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Differential release of histamine and eicosanoids from human skin mast cells activated by IgE-dependent and non-immunological stimuli

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1 Cells were dispersed from human foreskin using a mixture of collagenase and hyaluronidase and separated into mast cell-depleted (<1%) or enriched (>75%) preparations by density-gradient centrifugation.

2 Challenge of gradient fractions with ε-chain-specific anti-human IgE stimulated the release of histamine, prostaglandin D_2 (PGD_2) and leukotriene C_4 (LTC_4). The release of eicosanoids was significantly correlated with that of histamine, suggesting that they are derived from the mast cell population of the dispersate. In highly purified (76.2 ± 4.2%) mast cell preparations, maximum net release of histamine, PGD_2 and LTC_4 was 3432 ± 725, 84.9 ± 10.8 and 6.6 ± 1.2 pmol/10^6 nucleated cells.

3 The non-immunological stimuli substance P, vasoactive intestinal peptide (VIP), somatostatin, compound 48/80, morphine and poly-L-lysine released similar amounts of histamine to anti-IgE, but 12 to 21 fold less PGD_2 and LTC_4.

4 These studies suggest that IgE-dependent and non-immunological stimuli activate human skin mast cells by different secretory mechanisms, a hypothesis supported by our previous findings of differences in Ca^{2+} requirements and time-course of histamine release. Activation by the non-immunological mechanism may be of importance *in vivo* due to the close anatomical association between skin mast cells and dermal nerve-terminals containing neuropeptides.

Introduction

There is increasing evidence that inflammatory mediators released from skin mast cells are involved in a variety of pathological processes, including adverse reactions to drugs, allergic dermatitis and various forms of urticaria. The efficacy of histamine H_1-receptor antagonists in the treatment of these disorders suggests that this amine plays an important role in their underlying pathology. Intradermal injection of histamine mimics the wealing (plasma extravasation) and flaring (vasodilatation) frequently observed in skin disorders (Lewis, 1927) and in the early response of sensitised individuals injected intradermally with allergen (Prausnitz & Kustner, 1962).

The work of Ishizaka & Ishizaka (1968) gave a direct demonstration of the ability of skin mast cells to be activated *in vivo* by cross-linkage of their IgE-receptors. However, this is not the only mechanism by which these cells may be activated; recent studies have shown that mast cells dispersed from human skin differ from those dispersed from human lung, tonsil, adenoid and intestine in secreting histamine in response to a variety of non-immunological secretagogues including compound 48/80, poly-L-lysine, substance P, vasoactive intestinal peptide (VIP) and somatostatin (Church *et al.*, 1982; Benyon *et al.*, 1986; 1987a; Lowman *et al.*, 1988a, b; Rees *et al.*, 1988). Histamine secretion induced by these stimuli differs from that triggered by anti-IgE in being only partially dependent on extracellular Ca^{2+} and rapid in time-course, suggesting that the two types of stimuli utilise different mechanisms of cell activation. However, possible differences in eicosanoid release by these stimuli have not yet been investigated.

Human mast cells are capable of generating a variety of pre-formed and newly-generated mediators in addition to histamine. *In vitro* studies show that mast cells dispersed from human lung parenchyma (Holgate *et al.*, 1984; Peters *et al.*, 1984) or skin (Benyon *et al.*, 1987b; Robinson *et al.*, 1989) release both prostaglandin D_2 (PGD_2) and leukotriene C_4

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(LTC_4) after activation with anti-IgE or calcium ionophore A23187. When injected intradermally, these eicosanoids induce wealing and flaring and promote leucocyte accumulation (Bisgaard *et al.*, 1982; Camp *et al.*, 1983; Soter *et al.*, 1983) and, therefore, if liberated in sufficient quantities by mast cells within the dermis, may be important mediators of skin inflammation.

In this study, we have continued our investigations of the response of human dispersed skin mast cells to non-immunological stimuli. We provide the first demonstration that IgE-dependent and non-immunological stimuli differ in their capacity to activate the release of eicosanoids from human skin mast cells, which supports the concept that these stimuli utilise different secretory mechanisms.

Methods

Human skin mast cell dispersion

Mast cells were dispersed from human foreskin as previously described (Benyon *et al.*, 1987a). Briefly, foreskin, obtained by circumcision of patients aged 1–9 years, was chopped finely with scissors into 1–2 mm^3 fragments which were washed twice by centrifugation (500 *g*, 30 s, 25°C) in MEM containing 1·5% v/v FCS (MEM/FCS). Mast cells were passively-sensitised by incubation of the fragments for 2 h at 37°C in 5 ml of MEM/FCS containing 2 $\mu g\,ml^{-1}$ of human myeloma IgE (generously donated by Dr Teruko Ishizaka, Johns Hopkins Medical School, Baltimore, MD, U.S.A.). Fragments were washed and then digested for two intervals of 60 min at 37°C in MEM containing collagenase, 1.5 $mg\,ml^{-1}$, hyaluronidase, 0.75 $mg\,ml^{-1}$ and bovine serum albumin, 3.5% w/v. Dispersed cells were washed three times by centrifugation in MEM/FCS (500 *g*, 10 min, 25°C) and resuspended in 1 ml of MEM/FCS. Mast cell numbers and purity were assessed by optical microscopy after unfixed cell suspensions had been stained with Kimura's metachromatic stain (Kimura *et al.*, 1973).

Density-gradient separation of mast cells

Cell preparations depleted or enriched in mast cell content were obtained by density-gradient centrifugation as previously described (Benyon *et al.*, 1987a). Briefly, cells dispersed by the above enzymatic method and containing 5–8% mast cells were resuspended in 1 ml of MEM/FCS and layered over a five-step discontinuous gradient of 40–80% isotonic Percoll (density 1.051–1.100 $g\,ml^{-1}$). After centrifugation (500 *g*, 10 min, 25°C), cells which collected at the interfaces between layers of different density were aspirated and washed twice in MEM/FCS, before assessment of mast cell numbers and purity as described above. Lymphocytes constituted >90% of the cells contaminating mast cell-enriched preparations collected from high density (>70% Percoll) fractions of gradients.

Mast cell activation

Cells were washed once by centrifugation in HBSS and then resuspended in this buffer to a density of 5–10 $\times 10^4$ mast cells ml^{-1}. Aliquots of 360 μl were added to Eppendorf tubes and warmed at 37°C for 10 min before challenge with 40 μl of secretagogue. Aliquots of cells challenged with a relevant concentration of non-immune IgG or dimethylsulphoxide were included as controls for mediator release by anti-IgE or ionophore, respectively. After a 30 min incubation at 37°C, tubes were centrifuged (10,000 *g*, 30 s, 25°C) and aliquots of supernatant removed for storage at −20°C before measurement of eicosanoid and histamine content.

Assay of mediators

Radioimmunoassay of PGD_2 was performed as described previously (Holgate *et al.*, 1984) using a rabbit antiserum generously supplied by Dr L. Levine (Brandeis University, MA, U.S.A.). Immunoreactive LTC_4 was measured in unextracted incubation supernatants using an antiserum from Amersham International PLC, Buckinghamshire, with a claimed cross-reactivity of <1.6% with other leukotrienes and <0.15% with other eicosanoids. By use of high performance liquid chromatography we have established that PGD_2 and LTC_4 are the major eicosanoids released from human skin mast cells activated by anti-IgE or calcium ionophore (Robinson *et al.*, 1989). Supernatant and cell-associated histamine was measured by an automated spectrophotofluorimetric method.

Data presentation

Data are shown as mean ± s.e.mean of the number of experiments (*n*) indicated. Mediator secretion is expressed as the net release calculated after subtraction of unstimulated release occurring in the absence of stimulus. Significance of the difference between mean values was calculated by use of Student's *t* test for paired data. Correlation coefficients were calculated by unweighted least-squares linear regression analysis.

Materials

Collagenase (type 1), hyaluronidase (type 1), deoxyribonuclease (bovine pancreas), calcium ionophore

A23187 (dissolved as a 1 mM stock solution in dimethyl sulphoxide), compound 48/80, poly-L-lysine (average molecular weight 45,000–55,000 Daltons), substance P (human, synthetic), vasoactive intestinal peptide (VIP, porcine, synthetic), somatostatin (human, synthetic), N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES), gelatin and bovine serum albumin were from Sigma Chemical Company, Poole, Dorset. Foetal calf serum (FCS) and Eagle's minimum essential medium (MEM) containing 25 mM HEPES and L-glutamine were purchased from Gibco Europe Ltd., Paisley, Scotland. Percoll density gradient medium was from Pharmacia, Milton Keynes, Buckinghamshire. Morphine sulphate was from Evans Medical Ltd., Greenford, Middlesex. ε-Chain-specific goat anti-human IgE and non-immune IgG were prepared as described (Holgate *et al.*, 1984). HEPES-buffered salts solution (HBSS), pH 7.2, comprised (mM): NaCl 137, HEPES 20, D-glucose 5, KCl 2.7, NaH_2PO_4 0.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8 and gelatin 0.1% w/v. All reagents in this buffer were of analytical grade.

Results

Separation of enzymatically-dispersed skin cells according to buoyant density yielded cell preparations containing from 1% to 86% mast cells (Table 1). In four experiments in which highly-purified (73.2 ± 2.4%) preparations of skin mast cells collected from the higher density (70/80% and >80% Percoll) fractions of the gradient were activated with anti-IgE, there was a concentration-dependent release of histamine, PGD_2 and LTC_4 (Table 2). Release of all three mediators was significantly ($P < 0.05$) greater than spontaneous release over the range 0.83–25 $\mu g\,ml^{-1}$ anti-IgE and reached maxima of 3432 ± 725, 84.9 ± 10.8 and 6.6 ± 1.2 pmol/10^6 nucleated cells respectively at the highest concentration of anti-IgE used. Over the whole concentration-response curve of 0.25 to 25 $\mu g\,ml^{-1}$ anti-IgE, there were significant correlations between the net release of PGD_2 and histamine ($r = 0.871$, $P < 0.001$, $n = 20$) and LTC_4 and histamine ($r = 0.686$, $P < 0.001$, $n = 20$).

Table 1 Preparation of mast cell-enriched or depleted fractions by density-gradient centrifugation

Percoll concentration (%)	*Mast cell purity* (%)
Unseparated	9.5 ± 1.5
<40	1.0 ± 0.1
40/50	2.5 ± 0.1
50/60	20.1 ± 4.2
60/70	61.5 ± 7.3
70/80	86.0 ± 2.9
>80	86.8 ± 2.2

Enzymatically-dispersed skin cells comprising 9.5 ± 1.5% mast cells were fractionated on five-step discontinuous gradients of 40–80% Percoll as described. Results shown are from 8 separate gradients.

Activation of gradient fractions with anti-IgE, 25 $\mu g\,ml^{-1}$, stimulated the release of PGD_2, LTC_4 and histamine. When all gradient fractions in five experiments were assessed, the release of PGD_2 was significantly correlated with both histamine release ($r = 0.498$, $P < 0.01$, $n = 38$) (Figure 1a) and with numbers of mast cells ($r = 0.454$, $P < 0.01$, $n = 35$). Release by anti-IgE of LTC_4, was also significantly correlated with that of histamine ($r = 0.712$, $P < 0.001$, $n = 25$) (Figure 1b) and with numbers of mast cells ($r = 0.773$, $P < 0.001$, $n = 21$). These correlation studies suggest that activated skin mast cells are likely to be the major source of both eicosanoids in the skin dispersates.

In the same experiments, PGD_2 and histamine release induced by substance P were compared to

Table 2 Anti-IgE-induced release of mediators from human purified skin mast cells

	Net release of mediator (pmol/10^6 cells)			
Anti-IgE ($\mu g\,ml^{-1}$)	*PGD_2*	*LTC_4*	*Histamine*	*Histamine release* (%)
0.25	13.0 ± 2.7	0.2 ± 0.1	269 ± 62	1.1 ± 0.5
0.83	21.2 ± 3.9	0.8 ± 0.2	522 ± 149	2.3 ± 0.6
2.5	57.8 ± 10.7	3.2 ± 0.7	1310 ± 347	5.4 ± 1.2
8.3	66.7 ± 11.2	5.7 ± 1.0	2647 ± 735	12.0 ± 3.7
25	85.0 ± 10.8	6.6 ± 1.2	3430 ± 725	16.4 ± 4.3

Results are from 4 experiments in which mast cell purity was 73.2 ± 2.4%. Spontaneous releases of prostaglandin D_2 (PGD_2), leukotriene C_4 (LTC_4) and histamine were 4.4 ± 1.9, 0.9 ± 0.3 and 957 ± 336 pmol/10^6 nucleated cells respectively, the latter corresponding to 4.8 ± 1.1% of total histamine.

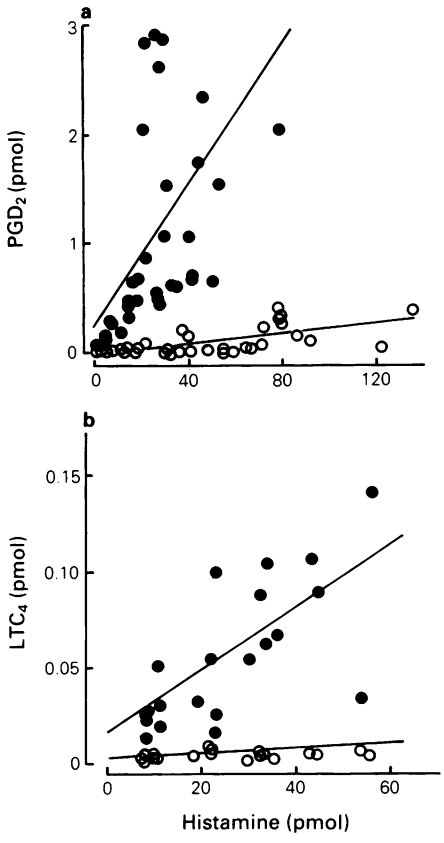

Figure 1 Relationship between net release of (a) prostaglandin D_2 (PGD_2) and histamine, (b) leukotriene (LTC_4) and histamine in skin cells separated by density-gradient centrifugation. Preparations containing 0.8–95.1% mast cells were activated with anti-IgE, 25 $\mu g ml^{-1}$ (●), or substance P, 30 μM (○). Release of PGD_2 and LTC_4 was measured in fractions from 5 and 3 density-gradients respectively.

that by anti-IgE (Figure 1a). Although this stimulus activated PGD_2 release which was significantly correlated with that of histamine ($r = 0.529$, $P < 0.01$), the shallower gradient of the correlation line demonstrated that the peptide was less effective than anti-IgE in stimulating release of this newly-generated mediator. Similar results were obtained when release of LTC_4 and histamine were compared for each stimulus (Figure 1b). Again, there was a significant correlation between release of the two mediators ($r = 0.441$, $P < 0.05$), but the greater effectiveness of the immunological stimulus in generating LTC_4 was demonstrated by the shallower gradient of the correlation line for substance P. The data from these experiments are summarised in Table 3. Whilst both stimuli induced the release of similar amounts of histamine per mast cell, as expected from the correlation analysis, substance P was less effective than anti-IgE in stimulating release of eicosanoids, the peptide releasing 21 fold less PGD_2 and 18 fold less LTC_4 than the immunological stimulus.

Several other non-immunological agents, which we have previously demonstrated to activate histamine release from skin mast cells (Benyon *et al.*, 1987a; Lowman *et al.*, 1988b), were also tested for their capacity to stimulate release of eicosanoids. In four experiments with skin cell preparations containing 76.2 ± 4.2% mast cells, comparisons were made of histamine and eicosanoid release following cell activation with anti-IgE or with substance P, VIP, somatostatin, compound 48/80, morphine and poly-L-lysine (Figure 2). Although histamine release was similar with each secretagogue, being in the range 3500–4500 $pmol/10^6$ nucleated cells, only the IgE-dependent stimulus released significant quantities of PGD_2 and LTC_4. In the same preparations, calcium ionophore A23187, like anti-IgE, stimulated release of all three mediators, a 1 μM concentration of this stimulus releasing 6882 ± 1065, 71.0 ± 6.6 and 7.7 ± 1.7 $pmol/10^6$ nucleated cells of histamine, PGD_2 and LTC_4, respectively.

Discussion

Mast cells dispersed from human skin resemble those of lung parenchyma in that both PGD_2 and LTC_4 are released in addition to histamine following

Table 3 Mediator release from density gradient-separated skin cells activated with anti-IgE (25 $\mu g ml^{-1}$) or substance P (30 μM)

	Net release (pmol/10^6 mast cells)		*Molar ratio of release*	
	Anti-IgE	*Substance P*	*Anti-IgE*	*Substance P*
Histamine	2897 ± 368	4132 ± 347	1000	1000
PGD_2	107 ± 15	7.1 ± 1.3	36.9	1.7
LTC_4	8.0 ± 0.6	0.7 ± 0.1	2.8	0.2

Results shown are from 20–35 fractions of 4–7 gradients. Net release of histamine by anti-IgE and substance P was 12.8 ± 2.1% and 17.9 ± 3.7%, respectively.

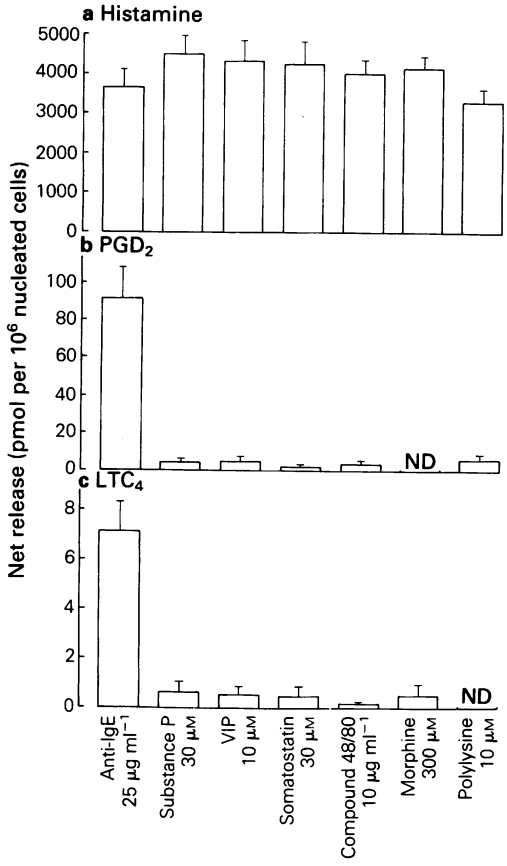

Figure 2 Comparison of the release of (a) histamine, (b) prostaglandin D_2 (PGD_2) and (c) leukotriene C_4 (LTC_4) from purified mast cells activated with anti-IgE or non-immunological stimuli. Results are from 4 experiments in which mast cell purity was $76.2 \pm 4.2\%$ and spontaneous releases of PGD_2, LTC_4 and histamine were 4.8 ± 0.7, 0.8 ± 0.4 and 910 ± 90 pmol/10^6 nucleated cells, respectively. Net release of histamine by each stimulus was: anti-IgE, $13.1 \pm 1.8\%$; substance P, $16.9 \pm 3.2\%$; vasoactive intestinal peptide (VIP), $15.8 \pm 2.9\%$; somatostatin, $17.2 \pm 4.1\%$; compound 48/80, $14.7 \pm 2.5\%$; morphine, $16.1 \pm 2.8\%$; poly-L-lysine, $13.3 \pm 3.2\%$. ND = not determined.

stimulation with anti-IgE. However, skin mast cells also release histamine after challenge with a variety of non-immunological stimuli to which lung mast cells, like those of tonsil, adenoid and intestine, are refractory (Lowman *et al.*, 1988a). These studies demonstrate that non-immunological stimuli differ from anti-IgE in being relatively poor activators of the release of PGD_2 and LTC_4 from human dispersed skin mast cells, despite inducing the release of similar amounts of histamine to the immunological stimulus.

Differential release of histamine and LTC_4 has been observed in human basophils activated with the complement fragment C5a (Schulman *et al.*, 1988) or hyperosmolar stimuli (Findlay *et al.*, 1981), although, to our knowledge, differential release of pre-formed and newly-generated mediators by IgE-dependent and non-immunological stimuli has not previously been demonstrated in human mast cells. Mast cells of human dispersed lung (Holgate *et al.*, 1984) and from patients with spleen mastocytosis (Robinson *et al.*, 1988) activated with anti-IgE release approximately twice as much PGD_2 per unit amount of released histamine compared to those stimulated by calcium ionophore A23187, but these differences are small compared to those between anti-IgE and non-immunological stimuli obtained in the present study. Our findings of large differences between anti-IgE and non-immunological stimuli in their ability to activate eicosanoid release from human skin mast cells, when considered together with their differing Ca^{2+}-dependency and time course of histamine release, would suggest that these two types of stimuli activate these cells by distinct biochemical mechanisms. Non-immunological stimuli may, therefore, be poor activators of mast cell cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism. As the activity of these pathways is primarily dependent on the supply of free arachidonic acid (Vonkeman & Van Dorp, 1968; Van den Bosch, 1980), it is possible that the two types of stimuli differ in their ability to activate the phospholipase A_2 enzymes responsible for the liberation of this fatty acid from membrane phospholipids.

The physiological implications of these findings with skin mast cells are uncertain but are worthy of consideration, as non-immunological stimuli, in the form of the substance P, VIP and somatostatin, have each been identified by histochemical techniques in mammalian dermal nerve endings (Hökfelt *et al.*, 1975; 1976; Hartschuh *et al.*, 1983; Johansson, 1986) and have been observed in close anatomical association with mast cells (Wiesner-Menzel *et al.*, 1981; Newson *et al.*, 1983; Skotfisch *et al.*, 1985). Ever since the proposal by Langley (1900) of an axon-reflex mechanism to explain the neurogenic spread of the flare component of the triple response, a large body of evidence has accumulated supporting the hypothesis that neuropeptides can increase dermal microvasculature blood-flow and, to a lesser extent, blood vessel permeability by activating mast cell histamine secretion (reviewed by Foreman *et al.*, 1987). The ability of skin mast cells to respond in different ways to IgE-dependent and non-immunological stimuli may allow them to have bi-functional roles in the dermis. The mounting of an effective tissue response to an allergic stimulus may require the release of the whole spectrum of mast cell mediators,

with their various chemotactic and vasoactive properties. For example, PGD_2 has several activities relevant to inflammation by potentiating IgE-dependent histamine release from basophils (Peters *et al.*, 1984) and stimulating leukocyte migration (Goetzl *et al.*, 1976). In contrast, neuropeptides appear to stimulate rapid release of a smaller variety of mediators including histamine, presumably in conjunction with other pre-formed mediators such as heparin and neutral proteases, which may have homeostatic rather than defensive functions such as control of blood flow (Schayer, 1962), angiogenesis (Marks *et al.*, 1986) or fibroblast proliferation (Pillarisetti *et al.*, 1983).

In conclusion, we have demonstrated that in human skin mast cells non-immunological stimuli, which include a variety of dermal neuropeptides, are of similar effectiveness to anti-IgE in releasing histamine but generate 10 to 20 fold less PGD_2 and LTC_4 from these cells. In combination with other differences such as their Ca^{2+}-dependency and time-course of histamine secretion (Benyon *et al.*, 1987a; Lowman *et al.*, 1988b), these findings suggest that these two types of stimulus have different mechanisms of mast cell activation. As activation of skin mast cells by neuropeptides may be involved in physiological and pathological processes, their detailed mechanism of mast cell activation would be a subject worth studying further.

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