

Histamine Release from Skin Mast Cells and Basophils in Patients with Urticaria Pigmentosa

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Histamine release from dispersed skin mast cells may be used for functional studies on the mast cell. However, technical difficulties have hampered such studies. In the present study a new fibreglass-based histamine assay was applied to previously described dispersion techniques, using excision biopsies from 7 patients with urticaria pigmentosa, 3 with psoriasis as well as 4 with urticaria. However, sufficient mast cell numbers for performing histamine release could only be obtained from patients with urticaria pigmentosa. The average mast cell yield was 935 ± 470 cells ($x \pm SD$) per mg wet weight of tissue. The skin mast cells from these patients responded with dose-dependent histamine release to anti-IgE, calcium ionophore A23187, and N-formyl-methionyl-leucyl-phenylalanine challenge without previous passive sensitization. The pattern of histamine release of mast cells and corresponding blood basophils did not indicate substantial differences between the two cell types. Key words: Fibreglass assay; Mastocytosis.

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Cutaneous mast cells are involved in both type I and IV hypersensitivity reactions. However, functional studies on dispersed human skin mast cells are few, due to technical limitations (1). Recently, new enzymatic dispersion techniques have been described, which allow examination of the secretory properties of human skin mast cells (2, 3, 4). In addition, the development of a new specific fibreglass-based histamine method has made it possible to investigate basophils and intestinal mast cells in a technically simple and rapid way (5–8). Therefore, it was of interest to examine if the fibreglass-based histamine assay was a convenient method for studies on dispersed skin mast cells.

MATERIALS AND METHODS

Patients and patient controls

Twenty-one subjects were included in the study after giving informed consent to participate. Seven patients suffered from

urticaria pigmentosa, confirmed by clinical symptoms and histopathological examination. As controls, 7 patients with atopic dermatitis, 3 with psoriasis and 4 with urticaria were enrolled. By venipuncture 5 ml EDTA-anticoagulated blood was collected from all subjects.

The study was approved by the Medical Ethics Committee of the County of Aarhus.

Reagents

Anti-IgE (461.620 U/ml) was obtained from Behringwerke (West Germany). Calcium ionophore A23187 and N-formyl-methionyl-leucyl-phenylalanine (FMLP) was obtained from Sigma. Collagenase from Boehringer-Mannheim. Hyaluronidase from Sigma. The histamine-releasing agents were diluted in PIPES-AMC buffer (8). Microfibreglass-prepared microtitre-plates were provided by Lundbeck Diagnostics (Copenhagen, Denmark).

Mast cell dispersion

Excision biopsies were taken from affected and non-affected skin, using lidocaine 1% as local anaesthesia. The skin sites had not been treated topically, systemically, or with UV-light within the last week. The skin biopsies with epidermis were immediately placed in RPMI-1640 medium following excision. Within 1 h the biopsies were chopped with scissors and dispersed during incubation for 120 min at 37°C in a buffered salt solution containing collagenase (0.015 U/ml), hyaluronidase (0.5 mg/ml), penicillin (75 U/ml), streptomycin (75 µg/ml), supplemented with 20% fetal calf serum. Following incubation, the cells were washed three times and pooled in 1.0 ml of PIPES-AMC. The basophil and mast cell count was determined by metachromatic staining and counting in a Burkert-Turk haematocytometer (6). For skin mast cell studies, 1500 mast cells suspended in 30 µl were added to each microtitre well.

Basophil and mast cell histamine release

In all subjects, histamine release from mast cells and basophils was measured as previously described (6, 9). Briefly, EDTA-anticoagulated blood was 'washed' four times to remove plasma. Aliquots of 50 µl 'washed' blood (containing approximately 1500 basophils) was placed on a fibreglass prepared microtitre plate. At the same time in the wells of another microtitre plate, 30 µl of the corresponding dispersates of mast cells was placed.

The samples were incubated for 60 min at 37°C at the following final dilutions: FMLP at 1×10^{-8} M, 10^{-5} M, and 10^{-3} M; anti-IgE at 1, 100, and 1000 U/ml; and calcium ionophore A23187 at 0.25, 0.50, and 1.00 µM. After incubation histamine was measured by the fluorimetric *O*-Phthalaldehyde method (8). The histamine release was calculated on the basis of a histamine standard curve included in all experiments (6).

Table I. Mean values of dispersed mast cell count and of their maximum histamine release obtained in each urticaria pigmentosa patient.

Patient	Mast cell count per mg tissue	Histamine release in ng/sample		
		Anti-IgE	A23187	FMLP
G. T.	1 762	1.7	0.8	0.0
E. M.	1 044	0.3	1.5	0.4
A. R.	977	0.3	1.5	0.2
C. R.	930	0.6	0.5	0.0
G. L.	1 055	0.0	0.0	0.0
E. J.	302	—	—	—
B. N.	475	—	—	—

Statistics

The results reported are calculated from the mean \pm SD for triplicate determinations.

RESULTS

Excision biopsies

Seven excision biopsies were taken from affected skin from the upper arm, lower leg or hip, with an average weight of 67 ± 17 mg ($x \pm$ SD). Among the rest of patients smaller biopsies of 18 ± 7 mg ($x \pm$ SD) were taken from the same skin areas as mentioned, as well as skin samples from non-affected skin. However, the number of mast cells (range: 0–245 mast cells per mg of tissue) obtained was too low to allow of functional histamine release studies from skin biopsies, except in patients with urticaria pigmentosa with skin lesions. In these patients, however, the average mast cell yield was 935 ± 470 cells ($x \pm$ SD) per mg wet weight of tissue. In 2 urticaria pigmentosa patients (E. J., B. N.) without skin lesions, sufficient numbers of mast cells for histamine release experiments could not be obtained.

Histamine release in patient controls

Basophil histamine release was carried out in all patients, but, no differences were found between the various groups of patients with respect to maximum histamine release or antigen concentration inducing histamine release.

Histamine release in patients with urticaria pigmentosa

Table I shows that dispersed skin mast cells from patients with urticaria pigmentosa responded to IgE-

mediated histamine release as well as non-IgE-mediated (A23187, FMLP). The histamine release was dose-dependent (results not shown) with an interindividual variation in the maximum histamine released. The corresponding basophils showed a similar release pattern (Table II).

DISCUSSION

In most functional studies on human skin, mast cells foreskin of infants have been used (1–4). These mast cells were passively sensitized before cell activation (1, 3). Skin mast cells from adults have only been carried out using skin samples obtained from mastectomies using grams of skin (2, 4). Thus, although skin mast cells can be dispersed into suspension, the amount and the specimen used seem to be very critical for the number of mast cells obtainable. Urticaria pigmentosa is characterized by excessive infiltration of the skin by mast cells (10). Hence, it is not surprising that sufficient mast cells numbers were obtained in these patients.

The study shows that skin mast cells from patients with urticaria pigmentosa responded with dose-dependent histamine release to IgE- and non-IgE-mediated stimuli without previous passive sensitization. The pattern of release was the same from basophils and corresponding mast cells, demonstrating that the two cell types share common characteristics with respect to IgE- and non-IgE-mediated histamine release. Thus, the fibreglass assay is a rapid and easy histamine assay with high sample performance for routine histamine analysis (6, 7, 8), but the number of skin mast cells obtainable by routine clinical skin biopsy are at present not sufficient in order to use this

Table II. Mean values of the maximum histamine release from basophils obtained in each urticaria pigmentosa patient

	Histamine release in ng/sample		
	Anti-IgE	A23187	FMLP
G. T.	2.0	0.3	0.2
E. M.	0.5	0.2	0.2
A. R.	0.4	0.4	0.3
C. R.	0.3	0.5	0.0
G. L.	0.5	0.4	0.2
E. J.	0.0	0.0	0.0
B. N.	0.6	0.3	0.0

assay for functional studies on human skin mast cells, since the assay requires between 1 000 and 1 500 mast cells per well.

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