

Basophil degranulation tests in atopic allergic states: A pilot study of ragweed pollen sensitive patients

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Of 22 patients with clinically active hay fever and positive skin tests to ragweed pollen extract, 27 per cent gave positive basophil tests with the use of the same extract. One half of these positive test reactors had received hyposensitization therapy. One of 12 nonallergic controls gave a positive basophil test with the same extract. The basophil test, as performed by the methods described, does not lend itself to practical application in the detection of the ragweed-sensitive patient.

A technique that would allow for identification of skin-sensitizing antibodies or reagins by means other than direct or passive transfer skin testing would be highly desirable from many standpoints. The recent precise immunochemical definition of the skin-sensitizing antibodies, associating them with the β_2A globulins,¹ and the development of sophisticated techniques for antigen purification² increase the likelihood that *quantitative* laboratory measurements of these antibodies may be feasible in the near future. For the present, a *qualitative* in vitro measure of immediate-type hypersensitivity states in humans, including those mediated by reagins, has already been applied on a large scale by Shelley.³ The adaptability of this relatively simple test to the detection of allergic reaginic activity in the blood of children with active hay fever is the subject of this report.

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Received for publication April 4, 1964.

Resubmitted Sept. 2, 1964.

MATERIAL AND METHODS

Selection of patients

The 22 children who constituted the patient groups were taken from Allergy Clinic and private practice populations. All subjects had classical pollinosis at the time the blood specimens were drawn. Intradermal skin tests were performed at the time of, or several days prior to, the withdrawal of the blood samples in 20 of the 22 patients. The skin test antigen was a buffered-saline extract of ragweed pollen used first in a concentration of 100 protein nitrogen units per milliliter and, if this test was negative, next in a concentration of 1,000 protein nitrogen units per milliliter.

The subjects used as controls were 7 children and 5 adults whose past and present histories were negative for allergic manifestations of any type and who gave negative skin-test reactions with ragweed pollen antigen in 1,000 protein nitrogen units per milliliter concentration.

Ten of the 22 hay fever sufferers had never received hyposensitization treatment. Of the remaining 12 patients, 3 had received such treatment in the past but not in the 2 years preceding the study. The other 9 had received treatment for varying lengths of time, which continued into the period of study.

Basophil test procedures

Two testing techniques were employed: The liquid-fixation and filtration technique as developed by Shelley,⁴ and the buffy-coat smear technique as described by Klopstock and his associates⁵ in 1962 with minor modifications as reported upon by Shelley in 1963.⁶ Both techniques make use of heparinized blood incubated with the same ragweed pollen extract used in skin testing. In the liquid-fixation and filtration technique, 0.2 ml. of the postincubation blood-antigen mixture, the proportion of blood to antigen being 10 to 1 (0.25 ml. blood to 0.025 ml. antigen), was ejected into chilled liquid fixative, shaken, and allowed to stand at freezer temperature overnight, while red blood cells disintegrated and leukocytes settled out. The sedimented leukocyte suspension was then filtered through a metal microfilter apparatus using a cellulose membrane filter which retained the leukocytes. The membrane filter and cells were stained with toluidine blue dye which was picked up selectively by the metachromatic granules of the basophils.

In the buffy-coat smear technique, approximately 1 drop of the blood-antigen mixture (in this case a 1 to 1 proportion, 0.25 ml. blood to 0.25 ml. antigen) was introduced into polyethylene tubing, and spun at 11,500 r.p.m. for 15 seconds in a microcapillary centrifuge. The buffy coat was clamped, cut off, and blown on a microscope slide, then smeared and stained with toluidine blue in an alcoholic solution. Twenty consecutive basophils were examined in both instances and classified as normal or in process of degranulation. Saline controls were used with each specimen tested. The highest antigen concentration which did not cause toxic degranulation of the basophils in the normal control subjects was used throughout, namely, 20,000 protein nitrogen units per milliliter.

Criteria for a positive test result

A wide variety of criteria for a positive test can be conceived of. The nature of our data do not appear to permit the selection of a criterion on the basis of strict statistical theory, nor is there a clear medical basis for choosing one criterion over another. Shelley has, on different occasions, defined his own criterion for a positive test differently. In this study, a test preparation is considered positive when 25 per cent or more of the 20 basophils counted in a preparation show evidence of degranulation over and above the degranulation noted in the saline control. This corresponds to a degranulation level of approximately two standard deviations beyond the mean of the saline control figures on all subjects tested, which was 6 degranulated cells of the 20 counted. For example, a test specimen received a 1+ positive rating only if at least 11 basophils were classified as abnormal after exposure to antigen. Morphologic classification was made according to a differential basophil count set forth by Shelley in his *Atlas*.⁴ (Figs. 1 and 2.)

Another possible criterion for a positive rating (and one used by Shelley⁷) is a variant on the above, which turns more upon the gap between the number of completely degranulated cells in the test and the control preparations. In this situation, at least 20 per cent of the cells in the test specimen must be abnormal, and the saline control must show a level of degranulation less than one-fourth of that seen with the test antigen. Application of this criterion to our set of data did not increase the percentage of positive results.

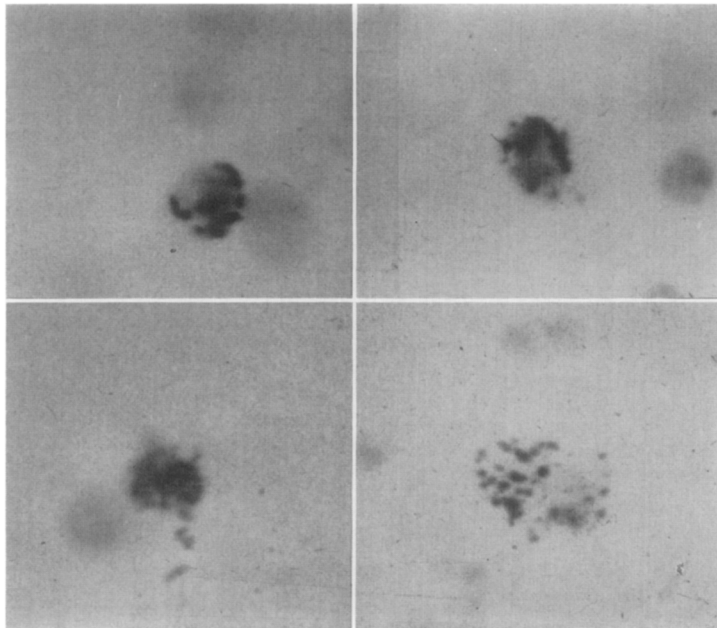
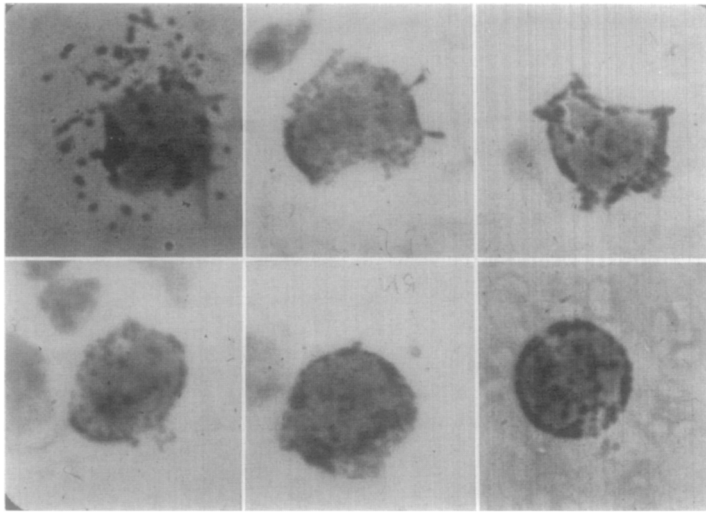


Fig. 1

Basophil degranulation employing the liquid-fixation and filtration technique of Shelley.⁴ Different basophils illustrating morphologic changes ranging from normal to almost complete extrusion of granules. ($\times 3,000$; reduced $\frac{2}{11}$.)

**Fig. 2**

Basophil degranulation employing buffy-coat smear technique of Klopstock.⁵ Different basophils illustrating morphologic changes ranging from normal to almost complete extrusion of granules. ($\times 3,000$; reduced $\frac{1}{3}$.)

All positive slides were read a second time by a different investigator. Correspondence of results obtained by the liquid-fixation filtration technique and the buffy-coat smear technique was good; the former technique providing preparations which could be read more rapidly.

Other techniques and other applications of basophil degranulation

Mention should be made of the fact that a pilot study, carried out prior to the ragweed season on 14 pollen-sensitive patients with positive skin tests, had made use of still a third basophil test technique. This was also devised by Shelley,⁸ and substitutes living rabbit basophils, passively sensitized by the patient's serum, for the patient's actively sensitized cells. This technique was abandoned eventually because of technical difficulties centered about the hemagglutination of rabbit red cells, fragility of rabbit basophils, and the inescapable element of subjectivity in interpretation of subtle morphologic changes in the cells.

Application of the basophil test to a different type of hypersensitivity had also been essayed for purposes of comparison. Mixed stinging insect venom had been used as antigen in testing specimens from 25 patients with immediate urticarial or anaphylactic reactions to the sting of a member of the Hymenoptera group of insects. Intradermal skin tests were also performed on 15 of these 25 subjects.

RESULTS

Applying the previously stated criteria for interpreting a test result as being positive, 27 per cent of 22 patients gave positive basophil tests. One

Table I. *Results of basophil and skin tests in patients manifesting active ragweed pollinosis*

	<i>Positive basophil test</i>		<i>Positive intradermal skin test*</i>	
	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>
Patients	6†/22	27	20/20	100
Nonallergic controls	1/12	8	0/12	0

*100 or 1,000 pnu per milliliter.

†Three had received hyposensitization therapy.

Table II. *Results of basophil and skin tests in patients with history of immediate urticarial or anaphylactic reactions to stings of Hymenoptera*

	<i>Positive basophil test</i>		<i>Positive intradermal skin test</i>	
	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>
Patients	7/25	28	12/15	80

half of these positive reactors had received specific hyposensitization therapy, and the other half had received none. Intradermal skin tests were positive in all 20 patients thus tested (Table I). One of 12 controls, all of whom gave negative ragweed skin tests, had a positive basophil test.

In regard to the 25 insect venom-sensitive patients tested by either the direct or indirect Shelley-test technique, 28 per cent gave positive basophil test results, as contrasted with 80 per cent who reacted positively on skin testing (Table II).

DISCUSSION

If one is prepared to accept the validity of the basophil test based on the reports by others of its performance in nonatopic hypersensitivity states,^{3, 5, 9} then the low percentage of positive test results in our group of patients with a clinically indisputable diagnosis of hay fever, reinforced by positive skin tests to ragweed antigen, raises two questions: Is the antibody involved in the degranulation of the basophil related in any respect to the skin-sensitizing antibody or involved in any fashion in the mediation of atopic allergies? If so, is it possible that the concentration of reagins in the blood of atopic individuals is too low to be detected by this test in most instances, and would the percentage of positive results be improved upon if quantitative adjustments were made in antigen-antibody ratios, or if a more highly-purified ragweed antigen were used?

Addressing ourselves to the first question, we are unable to elicit an answer from our data, but we have noted one interesting report in the literature on the use of this test in an atopic allergic state. This is the paper of Juhlin and Westphal¹⁰ which scrutinizes the *in vivo* basophil response to a provocation-by-ingestion test in an individual with localized atopic dermatitis exacerbated by milk and milk products. The patient was fed cheese on two occasions and

total, as well as differential, basophil counts were performed at 0, 30, 60, 90, and 120 minutes. A decrease in total basophils, and an increase in the percentage of degranulated basophils occurred and these changes were maximal between 10 and 50 minutes after provocation. The skin lesion, however, was not activated until 24 hours after provocation. Judging from the discrepancy in timing between maximum degranulation (and presumably maximum histamine release) and symptomatic flare-up in this case, the antibody responsible for basolysis was not the mediator of the atopic reaction. More studies along these lines are needed before a generalized statement can be made in this regard.

Addressing ourselves to the second question of the titer of skin-sensitizing antibodies and its relation to positive results, only an indirect answer suggests itself from our data: One patient with mild pollinosis had a blood specimen drawn immediately before skin testing with 0.05 ml. of 1,000 PNU per milliliter ragweed pollen antigen. The child developed immediate whealing with pseudopod formation and erythema at the skin-test injection site. Within 10 minutes' time, edema of the subcutaneous tissues beneath the wheal developed along with generalized pruritis, urticaria, angioedema of the eyelids, lips, and tongue, laryngeal edema, and asthma. Only after vigorous therapy with epinephrine, intravenous antihistamines, and theophylline did he become asymptomatic. Such a reaction presumes a high titer of skin-sensitizing antibody. This child, however, gave a negative basophil test.

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