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A SIMPLIFIED METHOD FOR MEASURING
BASOPHIL HISTAMINE RELEASE AND
BLOCKING ANTIBODIES IN
HAY FEVER PATIENTS. BASOPHIL HISTAMINE
CONTENT AND CELL PRESERVATION

By

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In recent years *in vitro* methods have been developed for the diagnosis of allergy. The intention was to avoid the risk of untoward reactions by testing, and to obtain precise information about the causative agent, the degree of allergy and improvement by therapy.

In the radioimmunosorbent test (RIST) the total concentration of IgE in serum is determined (1, 5, 6). Progress was made by the introduction of the radioallergosorbent test (RAST) in which allergen-specific IgE antibodies (reagins) are measured in serum (2, 15, 16).

However, it is the cell-bound IgE which reacts with allergen, resulting in release of histamine, that is responsible for the allergic symptoms. Therefore, basophil histamine release technique might be more profitable. The degree of allergy evaluated by this method is correlated to symptom score as well as to the RAST value in hay fever patients (11, 14). Also blocking antibodies can be measured by this technique and the titer increases by immunotherapy (9, 10).

Basophil histamine release technique is complicated and time-consuming. A simplified method has therefore been developed in the present study.

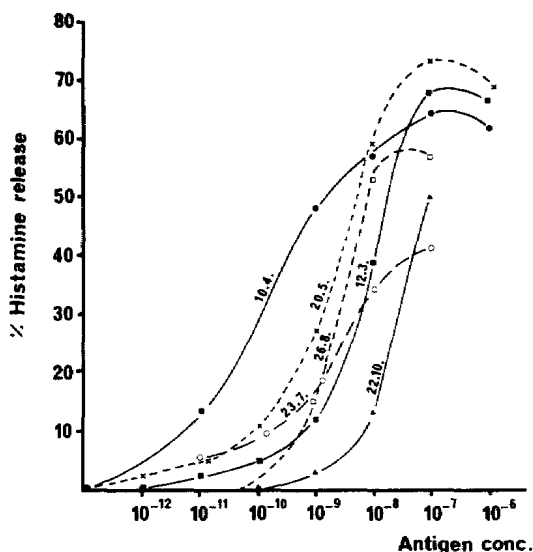


Fig. 1 a.

MATERIAL AND METHODS

Patients

The investigation comprises volunteers without a history of grass pollen allergy and with a negative RAST and patients between 22 and 28 years of age with a well-documented history of symptoms of grass pollen hay fever allergy. All patients showed a positive intracutaneous skin test reaction to timothy grass pollen extracts 1:10,000 with a weal and flare reaction of the same size or bigger than the reaction produced by a histamine standard of 0.1 mg/ml. The patients were sensitive also to other allergens, but grass pollen was considered the major allergen. The medical treatment included antihistamines but not corticosteroids. Specific hyposensitization therapy with Allpyral five-grass mix extracts including *Phleum pratense* (Dome Laboratories Ltd., England) was given by weekly injections from mid-March to the end of May.

Preparation of Leukocyte Suspension

By venipuncture 7.5 ml blood samples were collected in plastic tubes containing 0.5 ml 0.20 M EDTA (ethylene diamine tetraacetic acid) (pH 7.0). In some

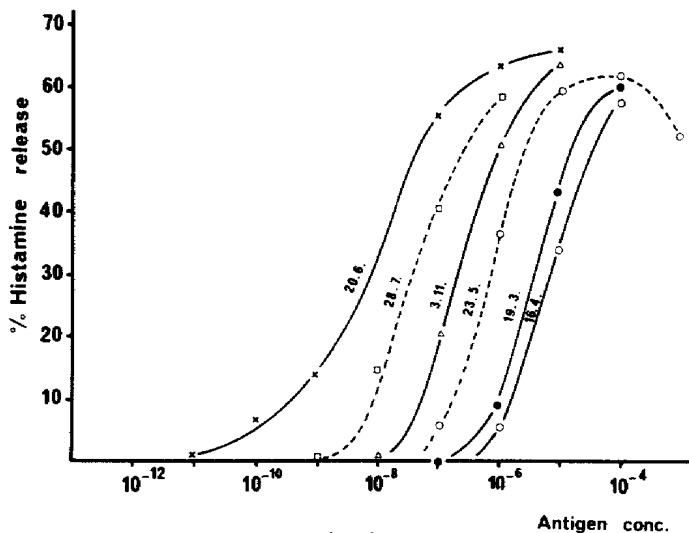


Fig. 1 b.

Fig. 1 a and b.

Release of histamine from leukocytes of grass pollen allergic patients on challenge with timothy grass pollen extract. a: patient receiving specific hyposensitization therapy, b: no hyposensitization therapy. Dates of determination are stated on the curves.

experiments the sample was preserved for transport by adding 8 ml cell culture medium (RPMI media serie 1640, Gibco Bio-Cult Ltd., Scotland) including L-glutamine 1.2 mM, heparine 0.1 I.E., penicillin G 4000 I.E. and streptomycin 4 mg base. 14.5 ml of 0.9 per cent NaCl was then added to preserved samples and 22.5 ml to non-preserved samples, and the leukocytes were isolated according to the method of Day (3). 10 ml Ficoll-Hypaque solution (12.8 g Ficoll + 21 g Hypaque, i.e. sodium diatrizoate; add H₂O up to 190-200 ml, the final solution has a density of 1.080 g/ml at 20° C) was placed below the mixture. The sample was centrifuged (400 g for 40 min) and the cotton wool-like interphase consisting of monocytes with approx. 2 per cent basophils was mixed with 15 ml Tris AMC (Tris (hydroxymethyl) aminomethane 25 mM at pH 7.6, NaCl 0.12 M, KCl 5 mM, CaCl₂ 0.6 mM, MgCl₂ 1.1 mM, human serum albumin 0.3 mg/ml and glucose 1 mg/ml). The mixture was centrifuged (107 g for 10 min) and the cell sediment was resuspended in 10 ml Tris AMC. The suspension was centrifuged (60 g for 10 min) and the supernatant containing thrombocytes

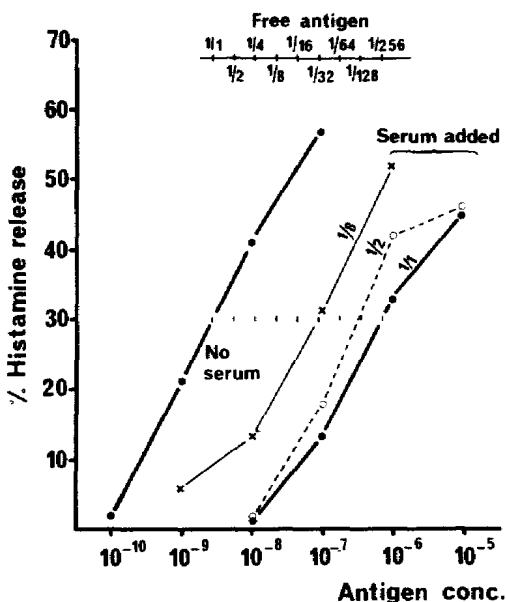


Fig. 2.

Antigen-neutralizing capacity of serum (undiluted and diluted 1/2 and 1/8) from a patient receiving specific hyposensitization therapy.

was discarded. The cell sediment was finally resuspended in 7.5 ml Tris AMC. Basophils were stained and counted according to the method of Kimura et al. (7).

Cell Sensitivity

400 μ l leukocyte suspensions containing 2000–8000 basophils were incubated in plastic tubes at 37° C for 40 min with 20 μ l timothy grass pollen extract at different concentrations. The extract was supplied by the Protein Laboratory, University of Copenhagen and manufactured according to Nielsen, Løwenstein & Weeke (13). Thereafter, 3 ml ice-cold Tris AMC was added to the samples and they were centrifuged at 2000 g for 10 min. The supernatant contains released histamine and protein, which interferes with the histamine analysis. It was therefore discarded and the residual histamine was determined in the cell sediment. To the cell sediment was added 200 μ l of a 0.02 per cent *o*-phthalaldehyde (Fluka) in 0.045 N NaOH (fresh solution) and the mixture was left

on ice for 40 min. 200 μ l 0.175 N H_2PO_4 was then added and the sample was centrifuged at 3000 g for 10 min. The histamine content in the supernatant was determined spectrofluorometrically in an Aminco-Fluoro-Colorimeter provided with a micro-flow cell. Samples are read at 355/455 nm (excitation/emission). The release of histamine was expressed as a percentage of the total histamine content of the sample. Correction was made for the spontaneous histamine release amounting to 1–10 per cent, except for Fig. 5.

Blocking Antibodies

Samples of 30 μ l timothy grass pollen extract were incubated at 37° C for 30 min with 200 μ l Tris AMC or with patient serum diluted 1:10 with Tris AMC. 400 μ l leukocyte suspension was then added and the mixture was incubated at 37° C for 40 min. 3 ml ice-cold Tris AMC was added and the sample was centrifuged at 2000 g for 10 min. The residual histamine was determined in the cell sediment as described above.

RESULTS

Histamine Content

The histamine content of a single basophil granulocyte was calculated to be 1.3 ± 0.1 pg (s.e.m.) histamine base in 12 normal persons and 1.2 ± 0.2 pg in 17 hay fever patients, indicating no significant difference between normal and allergic persons. The allergic persons include hyposensitized as well as non-hyposensitized patients, since no difference was found between these groups. All persons were examined in the month of October.

Cell Sensitivity

Fig. 1 shows the release of histamine from leukocytes of grass pollen hay fever patients. The leukocytes were challenged with timothy grass pollen extract. In the semilogarithmic plot increasing concentrations of allergen caused an increase in histamine liberation followed by a decrease. A parallel shift of the curve indicates a change of cell sensitivity to grass pollen allergen. Evaluation of the degree of allergy is based on the cell sensitivity. Fig. 1 a shows findings in a patient receiving preseasonal hyposensitization therapy. Cell sensitivity increased during immunotherapy, but decreased during the

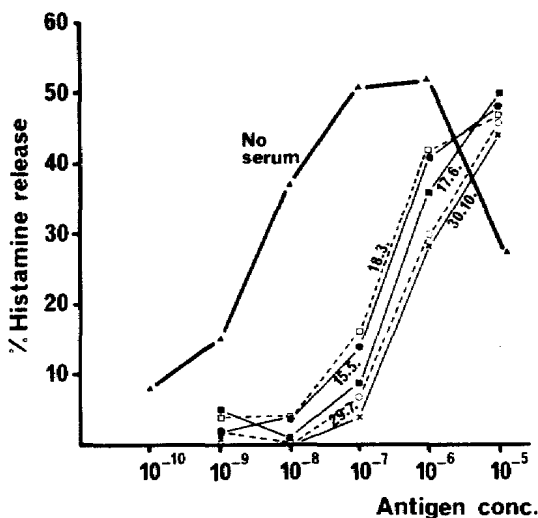


Fig. 3 a.

grass pollen season covering the period from the end of May to the end of September. In contrast, an untreated patient showed in the pollen season an increase of cell sensitivity (Fig. 1 b). No histamine liberation was obtained in five volunteers without a history of grass pollen allergy.

Blocking Antibodies

The level of blocking antibody was estimated as the antigen-neutralizing capacity of serum. In an excess of timothy grass pollen extract a part of the antigen was neutralized by pre-incubation with patient serum, and the remainder of free antigen was measured by histamine liberation in leukocytes from the same patient. Antigen-neutralizing capacity of allergic serum is shown in Fig. 2.

Leukocytes from a patient receiving specific hyposensitization therapy were challenged with timothy grass pollen extract in the absence of serum. The dose-response curve was not in-

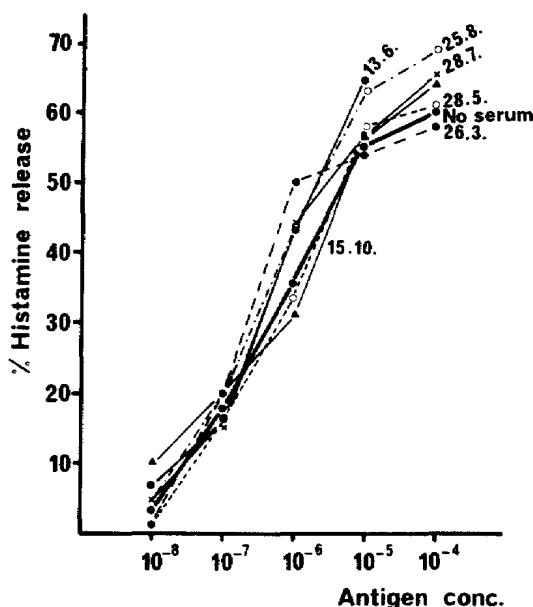


Fig. 3 b.

Fig. 3 a and b.

Antigen-neutralizing capacity of serum from a: patient receiving specific hyposensitization therapy, b: untreated patient. Both sera were measured during the period from March to October. Dates of determination are stated on the curves.

fluenced by preincubation of the timothy extract with serum from normal individuals (ABO type), but serum from the hyposensitized patient caused a parallel shift. A parallel shift of the dose-response curve to higher concentrations of timothy extract indicates antigen-neutralizing capacity. If 99 per cent of the antigen is neutralized only 1 per cent is free and available for induction of histamine release and a concentration of timothy extract a hundred times higher will be needed to obtain histamine liberation. The level of blocking antibody is 100, i.e. it is expressed by the ratio between the allergen concentrations causing a 30 per cent release in the presence or

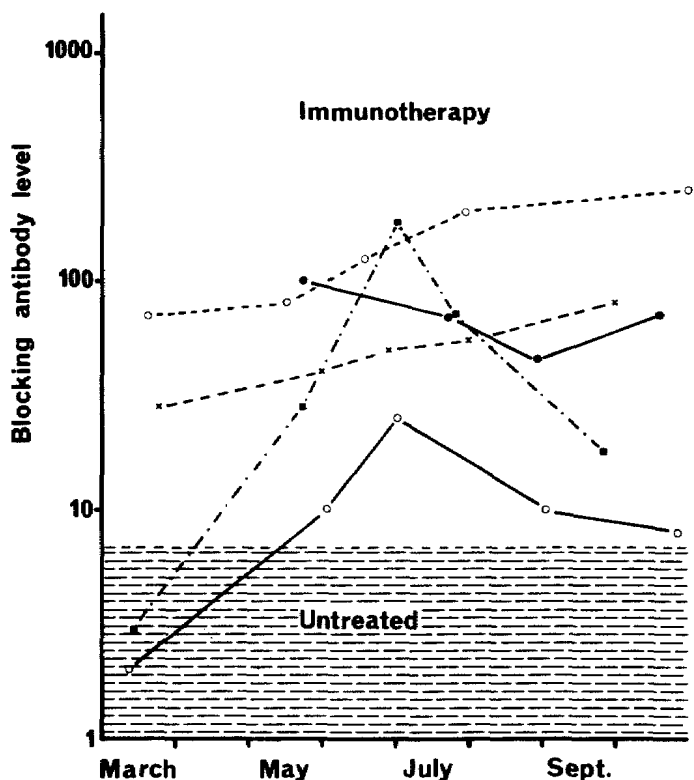


Fig. 4.

Level of blocking antibody in hyposensitized and non-hyposensitized patients.

absence, respectively, of patient serum in a final concentration of 3 per cent. In Fig. 2 the titer level of blocking antibody was 256. This is correct since no antigen-neutralizing capacity would be left in a $1/256$ dilution of the serum, which is in accordance with the calculations. Fig. 3 a shows the antigen-neutralizing capacity of serum from a patient receiving specific hyposensitization therapy. A parallel shift of the dose-response curve of about two decades indicates a level of blocking anti-

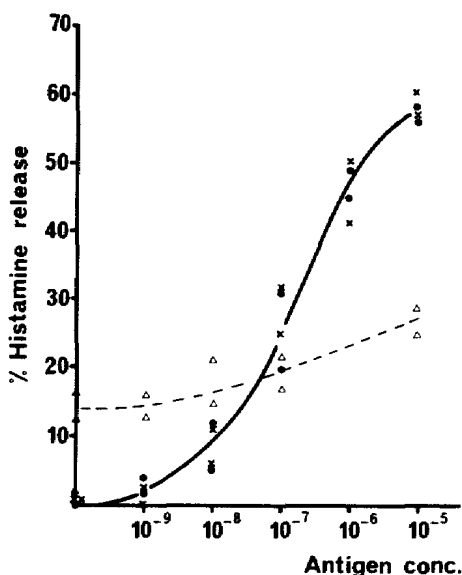


Fig. 5.

Preservation of blood for histamine release. Blood was divided for immediate assay on fresh unpreserved sample (●) and for assay after 48 h on preserved (x) and unpreserved (△) samples kept at 24° C.

bodies of approx. 200. A very small shift was obtained in an untreated patient (Fig. 3 b). A survey is given in Fig. 4. Untreated hay fever patients showed levels of blocking antibodies at seven and under, whereas higher levels were obtained in hyposensitized patients.

Preservation of Cells

Fig. 5 shows experiments in which blood was preserved for transport. Blood from a grass pollen hay fever patient was divided into three aliquots, one for immediate assay of histamine liberation and two for assay after 48 h at 24° C with and without preservation by cell culture medium. These two samples were shaken several times during the 2 days. Pre-

served blood showed a histamine liberation curve identical to that of the fresh sample, whereas unpreserved blood showed a reduced histamine release and a higher spontaneous release. The total content of histamine was trebled by preservation.

DISCUSSION

Basophil histamine release technique allows evaluation of both cell sensitivity and the level of blocking antibodies. Cell sensitivity seems to be useful for evaluating the degree of allergy in hay fever patients since it was found to be correlated to symptom score as well as to the RAST value (11, 14). By specific hyposensitization therapy changes occur in cell sensitivity which are in contrast to those obtained in untreated patients (Fig. 1). These changes, of which a survey has been given (14), involve a tendency to an initial increase in cell sensitivity at the beginning of the preseasonal hyposensitization therapy followed by a decrease during the pollen season, a decrease which is in contrast to findings in untreated hay fever patients, in whom an increase or no change in cell sensitivity is observed. The changes suggest that cell sensitivity could be valuable for monitoring hyposensitization therapy.

Basophil histamine release technique showed low levels of blocking antibodies in untreated hay fever patients and higher levels in hyposensitized patients (Fig. 4), which is in accordance with observations in ragweed hay fever patients (9, 10).

Thus, the basophil histamine release technique has several possible uses. The drawback is, however, that the method is time-consuming and complicated. Several steps are needed to purify the histamine by columns or organic solvent extractions before the spectrofluorometric determination can be made (8). The reason is that the presence of protein and other materials (allergen, serum, drugs, etc.) in the supernatant from the cell reaction mixture interfere with the estimation of histamine. In the present study these difficulties were overcome by discarding the supernatant and estimating the residual

histamine in the cell sediment. Without previous purification, histamine was then conjugated with *o*-phthaldialdehyde to form a fluorophore.

This simplified method showed a histamine content in the basophil granulocyte of 1.3 pg histamine base per cell in normal persons, which is in accordance with the results of 1.2 and 1.5 pg obtained by more specific methods for histamine assay (4, 12). The calculations are based on the fact that essentially all histamine in human leukocytes is present in basophil granulocytes (4, 12). A similar content was found in hay fever patients, indicating no significant difference between normal and allergic persons. However, the results are obtained in the month of October and they do not exclude a possible difference between the groups in the pollen season.

For routine work of the basophil histamine release technique in the clinic a preservation of blood to obtain correct histamine liberation is very important. A complete preservation for 48 h was obtained by adding cell culture medium to the blood. In addition, the total content of histamine was trebled by preservation, probably due to incorporation in basophils of L-histidine from the cell culture medium, a trick which could be used to increase the number of assays per blood sample.

SUMMARY

A simplified method for measuring basophil histamine release in grass pollen hay fever patients has been developed. Leukocytes were challenged *in vitro* with extracts of *Phleum pratense* (timothy) and the release of histamine was determined indirectly as the residual histamine in the cell sediment. Several steps to purify histamine thus became superfluous and histamine was directly conjugated with *o*-phthaldialdehyde to form a fluorophore. The simplified method showed a basophil histamine content which was in accordance with results obtained by more specific methods. No difference in basophil histamine content was found between normal and allergic persons. For

the histamine liberation assay blood could be adequately preserved for transport for 48 h at room temperature by adding cell culture medium. Basophil histamine release technique allows evaluation of cell sensitivity for determination of the degree of allergy as well as the level of blocking antibodies.

ACKNOWLEDGEMENTS

The timothy grass pollen extract was kindly supplied by Dr. Henning Løwenstein, Protein Laboratory, University of Copenhagen. This investigation was supported by Lundbeckfonden and P. Carl Petersens Fond (grant No. B. 913).

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