

Histamine-Dependent Allergy Blood Test

Clinical Evaluation

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Allergen-mediated histamine release from human leukocytes represents an important model for *in vitro* studies of allergic reactions. The purpose of this study was to determine whether the measurement of histamine released in allergic patients by radioenzymatic assay following mixing of their blood with common allergens represents a reliable index for diagnosis of atopic allergy. Three categories of allergens were used: 1) house dust and mite; 2) cat and dog dander; 3) trees, grasses and ragweed mixture. The presence of allergy was established by clinical history and intradermal skin testing in the study group of 150 patients. A significant allergen-mediated histamine release ranging from 4 to 65% of the total blood histamine content was observed in 96% of the patients with skin test sensitivity of $\geq 3+$. There was a significant correlation between skin testing and histamine release in terms of the allergens causing the response. Thus, the measurement of histamine by radioenzymatic technique following its release in blood in response to allergen challenge represents a clinically useful *in vitro* test for the diagnosis of atopic disease.

Key words: allergy; asthma; histamine; radioenzymatic; rhinitis.

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CLINICAL ASPECTS

A histamine-dependent allergy blood test is developed. The test was used to measure the released histamine of 150 patients with allergic disorders following the incubation of their blood with common allergens in three major categories. An excellent correlation was found between histamine released from whole blood and the response of skin mast cells to intradermal antigen administration. The availability of this test should be useful to various physicians to assist them in the diagnosis and management of their patients with allergic diseases.

Allergy is a major public health concern as large numbers of the American public, approximately 35 million, suffer from all sorts of allergic disorders. The cost involved in caring for the allergic sufferer is close to one billion dollars in the United States alone. More than two-thirds of the schooltime lost is a direct consequence of upper respiratory tract insults, including asthma and other respiratory tract allergies (7). These prominent concerns accentuated the need for specific and clinically reliable biochemical tests for the appropriate recognition and prediction of the severity of atopic disease.

Skin tests and provocative tests have been widely used for many years in providing useful confirmatory evidence for a diagnosis of specific allergy which has been made on clinical grounds (3, 8, 11). Recent reports by Imber (5), Reddy et al. (14) and Pascual et al. (13) challenged the reliability of various skin test techniques. The reasons for this are numerous and include the following: a) it is expensive, b) it requires a great deal of time on the part of the patient, c) there is a risk of anaphylaxis (it cannot be used to evaluate potent antigens that might produce anaphylaxis with even the minor exposure of skin testing), d)

dermographism and widespread skin disease interfere with testing, and e) it is a qualitative biological testing, as the interpretation of results depends entirely on the experience and the dexterity of the skin tester himself. These observations have stimulated others to seek far less invasive, safe and convenient methods for the identification of an inciting factor in an allergic patient.

For the most part, allergic reactions are a physiological response to the release of histamine (6). In 1966, Lichtenstein et al. (10) carefully assessed in patients with ragweed-sensitive allergic rhinitis, the release of histamine from isolated blood leukocytes when challenged with allergen. The test was reproducible and correlated with clinical symptoms. Even though this method is representative of a cellular IgE-mediated reaction comparable to the wheal and flare reactions following the release of the histamine from skin mast cells, it has not been widely accepted in clinical practice mainly because of the requirements of large blood volumes and inheritable complexities associated with the measurement of histamine.

In view of the importance of histamine in allergic disorders, we took the initiative to develop a radioenzymatic assay for histamine (4). The assay was used to study the released histamine of a limited number of atopic subjects following the incubation of their blood with a group of common allergens. A significant correlation was found between histamine released from whole blood, clinical history, and the response of the skin mast cells to intradermal antigen administration. Stimulated by this preliminary observation, we modified this assay in order to develop an *in vitro* histamine-dependent allergy test suitable for clinical routine application. This test was evaluated by the measurement of histamine released from whole blood of a diversified population of allergic patients as a result of the incubation of their blood with a mixture of common extrinsic allergens in three major categories.

MATERIAL AND METHODS

Patient population

One hundred and fifty patients ranging in age from 9 to 60 years admitted to an outpatient

allergy clinic were evaluated by history and intradermal skin tests. These patients were divided into four major categories: Group 1 consisted of 78 subjects with rhinitis, Group 2 consisted of 43 patients with asthma, Group 3 consisted of 27 patients with upper respiratory tract infection, and Group 4 was made up of 30 patients with combined symptoms of asthma and rhinitis.

Skin Testing:

The skin tests were performed with the same antigens used for *in vitro* histamine release. The concentrations used for skin testing were 1,000 PNU/ml for house dust, dog dander, and tree pollen, and 100 PNU/ml for house dust mite, cat dander, ragweed, and grass pollen. For skin tests, 0.02 ml of allergen was injected at each site on the upper arm with a disposable syringe and reactions read at 15 min. Skin-test grading was based on measurements of the greatest diameter of the wheal following the usual criteria: negative, (site no different from diluent control), 3+ (8–12 mm of wheal) and 4+ (> 12 mm wheal).

Histamine release from whole blood:

A schematic representation of the assay is shown in Fig. 1. Histamine release reactions were carried out in 12 by 75 mm plastic tubes. To each of the tubes were added 0.5 ml of Tris-albumin buffer, allergens (in PNU/ml: category I; house dust/house dust mite, 200:5; category II; cat/dog dander, 60:1000; and category III; trees/grasses/ragweed, 150:150:100) and 0.5 ml of heparinized blood. Control tubes were prepared by mixing buffer with blood alone. The tubes were incubated in a shaking water bath for 15 min at 37°C. This was followed by centrifugation at $500 \times g$ for 10 min. The plasma was separated and stored at -70°C until analysis. Samples for determination of total blood histamine were achieved by boiling 0.5 ml of whole blood with 1.5 ml of 0.05 mol/l phosphate buffer (pH 7.9) in a heating boiling water bath for 20 min. After centrifugation at $15,000 \times g$ for

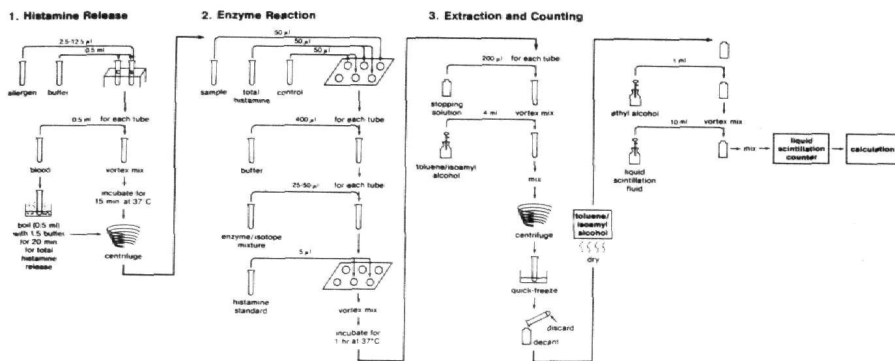


Fig. 1. Scheme of assay profile of histamine-dependent allergy blood test. C represents an allergen-free tube as a control and S denotes an allergen-containing tube.

30 min, the supernatant was removed and stored at -70°C until analysis.

Plasma histamine assay

The concentration of histamine released from blood was measured by radioenzymatic technique according to Faraj et al. (4). The method is based on the use of purified enzyme histamine-N-methyltransferase (HNMT) isolated from rat kidney, to transfer a radioactive methyl group from the cofactor [3H-methyl]-S-adenosyl-L-methionine (SAM) to an endogenous histamine acceptor molecule in the sample to form the radioactive metabolite [3H-methyl]-N-histamine. This was followed by selective extraction of the liberated metabolite and its subsequent quantification by liquid scintillation counting (Fig. 1). Lower level of sensitivity is 65 picograms of histamine per sample; intra- and interassay coefficients of variation were 5% and 7%, respectively.

Percentage of histamine release

The percentage of histamine released from 1 ml of blood by the allergen mixture in each of the three categories was determined from the following formula: $100 \times A/B = \% \text{ histamine release}$, where A = concentration of histamine in plasma following the incubation of 1 ml of

whole blood with allergens, minus concentration of histamine in plasma following incubation of 1 ml of blood alone; B = concentration of total histamine obtained from 1 ml of boiled blood minus blank sample.

RESULTS

Clinical observation

Of the 78 patients with rhinitis only 28 exhibited 4+ ($n = 10$) and 3+ ($n = 18$) skin test reactivity as compared to 50 patients with vasomotor rhinitis having negative skin test reaction when evaluated against a series of common allergens. In contrast, 30 of the asthmatic patients had extrinsic asthma since they demonstrated positive skin test reactivity against a variety of common inhalants (+4, $n = 10$; +3, $n = 20$) as compared to only 13 with negative skin test reactions (i.e. intrinsic asthma). A similar pattern was observed among those patients with recurrent upper respiratory infection ($n = 27$) and the asthma-rhinitis group ($n = 30$).

Histamine blood release

Allergen-mediated histamine release (in % of total blood histamine) from whole blood of the 150 patients is shown in Fig. 2. The results of

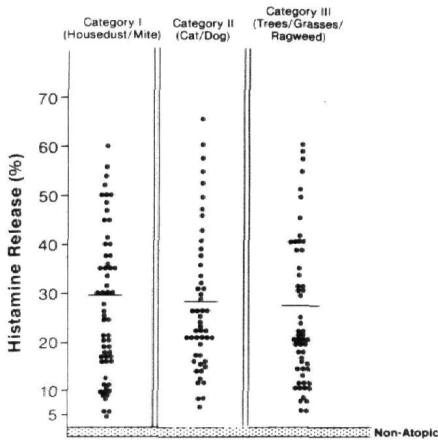


Fig. 2. Allergen-mediated histamine release (in % of total blood histamine) from whole blood of 150 patients with different allergic disorders following the incubation of their blood with common allergens in three major categories.

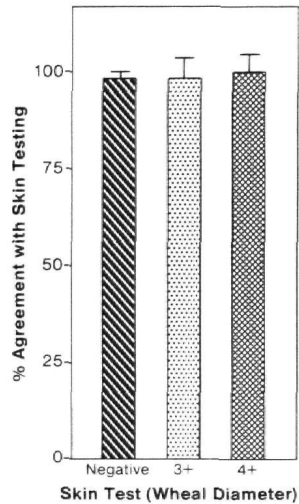


Fig. 3. Percent agreement between histamine blood release and skin test results.

this study showed 90–100% agreement between histamine blood release and skin testing (Fig. 3). Further, a significant correlation was noted between *in vitro* histamine release from whole blood and response of skin tests in these patients (Fig. 4). This was demonstrated by an average of 12–17% of histamine blood release following antigenic challenge with allergen mixture in each of three major categories when the skin test was 3+, compared with an average histamine blood release of 27–30% in patients with skin test results of +4 or greater. Non-allergic blood from donors with negative skin test released no histamine following incubation with the above allergens (Fig. 4).

DISCUSSION

In an earlier report (4) we demonstrated the development of a simple and sensitive radio-enzymatic assay for the direct measurement of histamine in plasma. This assay was used to formulate a histamine allergy blood test amenable to clinical routine use. We measured the amount of histamine released from whole blood of atopic patients after *in vitro* provocations with

suspected allergens in three major categories. We found that this method can be routinely applied and easily performed: a) The sensitivity of the system allows *in vitro* quantitative testing with allergens in three major categories with only 2.5 ml of blood; with 5 ml of blood the test can be expanded to include an additional five other categories (i.e., penicillin, insect venom, mold mixture, albumin and alternaria). b) Freshly prepared assay reagents including enzyme (histamine-N-methyltransferase), radio-labeled cofactor ($^3\text{H-SAM}$), buffers, and stopping solution are stable for at least 6 months if stored appropriately (4° to -70°C). c) The storing of a blood sample for 48 h at 4°C had no effect on the allergen-induced release of histamine from whole blood. This is a clear-cut advantage to both patient and physician. d) Many samples can be assayed per day and reporting of the results can be achieved within 24 h of receiving the sample. e) The test is inexpensive and easy to perform.

In agreement with previous observations (9, 12, 15, 16), an excellent correlation was noted between histamine released from whole blood of patients with allergic asthma, rhinitis and upper

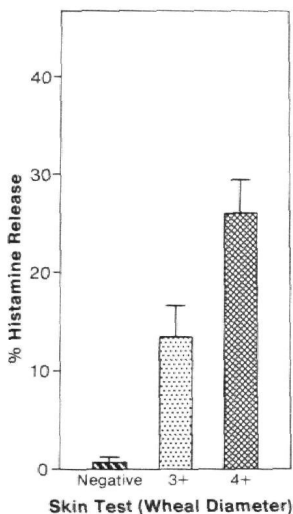


Fig. 4. Relationship between allergen-mediated histamine release and skin test results in patients with allergic disorders.

respiratory infection and the response of the skin mast cells to intradermal antigen administration. A significant amount of histamine was released, ranging from 25-30% of the total histamine content following incubation of the blood with allergens in any of the three categories. This effect was observed in 83-100% of the patients with skin test sensitivity of 4+ or greater to grasses, trees, house dust, ragweed and animal dander. There was good agreement between the intensity of the skin reaction and magnitude of histamine release, since patients with skin test sensitivity of 3+ had a histamine blood release upon antigen challenge *in vitro* of only 12 to 17%. This *in vitro* histamine-dependent allergy test is highly specific, since patients with vasomotor rhinitis and those with intrinsic asthma who were clinically non-atopic did not release histamine from blood.

The histamine-release results from this study compare favorably with published reports of the use of RAST technique in allergy diagnosis (1, 2). Several reports have shown that the RAST has an overall accuracy of about 70% when correlated with a patient's clinical history (12).

The present results showed that the histamine release was positive in over 90% of the patients who were allergic by clinical evaluation. Further, histamine release from whole blood probably is a biologically more sensitive index of a patient's sensitivity than RAST test because it is a cell-attached IgE that reacts with allergen, resulting in the release of allergic mediator histamine. The basophil histamine release test seems to be superior to RAST because no allergen coupling procedure is needed, and the same allergens can be used as for skin testing.

In summary, the data presented here outline the availability of a safe, quantitative, reliable and easy to perform histamine-dependent allergy blood test. This test should be useful to physicians with a wide range of specialties, including allergists, pediatricians, internists, anesthesiologists, ophthalmologists, and otolaryngologists, to assist them in the management of their patients with allergic symptoms. When acute symptoms are due to extrinsic agents, the histamine allergy blood test can be utilized to identify the offending allergens. The management of the patient could include allergen avoidance and pharmacological blockade of effector mechanisms.

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