

allergen antibody should preferably be an IgE antibody also, since then the biological situation (one allergen molecule cross-linking two IgE anti-allergen antibodies on the surface of the mast cell) would be closely mimicked. For practical reasons the second anti-allergen antibody will be an IgG antibody, either human or heterologous.

In system 5 (2), the IgG anti-allergen antibody, coupled to a solid phase, is incubated with the unknown allergen; IgE anti-allergen antibody is then added, and IgE binding to the solid phase is detected with labelled anti-IgE.

In system 6 (1), a mixture of the IgG antibody, the IgE antibody, and the allergen is incubated with some insoluble anti-IgG reagent; IgE-binding to this anti-IgG reagent is again detected with labelled anti-IgE. The latter test is suitable when the IgE and the IgG antibodies are present in the same human serum.

As anti-IgG reagent we have used rheumatoid factor, anti-IgG antibodies, or anti-IgG4 antibodies. In the grass pollen system a steeper dose-response curve was obtained with anti-IgG4 antibodies than with anti-IgG antibodies. This presumably indicates that the ratio: grass pollen-specific Ig to total Ig, is higher for IgG4 than for the other Ig subclasses. Comparison of system 5 with system 6 indicates a similar sensitivity; however, due to the extra washing step included in system 5, no prozone effect is found (Fig. 1).

Disadvantages of these systems are:

1. IgG anti-allergen antibodies are required.
2. For system 6, an anti-Ig reagent is required.
3. Aggregated allergens are possibly overestimated.
4. Non-specific effects, either increasing or decreasing IgE-binding, are theoretically possible.

Advantages are:

1. No allergen modification is required.

2. The requirement of a second anti-allergen antibody increases the specificity, especially if heterologous antibody is used.
3. Monovalent allergens will not be detected.

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In vitro Standardization of Basophil Histamine Release

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For many years it has been known that allergic mediators as histamine and SRS-A are responsible for the symptoms in allergic diseases such as asthma, rhinitis and urticaria. Investigations during the last 10 years have further elucidated the cellular mechanisms of the IgE-mediated release of these mediators from mast cells and basophils (1), and it is likely that an estimation of basophil histamine release would be of more diagnostic value than a RAST estimation, since it is the

cell-attached IgE that reacts with allergen, resulting in the release of allergic mediators.

The determination of histamine released from basophils after *in vitro* provocations with the suspected allergens is being increasingly used for diagnostics of type I allergic reactions, since the response is correlated to *in vivo* provocation (3). Investigations with patients with exogenous bronchial asthma have thus shown that *in vitro* provocation of the patient's basophil leukocytes

with the suspected allergen is the best single analysis for detection of the responsible allergen, since there is a good correlation with the bronchial provocation response with only a few false positive and negative responses (3).

The fluorometric analysis of histamine was originally described by Shore et al. (8) and has since then been modified in order to increase its specificity and sensitivity (2, 7). The extraction procedure for removing interfering compounds before the assay of histamine is, however, laborious and time consuming, and therefore the autoanalyzer has been used to facilitate the analysis (9, 10). The autoanalyzer method can be used in the clinic and has gained a limited foothold, since the analysis requires some experience, constant supervision and technical capacity.

In our laboratory, among others, an alternative facilitation and simplification of the analysis have been achieved by allergen provocation of isolated leukocytes instead of whole blood (11, 12). By this fractionation of the blood, interfering substances are removed so that histamine in the basophils can now be determined directly without the preceding long extraction procedure. Isolation of the leukocytes is made by the Ficoll-Hypaque gradient method, and the washed cells, which contain monocytes and approx. 2% of basophils, are suspended in Tris-AMC (11).

A 200- μ l cell suspension is incubated in plastic tubes at 37°C for 40 min with 10 μ l allergen extract at different concentrations. Allergen is omitted for spontaneous histamine release and for determination of the total histamine content; in the latter case the samples are left at 4°C. Thereafter 3 ml ice-cold Tris-AMC is added to the samples and they are centrifuged at 2000 *g* for 10 min. The residual histamine is determined in the cell pellets. A 200- μ l OPT mixture (2 mg *o*-phthalaldehyde, Fluka, dissolved in 1 ml methanol and 9 ml 0.05 *N* NaOH) is added to the cell sediment and the sample is left at room temperature for 4 min. Then 200 μ l 0.175 *N* H_3PO_4 is added and the sample is centrifuged at 3000 *g* for 10 min. The histamine content in the supernatant is determined spectrofluorometrically in an Aminco-Fluoro-Colorimeter provided with a micro flow cell and the samples are read at 355/455 nm (excitation/emission). The release of histamine is expressed as a percentage of the total histamine content of the sample. Correction is made for the spontaneous histamine release amounting to 1–5%.

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 24–48 h can be obtained by adding cell culture medium to the blood (11).

A simple and sensitive method for the determination of basophil histamine release, which is not based on fluorometric measurement but on a radioactive assay has been developed in our laboratory (13). The new

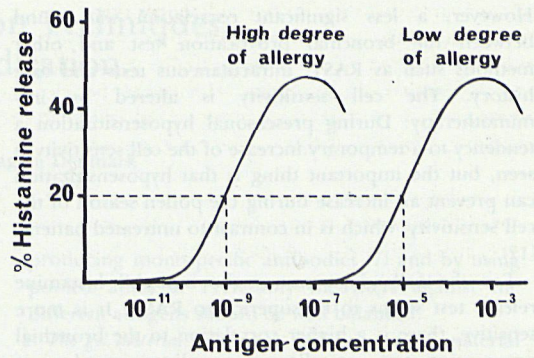


Fig. 1. Basophil histamine release curves in allergic patients.

principle is based on *in vitro* incorporation of ^3H -histamine in the basophils of the patient, whereafter release of labelled histamine is determined after provocation of the cells with the allergen under suspicion. Only 2 ml blood is sufficient for 50 determinations and one laboratory technician is able to determine 10–12 allergens in 8–10 patients per day. For comparison, the above-mentioned classical techniques require approx. 15 ml blood and only a couple of patients can be examined for up to six allergens per day. Therefore, this new assay seems to fulfil the demand for a rapid, simple and inexpensive method for large scale screening of patients under suspicion of type I allergy.

The interpretation of the histamine response by basophil histamine release technique is seen in Fig. 1. The left histamine release curve represents a patient with a high degree of allergy, since high degree of allergy causes a high cell sensitivity (i.e. the cell responds to a low allergen concentration), whereas a low degree of allergy is associated with a low cell sensitivity. In our laboratory the cell sensitivity is defined in terms of the allergen concentration causing a 20% histamine release (11, 12).

Fig. 1 indicates a cell sensitivity of 10^{-9} and 10^{-5} for the two patients with high and low degrees of allergy, respectively. Furthermore, we found that the cell sensitivity is dependent on the amount of antigen-specific IgE molecules on the basophil cell surface, since a high correlation between these parameters was found (14). In contrast to the cell sensitivity, the maximal release of histamine was found to be of no significance when evaluating the degree of allergy in pollinosis patients. However, in the case of intensive immunotherapy another pattern might appear with very little release of histamine, as found in children by Sadan et al. (5).

The cell sensitivity is well correlated to the bronchial provocation response in patients with exogenous bronchial asthma, and the basophil histamine release technique has been found to be the best single analysis for the detection of the responsible antigen (3).

However, a less significant correlation was found between the bronchial provocation test and other methods such as RAST, intracutaneous tests and case history. The cell sensitivity is altered by immunotherapy: During preseasonal hyposensitization a tendency to a temporary increase of the cell sensitivity is seen, but the important thing is that hyposensitization can prevent an increase during the pollen season of the cell sensitivity, which is in contrast to untreated patients (12).

For the following reasons the basophil histamine release test seems to be superior to RAST: It is more sensitive, there is a higher correlation to the bronchial provocation test, no allergen coupling procedure is needed, and finally this new test based on ^3H -labelled histamine is less expensive.

The basophil histamine release technique offers the following possibilities in the clinic. Precise information of the causative agent, evaluation of the degree of allergy and of the improvement by immunotherapy (12) and furthermore, estimation of blocking antibodies (11). The basophil histamine release has been found useful in the diagnosis of house dust, mite, animal dandruff, pollen and mould fungi (15). Investigations are now being made in our laboratory for testing drugs (6), foods, bacterial antigens (15) and autoimmune allergies (4) for diagnostics of type I reactions.

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