ALLERGOLOGY

Mite Allergen and Allergoid Stimulation of Histamine Secretion by Mast Cells

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Immunoglobulin E is an immunological trigger responsible for mast cell degranulation. The F_c-fragment of the IgE molecule tightly binds to high-affinity receptors of the mast cell surface. Exposure of an IgE-sensitized cell to polyvalent molecules of an allergen results in the formation of antigenic "bridges" connecting IgE molecules and the respective membranous F_c-receptors. This reaction leads to changes in membrane conformation and to the release of histamine and other transmitters from mast cells [10].

House dust mites are known to cause allergic disorders, *Dermatophagoides* mites being mainly responsible for sensitization [14]. Previously we revealed that an allergen obtained from dust mites *Dermatophagoides farinae* changed the intracellular pH in passively sensitized rat peritoneal mast cells, leading to activation of Na/H metabolism [4,8]. On the other hand, allergoid, a modified form of this allergen, virtually did not activate Na/H metabolism.

The aim of the present study was to investigate mast cell histamine secretion stimulated by an allergen capable of stimulating Na/H metabolism and to assess the activities of allergen and allergoid obtained from *D. farinae*. For this purpose we measured histamine secreted *in vitro* by sensitized rat peritoneal mast cells. An advantage of such an approach is the possibility of *in vitro* testing of a number of allergen lots and their standardization.

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MATERIALS AND METHODS

Allergens prepared from a *D. farinae* culture were used [2]. Allergoids were prepared from mite allergens by formaldehyde modification [11]. Specific activities of freeze-dried agents (allergens and allergoids) were tested by microdot enzyme immunoassay. The sera were selected according to the results of skin tests in patients sensitized to *D. farinae* (the +++ reaction).

Mast cells were obtained from rat peritoneal cavity. The resultant pool contained 5% mast cells, which were identified by toluidine blue staining. The cell suspension was centrifuged, and the pellet was resuspended in a balanced solution (10 mM Na-HEPES, pH 7.4, containing 145 mM NaCl, 2.7 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, and 0.05% serum albumin). A sample containing 100 μl mast cells (5×10^5 cells) was incubated for 5, 30, 60, and 120 min at 37°C with 10, 25, and 50% human serum with a high specific IgE titer. After serum activation of the cells, the suspension was incubated with 10 μl ligand for 10 min at 37°C. Mite allergen (0.1-50 μg/ml) and allergoid (10-100 μg/ml) were used as

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ligand. Mast cell secreting capacity was controlled with ConA (30 μg/min). Cells spontaneously secreting histamine were controls. The reaction was arrested with a three-fold volume of cold (4°C) buffer. After centrifugation the proteins were sedimented with 10% trichloroacetic acid solution and histamine was measured in the supernatant [15]. The sediment was resuspended in buffer and boiled in a water bath for 3 min. After centrifugation, histamine was measured in it. The percentage of secreted histamine was calculated according to the formula:

$$\frac{\text{Absolute volume of released histamine}}{\text{Total histamine content in sample}} \times 100\%$$

RESULTS

Since assessment of the *in vitro* activity of an allergen preparation by measurement of histamine release by mast cells was the purpose of our research, we used a heterogeneous system as a model: rat peritoneal mast cells passively sensitized by human serum with a high titer of specific IgE. The use of such a system was possible due to 60-90% homology detected during a comparison of human and rat IgE molecule F_c-fragments [1].

Hence, our first task was to define the optimal conditions for peritoneal mast cell sensitization.

The degree of mast cell sensitization is in proportion to the amount of IgE fixed on it. To induce histamine release from cells on which just a few IgE molecules are fixed, high doses of specific antigen should be used, and vise versa [7]. In our experiments the degree of mast cell sensitization depended on the serum dilution. We used serum in dilutions 1:10, 1:4, and 1:2. A 50% serum (diluted 1:2) exerted a proteolytic effect on the cell, inducing 27% histamine secretion. Serum diluted 1:4 resulted in 15% histamine release, which exceeds the spontaneous histamine secretion by approximately 5%. Such a negligible increase of secretion may also be due to the nonspecific effect of serum proteases on the cell. Cell sensitization with 10% serum resulted in the release of approximately half the amount of histamine in response to allergen (10 μg/ml) in comparison with the release after cell sensitization with 25% serum (Fig. 1).

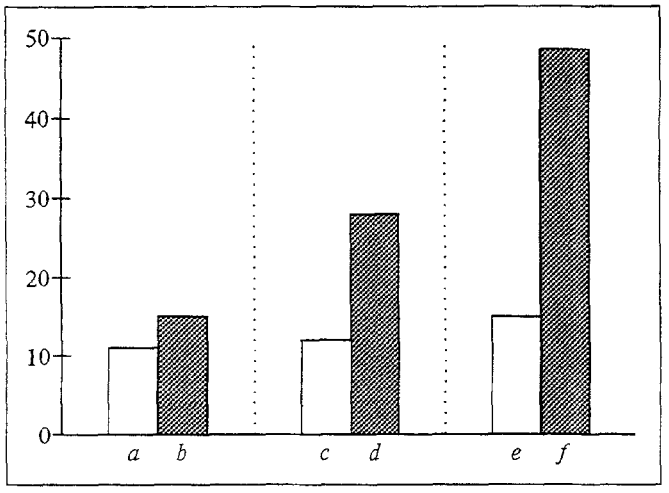

Fig. 1. Mite allergen (10 μg/ml) stimulation of histamine secretion by mast cells passively sensitized (for 120 min) with 10 and 25% sera. Percent histamine secretion: *a*) spontaneous; *b*) for allergen effect on intact mast cells; *c*) for 10% serum sensitization; *d*) for allergen effect on mast cells sensitized with 10% serum; *e*) for 25% serum sensitization; *f*) for allergen effect on mast cells sensitized with 25% serum; hatched bars show allergen addition.

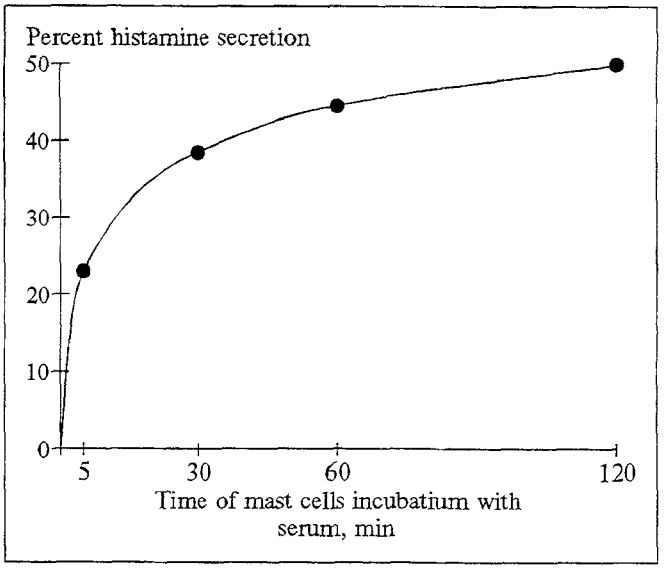

Fig. 2. Effect of prolonging cell incubation with 25% serum on histamine secretion induced by mite allergen (10 μg/ml).

IgE binding on the cell is a time-dependent process. The binding kinetics was assessed from histamine secretion by mast cells activated with mite allergen in a concentration of 10 μg/ml. Figure 2 shows that prolonging mast cell incubation with serum from 5 to 120 min increased the share of histamine release by mast cells more than two-fold (25 and 52%). These results indirectly indicate a sufficiently high intensity of IgE binding by cells during the first hour of incubation with the serum and a lower rate of this process during the following hour. The optimal sensitization is attained after approximately 120 min of incubation of cells with 25% serum, this correlating with the findings of Japanese scientists [6]. For this reason, mast cells spontaneously secreting histamine for 120 min served as controls in our further experiments. ConA, a lectin of plant origin which induces histamine secretion, was used in a concentration of

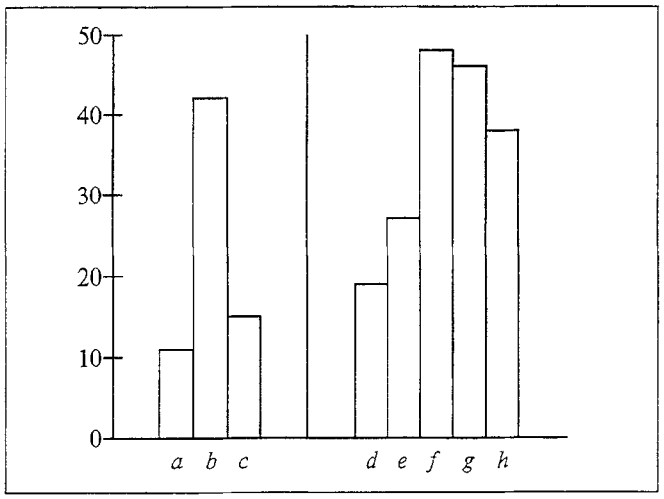

Fig. 3. Histamine release from mast cells stimulated by various concentrations of allergen. Histamine secretion, %: *a*) spontaneous; that during exposure to: *b*) ConA (30 μg/ml); *c*) 25% serum; allergen in concentrations: 0.1 (*d*); 1 (*e*); 5 (*f*); 10 (*g*); 50 μg/ml (*h*).

30 μg/ml in each experiment to test mast cell secretability.

Since our task was to assess mite allergen activity, we paid special attention to the relationship between various concentrations of the allergen preparation and histamine secretion by sensitized mast cells. The allergen in concentrations of 0.1 to 50 μg/ml induced histamine release from mast cells, the volume of released transmitter being related to the dose of inductor (Fig. 3). Addition of the allergen in a concentration of 10 μg/ml resulted in a 48% histamine release. Raising the dose of inductor to 50 μg/ml did not lead in the majority of cases to a further increase of histamine secretion by mast cells, and in some cases even a certain inhibition of this secretion was observed. The reduction of histamine secretion by mast cells at increased concentrations of the stimulating agent may be related to the "capping" phenomenon [13]. Receptor structures freely moving in the membrane form a compact aggregation or "cap," which then undergoes active endocytosis. Some believe that the capping phenomenon protects the cell, helping it to get rid of the accumulating antigen and thus prevent injury to its membrane [3]. It appears that the allergen concentration 10 μg/ml may be considered optimal, as it induces the maximal histamine release but is insufficient for receptor translocation and for the formation of the aggregation typical of capping. It is noteworthy that the effective allergen concentration, 0.1 μg/ml, is two orders of magnitude lower than the concentration inducing the maximal secretion.

Histamine secretion by mast cells stimulated with mite allergoid is of special interest. Allergoid is a formaldehyde-modified mite allergen characterized by reduced allergenicity but retaining the immunogenic properties at a sufficiently high level.

According to published data, several approaches are used to assess the allergenic characteristics of the two agents [12]. On the one hand, the difference in allergenic properties may be demonstrated as the difference in responses obtained for use of equal doses of the two agents. Allergoid and allergen in concentrations of 50 μg/ml induced 18 and 48% histamine release, respectively (Fig. 4); therefore, the allergenic characteristics of the modified preparation were reduced 2.5-fold in this case. On the other hand, allergenicity may be expressed as the relative dosage build-up necessary to make the responses to both preparations equal. Allergoid in a concentration of 50 μg/ml induced 18% histamine secretion, comparable to the secretion in response to allergen in a concentration of 0.1 μg/ml. Hence, the relative allergenic activity of allergoid is 500 times lower than the activity of mite allergen. It is possible that the inadequate mast cell histamine secretion in response to allergoid is caused by a changed spatial configuration of the IgE-binding determinants in the allergoid molecule, resulting from their chemical modification.

Thus, the model used in our study may be used to assess the allergenic activities of both allergens and their modified forms. Our results permit the conclusion that *D. farinae* allergen is a highly active preparation inducing dose-dependent histamine secretion by mast cells. At the same time, allergoid in concentrations comparable to those of the allergen virtually did not stimulate

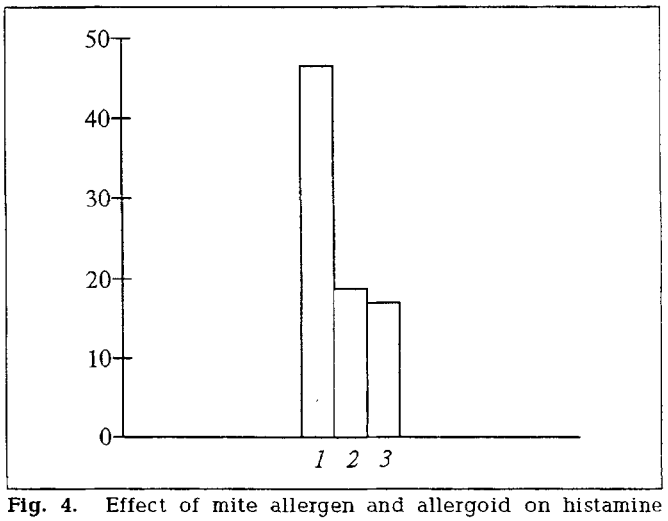

Fig. 4. Effect of mite allergen and allergoid on histamine secretion. Percent histamine secretion: for exposure to allergen in concentrations of 50 (*1*) and 0.1 μg/ml (*2*); to allergoid in a concentration of 50 μg/ml (*3*).

such secretion, this justifying its use in higher doses in specific immunotherapy.

Moreover, our previous data on the capacity of allergen, in contrast to allergoid, to stimulate the amyloride-sensitive system of Na/H metabolism in mast cells [4,8] and the literature on the ability of amyloride (a Na/H metabolism blocker) to inhibit histamine release from mast cells stimulated with calcium ionophore [5,9] permit us to postulate a relationship between the activation of Na/H metabolism and cellular secretion. It is possible that the switching on of the Na/H metabolism system in an IgE-mediated allergic reaction precedes or accompanies histamine secretion by mast cells.

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