New In-vitro Test for IgE-mediated Hypersensitivity in Man

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Summary
A new simple and sensitive in-vitro method for the diagnosis of type 1 (IgE-mediated) hypersensitivity in man is described. Sliced human skin is passively sensitized by reaginic serum from allergic patients and the presence of antigen-specific IgE on the sensitized slices is detected by assay of antigen-evoked histamine release. Serum from 12 out of 14 patients with clinical respiratory allergy and positive skin tests gave significant antigen-specific histamine release. This method, which is essentially an in-vitro model of the Prausnitz-Küstner reaction, should prove of value in the diagnosis of human reaginic hypersensitivity in man.

Introduction
Confirmation of the diagnosis of clinical reaginic (IgE-mediated) allergy (type 1, Gell and Coombs, 1968) normally involves intracutaneous testing with or without some form of provocation test. These procedures are unpleasant for the patient as well as involving significant risk in a highly sensitive subject. Recently we have devised a new in-vitro technique for study of the mechanisms of IgE-mediated hypersensitivity in human skin (Greaves et al., 1972). This method utilizes the fact that human skin passively sensitized in vitro by human reaginic serum will release histamine on subsequent challenge by specific antigen. Essentially the method is therefore an in-vitro model of the Prausnitz-Küstner reaction. In this paper we report application of the model to laboratory diagnosis of clinical reaginic hypersensitivity states in man.

Methods
Patients.—Fourteen young adult patients of both sexes with clinical respiratory allergy were studied. All were allergic to pollen antigen. In every patient the diagnosis was confirmed by pricking the antigen into the skin. The degree of sensitivity was roughly assessed by the magnitude of the weal, a scale from 0 to ++ + being used. All 14 patients gave a result of at least + + + on this scale.

Reaginic Sera.—Blood was withdrawn within a few days of prick-testing. After centrifugation at 4°C serum was stored at −20°C. All tests were carried out on serum obtained during November to January. The total IgE concentrations in 13 of the patients were determined by Dr. D. R. Stanworth and Dr. P. McLaughlan by a radioimmunosorbent method (McLaughlan et al., 1971). Nine of the values (Table) exceeded the upper limit of normal (375 ng/ml).

Antigens.—All antigen preparations used for both in-vivo and in-vitro tests were obtained from Bencard Ltd., Brentford, Middlesex. Most patients gave positive skin reactions to more than one antigen, and in these instances the antigen preparation giving the strongest positive skin reaction was the one selected for the in-vitro test.

Skin Samples.—Healthy-looking skin removed at mastectomy was used. Immediately after excision the subcutaneous fat was trimmed and the skin sample washed in Tyrode solution. In all experiments the skin was used within one hour of operation.

Preparation of Skin Samples for In-vitro Testing.—Skin was sliced into 500-μm thick slices with a hand microtome. At least 30 slices could be obtained from each specimen. The slices were placed on a filter paper moistened with ice cold Tyrode solution.

Passive Sensitization.—Each skin slice was incubated with 2 ml of reaginic serum for 120 minutes at 37°C. The serum dilutions used ranged from 1:5 to 1:40 with Tyrode solution diluent. “Spontaneous” histamine release during passive sensitization did not exceed 11.9% even with the lowest dilution of serum.

Antigen-evoked Histamine Release.—After washing in Tyrode solution the sensitized skin slices were incubated in Tyrode solution with specific antigen for 15 minutes at 37°C using a shaking water-bath. The reaction volume was 4 ml and the final concentration of antigen was 0.01% (100 Noon units/ml). The reaction was terminated by cooling and the histamine content of the supernatant Tyrode solution was determined by bioassay using the isolated atropinized guinea-pig ileum preparation and an automatic bioassay apparatus (Vickers Instruments Ltd.). All the smooth-muscle-contracting activity in the samples was due to histamine, since the response of the ileum preparation to the samples was completely abolished by the specific antihistamine mepyramine. Histamine release from each sample was expressed as a percentage of the total present in that sample by use of the following formula: percentage release = A/(A + B) × 100 where A = histamine content of supernatant Tyrode solution after incubation, and B = residual histamine (determined by boiling in 5 ml of Tyrode solution for five minutes followed by bioassay after cooling).

Controls.—All experiments included negative and positive control tests. Negative control samples were incubated with Tyrode solution instead of the same volume of reaginic serum during sensitization. These skin samples were then challenged by antigen as for serum-treated skin. The mean spontaneous release in these controls during incubation with antigen was 2.6±2.1 S.D. Positive control tests were carried out in every experiment involving different skin samples by including results using a reaginic serum of known ability to cause passive sensitization. Both control and test determinations were carried out in duplicate in all experiments.

Evaluation of Results.—In expressing results all antigen-induced histamine release was corrected by subtracting the corresponding spontaneous (negative control) histamine release. Calculation of the standard deviations of patient means for antigen-induced and “spontaneous” histamine release (±3.9 and ±1.2 respectively) and the standard error of the difference between means for antigen-induced and “spontaneous” release (±3.0) indicated that if a patient is not sensitive to a particular antigen 95% of differences (antigen-induced release and “spontaneous” release) would not exceed ±5.8%. Thus the criterion adopted for significant sensitization was a mean histamine release in the presence of antigen which exceeded spontaneous histamine release by at least 5.8%.

Results
The results are summarized in the Table. Sera from 14 allergic subjects were examined. Of these 12 (86%) gave a positive result with one of the dilutions of serum used. Eight gave positive histamine releases with the highest dilution of serum (1:40). Of the remainder a positive result was obtained by reducing the serum dilution to 1:20 in two and to 1:10 in a
Antigen-induced Histamine Release from Skin Passively Sensitized by Reaginic Serum for Patients with Allergic Respiratory Allergy. Results are Given as Percentages

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Ig E*</th>
<th>Serum Dilution</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1:5</td>
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</tbody>
</table>

Positive Result
1 950 
2 920 
3 655 
4 1120 
5 930 
6 1200 
7 1440 
8 1620 
9 350 
10 185 
11 79 
12 260 
13 644 
14 35 

Negative Result

Values for % histamine release given after subtraction of corresponding "spontaneous" release. 
= Not done. *Total Serum Ig E (ng/ml)

further two subjects. The remaining two sera proved negative with the 1:5 dilution of serum. More concentrated sera could not be used since they occasionally evoked histamine release from the skin slices during attempted passive sensitization.

Discussion

The present results indicate that measurement of antigen-evoked release of histamine from passively sensitized human skin is a valuable in-vitro test for the laboratory diagnosis of reaginic (IgE-mediated) hypersensitivity in man. This method is simple compared with the recently described radioallergosorbent test of Wide et al. (1967) and has the additional advantage that postoperative human skin is more widely available than human lung, which has been advocated for similar in-vitro tests by other workers (Assem and Schild, 1968).

That the observed histamine release in this model is due to union of antigen with IgE antibody is confirmed by the following evidence reported in detail elsewhere (Greaves et al., 1972).

(1) The sensitizing antibody is thermolabile, since heating to 56°C for one hour completely abolishes the ability of serum from allergic subjects to sensitize the skin samples. (2) Significantly more IgE is present on sensitized than non-sensitized skin, since reversed passive anaphylaxis experiments in vitro show that specific antihuman IgE serum evokes much greater histamine release from sensitized than non-sensitized skin. (3) Precipitation of human reaginic serum with specific antihuman IgE serum abolished the ability of the reaginic serum to cause passive sensitization of the skin samples. By contrast, when this experiment was carried out with anti-IgG serum no inhibition of activity of the reaginic serum was observed. Both this and other evidence (Greaves et al., 1972) establishes that the present method is a valid in-vitro model of the Prausnitz-Küstner reaction.

Serum from 12 out of 14 patients (86%) with proved reaginic hypersensitivity gave positive histamine releases when using dilutions of serum for passive sensitization ranging from 1:10 to 1:40. The order of magnitude of releases in these experiments is comparable with releases obtained in other laboratories using the same method with human lung tissue instead of skin (Parish, 1967; Assem and Schild, 1968; Orange, et al., 1971).

The method is highly sensitive since low concentrations of antigen-specific IgE are detectable. In 2 patients positive histamine releases were obtained following passive sensitization by diluted serum samples whose total IgE concentration was below 20 ng/ml. The test is therefore at least as sensitive as the Prausnitz-Küstner reaction itself (Stanworth, 1963).

Two of the sera tested gave negative results with the lowest dilution of sera used. The magnitude of histamine release in the present model depends on at least three factors—(1) ability of skin samples to absorb reaginic antibody, (2) concentration of antigen-specific IgE in the serum, and (3) ability of sensitized skin to respond by releasing histamine after challenge by antigen. The use in each experiment of a positive control in which slices of the skin sample are passively sensitized by a reaginic serum of known ability to cause sensitization excludes the third factor but does not entirely exclude the first, since poor sensitization by antigen-specific IgE may be related to the presence in the same serum of high concentrations of non-allergen-specific IgE. Thus either this factor or a low titre of antigen-specific IgE could explain the negative results in these two patients. That these subjects gave strongly positive weal reactions to prick tests with the same antigen does not necessarily indicate the presence of a high titre of circulating specific reaginic antibody (Colldahl, 1952), and it is possible that the present method gives a better indication of clinical hypersensitivity than prick tests. It would therefore be of great interest to compare results achieved with the present in-vitro model with results obtained by provocation tests in the same patients.

There are two further practical applications of this method. Firstly, by using a wider range of dilutions of antiserum than were employed in the present investigation it should be possible to make a rough estimate of antigen-specific IgE titre in the serum. Secondly, the method should also prove of great value in the investigation of penicillin and other drug hypersensitivity reactions. These possibilities are currently under investigation in this laboratory.

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References