

Histamine release from dispersed human intestinal mast cells

A method using biopsies from children and adults

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To study the human intestinal mast cell of children and adults, we combined a sensitive glassfibre-based histamine assay with the enzymatic and mechanical dispersion of surgical specimens or mucosal biopsies. The method yields between 1.2×10^3 to 4.6×10^3 mast cells/mg tissue constituting 1.2% to 5.3% of total cell count. The mast cell yield, however, depends on the intestinal tissue specimen used for dispersion. Aliquots containing 1500 mast cells per sample are sufficient for measuring significant amounts of histamine (≥ 0.15 ng histamine per sample), thus making it possible, to carry out approximately 75 tests for four mucosal biopsies of 10 mg each. The intestinal mast cell releases histamine in a dose-dependent manner on challenge with anti-IgE (6–600 U/ml), ionophore A23187 (0.25–1.0 μ M), and Concanavalin A (0.7–25.0 μ g/ml). The histamine release shows interindividual variation with a net histamine release between 0 to 2.5 ng/samples dependent on the secretagogue. In general, it is not necessary to passively sensitize the mast cells to obtain a sufficient histamine release response to anti-IgE challenge, indicating the presence of intact and functional cell-bound IgE. However, it is shown that four of 10 non-atopic intestinal mast cell samples could be passively sensitized with human plasma containing either mite- or grass-specific IgE without stripping off the IgE first. This indicates the presence of free and preserved Fc-receptors on the dispersed mast cells in some subjects. In addition, it is found that the phorbol ester TPA increases the histamine release response to A23187 and turns anti-IgE non-responding mast cells into responding mast cells, but TPA alone at 2 to 16 ng/ml has no histamine releasing effect. In patients with anti-IgE responding mast cells no additional effect of TPA is seen. Finally, no substantial differences between mast cells of children and adults are demonstrated.

Key words: histamine; histamine release; intestinal mast cell; passive sensitization; phorbol esters; TPA.

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The mast cell of the gastrointestinal tract is suspected to be an initiator of acute inflammation and a propagator of chronic changes as well (2, 9, 15, 16, 18, 19). However, time consuming as well as technical difficulties in the measurement of histamine from tissues have complicated the investigations. In addition, a major problem in human intestinal mast cells studies has been difficulties in obtaining sufficient numbers of mast

cells, and the methods described so far need grams of tissue (2, 4, 5, 10, 27). Therefore it is of interest to develop techniques which permit assessment of the mast cell function based on the relatively small numbers of mast cells that can be obtained from children and adults without surgical intervention.

We modified the specific and sensitive histamine assay previously described for human bas-

sophils and enhanced the sensitivity of the assay (20, 29). This assay now allows several release experiments to be made from a single biopsy obtained during endoscopy.

MATERIAL AND METHODS

Reagents

Hanks' balanced salt solution without Mg^{++} and Ca^{++} (HBSS 10 ×) HEPES (1 M), and $NaHCO_3$ (7.5%) from Gibco. RPMI-1640 with 20 mM HEPES, L-glutamine 200 mM, Penicillin 5000 IU/ml, streptomycin 5000 µg/ml (FLOW, Denmark). Medium-199 with 25 mM HEPES, Hanks' salts, and L-glutamine (Gibco). Collagenase 0.15 U/mg (Boehringer-Mannheim). Diamine oxidase (Histaminase) 0.14 U/mg (Sigma). Biotriron and renafix (Bie & Berntsen, Denmark). Phorbol 12-myristate-13-acetate (TPA) was obtained from Sigma and used at final concentrations of 2, 4, 8, and 16 ng/ml. Anti-IgE (461,620 U/ml) was obtained from Behringwerke (West Germany), and was used at final concentrations of 6, 60, and 600 U/ml. Calcium ionophore A23187 (Sigma) was used at final concentrations of 0.25, 0.50, and 1.00 µM. Concanavalin A (Pharmacia, Sweden) was used at final concentrations of 0.7, 1.5, 3.0, 6.0, 12.5 and 25.0 µg/ml. The histamine releasing agents were diluted in Pipes buffer (20). FITCH-conjugated anti-IgE was purchased from Behringwerke, West Germany and used as described by Stahl Skov et al. (30). Glassmicrofibre-prepared microtiter-plates were provided by Lundbeck Diagnostics (Copenhagen, Denmark).

Patients

Intestinal tissue from 10 children with a median age of 4 years (range 3–7) was taken from the proximal part of the jejunum or duodenum (5 surgical specimens and 5 biopsies) after informed consent of one or both parents. The samples were obtained for diagnostic purposes of malabsorption or during surgery for intestinal

obstruction. None of the patients was atopic and patients examined for malabsorption were found to be healthy as judged by light microscopy and the measurement of brush-border enzyme activities.

In 22 adults with a median age of 57 years (range 38–74) mucosal biopsies were taken from the third part of the duodenum during control gastro-duodenoscopy after peptic ulcers, and in three adults normal small bowel intestinal tissue was obtained from patients undergoing resections for carcinoma.

Dispersion of human intestinal mast cells

The biopsies and surgical specimens were immediately transferred to 5 ml Pipes-AMC at 4°C. Within 2 h the mucosal biopsies were chopped with a pair of scissors. However, the mucosa of the surgical specimens was first freed of elastic tissue before chopping. The fragments were placed in 15 ml (prewarmed to 37°C) HBSS (1 ×) buffered with HEPES (10 mM, pH 7.2–7.4) and $NaHCO_3$ (0.04%) containing collagenase (0.015 U/ml). Then penicillin (75 U/ml) and streptomycin (75 µg/ml) were added. The tissue was enzymatically dispersed during incubation for 90 min at 37°C in a shaking water-bath. Every 15 min dispersed cells were pooled in medium-199 (1 ×) with 10% fetal calf serum and antibiotics at 4°C. Thereafter the cells were washed three times at 4°C (600 g for 5 min) in Pipes-AMC. After the third wash, cells were resuspended in 3 ml of Pipes-AMC or RPMI-1640. Mast cells were counted by using a staining solution containing 0.05% toluidine blue, 0.03% light green, saturated solution of saponin white in 50% ethylalcohol and 1/15 M phosphate buffer (pH 6.4).

Passive sensitization of intestinal mast cells

When experiments of passive sensitization were performed, peripheral venous blood was collected from two atopic patients with high RAST values to house-dust mite and grass pollen. Passive sensitization was carried out by incu-

bating 700 μ l dispersed mast cell suspension (3×10^5 cells/ml) with 300 μ l plasma from either one of the atopics in RPMI-1640 medium containing 20 mM HEPES buffer, 10 % fetal calf serum, 2 mM L-glutamine, 75 IU/ml penicillin and streptomycin 75 μ g/ml in a shaking water-bath for 2 h at 37°C. After passive sensitization, the cells were washed twice in Pipes-AMC buffer. Prior to incubation for histamine release mast cells were counted and re-suspended. The cells were challenged with anti-IgE, A23187, grass pollen, and house-dust mite before and after passive sensitization.

Glassfibre-based histamine release assay

Thirty μ l cell samples of dispersed mast cells (6×10^4 cells/ml) were placed in glassfibre-prepared microtiter plates. To avoid trapping of the cells into the microfibrils 20 μ l of Pipes-AMC was added to the plates prior to addition of cell suspensions. The samples were incubated for 60 min at 37°C with 20 μ l of anti-IgE, A23187 or Concanavalin A. In the experiments with TPA 10 μ l was added to the samples at the final concentrations mentioned above. In experiments studying the influence of calcium, cells were suspended and incubated in calcium-free Pipes buffer. Where stated 0.1 mM EDTA was added to the Pipes buffer. During incubation released histamine was bound to the microfibrils and interfering substances and cells were removed by rinsing the plates for 90 s with deionized water. Further remaining residues of proteins were removed by incubating the microtitre plates with 50 μ l of an enzymatic washing detergent (1.0 mg/ml biotrinon and 5 μ l/ml renafix) for 30 min at 37°C, followed by another rinse with deionized water for 90 s.

Specific binding of histamine to the glassfibrils has previously been examined (29), but to further validate the assay using dispersed mast cells, we examined the susceptibility of apparent histamine to degradation by diamine oxidase (histaminase). When diamine oxidase degradation was performed, parallel experiments with authentic histamine standards were also carried out in the presence and absence of

heat-inactivated histaminase (100°C for 10 min). In the microtitre-wells apparent histamine bound to the fibrils was incubated (60 min, 37°C) with histaminase (1.0 U/ml, Pipes-AMC-buffer, pH 7.4) and residual histamine was determined after another rinse with deionized water for 90 s. Histamine was measured by the fluorometric O-phthalaldehyde method in a Perkin-Elmer LS 2 spectrophotofluorometer provided with a microtiterplate autosampler. None of the reagents used in these experiments affected the histamine assay under the conditions described. The histamine released from unstimulated samples (spontaneous release) was subtracted from the histamine released from stimulated samples to give the net stimulated histamine release. The net release of histamine was calculated on the basis of a standard histamine curve, where mucosal cell samples were incubated with 0.15, 0.30, and 0.60 ng histamine. Only net release of histamine ≥ 0.15 ng histamine per sample was considered significant.

Statistics

Mann-Whitney's test was used when comparing the results of children and adults. Wilcoxon signed rank test was used for comparing the values of each patient. The test of significance, coefficient of correlation, median, range, mean and standard deviation (SD) reported are calculated from the mean $\pm$ SD for triplicate determinations.

RESULTS

Biopsies

In three children mucosal biopsies of 300–400 mg wet weight were dissected by knife from surgical specimens of small bowel tissue. In two children intestinal tissue of 150 and 200 mg were taken from the jejunum and in five children nine mucosal biopsies of 10 ± 0.7 mg ($\times \pm$ SD) each were obtained from the third part of the duodenum. In three adults surgical

Table 1

Validation of the glassfibre assay procedure by enzymic degradation of added histamine and apparent released intestinal mast cell histamine

	Histamine (ng/sample) after treatment with:							
	Buffer				Histaminase			
A23187 [μ M]	0	0.25	0.50	1.00	0	0.25	0.50	1.00
Buffer control	-	0.4	0.6	0.6	-	< 0.1	< 0.1	< 0.1
Authentic histamine:								
0.3 ng	0.4	0.7	0.9	0.8	< 0.1	< 0.1	< 0.1	< 0.1
0.6 ng	0.7	1.1	1.2	1.2	< 0.1	< 0.1	< 0.1	< 0.1

Values are means of triplicate determinations based on three different experiments. SD < 0.1 in all experiments.

specimens of duodenal tissue, 1.0, 0.4, and 0.3 g were taken by knife. In 22 adults four mucosal biopsies with an average wet weight of 10 ± 0.8 mg/biopsy ($\times \pm$ SD) were obtained from the third part of the duodenum.

Average mast cell yield

Dispersed cells were pooled and the mast cell numbers were determined by light microscopy following metachromatic staining. Mast cells appeared granulated and structurally intact. By vital staining with Trypan blue more than 95% of the cells excluded the dye, indicating no fundamental cell damage.

Dispersed intestinal mucosal biopsies yielded an average of 87×10^3 ($\pm 5 \times 10^3$ SD) cells/mg wet tissue. As judged by light microscopy $4.6 \pm 0.7\%$ ($\times \pm$ SD) of these cells were mast

cells. In contrast, when dispersing the surgical specimens the average cell yield was higher with an average of 96×10^3 ($\pm 7 \times 10^3$ SD) cells/mg tissue, however, only $1.8 \pm 0.6\%$ ($\times \pm$ SD) of the cells were mast cells. By immunofluorescent staining with FITCH-anti-human IgE, it was found that 4.1% ($\pm 2.3\%$ SD) of the cells showed a patchy surface fluorescence distribution, substantiating the light microscopic finding and the presence of IgE on the cells.

Histamine release

The detectability of histamine was based on a sample volume of 20 μ l 5 ng histamine, HCl/ml corresponding to a sensitivity of 0.10 ng histamine ($3 \times$ SD). The assay has previously been found to show linearity of the fluorescence yield between 0 and 3.75 ng/sample (13). In this study known histamine standards were added linearly to the samples at 0.15, 0.30, 0.60, 1.25, and 2.5 ng/sample ($r = 0.99$, $P < 0.01$). The intra-assay variation in seven experiments was 2% and the day-to-day variation was 3% ($n = 7$). The binding of histamine to the fibres and the identity of histamine was determined by incubation with histamine standards and histaminase. Histaminase degraded both the added and "apparent" histamine (Table 1). In the presence of heat-inactivated histaminase the histamine standards were unaffected (results not shown). Table 2 further indicates that the material being measured was histamine, since in-

Table 2

Histamine release from increasing concentrations of intestinal mast cells

	Ionophore A23187 [μ M]		
	0.25	0.50	1.00
Mast cells/sample			
1,500	0.0 ± 0.02	0.2 ± 0.04	0.3 ± 0.04
2,500	0.2 ± 0.02	0.3 ± 0.05	0.5 ± 0.01
3,500	0.3 ± 0.06	0.4 ± 0.01	0.6 ± 0.04
4,500	0.3 ± 0.03	0.4 ± 0.02	1.0 ± 0.01

Mean $\pm$ SD of triplicate histamine determinations

cubating increasing numbers of mast cells with A23187 resulted in a dose-dependent increase in the amount of histamine being measured.

In response to challenge with A23187 mast cells from all patients released histamine, except one child. In adults and children 84 % (21/25) and 80 % (8/10) responded to anti-IgE, respectively. Concanavalin A (Con A) was less systematically examined, but 77 % (13/18) adults and 70 % (7/10) children released histamine on challenge.

Table 3 shows the net histamine release of the mast cell suspensions on challenge with ionophore A23187, Con A and anti-IgE. When challenged with A23187 (0.25 to 1.0 μ M) the mast cell suspensions released histamine in a dose-dependent fashion, maximal release being 0.5 ng/sample at a concentration of 1.0 μ M A23187. The cells were also responsive to Con A (0.7 to 25 μ g/ml) in a dose-dependent manner, but there were wide variations in the response of different individuals. The optimal concentration, however, was obtained at 1.5 to 6.0 μ g/ml reaching a plateau at 0.3 ng histamine/sample at 1.5 μ g/ml Con A. No correlation was found between the maximum Con A and the maximum anti-IgE induced histamine

release. The anti-IgE response showed a typical bell-shaped curve with a maximal histamine release at 60 U/ml. The cell reactivity was significantly higher among children than adults at 6 U/ml ($P < 0.05$). However, in all other concentrations used, and when comparing the different secretagogues in children and adults no significant ($P > 0.05$) differences were found with respect to cell reactivity or cell sensitivity (the concentration giving a 50 % histamine release of the maximal histamine release). Fig. 1 depicts the typical curves obtained with intestinal mast cells.

Enhancement of human intestinal mast cell histamine release by the phorbol ester TPA

Experiments in six adults with anti-IgE responding cells (3 patients) and anti-IgE non-responding cells (3 patients), i.e. cell samples releasing < 0.15 ng/sample, were carried out with TPA alone or in the presence of A23187 or anti-IgE. In all patients, the A23187 response was increased significantly ($P < 0.05$, Wilcoxon signed rank test) at all concentrations of A23187 between 2 and 4 ng/ml TPA. However, a con-

Table 3

Histamine release from human intestinal mast cells in adults and children induced by different secretagogues

Secretagogue	Histamine release (ng/sample)			
	Adults	(n)	Children	(n)
Anti-IgE [U/ml]				
6	0.1 (0.0-0.8)	(25)	0.4 (0.0-1.7)	(10)
60	0.5 (0.0-1.2)	(25)	0.4 (0.0-2.0)	(10)
600	0.2 (0.0-1.3)	(25)	0.1 (0.0-0.8)	(10)
Ionophore A23187 [μ M]				
0.25	0.2 (0.0-1.3)	(23)	0.2 (0.0-1.4)	(10)
0.50	0.3 (0.0-2.5)	(23)	0.4 (0.0-1.9)	(10)
1.00	0.4 (0.0-3.0)	(23)	0.5 (0.0-2.1)	(10)
Concanavalin A [μ g/ml]				
0.7	0.1 (0.0-0.9)	(18)	0.2 (0.0-0.7)	(10)
1.5	0.3 (0.0-1.2)	(18)	0.3 (0.0-0.7)	(10)
3.0	0.3 (0.0-0.7)	(18)	0.4 (0.0-0.7)	(10)
6.0	0.3 (0.3-0.4)	(6)		
12.5	0.3 (0.2-0.5)	(6)		
25.0	0.4 (0.2-0.4)	(6)		

Median and range for number of patients shown in parentheses

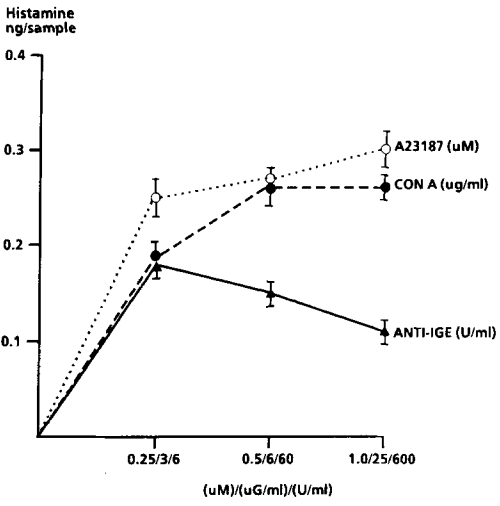


Fig. 1. Release of histamine from a surgical specimen by A23187, Con A, and anti-IgE. Values are means $\pm$ SD of triplicate determinations.

centration of >4 ng/ml TPA appeared to decrease the release, but was not statistically significant ($P>0.05$) (Table 4). The anti-IgE response was increased in two patients with non-responding mast cells in the presence of 2 to 8 ng/ml of TPA. At 8 ng/ml TPA a slight decrease of the maximal response was also found (Fig. 2). No additional effect of TPA was seen in patients with anti-IgE responding (results not shown), and TPA alone could not induce histamine release.

Influence of Ca^{++} on histamine release from intestinal mast cells

Anti-IgE induced histamine release of dispersed human intestinal mast cells was influenced by increasing concentrations of calcium. In the range from 0.25 to 1 mM, the histamine release was enhanced with an optimal concentration at 0.6 to 1 mM calcium. By increasing the concentration of calcium above 2 mM the release was significantly ($P<0.05$) decreased. Supramaximal concentration at 5 mM calcium abolished the response. Calcium-free medium did not inhibit histamine release completely, but in the presence of 0.1 mM EDTA no significant histamine release was measured (Fig. 3).

Table 4

Enhancement of ionophore A23187 induced histamine release in the presence of TPA

TPA	A23187 [μ M]		
	0.25	0.50	1.00
0 ng/ml	0.1 (0.0-0.6)	0.4 (0.1-1.2)	0.6 (0.3-1.3)
2 ng/ml	0.3 (0.1-1.2)	0.6 (0.4-1.6)	0.8 (0.5-1.8)
4 ng/ml	0.4 (0.1-0.9)	0.8 (0.4-1.9)	1.4 (0.7-1.9)
8 ng/ml	0.3 (0.1-0.5)	0.4 (0.3-0.9)	0.5 (0.4-1.0)
16 ng/ml	0.1 (0.0-0.3)	0.3 (0.1-0.6)	0.3 (0.1-0.4)

Median and range for six patients

Passive sensitization of human intestinal mast cells with plasma from allergics

Experiments of passive sensitization were carried out in 10 patients (7 adults and 3 children). However, only mast cells from four (3 adults and 1 child) were responsive to passive sensitization. Among the four "acceptors", the anti-IgE response after passive sensitization was enhanced resulting in an increase in cell reactivity and cell sensitivity. The relationship is demonstrated in Fig. 4, where the mast cells from two "acceptors" were sensitized with plasma from either a grass pollen or house-dust mite allergic patients. The mast cells were not able to respond to the allergen challenge before sensitization, but after passive sensitization with the appropriate plasma, the cells responded on challenge with the specific allergen solely, indicating the ability of these intestinal mast cells to bind the foreign allergen-specific IgE (Fig. 4b, 4d). All experiments also included challenge with A23187 before and after passive sensitization, but the procedure had no effect on the cellular response to the ionophore A23187 (results not shown).

DISCUSSION

Several methods, including HPLC, radioenzymatic assays and conventional fluorometric assays, can measure histamine with a high degree of specificity and sensitivity. However, the existing

methods are time-consuming, laborious, and often purification of tissue extracts prior to assay is necessary due to the lack of specificity. In the glassfibre assay the influence of possible interfering substances such as cadaverine, spermine, spermidine, histidine, arginine, serotonin and agmatine has previously been investigated. However, these substances did not interfere with the glassfibre method when they were tested alone and in combination with histamine. Further, the histamine derivatives 1-methyl-histamine, 3-methyl-histamine and *n*- α -methyl-histamine showed no interference with the method (29). In this study these investigations were extended to include enzymatic degradation with histaminase adding further evidence to the specificity of the assay.

Normal human duodenal mucosa contains approximately 20,000 mast cells/mm³ (21). Mast cells within the gastrointestinal layers vary in distribution, and the number of mast cells within the mucosa varies depending on the level of the gastrointestinal tract examined (21). However, in this study, the difference in the mast cell yield depending on the tissue speci-

men digested, probably reflects the efficacy of the mechanical procedure and the enzymatic dispersion in disintegrating the mucosal biopsy and the surgical specimen. In our hands the mucosal biopsies appeared more accessible for mechanical and enzymatic disruption, maybe due to the composition of the tissue, which contains only the mucosal layer. This factor and the anatomic site of our biopsies may in part explain our high mast cell yield compared with other investigators who use grams of tissue (from colon), several enzymes, and different disruption procedures (2, 4, 10, 11, 12).

The ability of human dispersed intestinal mast cells both from children and adults to release histamine when challenged with anti-IgE demonstrates that they are functionally intact, and that they have sufficient native IgE bound to their surface to respond to IgE-mediated signals. The demonstration of the dose-response curves, the cell response to A23187, as well as the effect of increasing the mast cell number, provides further evidence that the method leaves the mast cells respon-

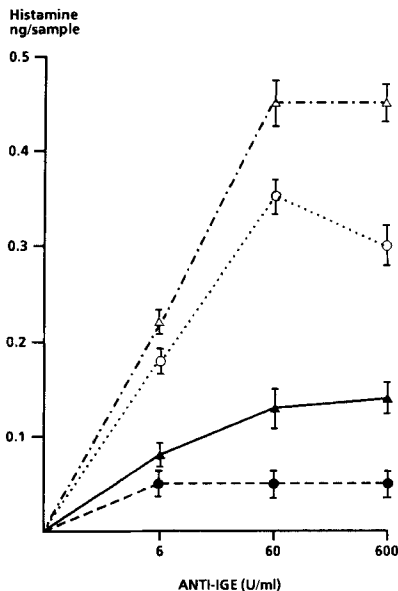


Fig. 2. Synergistic effect of TPA and anti-IgE in non-responding mast cells from one patient. 0 (●—●), 2 (▲—▲), 4 (△—△), and 8 (○—○) ng/ml TPA. Values are means $\pm$ SD of triplicate determinations.

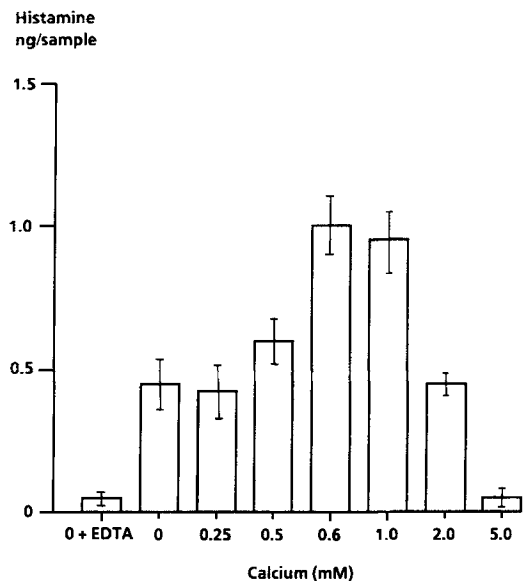


Fig. 3. Effect of Ca⁺⁺ from 0 to 5 mM on histamine release from human intestinal mast cells challenged with anti-IgE (40 U/ml). Values are the means from four different experiments in four patients, and vertical bars denote SD. Cells were also tested in calcium-free medium in the presence of 0.1 mM EDTA.

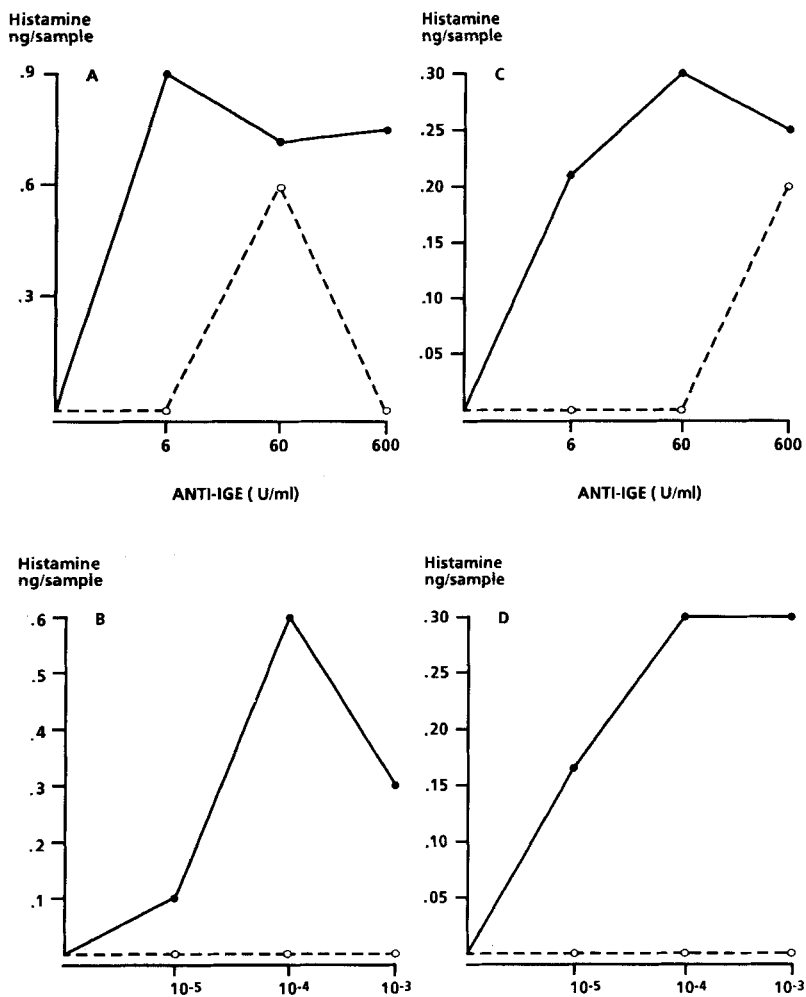


Fig. 4a, c. Anti-IgE induced histamine release before (open circles) and after (closed circles) passive sensitization of intestinal mast cells of two non-atopic adults with atopic plasma containing either mite-specific IgE (Fig. 4a + 4b) or grass pollen-specific IgE (Fig. 4c, d).

Fig. 4b. Mast cell response after passive sensitization when challenged with the appropriate allergen (mite allergen, closed circles) and inappropriate allergen (grass allergen, open circles). Values are presented as the mean of triplicate determinations and SD was < 0.05 ng/sample in all experiments.

Fig. 4d. Mast cell response after passive sensitization when challenged with the appropriate allergen (grass allergen, closed circles) and inappropriate allergen (mite allergen, open circles). Values are presented as the mean of triplicate determinations and SD was < 0.05 ng/sample in all experiments.

sive to immunological and non-immunological stimuli.

In studies of rodent intestinal mast cells and human mast cells from other tissues, the release process seems to be calcium-dependent (3, 6, 34). In this study, it was substantiated in the experiments with varying calcium concentra-

tions. The optimal secretion occurred at a calcium concentration of 0.6 mM. Surprisingly, histamine release in a calcium free medium persisted. Therefore experiments including 0.1 mM EDTA were performed. This abolished histamine release. The histamine release in calcium-free medium without 0.1 mM EDTA

could be explained by mobilization of intracellular stores of calcium, or due to the presence of trace calcium present on cells and tissue in the calcium-free medium. On the other hand, supra-maximal concentrations inhibited histamine release completely. This finding is interesting when compared with lung and cutaneous mast cells, where the inhibition at supramaximal concentrations is not as pronounced at 5 mM calcium, indicating differences in human lung, cutaneous, and intestinal mast cells (6, 26, 34).

TPA is thought to stimulate protein kinase C, which has been demonstrated to synergize the anti-IgE response in basophils (13, 24, 31). In our study, TPA alone failed to trigger histamine release. This suggests that activation of kinase C by itself is insufficient to cause release. In contrast to the response of basophil leukocytes, no increase was found in patients with anti-IgE responding cells. However, in subjects with anti-IgE non-responding mast cells an increase was found in two of the three patients tested. TPA was able to increase the A23187 response in all patients tested at concentrations ≤ 4 ng/ml. However, at higher concentrations the potentiating effect was reversed. Previous studies of rodent mast cells and basophils have also demonstrated both synergistic and inhibitory actions of TPA depending on the conditions of the experiments (8, 22, 25). Additionally, experiments with TPA also suggest that the release process of the intestinal mast cell is calcium-dependent.

In basophil leukocyte the cell reactivity of anti-IgE correlates well with the Con A-induced cell reactivity (28). This was not confirmed in our intestinal mast cell system. The decreased responsiveness (i.e. decreased frequency of responding subjects) to Con A and anti-IgE compared with A23187 could be related to a decreased responsiveness to immunologically dependent stimuli, since all our subjects were non-atopics, or related to the intestinal mast cell IgE receptor complex activation, since the calcium ionophore A23187 bypasses the receptor complex. Another explanation might be that proteolytic enzymes cleave the IgE-receptor molecule (4). However, several investigators

have been unable to confirm this observation in experiments with human lung and intestinal mast cells (10, 26). In some patients, we suggest the intestinal mast cells contain a smaller fraction of occupied IgE-receptors, indicated by the increased anti-IgE response following passive sensitization. Similar to findings in the basophil, cell sensitivity might be regulated by the number of antigen-specific molecules bound to the membrane (7, 14, 23, 32, 33). This is supported by the fact that the histamine release curve was shifted towards lower antigen concentrations, which reflects unoccupied IgE-receptors on the surface of the mast cells. This view is further supported by the ability of non-atopic mast cells to bind house-dust mite and grass pollen-specific IgE, and demonstrating histamine release to the specific antigens after passive sensitization. It also suggests that IgE-receptors are not significantly altered by the enzymatic dispersion or passive sensitization procedure. However, it does not rule out that the enzymatic procedure in some way can harm the cell-bound IgE. Passive sensitization also increased cell reactivity. Thus, by passive sensitization the mast cells achieve an "optimal" IgE concentration of membrane-bound IgE. Therefore, the inability of some mast cells ("non-acceptors") to passive sensitization could be related to mast cells with fully saturated Fc-receptors (14, 23, 32, 33). The A23187 response before and after passive sensitization was unaffected, which indicates that the procedure does not harm the histamine releasing activity of the cells.

In conclusion, the recent development of techniques permitting the dispersion of mast cells from human tissues and the development of a new histamine assay, make it possible to study mast cells from human intestinal biopsies and enable more definitive studies of intestinal mast cells. The method is quick and amenable to future investigations on the intestinal mast cell and its role in immediate hypersensitivity reactions and other inflammatory reactions in the intestine of children and adults.

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