

^3H -Histamine Release from Human Leukocytes

A New Method for Detecting Type I Allergy
Compared with Basophil Histamine Release Technique

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A rapid, simple, and inexpensive method for large scale screening of patients suspected of type I allergy has been developed. The method is based on *in vitro* incorporation of ^3H -histamine in the leukocytes of the patient, whereafter release of labelled histamine is measured after provocation of the cells with the suspected allergen. The new method was compared with the conventional basophil histamine release technique by *in vitro* provocation of six asthmatic patients under suspicion of type I allergy against animal dander, house dust, and mite, and an almost identical release of histamine was observed in both assays.

Key words: allergen; histamine release; *in vitro*; leukocytes; simple method.

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One of the problems in the treatment of bronchial asthma and other atopic diseases is the lack of investigation techniques sufficiently specific and sensitive and not implying any risk for the patient. Furthermore, there is a demand for a rapid, simple, and inexpensive investigation technique which would ensure a reduction of the number of patients waiting for a sufficient treatment.

The basophil histamine release technique can be used as a sensitive method for detecting type I allergy (1, 2). Investigations have proved this method to be superior in the detection of the responsible allergen in patients with extrinsic asthma, since the histamine release is well correlated to the bronchial provocation response, and the method shows only a few falsely positive and negative responses (3).

In the present study the method has been further developed to a rapid, simple, and in-

expensive investigation technique based on radioactive assay of ^3H -histamine release from leukocytes, and this method is compared with the classical basophil histamine release technique.

MATERIAL AND METHODS

The study comprised six asthmatic patients with a well-documented history of symptoms from known allergens. After provocation *in vitro* with the allergens, histamine release was estimated by the new method as well as by the classical basophil histamine release technique, which was based on the spectrofluorometric assay of histamine (4) omitting the extraction procedure according to Bergendorff & Uvnäs (5). By venipuncture a 15 ml blood sample was collected in a plastic tube containing 1 ml 0.20 M EDTA (pH 7.0). The sample was preserved for storage by adding 15 ml cell culture medium

(RPMJ media series 1640, Gibco Bio-Cult Ltd., Scotland) including L-glutamine 1.2 mM, penicillin G 4000 IE, and streptomycin 4 mg base. ^3H -histamine was incorporated in 1 ml of the blood-medium mixture by addition of 10 μCi freeze-dried (2,5- ^3H) histamine, 2 HCl (Amersham), whereafter the sample was left overnight at 37°C. The residual, 29 ml blood-medium mixture, intended for spectrofluorometric histamine assay, was also left overnight at 37°C. Enriched basophils consisting of monocytes with approximately 2% basophils were obtained from both samples by the Ficoll-Hypaque gradient method (1). The cell layer was transferred to a plastic tube and after washing the cells were finally resuspended in 8.5 ml Tris-AMC (1). For both assays 200 μl cell suspensions were incubated in plastic tubes at 37°C for 40 min with 10 μl allergen extract at different concentrations. Allergen was omitted for spontaneous histamine release and for determination of the total histamine content; in the latter case the samples were left at 4°C. Thereafter 3 ml ice-cold Tris-AMC was added to the samples and they were centrifuged at 2000 g for 10 min. In one assay the supernatant was discarded and the cell sediment was mixed with 100 μl water and thereafter with 1.2 ml Instagel, and the residual ^3H -histamine was determined in a Packard Prias PLD Liquid Scintillation Counter. In the other assay the supernatant was discarded and 200 μl of an OPT mixture (2 mg O-phthaldialdehyde, Fluka, dissolved in 1 ml methanol and 9 ml 0.05 N NaOH) was added to the cell sediment and the sample was left at room temperature for 4 min. 200 μl 0.175 N H_3PO_4 was then added and the sample was centrifuged at 3000 g for 10 min. The histamine content in the supernatant was determined spectrofluorometrically in an Aminco-Fluoro-Colorimeter provided with a micro flow cell. Samples were read at 355/455 nm (excitation/emission). In both assays the release of histamine was expressed as a percentage of the total histamine content of the sample. Correc-

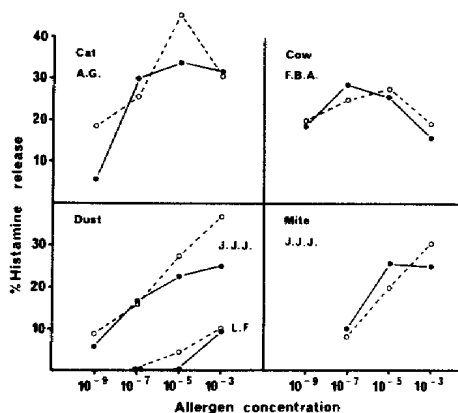


Fig. 1. Release of histamine from leukocytes of asthmatic patients on *in vitro* challenge with suspected allergens. The allergen concentration is given as the final w/v extract concentration. Radioactive assay ●—● and conventional basophil histamine release technique ○—○.

tion was made for the spontaneous histamine release amounting to 1–5%.

RESULTS AND DISCUSSION

A simple radioactive assay has been developed to detect type I allergy and to obtain precise information about the causative agents. The method was based on *in vitro* incorporation of ^3H -histamine in the leukocytes of the patient, whereafter the release of labelled histamine was measured as residual histamine after provocation of the cells with the suspected allergen. The method was compared with the classical basophil histamine release technique (1, 2) in six asthmatic patients with a well-documented history of symptoms from animal dander, house dust, and mite. In total 17 antigen provocations were investigated, and an almost similar histamine release was obtained in both assays. This is illustrated in Fig. 1, which shows the results from four patients.

Due to the almost identical histamine

release in the two assays it is reasonable to assume that the greater part of the labelled histamine is incorporated in the basophil leukocytes, from where it is released upon provocation with specific antigen.

With the radioactive assay a very sensitive method for detecting type I allergy has been achieved, since 40 samples could be obtained from 0.5 ml blood. For comparison, the classical basophil histamine release technique requires 14.5 ml blood. Furthermore, the method is simple, rapid, and inexpensive. Thus, the radioactive assay seems to fulfil the demand for a rapid, simple, and inexpensive method for large-scale screening of patients suspected of type I allergy.

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