

# Biological properties of human skin mast cells

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## Summary

Mast cells and basophils, although sharing many constitutive properties, are quite distinct in their development, functions and biological properties. Mast cell granules are composed of a macromolecular matrix of proteoglycan and neutral protease of which heparin and tryptase, respectively, are predominant. The distribution of the other major neutral protease, chymase, allows human mast cell subpopulations to be subdivided immunocytochemically. All human mast cells respond to IgE-dependent stimulation with the secretion of the preformed mediator, histamine, and the newly generated lipid-derived eicosanoids PGD<sub>2</sub> and LTC<sub>4</sub>. Although amounts of these products vary between mast cells dispersed from different tissues, it is uncertain whether this reflects true heterogeneity. Mast cells of the human skin, but not those of other tissues, are sensitive to stimulation by substance P, compound 48/80 and other basic non-immunological stimuli. The mechanism of mediator secretion induced by these agents is distinct from that induced by IgE-dependent stimulation. However, the morphological characteristics of degranulation are similar, suggesting that the distinct biochemical pathways merge into a common pathway before effecting degranulation.

## Development of mast cells

Mast cells and basophils are both involved in IgE-dependent allergic reactions. Superficially, these cells are very similar in that they both contain histamine and proteoglycan in their granules, bind IgE to the cell surfaces by a high-affinity FcεR1 and stain metachromatically with aniline dyes. However, they differ markedly in their distribution: basophils are predominantly a circulating leucocyte which infiltrates tissues on demand, whereas mast cells are essentially a tissue cell, developing from precursors in the tissue site.

The division of these cells into leucocytes and tissue cells is consistent with their differential development. Studies with basophils would suggest that they share a common precursor with eosinophils and other leucocytes [1,2] and that they have a life-span similar to eosinophils [3,4]. However, mast cells, unlike basophils, do not circulate in the blood as mature cells but develop following the deposition of blood borne precursors in the tissues. This has been elegantly demonstrated in mice by Kitamura *et al.* [5], who transplanted mast cell precursors into W/W<sup>v</sup> mice whose own precursor cells are unable to

respond to tissue derived growth factors due to their genetic deficiency of a *c-kit* receptor. Furthermore, these experiments strongly suggest that tissue-derived factors, probably emanating from fibroblasts, are essential for mast cell development and maturation.

Development of rodent mast cells has been shown to be dependent on products from T lymphocytes [6]. In addition, the deficiency of mast cells in T cell deficiency diseases in humans would suggest that the same may hold for human mast cells [7]. Attempts to culture mast cells has confirmed that murine mast cells require the presence of interleukin-3 (IL-3) for proliferation [8,9] and interleukin-4 (IL-4) for maturation [10]. Furthermore, co-culture of murine mast cells with 3T3 fibroblasts produces murine mast cells with the appearance of connective tissue type mast cells [11,12]. However, the quest of stimulate human mast cells to proliferate and mature in culture with IL-3 and IL-4 has resulted in the production of human basophils rather than mast cells [13]. The likely reason for this is that, unlike basophils or even murine mast cells, human mast cells do not possess cell surface receptors for IL-3 [14]. A more recent attempt to culture human mast cells using co-culture with 3T3 fibroblasts has met with some success [15], suggesting that human mast cells require tissue rather than T lymphocyte growth factors for proliferation.

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### Proteoglycan and protease heterogeneity in mast cells

The hypothesis that mast cells were not a single population of identical cells but consisted of at least two subpopulations of cells with distinctive properties was originally postulated by Maximow in 1906 [16]. However, it was not until the pioneering experiments of Enerback in 1966 [17,18] that the concept became firmly rooted. In these experiments, Enerback demonstrated that mast cells, rat skin and intestinal mucosa differed in their sensitivity to formaldehyde fixation and susceptibility to staining with alcian blue and safranin O dyes. These results reflect the charge differences of proteoglycans which constitute the granular matrix, that of rat skin and peritoneal mast cells being heparin [19] while that of intestinal mast cells is an over-sulphated chondroitin [20,21]. Whether human mast cells are heterogeneous with respect to their proteoglycan content is not yet entirely clear. The proteoglycan content of dispersed and purified human lung mast cells is comprised of approximately 65% heparin with the remainder being chondroitin sulphates A and E [22,23]. Although dissimilar in their protease content, preliminary experiments with human skin mast cells would suggest that they have a similar proteoglycan composition (unpublished observations). Although these findings would suggest a consistency of proteoglycan throughout human mast cells, experiments in which proteoglycans were extracted from whole colon biopsies suggested that mast cells at this site contain predominantly chondroitin E [24,25]. Thus an absolute definition of human mast cell proteoglycans must await studies with purified cells from a variety of tissues.

Closely associated with the glycosaminoglycan side chains of proteoglycans, neutral proteases provide another major macromolecular constituent of the granule matrix. In the rat the predominating proteases of the peritoneal mast cell, RMCPI (rat mast cell protease I), and of the intestinal mast cell, RMCPII, are both chymotrypsin-like and hence are termed chymases [26,27]. The use of antibodies to these proteases has established an immunocytochemical basis of mast cell heterogeneity in the rat.

The proteases of human mast cells are composed of at least three types on the basis of substrate activity, trypsin-like enzymes termed tryptases [28–30], chymotrypsin-like enzymes called chymases [31] and an enzyme with a profile similar to carboxypeptidase A [32]. Antibodies against the first two of these enzymes have been used widely to define mast cell heterogeneity at an immunocytochemical level [33–35]. All mast cells contain tryptase. In some, including most mast cells in the lung and those associated with the intestinal mucosa, tryptase is the only demonstrable protease. In contrast, the mast cells of the

skin and intestinal sub-mucosa contain chymase in addition. The distribution of these proteases led to the two sub-populations being termed MC<sub>T</sub> and MC<sub>TC</sub>, respectively [36].

### Functional heterogeneity of mast cells

#### *Eicosanoid production*

All mast cells of mammalian species bind IgE with high affinity to FcεR1 receptors on the cell surface. Cross-linkage of two or more IgE molecules brings their receptors into juxtaposition, thus providing the stimulus for exocytosis of preformed mediators and production of newly generated mediators. Also, introduction of calcium into the cell cytoplasm by the use of ionophores such as A23187 or ionomycin also provides a stimulus for mediator secretion. Experiments with rodent mast cells have suggested that there is marked heterogeneity in the ability of mast cells from different sites to produce the arachidonic acid-derived lipid mediators prostaglandin (PG) D<sub>2</sub> and leukotriene (LT) C<sub>4</sub>. For example, rat peritoneal mast cells produce predominantly PGD<sub>2</sub> while those from the intestinal mucosa produced less PGD<sub>2</sub> but significant levels of LTC<sub>4</sub> and LTB<sub>4</sub> in addition [37,38]. In order to examine whether this is the case in humans, we have examined the ability of mast cells dispersed from human skin, lung and intestine to produce eicosanoids on stimulation. In initial experiments [39], we demonstrated that skin mast cells incubated with [<sup>3</sup>H<sub>8</sub>]-arachidonic acid produce PGD<sub>2</sub> as their primary cyclo-oxygenase product and LTC<sub>4</sub> as their primary lipoxygenase product when examined by HPLC. Negligible amounts of other eicosanoids were produced. Quantification of eicosanoid production by radioimmunoassay in the absence of loading with arachidonate [39] demonstrated that following stimulation with anti-IgE, skin mast cells produced 3432 ± 725 pmol histamine, 84.9 ± 10.8 pmol PGD<sub>2</sub> and 6.6 ± 1.2 pmol LTC<sub>4</sub>/10<sup>6</sup> cells. In comparison, lung mast cells released 17027 ± 2072 pmol histamine, 153 ± 34 pmol PGD<sub>2</sub> and 55.3 ± 9.8 pmol of leukotrienes, of which 15% were LTC<sub>4</sub> and 85% were LTD<sub>4</sub>/LTE<sub>4</sub>, and intestinal mast cells produce 5797 ± 2881 pmol histamine and 121 ± 73 pmol PGD<sub>2</sub> per 10<sup>6</sup> mast cells [40]. Several observations may be made from these results. First, lung mast cells produce approximately nine times more leukotrienes than do skin mast cells suggesting a heterogeneity of eicosanoid production between populations of mast cells. Second, the majority of leukotrienes were present as LTD<sub>4</sub>/LTE<sub>4</sub>. As mast cells do not produce these products this presumably reflects the extracellular metabolism of LTC<sub>4</sub> [41]. Third, the mast cell populations released a surprisingly consistent amount of PGD<sub>2</sub> which in both skin and lung mast cells was higher than leukotriene

production. Thus,  $\text{PGD}_2$  production cannot be used as a basis to define mast cell heterogeneity whereas leukotriene production could be used. However, caution should be used in interpreting quantitative differences in leukotriene production as mast cell heterogeneity because of the potential variability of experimental techniques and cellular compositions of the incubates.

#### *Responsiveness to non-immunological stimuli*

A second major aspect of mast cell heterogeneity is the ability of subpopulations to respond differentially to non-immunological stimuli. For example, rat peritoneal mast cells respond with histamine secretion following incubation with a range of polybasic substances such as compound 48/80, poly-L-lysine and poly-L-arginine, opioids—including morphine, codeine, dynorphin and  $\beta$ -endorphin, neuropeptides such as substance P, vasoactive intestinal polypeptide (VIP), somatostatin and neurotensin and the complement peptides C3a and C5a [42–49]. In contrast, mast cells from the intestinal mucosa of rats are unresponsive to these agents [47,48,50].

We have extended these observations into human mast cells demonstrating that mast cells dispersed from skin, but not those dispersed by a similar enzymatic procedure from adenoids, tonsils, intestinal muscle and the region of the intestine comprising both the mucosal surface and the immediate sub-mucosal layers, are able to mount a secretory response to non-immunological stimulation with the neuropeptides, substance P, VIP and somatostatin as well as with compound 48/80, morphine and poly-L-lysine [51–53]. This provides clear evidence of functional heterogeneity between human mast cell subpopulations. Furthermore, unlike functional heterogeneity in rodent mast cells, that of human mast cells does not necessarily parallel immunocytochemical heterogeneity because both the skin and intestinal dispersates comprised mast cells mainly of the  $\text{MC}_{\text{TC}}$  subtype and yet they differ markedly in their susceptibility to neuropeptide stimulation.

#### **Differences between immunological and non-immunological stimulation in human skin mast cells**

One obvious difference between mediator release induced by immunological and neuropeptide stimulation is the time course of histamine release. Following stimulation with anti-IgE, histamine release progresses relatively slowly, release reaching completion approximately 6 min after challenge [51]. In contrast, histamine release from human skin mast cells induced by basic non-immunological stimuli of which substance P is archetypal, is rapid, being complete within 15–20 sec [51,54].

In experiments to determine eicosanoid release from

human skin mast cells with different stimuli [55], cells stimulated for 30 min with 25  $\mu\text{g/ml}$  anti-human IgE released  $2897 \pm 368$  pmoles of histamine,  $107 \pm 15$  pmoles of  $\text{PGD}_2$  and  $8.0 \pm 0.6$  pmoles of  $\text{LTC}_4/10^6$  mast cells, corresponding to molar ratios of 1000:37:3 [55]. In contrast, substance P at 30  $\mu\text{M}$  stimulated secretion of  $4132 \pm 347$  pmoles of histamine,  $7.1 \pm 1.3$  pmoles of  $\text{PGD}_2$  and  $0.7 \pm 0.1$  pmoles of  $\text{LTC}_4$ , giving molar ratios of 1000:1.7:0.2 [55]. Similar results occurred with VIP, somatostatin, morphine, poly-L-lysine and compound 48/80. These observations that neuropeptides and basic secretagogues caused minimal eicosanoid generation suggest a fundamental difference in the biochemical mechanisms leading to cell activation with anti-IgE and neuropeptides.

Elevation of intracellular calcium facilitates many biochemical processes including activation–secretion coupling. Following membrane stimulation with a physiological stimulus, calcium may be either stimulated to enter the cell through the membrane from the extracellular environment or mobilized intracellularly. For example, in rodent mast cells histamine release induced by IgE-dependent stimulation requires the influx of calcium from outside the cell whereas that induced by neuropeptides may proceed following only the mobilization of intracellular calcium [56,57]. In human skin mast cells we have examined the requirement of extracellular calcium for histamine release by measuring release in the presence of 1.8 mM extracellular calcium and when extracellular calcium had been chelated with 2 mM EDTA for 5 min before stimulation [51]. The results show that histamine release induced by anti-IgE has an obligatory requirement for extracellular calcium, release being negligible in the presence of EDTA. In contrast, histamine release from human skin mast cells induced by substance P, VIP, somatostatin, compound 48/80, morphine and poly-L-lysine is largely independent of the presence extracellular calcium [51], suggesting a mobilization of intracellular calcium in this process.

To confirm this hypothesis we used a fluorescent calcium probe, fura 2 [58], to monitor directly the changes in intracellular calcium levels following cell activation. Incubation of mast cells with anti-IgE in the presence of extracellular calcium histamine release was associated with a rapid elevation of intracellular calcium which reached a maximum at 20 sec and which decayed exponentially to baseline by 8 min. In the absence of extracellular calcium, however, there was neither a rise in intracellular calcium levels nor histamine secretion. This suggests that with IgE-dependent provocation, the calcium necessary for activation–secretion coupling is stimulated to enter the cell from the extracellular medium by the transient opening of calcium channels in the plasma

membrane. When substance P was used to activate mast cells, a similar calcium transient was seen which was more rapid in both onset and decay than that induced by anti-IgE, the peak being reached at approximately 12 sec and exponential to baseline occurring within 2 min. That both mediator release and calcium transient occurred similarly both in the presence and absence of extracellular calcium suggests that in contrast to immunological stimulation, activation with substance P stimulates human skin mast cells to mobilize the calcium necessary for activation-secretion coupling from intracellular stores, probably the endoplasmic reticulum. In other biological systems it has been suggested that generation of inositol 1,3,4,5-tetrakisphosphate (IP<sub>4</sub>) is responsible for regulating calcium entry through the plasma membrane [59], whereas mobilization of intracellular calcium is regulated by inositol 1,4,5-trisphosphate [60].

### Cyclic AMP involvement

The role of cyclic AMP in activation-secretion coupling in mast cells is controversial. In rat mast cells [61,62], human lung mast cells [63] and human basophils [64] immunological stimulation has been reported to induce a biphasic rise in intracellular cyclic AMP. The early peak, which occurs 5–15 sec after challenge, has been suggested to be an integral part of the immunological activation pathway [61,62]. The second smaller peak, which is seen at 1–2 min, is thought to be secondary to the stimulatory effects of PGD<sub>2</sub> released during secretion [65]. However, Peachell *et al.* [66] failed to find a significant elevation of cyclic AMP in human purified lung mast cells following immunological stimulation. Furthermore, in rat basophilic leukaemia cells, immunological challenge induces histamine release in the absence of cyclic AMP changes [34]. In addition, non-immunological stimulation of rat mast cells and human basophils by secretagogues such as calcium ionophore A23187, compound 48/80 or poly-L-lysine fails to elevate cyclic AMP levels despite inducing histamine release [64]. These results throw doubt on the obligatory role of cyclic AMP in activation-secretion coupling in mast cells.

In human skin mast cells of 85–90% purity, stimulation with anti-IgE in the presence of extracellular calcium induces a rise in cyclic AMP levels which peaks at 107% above a baseline of  $1.86 \pm 0.48$  pmol/10<sup>6</sup> mast cells 30 sec after challenge. Levels then decay rapidly and are followed by a smaller peak of 50% above baseline occurring 2 min after challenge. This is accompanied by a slow histamine release which begins approximately 1 min after challenge and is complete 5–6 min later. In the absence of extracellular calcium neither cyclic AMP changes nor histamine release are observed. Incubation of skin mast

cells with substance P in the presence of extracellular calcium, cyclic AMP levels rise to a maximum of 128% above a baseline of  $1.80 \pm 0.57$  pmol/10<sup>6</sup> mast cells 45 sec after stimulation. This decays more slowly than after immunological stimulation being still 47% above baseline at 5 min. In the absence of extracellular calcium the early part of the cyclic AMP rise was similar to that seen in its presence but the rise terminated earlier, at 30 sec, and at a lower level. A rapid histamine release was apparent both in the presence and absence of extracellular calcium indicating that influx of calcium from outside the cell is not necessary for either histamine release or cyclic AMP changes.

Studies on the mechanism(s) by which compound 48/80, substance P and mastopyran induce the release of histamine from rat mast cells has suggested a close association with regulatory G-proteins in the cell membrane [67,68]. In receptor mediated events, these G-proteins serve to couple receptors such as  $\alpha_2$ - and  $\beta_2$ -adrenoceptors, neurokinin NK<sub>21</sub>, NK<sub>2</sub> or NK<sub>3</sub> receptors or 5HT<sub>1A</sub> receptors to their respective catalytic units. Indeed, it has been suggested [69,70] that neuropeptides and related compounds interact directly with G-proteins possibly by mimicking the basic residue sequence in the third cytoplasmic loop of the receptor unit [71,72]. The observation of an elevation of cyclic AMP levels following substance P stimulation of human skin mast cells would support stimulation of the Ns protein which activates adenylate cyclase. However, our recent observation that pertussis toxin, an inhibitor of the Ni protein and possibly other G-proteins, reduces histamine release suggest that G-protein stimulation plays a key role in mast cell activation. In contrast, pertussis toxin does not reduce histamine release induced by anti-IgE, suggesting that immunological stimulation does not proceed by this mechanism. Similar results have been obtained in rat mast cells [9].

### Morphological aspects of mast cell degranulation

In dissociated human lung and rodent mast cells [73–76] and mast cells in human skin *in vivo* [77], immunological challenge results in the fusion of granule membranes with the cell membrane to form degranulation channels through which the preformed mediators are released into the extracellular environment. Also, granule membranes fuse with those of other granules to form large labyrinths (Fig. 1). This form of secretion is called compound exocytosis. We have recently established that mediator release from human skin mast cells progress by this mechanism regardless whether they are stimulated by anti-IgE or substance P, the only difference being the kinetics of degranulation channel formation [78]. These



**Fig. 1.** Compound exocytoses in a human skin mast cell 30 seconds after stimulation with substance P. Arrows indicate the opening of the degranulation channels to the extracellular space. Remnants of the proteoglycan-protease cores (c) of the granules are seen within the degranulation channels. These cores are less dense and do not have the crystalline structure of granules which are not undergoing secretion. Magnification  $\times 21\,000$ . Reproduced from [77] with permission.

results strongly suggest that the biochemical events leading to mast cell mediator release reach a common pathway, regardless of the mechanism of stimulation. This hypothesis is supported by our recent observations that inhibitors of protein kinase C are equally effective in inhibiting degranulation induced by anti-IgE and substance P (unpublished observations).

Morphometric analysis of human skin mast cells has revealed three further quantitative aspects of mast cell degranulation [78]. First, there is a linear relationship between histamine release and both loss of granules and formation of degranulation channels. Second, with concentrations of anti-IgE and substance P sufficient to produce  $10.2 \pm 1.7\%$  and  $21.4 \pm 4.0\%$  histamine release, respectively, degranulation channels occupied a mean of  $20.9 \pm 3.0\%$  and  $33.2 \pm 2.8\%$ , respectively, of the cytoplasmic volume. Furthermore, with both stimuli approximately 25% of the cells showed no increase in degranulation channel compared with control. These results strongly suggest that, while some cells do not respond, histamine release from the others is partial and graded and is not the result of a few cells being stimulated to release their entire content of histamine. Third, with both stimuli at least 75% of mast cells within the preparation showed some degree of degranulation. This strongly suggests that the cell population is not composed of subpopulations, one of which responds to immunological

**Table 1.** The effect of sodium cromoglycate on histamine release from human mast cells from different tissue sites

| Tissue site            | Drug activity | Tachyphylaxis |
|------------------------|---------------|---------------|
| Bronchoalveolar lavage | +++           | —             |
| Intestine              | +++           | —             |
| Adenoid                | ++            | —             |
| Tonsil                 | +             | +*            |
| Lung parenchyma        | +             | +*            |
| Skin                   | —             | —             |

—, no effect; +, weak effect; +\*, tachyphylaxis seen; ++, strong effect; +++, very strong effect.

stimulation and another which responds to basic secretagogues.

### Pharmacological modulation of mast cell mediator release

The effective inhibition of mediator release from mast cells has been the goal of many agents developed for the control of allergic diseases. Perhaps the drug most readily associated with this action is the so-called 'mast cell stabilizer', sodium cromoglycate. Although this drug is a potent inhibitor of mediator release from rat peritoneal and cutaneous mast cells [79,80], it is not so effective within different mast cell populations. For example, sodium cromoglycate has only a weak effect against mediator release from hamster mast cells and is devoid of activity in the mouse [81]. Similarly, while reducing histamine release from rat lung [82], it is ineffective in bovine, canine or guinea-pig lung [81,83,84]. Differences are also seen within a single species. For example, while mediator release from rat peritoneal mast cells is effectively inhibited by sodium cromoglycate, that released from mast cells of the intestinal mucosa is unaffected [81,85]. The effects of sodium cromoglycate on human mast cells are summarized in Table 1. As may be seen, there are broadly three types of activity. In mast cells obtained from the lung by bronchoalveolar lavage, sodium cromoglycate is an effective inhibitor of mediator release whose activity increases with pre-incubation time [86,87]. Results with intestinal and adenoidal mast cells show similar results (unpublished observations). In contrast, IgE-dependent histamine release from lung fragments [88] and mast cells dispersed from lung parenchymal tissue [89,90] are poorly responsive to sodium cromoglycate, concentrations of 100–1000  $\mu\text{M}$  being required to inhibit histamine release by up to 30%. Furthermore, these cells show rapid tachyphylaxis to this drug [89,90] similar to that seen with rat mast cells [91,92].

Different again are mast cells dispersed from human skin which are insensitive to the inhibitory effects of sodium cromoglycate [93]. These findings are supported by studies *in vivo* [94] and with human skin slices [95,96]. Similarly, human basophils are unresponsive to sodium cromoglycate [97].

Agents that elevate intracellular cyclic AMP levels also provide a mechanism to inhibit histamine release from human mast cells. The ability of  $\beta$ -adrenoceptor stimulants to inhibit histamine release from human lung fragments [88,98,99] and dispersed lung mast cells [99,100] is now well established. Unlike sodium cromoglycate, the effectiveness of  $\beta$ -adrenoceptor stimulants extends to other human mast cells including adenoids, tonsils and skin (unpublished observations). The effectiveness of  $\beta$ -adrenoceptor stimulants in human mast cells contrasts markedly with rat mast cells which are largely refractory to those agents [63].

### Conclusions

Mast cells are a heterogeneous population of tissue inflammatory cells. In their development and properties they are quite distinct from basophils which are in many respects their blood borne counterparts. The properties of mast cells summarized in this short review equip them for several roles in biology. The primary role of mast cells, particularly at mucosal surfaces, is thought by many to be one of host defence against parasitic infestation. In connective tissues, the close relationships between mast cells, blood vessels and nerves has led to the hypothesis that at this site they are involved in angiogenesis. More recently mast cells have been associated with tumours, scleroderma and wound healing. Clearly there are many positive functions of mast cells which may be balanced against their inappropriate activation which gives rise to many of the symptoms of allergic diseases in the nose, eyes, skin, intestine and lung.

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