

# Mast cell heterogeneity with special attention to cutaneous atopic disease

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Mast cells are the major primary effector cell type in diseases of immediate hypersensitivity, being activated by crosslinking of the high affinity receptors for IgE present in their plasma membranes. The array of mediators secreted by activated mast cells is directly responsible for the immediate response in such reactions and appears to be capable of initiating a more persistent inflammatory state, referred to experimentally as the late-phase response. Atopic dermatitis involves more than a derangement of immediate hypersensitivity alone, but mast cell involvement nevertheless appears to be an important component of the pathogenesis by virtue of affected individuals having high levels of IgE, having a high incidence of atopic rhinitis and asthma, and suffering exacerbations of their dermatitis together with histamine release at cutaneous sites and systemically with exposure to allergen. Two types of mast cells have been defined in humans based on different compositional and ultrastructural properties as well as T lymphocyte dependency (1-3). These are referred to as MC<sub>T</sub> (tryptase-positive and chymase-negative, mucosal-like) and MC<sub>TC</sub> (tryptase-positive and chymase-positive, connective tissue-like) cells on the basis of their distinctive neutral protease compositions. This review will summarize information relating to the characterization of these two mast cell types and their involvement in selected clinical conditions.

## *Characteristics of human MC<sub>T</sub> and MC<sub>TC</sub> cell types*

Trypsin-like (4) and chymotrypsin-like (5) activities each were initially detected in mast cells

within human cutaneous and gingival tissues by histochemical techniques. Later, one enzyme was found to be responsible for the major portion of each activity, called tryptase and chymase, respectively. Each was purified to homogeneity and characterized. Tryptase is a tetrameric neutral protease of 134 000 Mr. Each subunit of 31 000 to 35 000 Mr has an active site (6), apparently identical antigenic determinants (7) and the same N-terminal amino acid sequence (8), indicating that each subunit probably arises from the same gene. Tryptase is distinguished from pancreatic trypsin by its larger size and its resistance to inhibition by  $\alpha$ 2-macroglobulin and  $\alpha$ 1-trypsin inhibitor as well as to inhibition by plasma or urine. Tryptase is selectively concentrated in all mast cells; other cell types in blood, skin, bowel and lung contain no detectable enzyme by immunohistochemical techniques, except for basophils which contain only trace amounts. Based on a specific immunoassay the mean level of tryptase per mast cell from adult lung was 12 pg and from mature foreskin was 35 pg, whereas 0.04 pg were present per basophil (9, 10).

Human chymase is a monomeric neutral protease of 30 000 Mr (11). Chymase is immunologically and enzymatically distinct from bovine pancreatic chymotrypsin, human neutrophil cathepsin G, rat mast cell protease I and II and rat salivary gland tonin (12). Unlike tryptase, chymase is inhibited by protease inhibitors present in plasma. It is selectively concentrated only in mast cells of the MC<sub>TC</sub> type. By immunoassay there are 4.5 pg of chymase per mature foreskin-derived MC<sub>TC</sub> cell (10). This is approximately 10-fold higher than

Table 1

Selected characteristics of human basophils and MC<sub>T</sub> and MC<sub>TC</sub> cells

| Characteristic             | MC <sub>TC</sub> | MC <sub>T</sub> | Basophil |
|----------------------------|------------------|-----------------|----------|
| F <sub>ε</sub> I receptors | +                | +               | +        |
| Mediator                   |                  |                 |          |
| histamine                  | +                | +               | +        |
| tryptase                   | +                | +               | -        |
| chymase                    | +                | -               | -        |
| Morphology                 |                  |                 |          |
| nucleus                    | m                | m               | p        |
| granules                   | g,l<br>dc        | ds              | a        |
| T lymphocyte dependence    | -                | +               |          |

Key: +, present; -, absent; m, mononuclear; p, polymorphonuclear; g, grating; l, lattice; ds, discrete scroll; a, amorphus; dc, dense core.

the amount detected in dispersed human lung mast cells where the MC<sub>TC</sub> cell type accounts for about 10 % of the total number.

Two types of human mast cells were convincingly demonstrated by immunohistochemical techniques based upon apparent differences in neutral protease content, which were later confirmed by immunologic and enzymatic measurements of chymase and tryptase from dispersed cells containing a predominance of either the MC<sub>TC</sub> or MC<sub>T</sub> cell type (Table 1). The distribution of MC<sub>TC</sub> and MC<sub>T</sub> cell types in normal human tissues is summarized in Table 2. In both normal human skin and keloid, mast cells are localized throughout the dermis, concentrated superficially and near blood vessels, around hair follicles and between collagen bundles. Epidermis normally (and even in lesions of systemic mastocytosis) is devoid of mast cells. The MC<sub>TC</sub> cell is the dominant type in all regions of normal skin. Although 10 % to 20 % of the mast cells may be of the MC<sub>T</sub> type in breast skin, essentially all of the mast cells in foreskin are of the MC<sub>TC</sub> type. The MC<sub>T</sub> cell predominates in lung and bowel mucosa; the MC<sub>TC</sub> type predominates in bowel submucosa and skin; and a nearly even distribution of each type present in ocular conjunctiva and nasal mucosa (unpublished observations). It is impor-

tant to note that a mixture of MC<sub>TC</sub> and MC<sub>T</sub> cells resides in each location, making it almost impossible to define the mast cell type based on tissue location alone.

The distinguishing ultrastructural characteristics of MC<sub>TC</sub> and MC<sub>T</sub> cells only recently have been recognized (2). An indirect immunogold staining technique was developed using 15 nm gold particles conjugated to goat IgG anti-rabbit IgG (recognizing rabbit IgG antichymase) and 5 nm gold particles conjugated to goat IgG anti-mouse IgG (recognizing mouse monoclonal IgG antitryptase) to indentify MC<sub>TC</sub> and MC<sub>T</sub> in the same section. MC<sub>TC</sub> cells in skin and bowel submucosa stained strongly for tryptase and chymase over all granules, whereas MC<sub>T</sub> cells in lung and bowel mucosa stained strongly over all granules for tryptase alone. Thin sections adjacent to those stained by the immunogold technique were processed with uranyl acetate and lead citrate for optimal ultrastrutural detail. Granules of MC<sub>TC</sub> cells were larger and more homogeneous in shape and texture than those of MC<sub>T</sub> cells. The characteristic feature of MC<sub>TC</sub> granules was the presence of grating or lattice patterns only in granules of this cell type, whereas the presence of granules with discrete

Table 2

Distribution of human MC<sub>T</sub> and MC<sub>TC</sub> cells in normal tissues

| Tissue                  | % MC <sub>T</sub> | % MC <sub>TC</sub> |
|-------------------------|-------------------|--------------------|
| Dermis                  | -                 | ++                 |
| Respiratory tract       |                   |                    |
| alveolar wall           | ++                | -                  |
| bronchial epithelium    | ++                | -                  |
| bronchial subepithelium | ++                | +                  |
| nasal mucosa            | ++                | ++                 |
| Gastrointestinal tract  |                   |                    |
| small bowel             |                   |                    |
| mucosa                  | ++                | +                  |
| submucosa               | +                 | ++                 |
| large bowel             |                   |                    |
| mucosa                  | ++                | ++                 |
| submucosa               | +                 | ++                 |
| Conjunctiva             | +                 | ++                 |

++ = strongly present; + = present; - = nearly absent.

scrolls was characteristic only of MC<sub>T</sub> cells. A less common form of MC<sub>T</sub> cells contained granules with a particulate or beaded pattern. Granules without these distinguishing features also were commonly present within mast cells of each type. However, once a granule with a characteristic pattern could be identified in a given cell, all granules in that cell would have the same protease composition. Mast cells with discrete scrolls and grating/lattice features in the same cell have not been observed in normal human tissues. Thus, transition cells reflecting the possible interconversion of MC<sub>TC</sub> and MC<sub>T</sub> cells, if they occur *in vivo* at all, are quite rare in normal human tissues. Mast cells with particulate/beaded and discrete scroll features have been observed, indicating the close relationship of mast cells with these features and the rationale for referring to them as different forms of MC<sub>T</sub> cells rather than distinct cell types. In summary, the capability of correlating ultrastructural with compositional differences between mast cell types facilitated the recognition of characteristic morphological features that permit identification of MC<sub>TC</sub> and MC<sub>T</sub> cells by ultrastructural criteria alone.

Additional features of MC<sub>TC</sub> and MC<sub>T</sub> cells also may prove to be important for characterization. For example, the proteoglycan type has been evaluated chemically in dispersed lung mast cells, tissue fragments from bowel and lesional biopsies of systemic mastocytosis and histochemically in cutaneous and intestinal tissues. Evidence for heparin proteoglycan in skin (13) and lung (14, 15) and chondroitin sulfate E in lung (14, 15) and bowel (16) has been reported, but whether the distribution of these proteoglycans correlates in some way to the protease and ultrastructural differences noted above has not been clarified.

Although all mast cells and basophils undergo coupled activation-secretion mediated by IgE and by calcium ionophores (17-19), their responses to other secretagogues may differ. For example, the anaphylatoxin C5a and f-met-leuphe cause histamine release from basophils and skin-derived (MC<sub>TC</sub>) mast cells, but not from mast cells obtained from lung (mostly MC<sub>T</sub>) (19). Compound 48/80, morphine and sub-

stance P activate mast cells from skin, but not basophils or lung-derived mast cells (17-22). Thus, there initially appeared to be differences in the secretory response of these cells based on their designation as basophil, MC<sub>TC</sub> cell or MC<sub>T</sub> cell. However, more recent data comparing MC<sub>TC</sub> cells from skin and bowel showed that those from the bowel did not respond to compound 48/80 and substance P (23), suggesting that the local environment may influence the secretory response.

#### *Lineage of MC<sub>T</sub> and MC<sub>TC</sub> cells*

The lineage of mast cell subpopulations has been addressed experimentally in both human and rodent systems. Whether the fully differentiated subpopulations of mast cells in each species are derived along divergent or linear pathways, whether interconversions occur and, if so, by what mechanism, are issues that need to be resolved. In mice all mast cells are derived from a single hematopoietic precursor (23-25). That each type of mature mast cell develops from such a precursor by divergent pathways was suggested in mice by detection of distinct populations of committed precursors for mucosal and connective tissue types of mast cells (26), the growth *in vitro* of the mucosal type being supported by IL-3, that of the connective tissue cell type by IL-3 together with IL-4 (27). However, bone marrow-derived IL-3 dependent as well as peritoneal murine mast cells each give rise to both connective tissue and mucosal mast cells after being injected into mast cell deficient W/W<sup>v</sup> mice. Whether the mechanism for redirecting the lineage of these cells involves direct interconversion, transdifferentiation (dedifferentiation/redifferentiation) or both is controversial. Mouse bone marrow-derived mast cells co-cultured with mouse 3T3 fibroblasts produce primarily heparin rather than chondroitin sulfate and stain safranin-positive rather than negative, suggesting direct conversion of mucosal to connective tissue mast cells (29, 30). However, these bone marrow-derived murine mast cells are rather immature and may not yet be fully committed to a particular mast cell

lineage. *In vivo* data suggest that in W/W<sup>v</sup> mice injected mast cells dedifferentiate to regain the capacity to proliferate and redifferentiate (31).

Two studies have been performed in human tissues suggesting a separate lineage for MC<sub>TC</sub> and MC<sub>T</sub> cells. In humans, postmortem cases of combined immunodeficiency diseases and those of acquired immunodeficiency syndrome showed marked and selective decreases in MC<sub>T</sub> cell concentrations in bowel mucosa and submucosa whereas the distribution and concentration of MC<sub>TC</sub> cells was not altered (3). This suggested a dependence of MC<sub>T</sub> cells on T lymphocytes and indicated that development of MC<sub>TC</sub> cells proceeds independently of MC<sub>T</sub> cell development.

Additional support for separate maturational pathways for MC<sub>TC</sub> and MC<sub>T</sub> cells was obtained by an ultrastructural study of immature human mast cells (32). Immaturity was defined by morphological criteria and mast cell type was analyzed by immunogold staining as above. MC<sub>TC</sub> and MC<sub>T</sub> cell types could be clearly identified. Immature MC<sub>T</sub> cells (like mature cells) had granules with discrete scrolls, whereas immature MC<sub>TC</sub> cells had granules with electron dense circular cores surrounded by a less electron dense matrix. Occasionally, grating/lattice features could be seen within an electron dense core granule, indicating the probable derivation of one from the other. These compositional and ultrastructural differences between immature MC<sub>T</sub> and MC<sub>TC</sub> cells suggests that each one is on a distinct developmental path at or before the developmental stage when granule formation begins.

#### *Mast cells and cutaneous allergic reactions*

The skin chamber model has been useful for studies of cutaneous allergic reactions, because the presentation of antigen is better controlled in the skin chamber fluid than by injection and because the array of mediators released into the fluid can be assessed quantitatively and sequentially. Histamine appears maximally by 30 min after allergen challenge, tryptase by 60 min (33). Because tryptase and histamine are re-

leased together with degranulation, this delayed appearance of tryptase most likely reflects slower diffusion of tryptase (which is released as a complex with proteoglycan) through the cutaneous tissue. If the allergen challenge is interrupted by replacement of the fluid with buffer alone after the first hour, tryptase and histamine levels decline back to unstimulated levels within 4 h, and little, if any, evidence for a clinical late-response is apparent in spite of an influx of leukocytes, including basophils, after the first hour.

A clinically apparent late-response does occur if allergen remains continuously in the chamber fluid. Nevertheless, tryptase levels return to baseline as before (34). In contrast, histamine levels decline to a modestly elevated level after the first hour of allergen and remain elevated for the duration of the 6-h continuous challenge. A cellular influx is apparent, but basophils are rarely detected under these conditions. The most likely explanation for the persistent appearance of histamine without tryptase in the chamber fluid during the late-response to continuous allergen is that basophils are drawn into these lesions from the circulation, degranulate when exposed to the allergen and consequently lose their morphological and staining characteristics. An alternative possibility is that releasable tryptase (but not histamine) is depleted from local cells; however, codeine (which activates mast cells but not basophils) administered after 5 h of continuous allergen challenge releases tryptase and histamine (35). In addition, rechallenge with allergen after 1 h of allergen and 4 h of buffer results in the release of histamine but not tryptase, presumably due to activation of accumulated basophils without activation of local mast cells which had been desensitized. Against involvement of basophils is their apparent absence in late-phase skin chamber reaction sites, in spite of their ability to be easily detected after allergen-mediated activation-secretion *in vitro*. However, in other studies basophils have been detected at sites of cutaneous allergen challenge in atopic subjects (36), even though a selective accumulation of basophils has not been reported (37). Possibly, the combined chemotactic

and secretory stimuli encountered *in vivo* provokes a much stronger response than observed with allergen *in vitro*. I would predict that basophils may be a more active participant in late-phase allergic reactions than previously appreciated – including in atopic dermatitis – and that better reagents for identification of basophils and assessment of their activation will be needed in order to directly examine this hypothesis.

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