Clin Exp Immunol 1994; 95:343–350

Functional heterogeneity of mast cells isolated from different microenvironments within nasal polyp tissue

S. FINOTTO, J. DOLOVICH*, J. A. DENBURG, M. JORDANA† & J. S. MARSHALL† *Departments of Medicine, *Paediatrics and †Pathology, Molecular Virology and Immunology Programme, McMaster University, Hamilton, Ontario, Canada*

(Accepted for publication 25 October 1993)

SUMMARY

Nasal polyposis is a chronic inflammatory condition of the upper airways characterized by infiltration of activated inflammatory cells, including mast cells, both in the epithelium and in the stroma. The aim of this work was to study human mast cells derived from two different anatomical sites within the same nasal polyp tissue. To this end, we isolated two distinct mast cell populations, one from the epithelial and the other from the stromal layers of individual human nasal polyp tissues. We examined the mediator content of the two mast cell populations and found that stromal mast cells had a significantly higher content of tryptase compared with the epithelial mast cells from the same tissue. In addition, mast cells from the stromal compartment, but not those from the epithelium, released a significant amount of histamine after anti-IgE stimulation. By contrast, both populations released over 50% of the total histamine after non-specific stimuli (A23187 10^{-6} M). The content of mediators and the response to immunological activation were not significantly altered in patients receiving topical steroid therapy. It remains to be determined if the observed differences are the result of an intrinsic characteristic of the mast cell populations localized to separate tissue compartments, or reflect a different *in vivo* exposure to stimuli such as antigens, or different surrounding structural or infiltrating cells. In conclusion, these data provide evidence of functional heterogeneity and differences in mediator content between mast cell subpopulations from a single human tissue. The failure of release of epithelial mast cell mediators from an immunologic stimulus may have implications concerning acute effects of antigen exposure in nasal polyposis.

Keywords microenvironment histamine anti-IgE nasal polyp mast cell

INTRODUCTION

Nasal polyposis is a chronic inflammatory disease of the upper airways often associated with other respiratory disorders such as perennial rhinitis and asthma [1]. Mast cells infiltrate both the epithelium and the stroma of nasal polyp tissue [2,3]. Considerable evidence indicates that certain morphological features of mast cells are influenced by the tissue in which they reside. Mast cell heterogeneity has been defined in human gastrointestinal mucosa, submucosa, skin, lung and other tissues based on the presence of metachromasia after formaldehyde fixation, mediator release and other properties [4–8].

Human mast cells have further been classified both immunohistochemically and ultrastructurally. Mast cells containing only tryptase and others containing both tryptase and chymase have been designated MC_T and MC_{TC} respectively [9,10]. MC_T predominate in lung and bowel mucosa, while MC_{TC} mast cells predominate in bowel submucosa and skin. Recruitment of MC_T has been demonstrated in the acutely inflamed skin lesions of subjects with atopic dermatitis [11] and in the conjunctiva of subjects with vernal conjunctivitis [12]. Similarly to intestine, in nasal polyp tissue epithelial mast cells are predominantly MC_T, while in the stroma they may be of either type [13]. In addition, Kawabori *et al.* recently described pericellular localization of tryptase particularly in the stroma of the polyp, suggestive of ongoing mast cell degranulation [13].

Although both MC_T and MC_{TC} undergo IgE-mediated degranulation, the response of mast cell subpopulations to other secretagogues appears to be heterogeneous. For example, compound 48/80 has been reported to activate mast cells from skin, but not lung-derived mast cells [14–16].

This study addresses the influence of the anatomical location on mast cell responses and mediator content in nasal polyp tissue. This tissue provides an excellent opportunity to address directly the issue of mast cell heterogeneity. To this end, we isolated mast cell-containing cell suspensions from both the epithelium and the stroma of nasal polyp tissue and character-

Correspondence: Dr Jerry Dolovich, McMaster University, Department of Paediatrics, Room 3V41, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5.

ized mediator content and mast cell responses to both immunological and non-immunological stimuli.

SUBJECTS AND METHODS

Subjects

Tissues were obtained from patients with nasal polyposis referred to the Ear, Nose and Throat Clinic for polypectomy which was carried out under topical or general anaesthesia. These studies conformed to guidelines for human experimentation, and were approved by the ethics committee of Chedoke McMaster Hospitals. Before surgery, a detailed clinical history was taken. Allergy skin prick tests were performed with the 19 most prevalent allergens in the geographical area. The diameter of the wheal after 15 min was measured and the response was considered positive when the wheal diameter was >2 mm. The total serum IgE concentration was measured using a PRIST kit (Pharmacia, Uppsala, Sweden).

Reagents

The following reagents were purchased: collagenase class II (Worthington, Freehold, NJ); calcium Ionophore A23187 (Boehringer-Mannheim, Quebec, Canada); compound 48/80 (Sigma, St Louis, MO); rabbit immunoglobulin fraction (Dako, Ontario, Canada); immunoglobulin fraction of rabbit serum specific for human IgE ε chain (anti-IgE) (Dako); Zymed Streptavidin-Biotin kit for developing immunohistochemistry for mouse primary antibody (Zymed Lab, San Francisco, CA); normal rabbit serum (NRS; Dimension Lab., Ontario, Canada); mouse IgG1 from ascites fluid (MOP21) (Sigma). All other reagents were of analytical grade and obtained from BDH Chemicals Ltd (Toronto, Ontario, Canada). Murine monoclonal anti-tryptase termed G3 and murine monoclonal anti-chymase termed B7 were kindly provided by Drs Anne-Marie A. Irani and Lawrence B. Schwartz (Medical College of Virginia, Richmond, VA).

Buffer solutions were made up as follows: Tyrode's buffer (mM of each reagent) NaCl 136·8; KCl 2·6; $NaH_2PO_4 \cdot H_2O$ 0·36; $CaCl_2 \cdot 2H_2O$ 0·99; $MgCl_2 \cdot 6H_2O$ 0·98; HEPES 9·9 and glucose 5·5; 0·1% bovine serum albumin (BSA; Sigma) pH 7·2–7·4. Hanks' balanced salt solution (HBSS) (GIBCO, Ontario, Canada) calcium and magnesium free (HBSS-CMF) contains 20 mM HEPES (Boehringer). HBSS/EDTA contains 25 mM HEPES and 1·3 mM EDTA (Boehringer).

RPMI 1640 (GIBCO, New York, NY) with L-glutamine and without bicarbonate supplemented with 10% fetal calf serum (FCS; GIBCO, Toronto, Canada), 1% HEPES (GIBCO) pH 7·2–7·4.

The osmolarity of all buffers and medium was 300 ± 5 mOsmol.

Isolation of human nasal polyp mast cells

Polyp tissue obtained from polypectomy was transported on ice in HBSS-CMF. A piece of one polyp from each patient was separated and fixed intact in Carnoy's solution (Carnoy's) for histological assessment. Transport and washing solutions were filtered through a mesh stainless steel sieve (pore size 1 mm^2) and saved on ice (Fraction 1). The remaining tissue was incubated in HBSS-CMF containing 1·3 mM EDTA (HBSS/EDTA) with stirring at 22°C for 60 min, to separate the epithelium from the stroma. The supernatant was decanted and filtered through the sieve and the resulting cell suspension was kept on ice (Fraction 2). A piece of the tissue was then cut and fixed in Carnoy's for histological assessment in order to confirm complete removal of the epithelium and preservation of basement membrane. The remaining tissue was cut into pieces (0·3 mm^2) which were incubated for two 60-min periods in HBSS-CMF containing 190 U/ml of collagenase (60 ml/g of tissue) at 37°C with stirring. The supernatants were decanted and filtered through the sieve and saved on ice (Fractions 3 and 4 respectively). The four harvested cell suspensions were centrifuged at 100 ***g*** for 20 min, washed in RPMI 1640, pelleted again at 100 ***g*** for 20 min and resuspended in fresh medium. Fractions 1 and 2 were pooled and, as they were obtained before removal of basement membrane, they were considered epithelial cell suspension. Moreover, Fractions 3 and 4 were pooled and considered stromal cell suspension as they were obtained after removal of the epithelial layer. Total cell number and viability were assessed by counting on a haemocytometer following trypan blue (exclusion dye) staining. Furthermore, cytospins were prepared from each cell suspension stained with toluidine blue to count the number of mast cells recovered; other cytospins were stored at -20°C until used for immunocytochemical assessment. For analysis of tryptase and histamine, $0{\cdot}2–6 \times 10^5$ and 2×10^4 mast cells from each cell population were pelleted and stored at -20°C. For release studies, an appropriate cell number from each cell suspension was centrifuged at 100 ***g*** for 20 min and resuspended in Tyrode's buffer. Total and residual tissue histamine was determined by radioenzymatic assay [16] in sonicated and boiled samples, from which cell debris had been removed by centrifugation.

Histochemical staining

Toluidine blue. Cytosmears were air dried and fixed in Carnoy's (absolute methanol 60% v/v; chloroform 30% v/v; glacial acetic acid 10% v/v) for 10 min. The slides were then immersed in 66% ethanol for 10 min followed by 0·5% acetic acid for 1 min. Finally, slides were stained in 0·5% toluidine blue pH 0·5 (Sigma) for 6 min at room temperature.

Immunocytochemical staining

Additional cytosmears were air dried and stored at -20°C. Immediately before immunostaining, slides were fixed in Carnoy's for 5 min and incubated in 0·6% H_2O_2 in methanol for 30 min to block endogenous peroxidase. Cytosmears were washed with 0·01 M PBS (pH 7·2)/0·15 M NaCl/0·01% Triton X-100 for three 5-min periods. They were then incubated in undiluted heat-inactivated NRS for 3 h at room temperature to block surface Fc receptors. Excess NRS was removed and cytosmears were incubated with murine monoclonal anti-tryptase, termed G3 (1·5 μg/ml), or murine monoclonal anti-chymase, termed B7 (7·5 μg/ml) in 10% NRS overnight at 4°C. Cytosmears were washed in PBS and incubated with biotinylated rabbit anti-mouse immunoglobulins (Zymed kit) for 30 min at room temperature. Slides were then washed in PBS and incubated with peroxidase-conjugated streptavidin (Zymed kit) for 10 min at room temperature. Samples were washed in PBS and the chromogen solution of 3-amino-9-ethylcarbazide (AEC) (Zymed kit) was applied for 15 min. After washing in water, slides were counterstained with Gill's haematoxylin for 2 min and mounted in Glycerol Poly Vinyl mount (GVA mount). Controls for non-specific staining were incubated with the same concentration of mouse IgG1 from ascitic fluid in place of

specific antiserum, but were otherwise treated identically. Human intestine and skin sections fixed in Carnoy's were used as positive controls for anti-tryptase and anti-chymase staining respectively.

Mediator assays

Histamine was measured by a previously described radioenzymatic technique [17] in samples which had been boiled for 5 min to inactivate any endogenous histaminase activity. This assay is sensitive to below 0·1 ng/ml, and is not subject to non-specific protein inhibition. The percentage of tryptase-positive cells and the total cell number were similar in both epithelial and stromal cell suspensions. Before assay, tryptase was solubilized from mast cells by resuspending cell preparations in 1 ml of PBS followed by sonication on ice with Braun-Sonic 2000 sonifer at 50% pulse cycles, three times for 20 s. Solubilized material was obtained from the supernatant after centrifugation of the sonicant at 100 *g* for 20 min at 4°C. Immunoreactive tryptase was measured by a specific sandwich radioimmunoassay (RIA) kit (Pharmacia). The sensitivity of this method is 0·5 U/*l*. The tryptase standards in the kit are calibrated against tryptase derived from human lung which has been purified to homogeneity. One microgram of this preparation has been assigned a value of 1 U.

Histamine release assay

For release studies duplicate aliquots of between 0·3 and $7{\cdot}6 \times 10^4$ mast cells in 1 ml of Tyrode's buffer were challenged for 10 min at 37°C with rabbit anti-human IgE (ε chain) (0·78, 7·8 and 78 μg/ml), rabbit immunoglobulin fraction (7·8 μg/ml), the calcium ionophore A23187 (10^{-6} M) or compound 48/80 (2 μg/ml). Spontaneous release of histamine was assessed by the addition of buffer instead of potential secretagogue. Each compound was diluted in Tyrode's buffer. After incubation, samples were centrifuged at 4°C for 10 min at 100 *g* and the supernatants separated from the pellet and stored at −20°C. The cell pellet was boiled for 5 min to inactivate any residual histaminase activity. The pellets were then resuspended in 1 ml of Tyrode's buffer. Histamine release was expressed according to the formula:

$$\frac{\text{histamine in supernatant}}{(\text{histamine in supernatant} + \text{residual histamine in cells})} \times 100$$

LDH assay

LDH was assayed by a standard clinical laboratory method adapted for use with an enzyme rate analyser (EKTACHEM 700 analyser). LDH release was evaluated in unboiled supernatants from histamine release assays. The test was performed on Kodak EKTACHEM Clinical Chemistry Slides (LDH) according to the manufacturer's instructions. The rate of change in reflection density measured in a linear region was then converted to enzyme activity in U/*l*. LDH release is expressed as $U/10^8$ cells.

Histamine recovery

To examine the recovery of histamine from the original tissue we evaluated the distribution of histamine at different experimental stages. A piece of the original polyp was weighed, sonicated and boiled. Histamine content from samples was evaluated at every stage in the cell suspension procedure, and all supernatants were pooled and analysed for histamine content.

Table 1. Patient data

Patient no.	Sex	Age (years)	Atopy	Steroids local treatment	IgE (U/ml)	Anaesthetic
1	M	81	−	−	7	L
2	F	72	−	+	285	G
3	F	67	+	−	195	G
4	M	60	−	+	15	G
5	F	27	+	+	41	L
6	F	62	+	+	69	G
7	M	65	−	−	26	G
8	M	47	+	+	99	L
9	M	64	+	−	800	G
10	M	49	+	+	16	G

Atopy was considered + when at least one allergy skin test was positive from a range of allergens, including egg white, milk, wheat, peanut, nut mix (no peanut), dog epidermis, cat epidermis, horse epidermis, feather mix, dictyoptera, *Dermatophagoides farinae, D. pteronyssinus,* Alternaria, Hormodendrum, *Aspergillus fumigatus*, tree mix, grass mix, ragweed mix, weed mix (no ragweed).

L, Local; G, general.

Statistical analysis

Linear regression analysis was used to determine correlation coefficients (*r*). Differences between determinations between groups were assessed by paired Student's *t*-test.

RESULTS

Patient characteristics

Ten tissues from patients (mean age 60, range 27–81 years) with nasal polyposis were studied. The characteristics of these patients are presented in Table 1. Briefly, four patients had a history of both allergic rhinitis and allergic conjunctivitis, and two of these also had asthma. Six patients were on nasal steroid treatment at the time of surgery, while four had not taken any medication for the 30 previous days. Six patients had at least one positive allergy skin test. One patient had a high serum concentration of IgE (800 U/ml), while two others had serum IgE concentrations of greater than 100 U/ml. No correlation was found between pharmacological treatment of the patients, skin test positivity or serum IgE level and mast cell mediator content in either the epithelial or stromal cell suspension derived from nasal polyp tissues.

Cell yields and purity

An average of 2·6 g (range 0·6–4·9) of nasal polyp tissue was obtained from each patient. Two cell suspensions, one from the epithelial layer and the other from the stroma, were prepared from each tissue. Both cell suspensions would be expected to contain few if any contaminating cells from the other tissue compartment, as demonstrated by the complete removal of the epithelial layer and preservation of an intact basement membrane after EDTA treatment (Fig. 1). Collagenase proved to be effective in the dissociation of the stroma of human nasal polyp

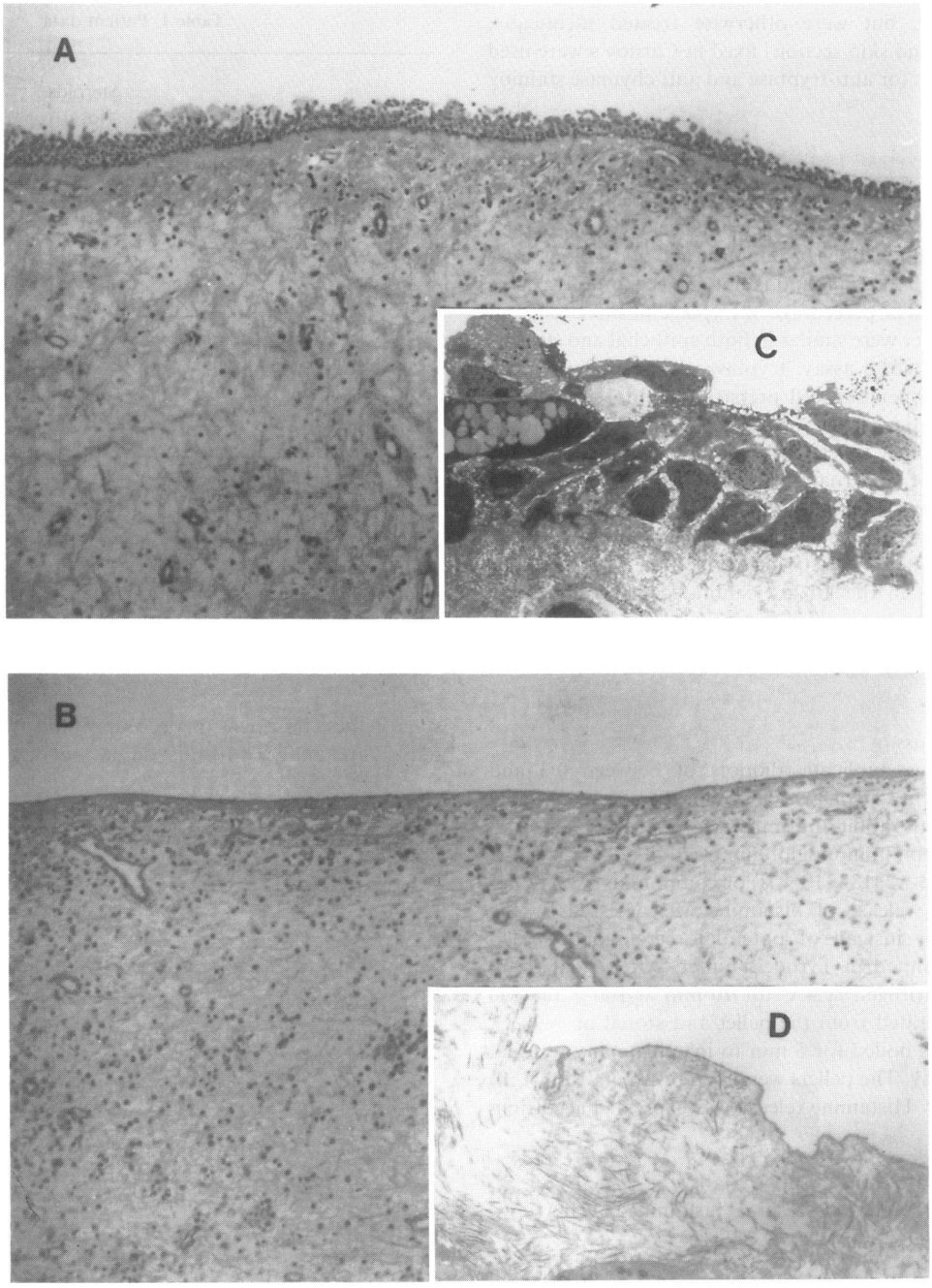

Fig. 1. Histological assessment of the efficiency of epithelium removal by EDTA treatment. (a) Section of human nasal polyp tissue fixed in Carnoy's, embedded in paraffin and stained with periodic acid-Schiff (PAS). (b) By comparison the respective tissue after EDTA treatment (× 330). In inserts (c and d) the same sections have been represented at the electron microscopic level. Note the removal of the epithelial layer by EDTA treatment and preservation of the intact basement membrane. (× 1500 and × 5000 (c and d insert respectively).)

tissue. The cells were recovered in high yield and viability ($80{\cdot}2 \pm 1{\cdot}9$). Both cell suspensions contained mast cells as determined by toluidine blue staining. Mast cell yields per gram of nasal polyp tissue were $0{\cdot}35 \pm 0{\cdot}07 \times 10^6$ (epithelial) and $1{\cdot}16 \pm 0{\cdot}34 \times 10^6$ (stromal). The proportion of mast cells from the epithelial and stromal cell suspensions, after toluidine staining, was $3{\cdot}4 \pm 0{\cdot}7\%$ and $2{\cdot}8 \pm 0{\cdot}6\%$ respectively. The percentage of cells positive to anti-tryptase was similar in the two cell suspensions ($3{\cdot}9 \pm 1{\cdot}1\%$ and $3{\cdot}5 \pm 0{\cdot}5\%$), and these values did not significantly differ from the respective values from toluidine blue staining. Human intestine frozen sections stained positively for tryptase in the parallel experiments.

To evaluate the percentage of cells positive for mast cell chymase further cytospin preparations of the mast cell-containing suspensions were stained immunohistochemically using the murine monoclonal anti-mast cell chymase termed B7. Both

Table 2. Effect of further collagenase or EDTA treatment on histamine release in two isolated populations of mast cells derived from human nasal polyp tissue

Cell population (cross-treatment)	Epithelial (collagenase)		Stromal (EDTA)	
	Before	After	Before	After
Spontaneous*	7·9±2·5	8·9±0·8	7·5±2·5	4·7±1·5
Anti-IgE (7·8 μg/ml)	10·6±2·5	9·2±1·6	29·5±5·8	32·5±5·3

Values are mean±s.e.m. of three different preparations after tissue digestion and before or after cross-treatment, and they are expressed as per cent of histamine release.

* Spontaneous release refers to the per cent histamine release from mast cells treated in parallel with diluent media alone in place of anti-IgE antibody.

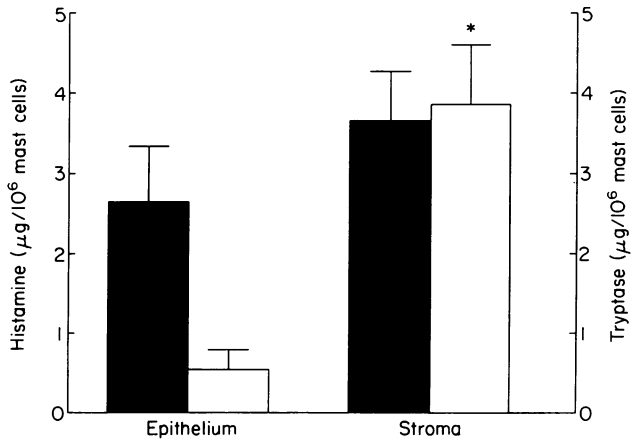

Fig. 2. Histamine and tryptase content of mast cells isolated from two different sites in the human nasal polyp: epithelium and stroma. Each value represents mean±s.e.m. from nine different preparations. * Significant difference ($P<0·01$) between the tryptase content of the two mast cell populations. ■, Histamine; □, tryptase.

human skin sections and intestine sections from a patient with diverticulitis showed numbers of chymase-postive cells in good agreement with those reported in the literature using this technique. We observed that very few mast cells appeared to be chymase-positive, and significant numbers of chymase-positive cells were observed only in the stromal-derived population. Within the epithelial compartment 0·01±0·01% of cells were chymase-positive, which corresponds to an equivalent of 0·02% of the number of tryptase-positive cells observed ($n=8$). In contrast, 0·4±0·2% chymase-positive cells, equivalent to 11·4% of the tryptase-positive cells, were observed in the stromal population.

Effect of enzymic/EDTA treatment

To investigate the effect of the EDTA and enzymatic methods of dispersion on our results we performed experiments ($n=3$) in which the EDTA isolated epithelial cell population was incu-

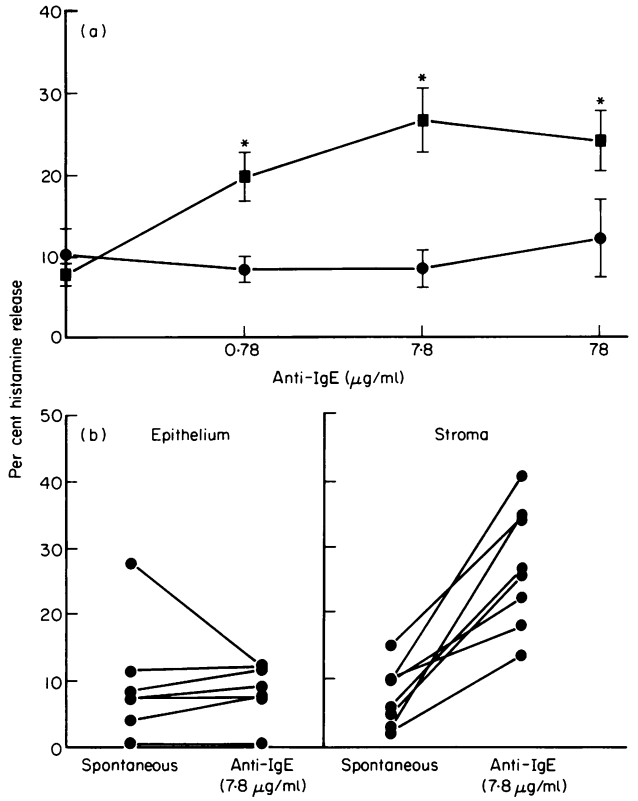

Fig. 3. Per cent of histamine release in response to rabbit anti-human IgE from two populations of mast cells dispersed from human nasal polyp tissue. (a) Dose response curves are represented in a semilogarithmic graph. Each point represents the mean of six experiments performed in duplicate; error bars represent s.e.m. * Statistical difference ($P<0·01$) from the level of spontaneous release. ●, Epithelium; ■, stroma. (b) Single experimental points of histamine release at a single concentration taken from the dose responsive curve to anti-IgE.

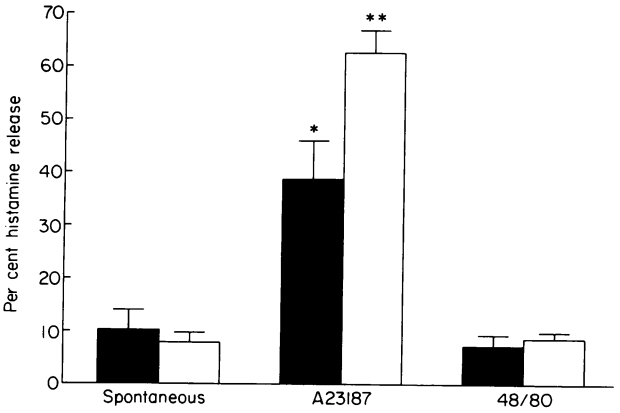

Fig. 4. Spontaneous and induced histamine release of mast cells derived from nasal polyp. Stimuli: A23187 (10^{-6} M) and compound 48/80 (2 μg/ml). The values are means±s.e.m. of duplicate determinations from six experiments. *, ** Significant difference ($P<0·05$ and $P<0·001$, respectively) compared with spontaneous release. ■, Epithelium; □, stroma.

bated for 2 h with collagenase, while the collagenase-dispersed stromal cells were incubated in HBSS/EDTA 1·3 mM at room temperature for 60 min before the cytochemical and functional features were assessed. Our results show that neither EDTA nor collagenase treatment significantly affected the levels of either of the preformed mediators or functional cellular responses (Table 2).

Mediator content

Figure 2 shows the content of preformed mediators in the two cell suspensions. Mast cells in the stromal population had a significantly higher content of tryptase than in the epithelial population ($P < 0{\cdot}01$). In contrast, the histamine content did not differ significantly between the two cell preparations. Histamine and tryptase content correlated with each other only in the epithelial cell suspension ($r = 0{\cdot}84$; $P < 0{\cdot}002$). The lack of observed correlation in the stromal cell population ($r = 0{\cdot}46$) could be due to heterogeneity in the cells containing one or both of these mediators, or to ongoing production and secretion of tryptase by a proportion of these cells.

Histamine release

Functional studies were performed in six consecutive experiments. Mast cells in the two cell suspensions showed low spontaneous histamine release, suggesting good cell preservation. Both cell suspensions were then challenged with immunoglobulin fraction of rabbit serum-specific human IgE ε chain (anti-IgE). Stromal mast cells, incubated in the presence of 0·78–78 μg/ml of anti-IgE, released significant amounts of histamine within 10 min (Fig. 3a). A mean histamine release of $26{\cdot}9 \pm 3{\cdot}8\%$ was observed after stimulation with 7·8 μg/ml of anti-IgE. In contrast, mast cells from the epithelial cell suspension did not release further histamine after anti-IgE stimulation. The single experimental values of histamine release after challenge are shown in Fig. 3b. These data demonstrate that stromal mast cells from six patients consistently released histamine in response to anti-IgE while epithelial mast cells did not. Controls for non-specific release with a rabbit immunoglobulin fraction at 7·8 μg/ml gave values comparable to the spontaneous release in both cell suspensions ($8{\cdot}53 \pm 0{\cdot}93$ epithelial; $10{\cdot}75 \pm 1{\cdot}4$ stromal). To examine the response of these cells to non-immunological stimuli, challenges with calcium ionophore A23187 and compound 48/80 were studied. Stimulation by A23187 (10^{-6} M) caused mast cells from both the stromal and the epithelial compartments to release significantly greater amounts of histamine compared with the spontaneous release ($P < 0{\cdot}001$ and $P < 0{\cdot}02$ respectively). The release after A23187 (10^{-6} M) from the stromal compartment was significantly higher compared with that from the epithelial ($P < 0{\cdot}02$). In four experiments we tested LDH release as a marker of cell integrity, and found that histamine release induced by A23187 10^{-6} M was not accompanied by increased release of this cytoplasmic enzyme in either cell population (spontaneous *versus* A23187 10^{-6} M: $7{\cdot}6 \pm 1{\cdot}1$ *versus* $7.35 \pm 0{\cdot}9$ and $6{\cdot}35 \pm 0{\cdot}73$ *versus* $6{\cdot}6 \pm 0{\cdot}6$ U/10^8 cells, in the epithelial and in the stromal population, respectively). The classical secretagogue compound 48/80 did not induce histamine release above that released spontaneously from either cell population (Fig. 4). No differences were observed between patients who received topical *versus* general anaesthesia with respect to histamine release or mediator content of the mast cells in either compartment.

Histamine recovery

In two representative experiments, the total histamine content was measured before and after each treatment both in the pellets and in the supernatants. An average of 11·38 μg of histamine per gram of tissue was obtained by sonication of starting tissues. On average, 52% of the total histamine content remained associated with the recovered cell populations. Histamine recovered at the end of the experiment was $\geqslant 100\%$ if the histamine contents of all the washing solutions and recovered cells were taken into account, suggesting that there was little histamine degradation occurring during the cell isolation procedure.

DISCUSSION

Mast cells have been implicated as important effector cells in a variety of allergic disorders and chronic inflammatory diseases. It is well recognized that mast cells with distinct functional and histochemical properties can be isolated from human tissues [7–9,18]. Differences in mediator content, functional responses and responses to pharmacologic agents such as the mast cell stabilizing agent disodium cromoglycate have been described [19]. These differences have been explained on the basis of microenvironmental control. Support for this concept has been obtained from rodent models where 'connective tissue type' mast cells from one microenvironment such as the peritoneal cavity have been demonstrated to acquire 'mucosal mast cell' characteristics when introduced into the stomach wall [20].

In the human, the cell sources used to date for studies of heterogeneity have been obtained from separate patient populations. The methods used to isolate mast cell from different tissues also vary considerably. The present study demonstrates that within nasal polyp tissue from individual patients two distinct mast cell populations can be isolated, one from the stroma and the other from the epithelial compartment. Differences in mediator content and functional characteristics have been noted betwen these two mast cell suspensions which cannot be accounted for by differences in isolation procedure, since treatment of cells isolated by each procedure with the agents necessary for isolation of the other cell population does not significantly alter their characteristics (Table 2).

The most widely studied marker of mast cell activation is histamine, the release of which has been shown to correlate with mast cell activation in a number of circumstances [21]. More recently, mast cell tryptase has been employed to examine mast cell activation during disease in a clinical setting [22]. The histamine content of the mast cell populations examined did not differ significantly while, in contrast, the tryptase content of the stromal mast cells was substantially greater than that of the epithelial mast cell population. Thus the ratio of tryptase/histamine content differed considerably between the two anatomical locations. This is of particular interest given that both mediators are thought to be stored within the same granule structure. The degree of difference observed is probably an underestimate of the quantity of cell-associated tryptase in the stromal compartment, since we have previously shown that stromal mast cells exhibit pericellular tryptase staining on immunohistochemical analysis of intact tissue [4]. This staining is suggestive of ongoing tryptase production or secretion by polyp stromal mast cells. Perhaps partly as a result of this, the levels of tryptase per mast cell for both stromal and epithelial mast cell populations are lower than those reported in the

literature for human lung mast cells [23,24]. In addition, we believe our tryptase data are not the result of an incomplete extraction, since it was performed under conditions similar to those reported in literature [23] and in a buffer whose NaCl molarity (0·1 M) allowed complete dissociation of tryptase from its tetrameric form into the monomeric form [25] which is recognized by the antibody used in our assay.

Mast cells derived from the stroma of polyps released histamine in response to anti-IgE *in vitro*, whereas mast cells from the epithelial compartment of the same tissue were unresponsive to this stimulus. Both populations released histamine after treatment with the calcium ionophore A23187. There was no significant release of histamine by either population after treatment with the classical CTMC secretagogue compound 48/80. The responses to compound 48/80 are of particular interest in view of previous reports that human skin mast cells (predominantly MC_{TC}) can be activated with this agent [26,27], while cells from other anatomical locations in which MC_T are found in greater numbers were shown to be unresponsive to this agent [28,29]. The ability of human enzymatically dispersed stromal polyp mast cells, without prior passive sensitization, to release histamine when challenged with anti-IgE demonstrates that, even after collagenase treatment, they are functionally intact and that they have sufficient native IgE bound to their surface to respond to immunological activation. The per cent release of histamine following anti-IgE activation is similar to that reported for human mast cells isolated from other tissue sites [30].

A possible explanation for the apparent lack of responsiveness of the epithelial mast cells to immunological stimuli is their prior activation *in vivo* by antigen exposure or mediator exposure within the nose. The epithelial surface of the mucosa in the nasal polyp surrounds the whole stroma. Antigen in the upper airways would be expected to first come into contact with intraepithelial mast cells, potentially triggering their degranulation via an IgE-dependent or other mechanism. Evidence in support of this comes from studies by Okuda *et al.* examining the localization of allergen following nasal challenge [31,32]. Functional studies of mast cells derived from the nasal surface and nasal lamina propria in normal subjects by other laboratories have demonstrated degranulation of both mast cell populations in response to anti-IgE and calcium ionophore. These results might suggest that the inability of nasal polyp epithelial mast cells to respond to anti-IgE is a consequence of an ongoing immunological and inflammatory process. It is interesting to note in this regard that the epithelial mast cells from steroid-treated patients had similar functional characteristics to those isolated from patients not receiving such therapy. We considered the possibility that the mast cells within the epithelial layer were non-responsive due to poor preservation or functional viability as a result of the isolation procedure. However, electron microscopic examination of the cells from both compartments revealed well preserved mast cells and cells from both the epithelium and the stroma degranulated in response to non-cytotoxic concentrations of the calcium ionophore A23187.

These findings demonstrate heterogeneity in the functional properties and content of preformed mediators between human mast cells residing in the same inflammatory tissue but exposed to different microenvironments. The basis for human mast cell functional heterogeneity, the environmental forces that dictate responsiveness, and the impact of pharmacologic agents and disease on mast cell responses are important areas for future study.

ACKNOWLEDGMENTS

We thank Drs Anne-Marie A. Irani and Lawrence B. Schwartz for providing monoclonal antibody; Jan Switzer, Susan Evans, Lynne Larocque and Laurie Nielsen for their excellent technical assistance; Drs Jerry Grabec, Paul Lapp and Donald Hitch for providing human nasal polyp tissue; Mary Conway for collecting the patient data; the clinical laboratory of McMaster Hospital for performing LDH assay; and Mary Kiriakopoulos for her excellent secretarial assistance. This work was supported by the Medical Research Council of Canada. J.S.M. and M.J. are holders of Medical Research Council of Canada Scholarship Awards.

REFERENCES

1 Settipane GA, Chafee FH. Nasal polyps in asthma and rhinitis: a review of 6037 patients. J Allergy Clin Immunol 1976; **59**:17–21.
2 Cauna N, Hinderer KH, Manzetti GW, Swanson EW. Fine structure of nasal polyps. Ann Otol Rhinol Laryngol 1972; **81**:41–58.
3 Connell JT. Cellular molecular and clinical aspects of allergic disorders. In: Gupta S, Good RA, eds. Comprehensive immunology. New York: Plenum Publishing Co, 1979; **6**:397–415.
4 Barrett KE, Metcalfe DD. Mast cell heterogeneity: evidence and implications. J Clin Immunol 1984; **4**:253–61.
5 Strobel S, Miller HRP, Ferguson A. Human intestinal mucosal mast cells: evaluation of fixation and staining techniques. J Clin Pathol 1981; **34**:851–8.
6 Benyon RC, Church MK, Clegg LS, Holgate ST. Dispersion and characterization of mast cells from human skin. Int Arch Allergy Appl Immunol 1986; **79**:332–4.
7 Tharp MD, Sobotka AK, Fox CC, Maroe G, Lichtenstein LM, Sullivan TJ. Functional heterogeneity of human mast cells from different anatomic sites: *in vitro* responses to morphine sulface. Clin Immunol 1987; **79**:646–53.
8 Lowman MA, Rees PH, Benyon RC, Church MK. Human mast cell heterogeneity: histamine release from mast cells dispersed from skin, lung, adenoids, tonsils, and colon in response to IgE-dependent and non-immunologic stimuli. J Allergy Clin Immunol 1988; **81**:590–7.
9 Irani AA, Schechter NM, Craig SS, DeBlois G, Schwartz LB. Two human mast cell subsets with distinct neutral protease compositions. Proc Natl Acad Sci USA 1986; **83**:4464–8.
10 Craig SS, Schwartz LB. Tryptase and chymase, markers of distinct types of human mast cells. Immunol Res 1989; **8**:130–48.
11 Irani AA, Sampson HA, Schwartz LB. Mast cells in atopic dermatitis. Allergy 1989; **44**:31–34.
12 Irani AA, Butrus SI, Tabbara KF, Schwartz LB. Human conjunctival mast cells: distribution of MC_T and MC_{TC} cells in vernal conjunctivitis and giant papillary conjunctivitis. J Allergy Immunol 1990; **86**:34–40.
13 Kawabori S, Denburg JD, Schwartz LB *et al.* Histochemical and immunohistochemical characteristics of mast cells in nasal polyps. Am J Respir Cell Mol Biol 1992; **6**:37–43.
14 Church MK, Pao K, Holgate ST. Characterization of histamine secretion from dispersed human lung mast cells: effects of anti-IgE, calcium ionophore A23187, Compound 48/80 and basic polypeptides. J Immunol 1982; **129**:2116–21.
15 Lawrence ID, Warner JA, Cophan VL, Hubbard WC, Kagey Sobotka A, Lichtenstein LM. Purification and characterization of human skin mast cells. Evidence for human mast cell heterogeneity. J Immunol 1987; **139**:3062–9.
16 Tharp MD, Suvunrungsi RT, Sullivan TS. IgE mediated release of histamine from human cutaneous mast cells. J Immunol 1983; **130**:1896–901.

17 Beaven MA, Jacobsen S, Hora'kova'Z. Modification of the enzymatic isotopic assay of histamine and its application to measurement of histamine in tissues, serum and urine. Clin Chim Acta 1972; **37**:91–103.

18 Enerback L, Pipkorn U, Aldenborg F, Wingren U. Mast cell heterogeneity in man: properties and function of human mucosal mast cells. In: Galli SJ, Austen KF, eds. Mast cells and basophils differentiation and function in health and disease. New York: Raven Press, 1989:27–37.

19 Pearce FL, Flint KC, Leung KBP *et al.* Some studies on human pulmonary mast cells obtained by bronchoalveolar lavage and by enzymic dissociation of whole lung tissue. Int Archs Allergy Appl Immun 1987; **82**:507–12.

20 Kobayashi T, Nakano T, Nakahata T *et al.* Formation of mast cell colonies in methylcellulose by mouse peritoneal mast cells and differentiation of these cloned cells in both the skin and gastric mucosa of W/WV mice. Evidence that common precursor can give rise to both connective tissue type and mucosal mast cells. J Immunol 1986; **136**:1378–84.

21 Togias AG, Naclerio RM, Proud D *et al.* Nasal challenge with cold, dry air results in release of inflammatory mediators. Possible mast cell involvement. J Clin Invest 1985; **76**:1375–81.

22 Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicator of mast cell activation in systemic anaphylaxis and mastocytosis. N Engl J Med 1987; **316**:1622–6.

23 Schwartz LB, Lewis RA, Austen KF. Tryptase from human pulmonary mast cells: purification and characterization. J Biol Chem 1981; **256**:11939–43.

24 Schwartz LB, Irani AMA, Roller K, Castells MC, Schechter MM. Quantitation of histamine, tryptase, and chymase in dispersed human T and TC mast cells. J Immunol 1987; **138**:2611–5.

25 Alter SC, Metcalfe DD, Bradford TR, Schwartz LB. Regulation of human mast cell tryptase. Effect of enzyme concentration, ionic strength and the structure and negative charge density of polysaccharides. Biochem J 1987; **248**:821–7.

26 Lawrence ID, Warner JA, Hubbard WC, Kagey-Sobotka A, Lichtenstein LM. Purification and characterisation of human skin mast cells. J Immunol 1987; **139**:3062–9.

27 Benyon RC, Lowman MA, Church MK. Human skin mast cells: their dispersion, purification and secretory characterization. J Immunol 1987; **79**:646–53.

28 Church MK, Pao GJK, Holgate ST. Characterization of histamine secretion from mechanically dispersed human lung mast cells: effects of anti-IgE, calcium ionophore A23187, compound 48/80 and basic polypeptides. J Immunol 1982; **129**:2116–21.

29 Flint KC, Leung KBP, Pearce FL, Hudspith BN, Brostoff J, Johnson NM. Human mast cells recovered from bronchalveolar lavage: their morphology, histamine release and the effects of sodium cromoglycate. Clin Sci 1985; **68**:427–32.

30 Schulman ES, MacGlashan JR DW, Peters SP, Schleimer RP, Newball HH, Lichtenstein LM. Human lung mast cells: purification and characterization. J Immunol 1982; **129**:2662–7.

31 Okuda M. Mechanism and pathogenesis of ragweed pollen nasal allergy. Wakayam Med Rept 1972; **15**:143–52.

32 Okuda M, Otsuka H, Kawabori S. Characteristics and role of the surface basophilic cells in nasal allergy. Veldman JE, ed. Immunobiology, autoimmunity and transplantation in otorhinolaryngology. Amsterdam: Kurgler Pub, 1985: 267–72.