine release response induced by the three allergens did not differ significantly ($P > 0.05$) as computed by the absolute histamine values.

Control experiments using acid treatment and passive sensitization of non-atopic donor cells with own plasma did not cause allergen-induced histamine release.

Passive sensitization of mite-allergic donor basophils

The untreated mite allergic donor cells only responded to house dust mite challenge (Table 2). The acid treatment effectively abolished allergen-mediated histamine release. When the mite allergic donor cells were passively sensitized with plasma from the atopic children the donor cells released more histamine at allergen (grass, mite, and cat) challenge compared with non-atopic donor cells passively sensitized with the same plasmas ($P < 0.05$). When cells from

the mite-allergic donor were passively sensitized with plasma from 10 mite-allergic children the mite-induced histamine release was significantly increased ($P < 0.05$) compared with untreated donor cells as well as donor cells passively sensitized with own plasma. As demonstrated with non-atopic donor cells the histamine release response induced by the three allergens did not differ significantly ($P > 0.05$).

TPA-induced histamine release from non-responding basophils

Table 3a shows that no histamine release was observed from the non-responding basophils at day 1. However, in the presence of 10 ng/ml TPA seven of 10 children released histamine to anti-IgE and four of six atopic children at allergen challenge. The non-responsiveness was not related to low amounts of circulating IgE antibodies since the allergic patients all expressed high RAST and total IgE values with the exception of pt. No. 4.

In non-atopics, however, despite low total IgE, anti-IgE induced histamine release in the presence of TPA.

In all subjects the non-responsiveness of the basophils was a consistent phenomenon for a 90

Table 3a
Histamine release from non-responding basophils in absence and presence of 10 ng/ml TPA

Blood samples from:	Allergic to:	Total IgE [kU/L]	RAST [PRU]	Histamine release in ng/sample		
				TPA: 0 ng/ml	10 ng/ml	10 ng/ml
				Day: 1	1	90
Atopics				E/A	E/A	E/A
Pt No.	1 Grass	540	7	0.0/0.0	0.0/0.0	0.4/0.5
	2 Grass	215	12	0.1/0.0	0.5/0.3	0.7/1.4
	3 Grass	490	20	0.0/0.0	0.0/0.0	0.6/1.3
	4 Cat dander	95	3	0.0/0.0	0.4/1.9	0.6/1.2
	5 Grass	294	11	0.1/0.0	1.0/0.8	1.2/1.1
	6 Mite	510	26	0.0/0.0	1.8/1.9	1.8/1.9
Non-atopics						
Pt No.	7 -	< 150	0	0.0/0.0	0.0/0.0	0.0/0.0
	8 -	< 150	0	0.0/0.0	0.4/0.0	0.5/0.0
	9 -	< 150	0	0.0/0.0	0.4/0.0	0.6/0.0
	10 -	< 150	0	0.0/0.0	0.5/0.0	0.5/0.0

Values represent maximal histamine release in ng/sample induced by either anti-IgE (E) or allergen (A). SD < 0.1 ng histamine/sample in all experiments

Table 3b

Histamine release from donor basophils passively sensitized with plasma from non-responding patients					
Plasma samples from:	Allergic to:	Non-atopic donor cells		Atopic donor cells	
		Anti-IgE	Allergen	Anti-IgE	Allergen
Atopics					
Pt No.	1 Grass	0.5	0.8	0.5	1.0
	2 Grass	0.3	0.6	0.8	1.0
	3 Grass	0.6	0.7	0.9	0.9
	4 Cat dander	0.0	0.0	0.0	0.0
	5 Grass	0.5	1.0	0.7	1.1
	6 Mite	0.6	0.6	0.9	1.3
Non-atopics					
Pt No.	7 -	0.0	0.0	0.0	0.0
	8 -	0.4	0.0	0.4	0.0
	9 -	0.3	0.0	0.3	0.0
	10 -	0.3	0.0	0.3	0.0

Values represent maximal histamine release in ng/sample. SD < 0.1 ng histamine/sample in all experiments

days' observation period (results only shown for day 1). However, two patients (Nos 1 & 3), who initially did not release histamine in the presence of TPA, turned responsive in the presence of TPA 90 days later. One non-atopic child (No. 7), however, never turned responsive to anti-IgE challenge despite addition of TPA.

To exclude TPA-induced unspecific histamine release it was demonstrated that the addition of TPA did not make the non-atopics responsive to allergens.

Passive sensitization with plasma from non-responding basophils

Table 3b reports the effect of using plasma from 10 subjects with non-responding basophils for passive sensitization of basophils from the non-atopic and mite allergic donor. Plasma from eight of the non-responding subjects could sensitize the donor cells as demonstrated by histamine release to anti-IgE. Allergen challenge induced histamine release in five of six atopic children. However, plasma from two subjects (Nos. 4 & 7) were not able to sensitize the donor cells since histamine release was not observed after passive sensitization.

The maximal anti-IgE-mediated histamine release was the same from atopic and non-atopic

donor basophils passively sensitized with plasma samples from non-atopic non-responders. In contrast, the maximal anti-IgE and allergen-mediated histamine releases from atopic donor basophils were increased compared with the histamine release from non-atopic donor basophils.

DISCUSSION

Recently, we have used passive sensitization of human basophils and mast cells with or without previous acid treatment as a useful experimental procedure for investigating the functional ability of IgE antibody to regulate histamine release (7, 8, 9, 14).

In the present study we further investigated whether there was an additional functional heterogeneity with respect to cellular properties of basophils of two different donors (one allergic and one non-atopic) in passive sensitization experiments.

To exclude the effect of donor's own IgE on the cell response all cells were acid-treated to remove membrane-bound IgE (7, 11). In addition, it has previously been shown that acid treatment rendered the donor cells more responsive to passive sensitization (14, 18). It was established that acid treatment abrogated histamine release to anti-IgE, indicating that the donor basophils lost membrane-bound IgE. By

passive sensitization the histamine release response was reestablished, indicating cell function and Fc-receptors were unaffected by the acid treatment. Since FMLP activates a specific membrane receptor (13) different from the IgE-Fc receptor it is not surprising that the FMLP-mediated release was unaffected by acid treatment and passive sensitization. However, these data substantiate that removal of IgE from its receptor is consistent with the functional integrity of the basophils. It also indicates that membrane receptors are not affected by the acid treatment.

It appears that acid-treated cells were able to release some histamine at Concanavalin A challenge. Because Concanavalin A acts by binding to carbohydrates on the IgE molecule (12) it may also bind other carbohydrates present on the cell membrane which can initiate histamine release. Hence, it is possible that in acid-treated cells non-IgE-mediated trigger mechanisms were activated by Concanavalin A.

The secretagogues (Concanavalin A, anti-IgE or FMLP) induced the release of different quantities of histamine from the cells (Table 1). However, the various plasmas used for passive sensitization did not change the histamine release response to the secretagogues. Hence, the response to these secretagogues seems to be independent of the types of IgE bound to the cell membrane.

The same non-atopic donor cells passively sensitized with different types of specific IgE (cat dander-, mite-, and grass-specific IgE) from different plasma samples containing approximately the same amount of specific IgE (Table 2) responded similarly to the same dilutions of each extract. Hence, in allergen-specific histamine release the biological activity of each allergen extract as expressed in biological activity (BU/ml) seems to correspond well to a particular cellular property rather than with the type of IgE molecules bound to the cell membrane.

In subsequent passive sensitization experiments atopic donor cells were sensitized with the same plasmas. However, the atopic cells released more histamine than the non-atopic donor cells. Several possible explanations may be considered. The difference may be a higher

histamine content of the atopic donor cells in connection with an increased "releasability" (i.e. biochemical events in the basophil-inducing histamine release and not only the density of membrane-bound IgE). Stahl Skov et al. (14, 18) have suggested that increased histamine release to specific antigen after passive sensitization of acid-treated cells could be related to additional binding to free cell receptors as well as partial replacement of bound IgE. Hence, increased numbers of Fc-receptors on atopic cells may bind more specific IgE than the non-atopic cells. In contrast, Conroy et al. (2) did not observe any relationship between histamine release and the quantity of basophil bound IgE. In this study, however, increased histamine release due to acid treatment is less likely, since an increased histamine release was not demonstrated in the passive sensitization experiments with donor's own plasma. Finally, the plasma of the atopic patients may contain histamine releasing factors which interact with a subset of IgE molecules (designated IgE+) which occur in atopic patients only (5, 11). The histamine releasing factors may interact with the basophils of atopic patients only (5, 11). Hence, this may explain why passive sensitization of acid-treated atopic donor basophils with own plasma did not change the release pattern compared with the untreated cells, whereas, plasma from various mite allergic patients (e.g. containing histamine-releasing factors and IgE+) showed an increased histamine release from atopic donor cells.

Non-responding basophils

The percentage of patients in an allergic population who do not respond with histamine release after antigen challenge is reported to be 6-12% (10, 19). This group of patients is of interest because despite high specific IgE values and clinical symptoms of allergy they give rise to false negative test results in the histamine release assays used for allergy diagnosis (6, 7, 10, 19).

The non-responsiveness was reproducible in all patients within a 3 - month period and none

of the patients was taking medicine, nor had ever received previous hyposensitization treatment. Thus, a possible inhibiting effect of IgG-, and subclass IgG₄-antibodies, or medicine is less likely.

The atopic patients all had high amounts of allergen-specific IgE in serum and the IgE from the non-responders was demonstrated to be functionally intact since passive sensitization of responding basophils could initiate histamine release at allergen challenge. However, plasma from patients 4 and 7 could not passively sensitize donor cells. Therefore, these plasmas probably contained too low amounts of IgE for sufficient passive sensitization.

The two non-responders (Nos. 1 & 3) who became responsive in the presence of TPA after the 90-day period may be explained by a change in cellular function due to unknown effects perhaps related to the grass pollen season. Thus, the non-responsiveness of mast cells and basophils appears to be related to subcellular alterations in function and not to heterogeneity of IgE (9, 15). In passive sensitization experiments with plasma from non-responding patients the basophils from the atopic donor also released more histamine at anti-IgE and allergen challenge compared with the non-atopic donor cells. Hence, these data seem to further confirm that the atopic donor cells either contain more histamine, possess an increased "releasability", or have been passively sensitized with a certain type of IgE in the presence of histamine-releasing factors. In some patients the non-responsiveness of basophils is due to an impaired cell function which can be reverted by stimulating protein kinase C. The non-responsiveness is not related to serum IgE-levels or the heterogeneity of IgE bound to the cell membrane. These findings may explain some of the discrepancies sometimes observed between clinical symptoms, serological IgE-antibody measurements and histamine-release testing in allergic patients.

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