

Histamine release from leukocytes of hypersensitive individuals

I. Use of several antigens

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Histamine release has been obtained from washed leukocytes of allergic individuals after incubation of the cells with a variety of specific antigens. With leukocytes from highly sensitive individuals who also had positive cutaneous reactions, a large proportion of the cellular histamine was released. Binding of labeled ragweed antigen did not appear to be closely related to the other parameters studied. Individual cells may be sensitive to more than one antigen but they may not be equally sensitive to all of the antigens with which they react. Virtually complete lysis of erythrocytes by brief suspension in distilled water does not affect histamine release by the leukocytes.

Histamine is released from leukocytes of allergic individuals after incubation of whole blood with specific antigens.^{1, 2} Refinements of the technique by Lichtenstein and Osler³ allowed them to study several parameters of the reaction including cation requirements, time of incubation, temperature, pH, and some aspects of the mechanism. The present study utilizes the improved procedure to confirm and extend the earlier observations of Noah and Brand² on histamine release by multiple antigens. Various systems of experimental study of atopic sensitivity include skin testing, passive transfer cutaneous testing, direct aerosol antigenic challenge, in vitro measurement of antibodies in atopic subjects, and spontaneously, passively, and actively induced hypersensitivity in

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Supported in part by United States Public Health Service Research Grant No. A106139-03 from the National Institute of Allergy and Infectious Diseases, and by the Ernest S. Bazley Asthmatic Research Fund, Chicago Wesley Memorial Hospital.

Received for publication April 6, 1966.

*Career Development Investigator Awardee, United States Public Health Service Research Grant No. 5-K3-19, 460-02.

animals. Histamine release from sensitive leukocytes has the advantage of being an *in vitro* specific, reproducible response of cells to an antigen which can be determined by quantitative techniques. This system has the same specificity but may lack the sensitivity of cutaneous testing, and it is an atopic antigen-antibody reaction which is probably initiated on the surface of a cell.

MATERIALS AND METHODS

Antigens

Purified ragweed antigen, King fraction IV-C⁴, was dissolved in 0.15M saline and stored in aliquots at -20° C. Egg albumin, two times crystallized (Pentex), was dissolved in 0.15M saline. A commercially prepared extract of house dust (Endo Laboratories, Inc.) was used, while other aeroallergens were obtained in the dry state (Hollister-Stier Laboratories) and extracted in buffered saline containing 0.5 per cent phenol and 50 per cent glycerine.⁵ The stock 2 to 5 per cent extracts were diluted in buffered saline just before use.

Histamine release from human leukocytes

The procedure was adapted from that of Lichtenstein and Osler.³ One volume of blood was drawn from allergic individuals who had histories of allergic symptoms following exposure to various allergens. It was mixed with one-half volume ethylenediaminetetraacetic acid (1.5 per cent in 0.7 per cent saline). One and one-half volume of 6 per cent clinical dextran in saline solution was added, and the red cells were allowed to settle for 60 minutes. The upper layer was removed and centrifuged at 110 g. The packed cells obtained were washed twice with a Tris-buffered salt solution³ and resuspended in aliquots of 5 ml. each, with the addition of Ca^{++} and Mg^{++} to final concentrations of $6 \times 10^{-4}\text{M}$ and 10^{-3}M , respectively. Various antigens were then added to separate aliquots of cells. Ragweed antigen was used in serial tenfold amounts from 10^{-1} to 10^{-4} μg and egg albumin in serial hundredfold amounts of 10^{-1} to 10^{-7} μg . Extracts of other antigens were added at a final single concentration of 0.0004 per cent, but in some experiments further serial tenfold dilutions of antigen were used. After incubation at 37° C. for 45 minutes, the cells and supernatant fluid were separated. The quantity of histamine in both fractions was determined, and the percentage of the total cell histamine that was present in the cell supernatant was recorded. Histamine was determined by a modification of the method of Shore, Burkhalter, and Cohn.⁶ Five milliliters of a 0.4N perchloric acid solution of cell or supernatant histamine saturated with sodium chloride was mixed with 0.5 ml. of 7.4N sodium hydroxide, and the mixture was extracted with 5 ml. n-butanol. The butanol layer to which 2.5 ml. n-heptane was added was extracted with 2.3 ml. of 0.1N hydrochloric acid. Two milliliters of the aqueous phase was made alkaline by the addition of 0.1 ml. of 4N sodium hydroxide; 0.1 per cent o-phthalaldehyde, 0.1 ml., was added and, after exactly 4 minutes, 0.2 ml. of 3N hydrochloric acid was used to stop the reaction. The fluorescence was measured in a Turner photofluorometer. The histamine content of the samples was recorded as the free base, although calibration was made with the dihydrochloride.

¹²⁵I ragweed binding

Aliquots of 0.05 ml. of human serum or of 0.25 ml. of the fivefold diluted plasma, obtained after removal of the leukocytes used for histamine release, were incubated with 1.25×10^{-4} μ g of ¹²⁵I ragweed antigen N, and the bound antigen coprecipitated by the addition of rabbit antihuman Cohn Fraction II. The percentage of labeled antigen bound by each serum was determined. The procedure and the calculations used have been described.⁷ The sera of nonsensitive controls bound less than 10 per cent of the antigen added. A single point may be used to estimate relative antibody content if the isotope coprecipitation curves for different sera are parallel in the region of antigen excess and if the values are between the maximum and minimum binding levels of 78 and 10 per cent. Previous experiments with 42 sera showed that the binding curves were essentially parallel.

Cutaneous tests

Cutaneous tests were performed by standard "prick" type techniques.⁸ Positive reactions were rated from 1 plus to 4 plus, and negative reactions were designated by a dash.

RESULTS

The clinical histories of the 12 individuals studied are presented in abridged form in Table I. Table II shows the maximum percentage of histamine released by a variety of antigens from the leukocytes of the 12 subjects. In parentheses are the results of cutaneous testing with the same antigen. Also recorded is the per cent of ¹²⁵I ragweed bound by 0.05 ml. undiluted serum with the use of 1.25

Table I. *Clinical history of allergic subjects studied*

Sub- ject	Age	Sex	Duration of symptoms (years)	Type of symptoms	Season of symptoms*	Clinical impression of etiologic factor†
Ja	24	M	5	Rhinitis	Perennial	Ragweed, grass, mold, house dust
Bo	23	M	10	Rhinitis, Con- junctivitis	June to October	Grass, ragweed
Me	23	M	17	Rhinitis, asthma	June	Grass, egg
Fe	28	M	22	Rhinitis	August	Ragweed
Se	25	F	5	Rhinitis, asthma	Perennial	Cat and dog dander, ragweed
Ho	24	M	7	Rhinitis	Perennial	Ragweed, mold, grass, tree
La	24	F	4	Rhinitis	June	Grass
Ar	23	M	7	Rhinitis	Perennial	Grass, mold, ragweed
Sb	25	M	8	Rhinitis, Con- junctivitis	June, August	Grass ragweed
Fa	24	M	20	Rhinitis, Con- junctivitis	April to June	Tree, grass
Sn	22	M	16	Asthma	April to September	Tree, grass, mold, ragweed
Ni	23	M	18	Rhinitis	June to September	Grass, mold, ragweed

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†By history and correlated skin tests.

Table II. *Comparison of histamine release, cutaneous reactions, and serum antibody in allergic subjects*

Sub- ject	Maximum histamine released (%)							Ragweed bound* (%)
	Ragweed	Molds	Timothy	Trees	Cat	Dust	Egg albumin	
Ja	10 (2+)†	43 (4+)	18 (4+)			11 (2+)		57
Bo	45 (4+)	28 (3+)	8 (—)					16
Mc	47 (4+)		67 (4+)			24	56 (4+)	34.5
Fe	63 (4+)	8 (3+)	11 (3+)			10 (2+)		18.5
Sc	40 (4+)		72 (4+)		77 (4+)	5 (3+)		10
Ho	85 (4+)	82 (3+)	92 (4+)	54 (3+)				47.5
La	52 (3+)		81 (4+)	3 (4+)		37 (—)		44.5
Ar	35 (4+)	(3+)	68 (3+)					51.5
Sh			79 (4+)	46 (4+)				
Fa	68 (4+)	12 (3+)	76 (4+)	66 (4+)				43
Sn	36 (3+)	67 (4+)			51 (4+)	35 (3+)		53
Ni	60 (4+)	63 (4+)	24 (3+)					38

*Per cent I¹²⁵ ragweed (1.25×10^{-4} μ g N) coprecipitated with 0.05 ml. undiluted serum or plasma.

†Maximum histamine released with scratch test given in parentheses.

$\times 10^{-4}$ μ g antigen N. In most instances where significant release of histamine was demonstrated, cutaneous tests were strongly positive. In 7 cases, a 3 or 4 plus skin reaction was obtained with antigens other than ragweed in individuals whose leukocytes did not release histamine when incubated with the specific antigen. In only one instance was a significant proportion of histamine released from the cells of an individual who had a negative cutaneous test. Twice re-crystallized egg albumin effected the release of histamine from leukocytes of an egg-sensitive individual. Not tabulated is the release of 54 per cent of the histamine from white cells of a tetanus toxoid-sensitive individual using fluid tetanus toxoid, and of 75 per cent of the histamine from cells of a horse serum-sensitive patient by horse serum albumin. The histamine content of leukocytes from 6 nonallergic individuals averaged 0.12 μ g per 5 ml. blood sample, and less than 0.01 μ g or 10 per cent was released in the presence of ragweed antigen. This level of histamine in supernatant solutions of control cells is considered to be a nonspecific release due to mechanical trauma during handling of cells.

The total quantity of histamine base found in leukocytes from 5 ml. blood of allergic patients was usually around 0.1 μ g, but in some instances it approached 0.5 μ g. Assay at different times indicated that the value remained relatively constant in untreated individuals. The percentage of the total histamine released in the presence of varying amounts of antigen was also similar in samples taken at different times.

Since the cells obtained in this study contained 3 to 5 times as many erythrocytes as leukocytes, a leukocyte suspension free of red cells was prepared. Washed cells from 5 ml. of blood were suspended for 25 seconds in 6 ml. distilled water and restored to isotonicity by the addition of 2 ml. of 3.4 per cent saline. This process was repeated as necessary to remove the last grossly visible traces of red cells. Washed leukocytes obtained after red cell lysis were as sensitive to antigen as untreated cells. The small reduction in total histamine observed was probably due to hypotonic destruction of a few leukocytes.

Snake (*Naja naja*) venom incubated with human leukocytes caused histamine to be released into the medium in confirmation of the report of Finke, Philips, and Middleton.⁹ In white cell preparations containing erythrocytes, hemoglobin was also released by the venom. In histamine release by antigen, however, extracellular hemoglobin has never been observed with the use of mixtures of leukocytes and erythrocytes.

DISCUSSION

The finding of sensitivity to several antigens of leukocytes from a single patient is consistent with the multiple clinical sensitivities and the multiplicity of positive skin tests seen in some allergic individuals. With separate aliquots of cells, a large proportion of the total cellular histamine was released by each of the antigens incubated with leukocytes from one individual (Ho). This is possible only if some cells were sensitized to more than one antigen and if they were capable of releasing their histamine upon incubation with any of the antigens. With the leukocytes of most subjects (Table II), few antigens effected the release of the same maximal percentage of the total histamine. This means that although cells may be sensitive to more than one antigen as shown above, they are not equally sensitive to all of the antigens tested. At least two mechanisms may account for differential sensitivity of white cells. All cells may be sensitive to all of the specific antigens used, but the degree of sensitivity to different antigens may vary. Individual cells would release histamine in proportion to their sensitivity. On the other hand, a heterogeneous population of sensitive cells might be present. Some leukocytes might be sensitive to certain antigens and not to others. Sensitive cells would release all of their histamine in the presence of antigen and nonsensitive cells, none. In either case, the differential sensitivity of leukocytes is probably a reflection of the differences in concentration of individual antibodies in the blood of the allergic patient.

The variations in I¹²⁵ ragweed binding of the plasmas do not closely parallel the variations in histamine release. This lack of correspondence may result from the presence of heterogeneous populations of antibodies capable of binding antigen but not all capable of sensitizing cells.

Distilled water lysis of red cells under the conditions used resulted in minimal effect on the leukocytes and did not alter the reactivity of the white cells in histamine release. The lysis of both erythrocytes and leukocytes by *Naja naja* venom, presumably through enzymatic action, indicates a lack of specificity of the venom in attacking cell membranes. Allergen-induced histamine release, on the other hand, is specific since red cells are not lysed, and all leukocytes do not invariably release their histamine. They must be specifically sensitized and in contact with sufficient quantities of specific antigen. Leukocytes from non-allergic patients do not release histamine in the presence of allergens but do so in the presence of venom. The extracellular action of venom does not appear, therefore, to be a good model of a possible enzymatic mechanism in histamine release, since the latter process is specifically activated by antigen and, furthermore, it is intracellular.

The diagnosis of etiologic factors in inhalant respiratory allergic disease

is largely dependent on the clinical history, with the use of skin tests for confirmation. The present study indicates that a strongly positive cutaneous reaction was usually found in allergic individuals whose leukocytes released a large proportion of their histamine. Histamine release might, therefore, appear to be useful as a confirmatory diagnostic test. However, histamine release may be less sensitive than the skin test. A low release was found in seven instances where highly positive cutaneous tests were obtained, while in only one instance was a significant release observed with cells from a patient with a negative cutaneous reaction. This and the relative ease of performance of the cutaneous test make it impractical to determine histamine release routinely. It may, however, be an indicator of changes occurring during prolonged and effective hypersensitization.¹⁰

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