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Tissue-specific expression of mast cell granule serine proteinases and their role in inflammation in the lung and gut

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SUMMARY

Serine proteinases with trypsin-like (tryptase) and chymotrypsin-like (chymase) properties are major constituents of mast cell granules. Several tetrameric tryptases with differing specificities have been characterized in humans, but only a single chymase. In other species there are larger families of chymases with distinct and narrow proteolytic specificities. Expression of chymases and tryptases varies between tissues. Human pulmonary and gastrointestinal mast cells express chymase at lower levels than tryptase, whereas rodent and ruminant gastrointestinal mast cells express uniquely mucosa-specific chymases. Local and systemic release of chymases and tryptases can be quantified by immunoassay, providing highly specific markers of mast cell activation. The expression and constitutive extracellular secretion of the mucosa-specific chymase, mouse mast cell proteinase-1 (mMCP-1), is regulated by transforming growth factor-β_1 (TGF-β_1) *in vitro*, but it is not clear how the differential expression of chymases and tryptases is regulated in other species. Few native inhibitors have been identified for tryptases but the tetramers dissociate into inactive subunits in the absence of heparin. Chymases are variably inhibited by plasma proteinase inhibitors and by secretory leucocyte protease inhibitor (SLPI) that is expressed in the airways. Tryptases and chymases promote vascular permeability via indirect and possibly direct mechanisms. They contribute to tissue remodelling through selective proteolysis of matrix proteins and through activation of proteinase-activated receptors and of matrix metalloproteinases. Chymase may modulate vascular tissues through its ability to process angiotensin-I to angiotensin-II. Mucosa-specific chymases promote epithelial permeability and are involved in the immune expulsion of intestinal nematodes. Importantly, granule proteinases released extracellularly contribute to the recruitment of inflammatory cells and may thus be involved in innate responses to infection.

INTRODUCTION

Mast cells are particularly rich in neutral serine endopeptidases that are stored in and released from the secretory granules. At the time of writing this review, over 50 mast cell-derived serine endopeptidases in 11 species have been identified (see the SWISS-PROT and TrEMBL databases; ref. 1). The vast majority of these enzymes have trypsin- or chymotrypsin-like activities that are highly selective for different target substrates. The purpose of this review is to describe some recent developments in our understanding of the functions of these abundant proteolytic enzymes and how their expression is regulated, with particular emphasis on mast cells at mucosal surfaces of the lung and gut.

For convenience, the mast cells found in the lamina propria or within the epithelium of mucosal surfaces will be referred to as mucosal mast cells (MMC).[2] When compared to serosal mast cells (SMC) or to connective tissue mast cells (CTMC) in skin and skeletal muscle, MMC in rodents are morphologically[3] and functionally[4] atypical, with distinctive fixation and histochemical properties[3] as well as a distinctive content of granule proteinases.[2] Although the phenotypic and functional differences between MMC and CTMC are less distinct in humans, human mast cells are heterogeneous in their expression of granule proteinases in that there is differential expression of tryptase (with trypsin-like

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activity) and chymase (with chymotrypsin-like activity) by mast cells in different tissues.[5] This heterogeneous expression of granule proteinases may be regulated by the local environment in a 'tissue-specific' manner[2,6,7] and by differences in genetic background, as demonstrated between inbred strains of mice.[7,8]

The tissue specificity of proteinase expression by mast cell subsets[6,9] suggests that specific inhibitors and target substrates for the proteinases vary from tissue to tissue. Thus, proteinases released by MMC located within gut epithelium during nematode infection[10] are initially likely to encounter lower concentrations of plasma-derived proteinase inhibitors such as α_2-macroglobulin and serpins[11] when compared with CTMC in the vicinity of small blood vessels. Extravasation of plasma that is rich in inhibitors would rapidly inactivate chymases released by CTMC, but it may take longer for the inhibitors to diffuse into the epithelium and to reach sequestered MMC. The *in vivo*, extracellular functions of mast cell granule proteinases will therefore be governed by:

- the specificity of the proteinase;
- the efficacy of inhibition and the ratio of proteinase to inhibitor;
- the solubility and stability of the proteinase itself; and
- the accessibility of target substrates and their susceptibility to proteolysis.

MMC play key roles in airway and gastrointestinal pathologies,[12] including atopic asthma[13] nematode infections[14] stress-induced enteropathies[15] and reperfusion injuries.[16] MMC both infiltrate and migrate through mucosal epithelia,[17] which is consistent with data suggesting that they are involved in the pathogenesis of inflammatory changes within the epithelium itself.[18] Such a notion is supported by recent studies showing that targeted deletion of the MMC-specific chymase, mouse mast cell proteinase-1 (mMCP-1),[19] expressed by predominantly intraepithelial mast cells, leads to delayed expulsion of the intestinal nematode, *Trichinella spiralis*[14] which is, itself, an intraepithelial parasite. Furthermore, the range of mast cell granule serine proteases with distinct chymotryptic, tryptic and dual tryptic/chymotryptic specificities (Table 1) suggest that these cells have diverse and potentially significant proteolytic functions.

VARIANT EXPRESSION OF GRANULE PROTEINASES AND PROTEOGLYCANS IN MAST CELLS

Classical histochemistry in the 1950s and 1960s established that CTMC were rich in esterases,[47] and rat mast cell proteinase-1 (rMCP-1) was the first chymase to be isolated from CTMC granules.[48,49] A second, much more soluble rat mast cell chymase, originally described as an intracellular

Table 1. Properties of selected mammalian mast cell granule serine proteinases

Species	Proteinase	Specificity	SWISS-PROT	MW*	PI*	Reference
Man	Tryptase-αI	T	P15157	27 701	6·20	20
	Tryptase-βI	T	Q15661	27 444	6·30	21,22
	Tryptase-βII	T	P20231	27 458	6·46	22,23
	Tryptase-γI	T	Q9NRR2	30 230	6·24	24,25
	Chymase	Cα	P23946	25 030	9·60	26,27
	Cathepsin G	T/C	P08311	25 441 (Ile21–Ser244)	11·51 (Ile21–Ser244)	28
Mouse	MCP-1	C	P11034	24 956	8·46	29
	MCP-4	C	P21812	25 146	9·67	30
	MCP-5	Cα	P21844	25 343	9·51	31,32
	MCP-6	T	P21845	27 483	6·21	33
	MCP-7	T	Q02844	27 411	5·69	34
	Transmembrane tryptase	T	Q9QUL7	29 788	5·88	25
Rat	MCP-1	C	P09650	25 191 (Ile21–Asp247)	9·77 (Ile21–Asp247)	35
	MCP-2	C	P00770	25 044	8·70	36,37
	MCP-6	T	P50343	27 473	5·90	38
	MCP-7	T	P27435	27 432	5·89	39,40
Dog	Tryptase	T	P15944	27 153	6·37	41
	Chymase	Cα	P21842	25 461	9·93	42,43
Sheep	Tryptase-1	T	Q9XSM1	27 376	5·52	9
	Tryptase-2	T	Q9XSM2	27 494	5·68	9
	MCP-1	T/C	P80931	24 952	8·90	44
Cow	Tryptase	T	Q29464	27 302	8·12	45
	Duodenase†	T/C	P80219	25 051	9·03	46

*For theoretical core protein.
†Detected in bovine intestinal mast cells (A. D. Pemberton & T. S. Zamolodchikova, unpublished).
Cα, α-chymase; C, other chymase; MCP, mast cell proteinase; MW, molecular weight; PI, isoelectric point; T, tryptase.

'group-specific' protease and isolated from intestinal mucosa,[36] was subsequently shown to be of mast cell origin[50] and was categorized as rMCP-2. A wide variety of mast cell granule chymases have now been identified and in rodents they are numbered (Table 1) according to the chronology with which they were discovered. Studies on phylogeny from aligned amino acid sequences show that rMCP-1 and -2 belong to the beta chymase group and are in a different evolutionary branch from the family of mast cell granule alpha chymases.[51] The latter include human[52] and dog[43] chymases, rat mast cell proteinase-5 (rMCP-5)[40] and mouse mast cell proteinase-5 (mMCP-5);[31,53] (see below and Table 1).

A trypsin-like histochemical activity was also described in mast cells[48] and human tryptase was later purified from pulmonary mast cells.[54] This neutral proteinase is unusual in that it functions as a tetramer (see below) that is stabilized by granule heparin[13] and is ubiquitously expressed in human (≈35 pg/cell), canine and ruminant mast cells,[9,13,55] but is selectively expressed in subpopulations of mast cells in rodents.[56] Tryptases, like chymases, comprise a large family of genes[24,57] and there is increasing evidence of different tryptase specificities as well as selective expression of tryptase genes.[24] Sequencing of human chromosome 16p has revealed at least three functional tryptase genes, with tryptases αI, αII and βI, βII and βIII, and γI, γII and transmembrane tryptase, being apparent allelic variants at these three loci.[21,24,25] Other neutral proteinases in mast cells include cathepsin G[58] (Table 1) and a tryptase-like monomer, dog MCP-3.[59]

Heterogeneous expression of granule proteinases by mast cell subpopulations was initially described in rodent mast cells where it was shown, using specific antibodies, that rat MMC expressed the highly soluble beta chymase rMCP-2, but lacked the insoluble and strongly basic beta chymase, rMCP-1.[60] Conversely, CTMC contain rMCP-1 and lack rMCP-2[60] and this was later confirmed through analysis of mRNA transcripts[61] and by immunohistochemistry and two-dimensional sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of isolated rat MMC.[62] An homologous, soluble chymase, mMCP-1,[29] is uniquely expressed in mouse MMC that are predominantly located within mucosal epithelia.[63–66] Similarly, in normal sheep, sMCP-1, a dual-specific chymase/tryptase (Table 1) is expressed by MMC in the gut, but not by mast cells in the adjacent submucosa.[9] Thus, in rodents and sheep, intestinal MMC have a distinct proteinase phenotype.

Levels of mMCP-1, rMCP-2 and sMCP-1 are substantially increased in nematode parasite infections of the gut where there is hyperplasia of MMC.[67–69] Nematode infection is also associated with altered profiles of expression of proteinases by MMC such that, in mouse, mMCP-4, -5, -6 and -9 are expressed to varying degrees at different time-points after infection.[70,71] Rat MMC may also express the putative proteinases, rMCP-3, -4, -8, -9 and -10,[40,72] and low levels of the homologue of human chymase, rMCP-5[38] but they apparently lack tryptase.[38,56] An interesting subtext to the concept of 'tissue-specific' expression of mast cell proteinases, is evidence of strain-specific expression of the putative chymase, mMCP-2,[7,71] and this reflects the probable differential expression of transcription factors in different strains of mice.[8]

Analysis of mast cell granule proteinases in human tissues indicated that while all mast cells expressed tryptase, the majority of mast cells in the gut express relatively little chymase[73] and similar observations were reported for canine enteric mast cells.[74] However, more recent studies suggest that the majority of human enteric mast cells do, in fact, contain chymase.[75,76] The degree of expression of chymase relative to tryptase in the human gastrointestinal tract may therefore be low, but there does not appear to be a unique MMC-specific proteinase phenotype in human intestine. Mast cells in the mucosae of human, rat, canine and ruminant intestines, when compared with populations in other tissues, are relatively numerous and in the dog this is reflected by the higher concentrations of tryptase in intestine than in any other organ.[77] In contrast, mast cells in normal mouse intestine are rare.[78]

Mast cells are also rare in pulmonary parenchyma and airways in the mouse[64] and are located predominantly around the main-stem bronchi and have a CTMC-like phenotype (Fig. 1).[79] Thus, apart from the occasional intraepithelial MMC expressing mMCP-1,[64] granule proteinases released in mouse lung will probably be from mast cells around the major airways. This contrasts with larger vertebrates, including primates, where mast cell density in the peripheral bronchioles is substantially greater than around the conducting airways (Fig. 1).[80–82] In rats, sheep, cattle and humans pulmonary mast cells all express tryptase,[9,38,56,83,84] but the expression of chymase is not ubiquitous in human lung;[85] a significant proportion (73%) of mast cells close to glands contain chymase whereas, in smooth muscle, this decreases to 14%.[85] Chymase expression in rat and ovine lung is modified by nematode infection[86,87] and by allergic sensitization and challenge.[88]

In addition to the proteinase heterogeneity described above, there is heterogeneity of granule glycosaminoglycans (GAG).[3] For example, rat MMC granules contain chondroitin sulphates E and di B, and dermatan sulphate,[89,90] and human MMC apparently contain heparin with a lower degree of sulphation than that of the granule heparin in CTMC.[3] The presence of proteoglycans, such as heparin, in the granules is essential for the storage of chymases and histamine, as demonstrated by the absence of these granule constituents in heparin-deficient mice.[91,92] Heparin is also a key contributor to the stabilization of the tryptase tetramer that, in the absence of heparin, dissociates into four non-functional subunits (see below).

MMC are unaffected by the targeted deletion of the heparin-synthesizing enzyme *N*-sulphotransferase, whereas CTMC are unable to store mMCP-4 and -5.[91] This is consistent with modelling studies which suggest that the negative charge of GAG side-chains on the proteoglycans regulate the storage of those neutral serine proteinases with positively charged domains.[93] Thus, in terms of function, the relationship between negatively charged proteoglycans and positively charged residues on the proteinases are

of critical importance.[94] This was further confirmed when, during immunoglobulin E (IgE)-mediated systemic anaphylaxis, it was shown that mMCP-6, a tryptase with a lysine/arginine-rich domain distant from its active site, was retained in the vicinity of degranulated mast cells in association with granule heparin.[95] In contrast, the tryptase, mMCP-7, that lacked this positively charged domain, was released from the cells and was found in the bloodstream.[95] The lack of heparin in rodent MMC may also account for the high solubility of mMCP-1 and rMCP-2 and for the rapid, concomitant release of rMCP-2 and GAGs into peripheral blood during systemic anaphylaxis.[96] The heterogeneity of the GAGs of mast cells at sites of inflammation,[97] as well as the strong net positive charge of most chymases (Table 1) and the positively charged domains on tryptases, indicate therefore that patterns of storage and release of these proteolytic enzymes will differ from tissue to tissue.

SYSTEMIC RELEASE OF MAST CELL GRANULE PROTEINASES, MARKERS OF MAST CELL ACTIVATION

The expression of rMCP-2 in the gastrointestinal tract and the fact that it is such a soluble and abundant enzyme[67] suggested that, when released from MMC granules during intestinal allergic responses, it might be detectable systemically in peripheral blood. This was confirmed experimentally in rats infected with enteric nematodes,[98] and enzyme-linked immunosorbent assays (ELISA) were developed to quantify rMCP-2, mMCP-1 and sMCP-1 in peripheral blood and lymph[10,99,100] and established the involvement of MMCs in intestinal allergic responses[101] in reperfusion injuries[16] and in enteric neuroendocrine responses.[102–104] Levels of mMCP-1 and rMCP-2 in the blood of nematode-infected rodents can reach 5–10 μg/ml,[10,99] and up to 1 mg of rMCP-2/ml of plasma has been detected in rats during anaphylactic shock.[101]

Antibodies that permit the detection of α-tryptase were initially used to quantify tryptase in plasma from allergic patients[105,106] and more recently it has proved possible, using different monoclonal antibodies, to distinguish between α- and β-tryptases.[106] Elevated levels of tryptase have been reported in bronchoalveolar lavage fluid,[107,108] synovial fluid,[106] tears[109] and nasal secretions.[110] These levels are rarely >500 ng/ml as compared with the microgram quantities of mMCP-1 and rMCP-2 in rodent plasma. In some instances the locally detected release of tryptase correlated well with other signs of allergic responses, such as vascular permeability.[110] The tryptase assay may be less reliable in the retrospective diagnosis of systemic allergic responses when compared with measurement of plasma histamine levels,[111] although systemic histamine levels may indicate the participation of non-mast cell effector cells such as basophils. However, the timing of tryptase measurement may be crucial as reliable and repeatable increases in plasma tryptase have been reported 1 hr after allergen inhalation,[112] and release of tryptase into the gut lumen appears to be a reliable indicator of both cold pain stress and, in food-allergic patients, response to antigen challenge.[113] Because tryptase is apparently unaffected by plasma proteinase inhibitors,[114] the tryptase assay can be used postmortem although, again, other parameters seem more reliable for retrospective diagnosis of anaphylactic deaths.[115] Only low levels of tryptase have been described in human basophils[116] and it is clear that when tryptase is released into peripheral blood, the source is mast cells.[117]

MECHANISMS GOVERNING THE VARIANT EXPRESSION OF GRANULE PROTEINASES

The mechanisms underlying differential expression of granule proteinases and the consequent heterogeneity of mast cells in the intestine and connective tissues are not fully understood. Kitamura and colleagues adoptively

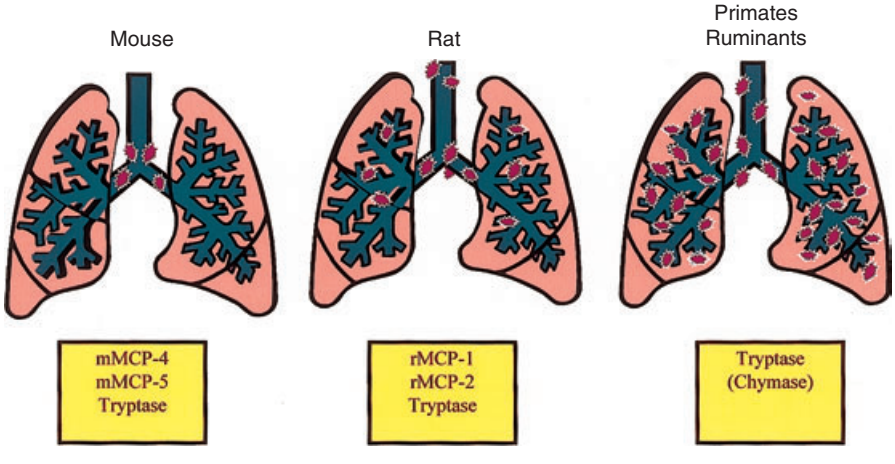

Figure 1. Schematic representation of the distribution of mast cells in the lungs of mouse, rat, primates and ruminants. Note that the larger mammals have a substantial proportion of mast cells in the lung parenchyma, whereas in the mouse, the mast cells are located predominantly adjacent to the major airways. The proteinases that are predominantly expressed in the airways are shown in the boxes below each diagram. mMCP, mouse mast cell proteinase; rMCP, rat mast cell proteinase.

transferred SMC into the gastric wall of mast cell-deficient W/W^V mice and noted that phenotype was, to a large extent, governed by the tissue in which mast cells were located.[118,119] Histochemical analysis of the proteoglycan content of the transferred cells suggested that SMC produced heparin proteoglycan when transferred into connective tissues such as the gastric submucosa, but switched production to non-heparin proteoglycans when they entered the gastric mucosa.

Subsequent studies examining the tissue- and strain-specific expression of the chymases, mMCP-2 and -4, and the tryptase, mMCP-6, suggested a more complex process.[71] In essence, cultured mast cells derived from the bone marrow of $WBB6F_1^{+/+}$ mice and implanted into the gastric wall of mast cell-deficient $WBB6F_1$-W/W^v mice expressed the granule chymase mMCP-2 when located in the mucosa, but not in the muscularis. In contrast, implanted SMC expressed mMCP-2, regardless of their location in the stomach. Additional experiments, in which the numbers of implanted SMC were varied, showed that differences in chymase expression, including the expression of mMCP-1, occurred when the cells proliferated after implantation.[120] This result, supported by additional observations on the expression of mMCP-4 and -6, provided convincing evidence that extracellular factors regulated proteinase expression *in vivo*.[71] These experiments[71] also confirmed previous studies showing that the expression of mMCP-2 was strain-dependent.[7]

Regulation of mast cell granule chymase expression by extrinsic factors *in vitro* was reported when rat bone marrow cells, cultured in T-cell conditioned medium, were found to express abundant rMCP-2.[121] Since then, a variety of cytokine combinations have been used to investigate the expression of mast cell granule proteinases in cultures of rodent, human and ovine bone marrow cells.[122–127] As yet there are no obvious clues as to why human mast cells express tryptase with variable expression of chymase. Human mast cells derived by culturing adult bone marrow, peripheral blood leucocytes or fetal cord blood cells vary in the level of chymase and tryptase expression, depending on the source of cells, on the growth factors added to the culture medium[128,129] and on inherent, clonally regulated expression of chymase.[130] Most studies show the absolute requirement for stem cell factor (SCF) to initiate and maintain mast cell growth from bone marrow or cord blood cells, and the differentiating mast cells express tryptase after several weeks.[125,131] Supplementation with interleukin (IL)-6 enhances mast cell growth with concomitantly increased expression of tryptase[125] and there is a suggestion that expression of tryptase precedes that of chymase,[131] but the addition of recombinant human IL-4 did not significantly alter proteinase expression.[131] Conditioned medium from a human mast cell line did, however, upregulate chymase expression and generated tryptase-negative/chymase-positive cells.[128] The mechanisms that might regulate the *in vivo* expression of chymase and tryptase in human tissues are not therefore readily resolved from these *in vitro* studies.

The expression of mMCP-1 by implanted SMC after they have proliferated in the gastric mucosa[120] is consistent with the results of an *in vitro* study of cultured rat SMC showing that IL-3 and SCF promote expression of the MMC-specific chymase, rMCP-2, in a subpopulation of proliferating SMC.[121] *In vivo* analysis of MMC hyperplasia during nematode infection in the mouse, showing that mMCP-1 is expressed very early during differentiation,[132] is substantiated by recent *in vitro* studies on the expression of mMCP-1 and its regulation by the multifunctional cytokine, transforming growth factor-β_1 (TGF-β_1).[133,134] The addition of recombinant TGF-β_1 to mouse bone marrow mast cells (mBMMC) promotes the expression of mMCP-1, and kinetic analysis shows that, within 4 days of initiating a bone marrow culture in the presence of SCF, IL-3, IL-9 and TGF-β_1, $\approx 40\%$ of the cells are mMCP-1-expressing mBMMC and, by day 7 of culture, $>85\%$ of the cells are mMCP-1 positive.[133] Supplementation with TGF-β_1 promotes the extracellular release of mMCP-1 into the culture supernatant in a dose-related response[134] and this observation is consistent with the concept of a non-IgE-mediated systemic release of mMCP-1 during nematode infection.[2] *In vitro*, mMCP-1-positive mBMMC express the integrins $\alpha_\varepsilon\beta_7$, the membrane tyrosine kinase receptor for SCF, c-*kit*, and the high-affinity receptor for IgE.[133] The morphology of these mBMMC with their large, variably shaped mMCP-1-positive granules, and the fact that they express the integrin α_E (Fig. 2), suggests that they are true homologues of MMC.[135,136] In this respect they are very similar to the rat BMMC grown in the presence of T-cell conditioned medium[121] that are biochemically and functionally identical to isolated rat MMC.[62]

The expression of mMCP-1 by intraepithelial mast cells in parasitized mice[64] indicates that TGF-β_1 is probably a differentiation factor for MMC sequestered in the epithelium. As this cytokine is secreted by epithelium,[137] the question is how it is converted from the latent to the mature and functionally active form in this location. One probable mechanism is through the integrins $\alpha_v\beta_6$ that are expressed by epithelia and that bind RGD motifs on the latency-activated peptide (LAP) of TGF-β_1.[138,139] Once binding of TGF-β_1-LAP has occurred, sites on the β6 cytoplasmic domain become accessible for binding to the actin cytoskeleton and this results in the activation of TGF-β_1 and its presentation as a cell surface-bound cytokine that will interact with cognate receptors on adjacent cells.[139] Preliminary studies using β6 knockout mice[139] infected with the intestinal nematode, *Nippostrongylus brasiliensis*, suggest that this integrin is essential both for the recruitment of mast cells and for the expression of mMCP-1 (P.A. Knight *et al.*, unpublished). This preliminary finding supports the concept that the activation of TGF-β_1-LAP and its cell-surface presentation via $\alpha_v\beta_6$ is a key event in the expression of mMCP-1 (Fig. 3). This epithelially regulated mechanism is also consistent with the expression of SCF and IL-9 by epithelial cells[140] (Fig. 3), but may be unique to the mouse because, in other species, MMC hyperplasia occurs both in the lamina propria and epithelium.[2]

An alternative mechanism of activation of TGF-β_1-LAP, which in the rat is stored in the granules of SMC, is the cleavage of the latent form by rMCP-1 after both have

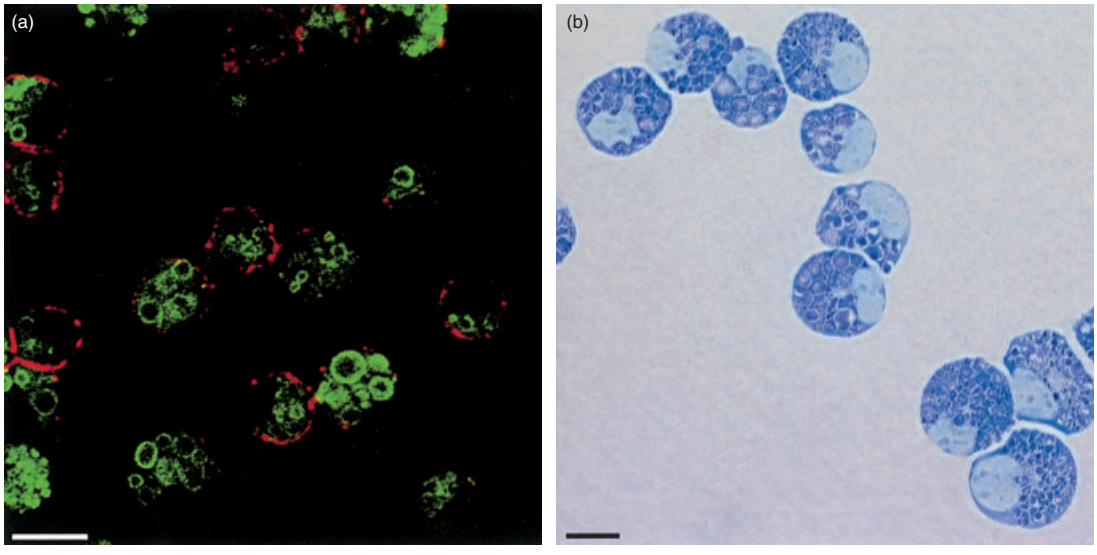

Figure 2. Confocal image (a) of 14-day-old-mouse bone marrow cultures demonstrating the presence of mature mast cells with abundant granules containing mouse mast cell proteinase-1 (green fluorescence) and expressing the integrin α_E (red fluorescence) on their surface membranes. A light micrograph (b) of the mouse bone marrow mast cells (mBMMC) stained with Leishman's shows that they are mature, heavily granulated cells. The cells were grown in medium containing recombinant mouse interleukin (IL)-3, IL-9 and stem cell factor (SCF) supplemented with recombinant human transforming growth factor-β_1 (TGF-β_1), as described in detail by Miller *et al.*[134] (Horizontal bars represent 10 μm.)

been released from the granules following degranulation.[141] Human chymase similarly will cleave the latent form of TGF-β_1.[141] Activated TGF-β_1, released during degranulation, stimulated macrophages expressing the TGF-β_1 receptors, TGFR-I and -II, but not the SMC that lacked these receptors.[141] These results again demonstrate a probable functional difference between SMC and MMC in rodents.

SPECIFICITIES OF MAST CELL GRANULE PROTEINASES AND THEIR NATIVE INHIBITORS

Proteolytic specificities

Mast cell chymases, and some granzymes normally expressed by T cells, belong to a group of evolutionarily related serine proteinases with a characteristic 'missing' Cys191-Cys220 disulphide bond.[28] Mutations in the substrate-binding region of serine proteinases of this family appear to have more profound effects on specificity than in the trypsin model. Phylogenetic analysis[51] shows the evolution of chymases from an ancestral α-chymase with conservation of the ability to convert angiotensin-I to angiotensin-II. Examples of homologous α-chymases have been demonstrated in primates[142] dog[143] and rodents[32,40] However, in the β-chymase group of rodent proteinases (e.g. rMCP-1 and -2; mMCP-1 and -4) that also evolved from this ancestor, the angiotensin-converting specificity is not mandatory.[144] A related group of proteinases, typified by the cytotoxic T-cell enzyme granzyme B,[145] contains members expressed by mast cells. By mutations at residue 226 (chymotrypsinogen numbering), these enzymes have acquired a variety of different primary specificities, such as the dual tryptase-chymase specificities of cathepsin G, sMCP-1 and duodenase,[44,146,147] and the putative granzyme B-like activity of mMCP-8,[148] i.e. cleavage C-terminal to Asp residues.

The trypsin-like primary specificity of tryptases is fixed owing to the invariant Asp residue at position 189 (chymotrypsinogen numbering) in the substrate-binding pocket. The one exception to this is bovine tryptase, with Asn-189 but still retaining trypsin-like activity.[45] Therefore, all cleavages occur C-terminal to Arg and Lys residues. However, tryptases have a more developed substrate-binding cleft than trypsin, being able to sample side-chains of several amino acid residues on either side of the scissile bond of the substrate.[149] The ability of tryptases to cleave substrates is further restricted by their natural association (in most cases) as tetramers. The crystal structure of human tryptase-βII tetramer[149] shows the active sites angled towards a central oval pore of diameter 50×30 Å. Tryptase will probably function most efficiently with peptide substrates, or as a processor of protrusions of larger proteins. As tryptases occur as multigene families, the existence of small differences in the substrate-binding region between tryptase forms may result in differential affinities for important substrates. For example, human tryptase-αI has lost the ability to cleave fibrinogen owing to a Gly→Asp mutation at residue 215.[150]

Native inhibitors and substrates

Examples of native substrates and some of the known inhibitor/inactivators are shown in Table 2. The activity of MCPs may be controlled *in vivo* by plasma-derived

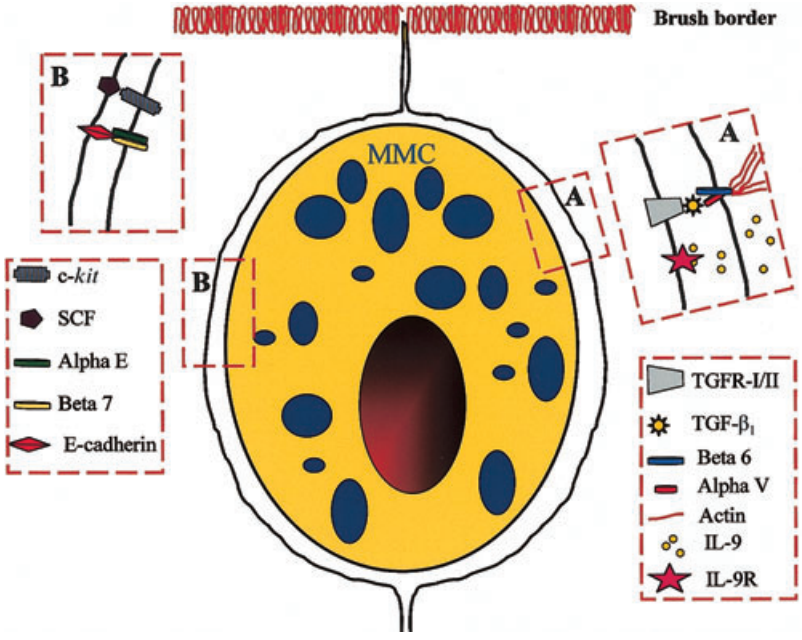

Figure 3. Diagrammatic representation of a mouse mucosal mast cell (mMMC) within the intestinal epithelium with the postulated receptor–ligand interactions between the two cell types illustrated in boxes A and B. In Box A the epithelial cell-specific integrins $\alpha_V\beta_6$ are shown binding an activated transforming growth factor-β_1 (TGF-β_1) molecule and presenting it to its receptor on the mast cell surface. The probable interaction between the integrins and actin fibres in the epithelial cytoskeleton is also illustrated. We speculate that interleukin (IL)-9 is produced by the epithelium, as indicated by published studies,[140] and that it binds to its receptor on the mast cell surface. In box B the interaction between epithelially expressed stem cell factor (SCF) and its tyrosine kinase receptor c-*kit* is shown together with the probable interaction between the integrins $\alpha_E\beta_7$ on the mast cell surface and epithelially expressed E-cadherin. The receptor–ligand interactions illustrated here are consistent with *in vitro* studies showing that IL-9, SCF and TGF-β_1 are important growth and differentiation factors. We speculate that the constitutive secretion of mouse mast cell proteinase-1 (mMCP-1), induced by TGF-β_1,[134] exerts a modulatory effect on these receptor–ligand interactions through, for example, the proteolytic degradation of ligands such as SCF or of the cytokines in the intercellular milieu. This hypothesis might explain the augmented mast cell hyperplasia in mMCP-1$^{-/-}$ mice lacking this proteinase.[14,19]

proteinase inhibitors such as the pan-specific 720 000-molecular weight (MW) plasma proteinase inhibitor, α_2-macroglobulin,[173] which inhibits chymase[166] and sMCP-1[172] (Table 2). Another important plasma-derived inhibitor is the serpin, α_1-proteinase inhibitor (α_1-PI, also known as α_1-antitrypsin). While the main target enzyme for this inhibitor appears to be neutrophil elastase[174] it is also an effective inhibitor of human chymase.[167] The serpin α_1-antichymotrypsin (α_1-AC) inhibits chymase,[167] and α_1-PI and α_1-AC may serve as substrates for chymase, with the cleavage:inhibition ratio being sensitive to pH.[175] Related serpins in the rat and sheep inhibit rMCP-2[170] and sMCP-1,[172] respectively (Table 2).

Secretory leucocyte protease inhibitor (SLPI), an 11 700-MW inhibitor of neutrophil elastase[176] secreted onto mucosal surfaces, appears to be an important native inhibitor of mast cell proteinases. It is an effective native inhibitor of human chymase (Table 2),[168] and, in the presence of heparin, a 10-fold increase in association rate is observed. Mouse and rat SLPIs[177] may have a selective role in controlling the activity of β-chymases, as human SLPI is a highly efficient inhibitor of rMCP-1, but not of rMCP-2.[171] It is important to note, however, that chymases in association with heparin proteoglycan or granule remnants, may be more resistant to inhibition than isolated chymases.[178] For example, human chymase is resistant to α_2-macroglobulin inhibition in the presence of heparin proteoglycan.[179] In contrast to chymases, mast cell tryptases appear refractory to most native inhibitors. Human lung tryptase is stable in the presence of high concentrations of plasma proteinase inhibitors.[180] However, rat (rMCP-6) and bovine tryptases can be inhibited by aprotinin[39,160] (Table 2). It appears that an important mechanism controlling tryptase activity is the sequestration of heparin, which stabilizes the tetramer at physiological salt concentrations.[181] Lactoferrin and myeloperoxidase, released from activated neutrophils, are highly efficient heparin scavengers[161,162] (Table 2). Following removal of heparin, tryptase rapidly dissociates into inactive monomers.[182]

FUNCTIONS OF MAST CELL GRANULE SERINE PROTEINASES

Vascular permeability

Tryptase may contribute to vascular permeability by the direct or indirect generation of bradykinin from kininogens.

Table 2. Examples of native substrates and inhibitors for different classes of mast cell proteinases*

Enzyme type	Examples of native substrates	Reference	Known native inhibitors/inactivators	Reference
Tryptase	VIP	151	SLPI	159
(e.g. human tryptase-βII)	HMW kininogen	152, 153	Aprotinin	39,160
	Pre-kallikrein (activation)	154	Lactoferrin	161
	Fibrinogen	155	Myeloperoxidase	162
	Fibronectin	156		
	PAR-2 (activation)	157		
	MMP-3 (activation)	158		
α-chymase	Angiotensin-I	51	α_2-macroglobulin	166
(e.g. human chymase)	(conversion to angiotensin-II)		α_1-proteinase inhibitor	167
	Pro-collagen-1 (activation)	163	α_1-antichymotrypsin	167
	VIP	151	SLPI	168
	Substance P	151		
	MMP-1 (activation)	164		
	MMP-9 (activation)	165		
β-chymase	MMP-3 activation	169	α_1-proteinase inhibitor	170
(e.g. rat MCP-1)			SLPI	171
'Janus-faced' dual-specific mast cell proteinase	Fibrinogen	172	α_2-macroglobulin	172
			α_1-proteinase inhibitor	172
(e.g. sheep MCP-1)			SLPI	171

*Note that these examples do not necessarily apply to all members of each proteinase class: see text for details.
HMW, high molecular weight; MCP-1, mast cell proteinase-1; MMP-3, matrix metalloproteinase-3; PAR-2, proteinase-activated receptor-2; SLPI, secretory leucocyte protease inhibitor; VIP, vasoactive intestinal peptide.

Mast cell tryptase, originally shown to degrade high-molecular-weight kininogen,[152] may generate bradykinin at low pH.[153] It also activates the kininogen-processing enzyme, kallikrein,[154] and co-operative hydrolysis of kininogen by tryptase and neutrophil elastase generates bradykinin with a yield comparable to that obtained by kallikrein.[183] In support of their roles in increasing vascular permeability, human and mouse tryptases (mMCP-7) inactivate fibrinogen,[155,184] preventing thrombin-induced clot formation. Similarly, sMCP-1 degrades fibrinogen[172] by rapidly and specifically cleaving α- and β-chains when added to plasma. Cleavage of fibrinogen β-chain by both sMCP-1 and human tryptase[44,172,185] occurs C-terminal to, and removes, the thrombin activation site. The α-chain target of human tryptase is the RGD domain, thus disrupting binding to cell-surface integrins.[185] Another mechanism by which tryptase promotes microvascular permeability appears to involve direct activation of mast cells. For example, induction of guinea-pig dermal microvascular permeability by human tryptase is down-regulated by histamine receptor antagonists, and tryptase causes histamine release from dispersed skin and lung mast cells *in vitro*, with tryptase apparently acting as an amplification signal.[186] Dermal microvascular permeability to injected tryptase in the sheep is sensitive both to histamine receptor antagonists and the synthetic tryptase inhibitor APC 366.[187] In contrast, human chymase stimulates a histamine-independent and more prolonged microvascular leakage in guinea-pig skin.[188] Thus, the two proteinases appear to promote vascular permeability via two distinct mechanisms.

Tissue and vascular remodelling

Mechanisms of tissue remodelling may involve the direct activity of granule proteinases, because tryptase cleaves fibronectin,[156,189] both tryptase and chymase degrade type VI collagen microfibrils[190] and chymase proteolytically activates type I procollagen, initiating fibril formation.[163] However, MCPs arguably contribute more to matrix turnover via activation of matrix metalloproteinases (MMPs). MMP-1 (collagenase-1) is activated by human chymase,[164,191] although not directly by tryptase,[191] but indirectly via tryptase-mediated MMP-3 activation.[158] Pro-MMP-9 is activated by canine chymase, but not tryptase,[165] whereas tryptase activates a 72 000-MW gelatinase of fibroblast origin.[156] Pro-stromelysin (MMP-3) is activated by tryptase,[158] chymase[191] and the rat β-chymases rMCP-1 and rMCP-2.[169] Despite this potentially wide range of *in vitro* tissue-remodelling activities of mast cell neutral proteinases, the *in vivo* significance of these findings has yet to be determined.

Tissue remodelling may also occur when tryptase triggers proteinase-activated receptor-2[157] (PAR-2) – a G-protein coupled receptor with seven transmembrane regions and an extracellular 'tethered ligand'. Cleavage of the ligand by tryptase or trypsin generates a new N-terminus that binds to the receptor, initiating intracellular signalling

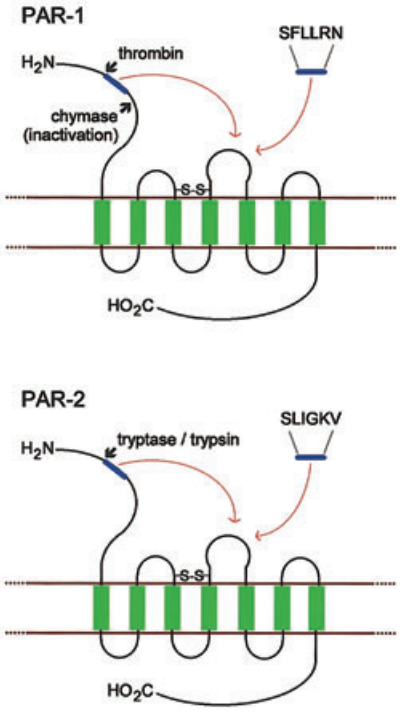

Figure 4. Schematic representation of human proteinase-activated receptor-1 (PAR-1) and PAR-2. The N-terminus is extracellular, the C-terminus is intracellular and transmembrane regions are shown in green. Activation of PAR-1 by thrombin and PAR-2 by tryptase or trypsin exposes the tethered ligand region (shown in blue). This docks into the binding region of extracellular loop 2, which can also be activated by a synthetic hexapeptide representing the new N-terminus. The inactivation of PAR-1 by chymase is also represented, which is presumed to be via cleavage C-terminal to the tethered ligand region.

(Fig. 4).[192] PAR-2 activation in airway smooth muscle cells occurs through calcium mobilization and phospholipase C-mediated activation of the inositol triphosphate pathway[193] with subsequent proliferation,[194] and similarly, tryptase-induced activation of lung fibroblasts proceeds via PAR-2 activation.[195] In contrast, dermal fibroblasts that lack PAR-2 are activated by tryptase through an unknown alternative mechanism[196] and chymase degrades PAR-1, the thrombin receptor, by inappropriate cleavage of the tethered ligand (Fig. 4).[196]

Angiotensin conversion by α-chymases may modulate not only blood pressure, but also vascular remodelling and cardiac hypertrophy.[197] Targeted overexpression in transgenic mice of a rat vascular chymase with angiotensin-converting properties and 80% identity to rMCP-2, resulted in hypertensive arteriopathy.[198] This supports the view that chymase represents a valid therapeutic target in treating hypertension. Human chymase was an angiogenic factor in a hamster sponge implant model, apparently acting via angiotensin-II generation.[199] The chymase mMCP-4 is also implicated in angiogenesis at the invading fronts of squamous carcinomas in mice.[200]

Allergic reactivity

Aerosolized tryptase causes bronchoconstriction in allergic sheep lung, apparently via histamine release, which further supports the concept that tryptase is amplifying reactivity through mast cell activation.[201] It is interesting that *Ascaris suum* 'sensitized' sheep were used in this study[201] where, presumably, increased airway permeability facilitated the access of tryptase to airway mast cells. Again, using the *Ascaris* model of allergic lung disease, pretreatment of allergic sheep with the tryptase inhibitor, APC 366, significantly reduced late-phase and hypersensitivity responses to inhaled allergen.[202] A similar protective effect was observed using aerosolized SLPI,[159] although it should be noted that SLPI might not only compete for tryptase-associated heparin but also target sMCP-1,[171] which is expressed in sensitized lung.[88] The recruitment of inflammatory cells is another important feature of allergic reactivity for which mast cell proteinase activity may be responsible. For example, intraperitoneal injection of the tryptase, mMCP-6,[203] in mice generated a marked neutrophilia, as did human tryptase, where co-injection of histamine induced a concomitant eosinophilia.[204] Tryptase-mediated neutrophilia is probably caused, at least in part, by its ability to induce release of the chemokine IL-8 from epithelial[205] and endothelial[203,206] cells. Human chymase also recruited neutrophils and eosinophils when injected into the skin of guinea-pigs.[207]

In the context of airway and gut allergic reactivity, tryptase efficiently hydrolyses the neuropeptide vasoactive intestinal peptide (VIP), but not substance P, whereas chymase cleaves both peptides,[151] raising the possibility that mast cell proteinases can modulate neurogenic inflammatory reponses. Another important feature of allergic disease is altered epithelial permeability and this is well described, for example, in nematode infections and involves MMC.[2] Increased intestinal epithelial paracellular permeability occurs in rat intestine within minutes of introducing rMCP-2 into the perfusate during *ex vivo* perfusion of the intestinal vasculature or following the anaphylactic release of rMCP-2 by intestinal MMC.[104] No gross pathology is associated with this increased permeability and concomitant translocation of rMCP-2 into the gut lumen.[104] *In vitro* studies suggest that rMCP-2 opens the epithelial barrier by disrupting the tight juctional complex.[208] Integrity of epithelial tight junctions may be important therefore during intestinal infection with the nematodes *N. brasilensis* and *T. spiralis* in mMCP-$1^{-/-}$ mice. Infection is associated with a more pronounced intraepithelial mast cell hyperplasia[14,19] in mMCP-$1^{-/-}$ mice, together with delayed expulsion of *T. spiralis*, when compared with mMCP-$1^{+/+}$ controls.[14] It is possible that the egress of MMC into the gut lumen, as described in detail in parasitized sheep,[209,210] is compromised by the absence of mMCP-1 in mMCP-$1^{-/-}$ mice with relatively intact tight junctions. An alternative explanation is that extracellular mMCP-1, released during infection,[69] downregulates mast cell hyperplasia in mMCP-$1^{+/+}$ mice by degrading SCF, c-*kit*, or the TGF-R/TGF-β_1/β_6 complex

(Fig. 3). In the absence of the proteinase there is unregulated expansion of the MMC population.

Innate immunity

Serosal mast cells play a key role in maintaining peritoneal integrity and are involved in the early recruitment of neutrophils following experimental caecal ligation and puncture in mice. This recruitment does not rely entirely on the release of tumour necrosis factor-α (TNF-α)[211] and apparently protects the mice from fatal septicaemia. In a similar context, recombinant human tryptase βI, but not tryptase αI, induces airway neutrophilia when instilled into mouse lung in a process that is apparently independent of PAR-2 activation.[212] Importantly, tryptase βI instilled into the airways of mast cell-deficient W/W^v mice significantly reduces pulmonary bacterial load following challenge with *Klebsiella pneumoniae*.[212] This process, where airway reactivity is unaltered, suggests that tryptases can contribute significantly to innate immunity against bacterial infection.[212] It is also possible that tryptases released by the serosal mast cells are involved in neutrophil recruitment and protection after caecal puncture.

CONCLUSIONS

Neutral proteinases are important contributors to mast cell-related inflammatory responses in the lung and gut. Many of the current concepts on the functions of chymases and tryptases are being revised as new proteases are discovered and analyses of proteolytic specificity reveal subtle, but important, differences in function and, consequently, of potential *in vivo* activities. For the future, mechanisms governing heterogeneity of proteinase expression must be linked to proteinase function in tissues where there may be selective proteolysis of a limited range of target substrates, including a family of protease-activated receptors, in the presence of inhibitors or of other factors that regulate proteolysis. Targeted, tissue-specific and inducible deletion of proteinases and of their inhibitors will be necessary to further dissect the complex, but potentially important, *in vivo* functions of mast cell granule proteinases.

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