MINIREVIEW

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Mast Cell-restricted Tryptases: Structure and Function in Inflammation and Pathogen Defense*

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Mast cells (MCs) are highly specialized immune cells present in mammals and in lower organisms that predate the development of adaptive immunity. The strong evolutionary pressure to retain MCs for >500 million years suggests critical roles for these cells in our survival. In support of this conclusion, no human has been identified to date that lacks MCs, despite the adverse roles of MCs in systemic anaphylaxis and varied inflammatory disorders. MCs express numerous lineage-restricted neutral proteases, and four members of the chromosome 17A3.3 family of tryptases are preferentially expressed in mouse MCs. The anatomical location of MCs at host-environment interfaces has raised the possibility that some of these enzymes are evolutionally conserved because they are needed for combating infectious organisms. Here we review recent insights into the structure and function of MC tryptases in inflammation and host defense against bacteria and other infectious organisms.

MC-restricted Granule Proteases and Proteoglycans

A characteristic morphologic feature of mammalian mast cells (MCs)[3] is their electron-dense secretory granules, which contain serglycin proteoglycans (1, 2) bearing heparin (for review see Ref. 3) and chondroitin sulfates diB and E (4, 5). Heparin is the most negatively charged molecule in the body, and each rat and mouse peritoneal MC contains ~25 pg of this glycosaminoglycan, thereby explaining the avidity of MCs for cationic dyes (6). The repeating Ser-Gly sequence in serglycin where its glycosaminoglycans are attached cannot be cleaved by any known protease. This protease resistance feature is biologically relevant because the primary function of serglycin proteoglycans in MCs is to provide a scaffold for formation of macromolecular complexes with the cell's varied granule proteases. When properly folded, a positively charged face forms on the surface of each granule protease that allows it to bind tightly to the proteoglycan's negatively charged glycosaminoglycans (7–9).

The proteases packaged in the secretory granules of mouse MCs include carboxypeptidase A3 (CPA3), granzyme B, cathepsin G, neuropsin, transmembrane tryptase/tryptase γ/protease serine member S (Prss) 31, mouse MC protease (mMCP) 1–10, and mMCP-11/Prss34. Definitive evidence of the importance of heparin-containing serglycin proteoglycans in the packaging of specific cassettes of proteases in the MC's granules came with the characterization of *N*-deacetylase/*N*-sulfotransferase-2 (NDST-2)-null mice (10, 11). NDST-2 is essential for heparin biosynthesis and is preferentially expressed in MCs. Because of the loss of fully sulfated heparin, the MCs in the skin and peritoneal cavities of NDST-2-null mice store reduced amounts of mMCP-6 and almost no CPA3, mMCP-4, and mMCP-5, even though all four MC-restricted protease genes are transcribed at normal rates. The accumulated data led to the current view that serglycin proteoglycans are essential for the post-translational processing and granule accumulation of most, if not all, proteases stored in the MC's secretory granule. The type of glycosaminoglycan attached to this intracellular proteoglycan also plays a critical role in selecting which proteases will be stored in the cell's granules.

Four of the 15 serine proteases in mouse MCs are tryptases (designated mMCP-6 (12, 13), mMCP-7 (14), Prss31 (15), and Prss34 (16)). Their genes reside at chromosome 17A3.3 within a larger ~11.8-megabase serine protease gene cluster that encodes 13 functional tryptic-like proteases. mMCP-6, mMCP-7, and Prss31 are abundant in numerous tissue MCs. Although Prss34 is expressed by interleukin (IL) 3-differentiated mouse bone marrow-derived MCs (mBMMCs), it remains to be seen whether tissue MCs express this tryptase. The syntenic human tryptase locus resides ~1.2 megabases from the telomeric end of chromosome 16p13.3 (17–20). This region has been difficult to sequence because of its high rate of mutation and recombination (21). A comparison of the mouse and human tryptase loci revealed that a chromosomal break and inversion event occurred downstream of the *Prss27* gene after mice and humans diverged in evolution (16). Thus, it is likely that this recombination event contributes to the instability of the tryptase locus in humans. The fact that some of the initially isolated hTryptase cDNAs are nearly identical added to the confusion as to how many tryptase genes are present in the human genome. It tentatively has been concluded that two adjacent genes on chromosome 16p13.3 give rise to the *hTryptases* α, β1, β2, and β3 transcripts which encode tetramer-forming proteases that are 93–99% identical (17, 22–24).

Sequence analysis of mouse chromosome 17A3.3 led to the identification of the *Prss31* gene (18), which resides 2.3 kb downstream of the *mMCP-6* gene. Cloning of its human

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[3] The abbreviations used are: MC, mast cell; CPA3, carboxypeptidase A3; hTryptase, human tryptase; IL, interleukin; mBMMC, mouse bone marrow-derived mast cell; MITF, microphthalmia transcription factor; mMCP, mouse mast cell protease; NDST-2, *N*-deacetylase/*N*-sulfotransferase-2; Prss, protease serine member S; *tg/tg*, WBB6F$_1$-*tg/tg*; TNF-α, tumor necrosis factor-α; *W/W^v*, WBB6F$_1$-Kit^W/Kit$^{W\text{-}v}$.

ortholog followed shortly thereafter, and expression studies confirmed its localization in mouse and human MCs (15, 18, 20). Fifteen single nucleotide polymorphisms have been identified in the *hPrss31* gene, which result in amino acid changes at residues 23, 123, 202, and 252 in the protease's mature domain. Residues 23 and 202 are predicted to reside in the protease's substrate-binding cleft (18) and residue 123 in the Trp-rich domain (7) that is required for activation of tryptase zymogens (25). It therefore is likely that some allelic variants of the *hPrss31* gene encode defective or functionally distinct tryptases.

Analysis of the corresponding tryptase locus on human chromosome 16p13.3 led to the identification of the *hTryptase* δ gene (17), which encodes a truncated mMCP-7-like protease. Although numerous MCs contain hTryptase δ mRNA (26), this serine protease is less enzymatically active than mMCP-7 and hTryptase β1. It therefore remains to be determined whether hTryptase δ is functional *in vivo*. Complicating the studies on this human protease, 22 single nucleotide polymorphisms already have been identified in the 2.2-kb *hTryptase* δ gene that result in 12 amino acid changes.

Expression of Tryptase Genes in MCs

All mouse strains examined to date express mMCP-6. In contrast, mMCP-7 and Prss31 are expressed in strain-dependent manners (18, 27, 28). C57BL/6J (27) and CNZW/LacJ mice lack mMCP-7 because of a point mutation at the gene's exon 2/intron 2 splice-site, whereas MOLF/EiJ mice lack mMCP-7 because of a point mutation at the gene's exon 3/intron 3 splice-site. Inactivating mutations have not yet been identified in the mouse or human *Prss31* gene. Nevertheless, Prss31 is considerably more abundant in the MCs of C57BL/6J mice than those of BALB/cJ and 129/SvJ mice (18). These three MC tryptases also can be differentially expressed within specific tissues of the same mouse strain. For example, the safranin$^+$ MCs in the skin of BALB/cJ mice contain abundant amounts of mMCP-6 and mMCP-7, whereas the safranin$^+$ MCs in the peritoneal cavity of this mouse strain express mMCP-6 but not mMCP-7 (29). The intestines of C57BL/6J mice also contain more Prss31 than mMCP-6 or mMCP-7 (15). These three tryptase family members therefore are not coordinately expressed in mouse MCs even though their genes are adjacent to one another on chromosome 17A3.3.

On a weight basis, the predominant serine proteases stored in the secretory granules of human MCs are the tetramer-forming α/β tryptases (30). Human lung and skin MCs contain 12–35 pg of these serine proteases, and immunohistochemical studies have led to the conclusion that virtually all human MCs express at least one tetramer-forming tryptase (31). Because human MCs contain substantial amounts of α/β tryptases, detection of these proteases in blood is often used to identify patients who have experienced a systemic anaphylaxis reaction (32). Information on the expression of hPrss31 is limited, but the MCs in the intestine, skin, and airway submucosa store this tryptase in their secretory granules (15, 18, 20).

Structural Analysis of MC Tryptases

Although all mouse and human tryptases are initially translated as inactive zymogens, they are stored in the MC's secretory granules in their mature, enzymatically active forms. The low pH (~5.5) of the MC's secretory granules ensures that the proteolytic activities of the tryptases are low, presumably to minimize their autolysis. The spatial constraints imposed by the associated serglycin proteoglycans also help prevent their catabolism. Using mMCP-6 as an example, the initially translated zymogen contains a 21-mer signal peptide, 10-mer propeptide, and 245-mer mature domain (12, 13). The proteolytic activation of pro-mMCP-6 by an undefined intracellular processing enzyme results in loss of the zymogen's propeptide. The amino acid sequence at the proteolytic processing site of pro-Prss31 is very different from that in pro-mMCP-6 and pro-mMCP-7, suggesting a distinct mechanism of activation. Mature Prss31 also differs from mature mMCP-6 and mMCP-7 in that its 18-mer propeptide remains covalently bound to its 284-mer functional domain via a novel disulfide bond (15).

The tetramer-forming mouse tryptases mMCP-6 and mMCP-7 are ~75% identical, whereas hTryptases α, β1, β2, and β3 are 93–99% identical. hTryptases α and β1 differ in 18 of their 245 amino acids, but surprisingly 9 of these variable residues are in the substrate-binding cleft. Presumably, evolutionary pressure caused the preferential mutation of the substrate-binding clefts of these tryptases to generate enzymes with different substrate specificities. In this regard, biochemical and structural studies revealed that hTryptase α differs functionally from hTryptases β1, β2, and β3 in large part because of a Gly → Asp mutation at residue 215 in the enzyme's S1 pocket (33–35). As noted in this example, a change of a single amino acid in the substrate-binding cleft of a tryptase can have profound consequences in the regulation of its enzymatic activity and/or substrate preference. Because rhesus monkeys (see Ensembl ENSMMUG00000015103) and chimpanzees (see Ensembl ENSPTRG00000007584) have genes that encode tryptases that have the same Asp^{215} mutation, hTryptase α probably is biologically important in humans due to its conservation in primates.

The conserved residues that form the catalytic triad amino acids of pancreatic trypsin/Prss1 and other serine proteases are present in all MC tryptases, including hTryptase δ. However, MC tryptases have proteolytic activities that are considerably more restricted than that of trypsin. The crystal structure of hTryptase β2 deduced by Bode and co-workers (36) revealed that this serine protease exists as a tetramer because of novel interactions between conserved Tyr and Pro residues residing in six loop segments. The active site of each monomer faces inward toward the 50 × 30-Å pore in the center of the donut-like structure, thereby explaining in structural terms why few proteins are susceptible to hTryptase β2. MC tryptases also have a conserved Trp-rich domain that is essential for their enzymatic activities (7, 25). Activation studies carried out on recombinant pro-mMCP-6 (37), pro-mMCP-7 (38), and pro-hTryptase β1 (39) revealed that these zymogens spontaneously form tetramers when their propeptides are removed. Heparin is required for maintaining the enzymatic activities of naturally

occurring (40) and recombinant (39) human β tryptases, and this glycosaminoglycan also regulates the substrate preference of recombinant mMCP-6 (37). Nevertheless, enzymatically active mMCP-7 homotetramers are present in the circulation of the V3 mastocytosis mouse free of serglycin proteoglycans (41). Enzymatically active mMCP-6 (37) and mMCP-7 (38) also can be generated *in vitro* in the absence of heparin. Thus, mMCP-6 and mMCP-7 are less dependent on serglycin proteoglycans than hTryptases β1, β2, and β3.

Human and mouse Prss31 are ~75% identical to each other but are <50% identical to mMCP-6, mMCP-7, and hTryptase β1. The amino acids that form the substrate-binding cleft of Prss31 are unique, and recombinant Prss31 cannot cleave some trypsin- and hTryptase β1-specific peptide substrates (15). However, in contrast to all mouse and human tetramer-forming tryptases, recombinant Prss31 is rapidly inactivated by α1-antitrypsin/SERPINA1. Prss31 also differs from its other MC family members in that it remains monomeric when activated (15) because it lacks the Tyr- and Pro-rich domains that are needed for creation of the tetramer unit. Prss31 also possesses a novel membrane-spanning domain at its C terminus that anchors it to the outer leaflet of the plasma membrane when MCs degranulate. Mouse and human Prss31 also possess an additional disulfide bond that links Cys^{-12} in the propeptide with Cys^{108} in the enzyme's mature domain. Prss31 is therefore a 2-chain protease when activated.

Bioactivity and Substrate Preferences of MC Tryptases

The MC's tryptases increase their proteolytic activity when exocytosed into neutral pH environments. Studies carried out on BALB/cJ and V3 mastocytosis mice have revealed that mMCP-6 and mMCP-7 are both packaged in the MC's granules ionically bound to serglycin proteoglycans. However, much of exocytosed mMCP-7 rapidly dissociates from its proteoglycan after degranulation (41) (Fig. 1). In contrast, nearly all exocytosed mMCP-6·serglycin proteoglycan complexes remain intact in extracellular matrices near the degranulated MCs for >1 h. Recombinant mMCP-6 and mMCP-7 can form heterotetramers *in vitro* (25). Because not all exocytosed mMCP-7 leaves the inflammatory site rapidly in IgE/antigen-challenged mice (41), it is likely that some mMCP-6·mMCP-7 heterotetramers exist *in vivo*; but the functional consequences of these complexes have not been evaluated.

Data obtained from site-directed mutagenesis studies have revealed that the reason mMCP-7 homotetramers rapidly dissociate from their exocytosed macromolecular complexes at neutral pH is because this tryptase's proteoglycan-binding domain is His-rich (9) rather than Lys/Arg-rich as occurs in mMCP-6 and hTryptases β1, β2, and β3 (41). This three-dimensional structural feature allows mMCP-7 homotetramers to leave inflammatory sites, and substantial amounts of these homotetramers can be found in the circulation 15 min after inducing systemic anaphylaxis in V3 mastocytosis mice. More than 10% of the proteins in mouse blood are protease inhibitors, including various serpins (*e.g.* α1-antitrypsin) that inhibit tryptic proteases. The fact that circulating mMCP-7 homotetramers are enzymatically active (41) in V3 mastocytosis mice indicates how resistant mMCP-7 homotetramers are to circulating

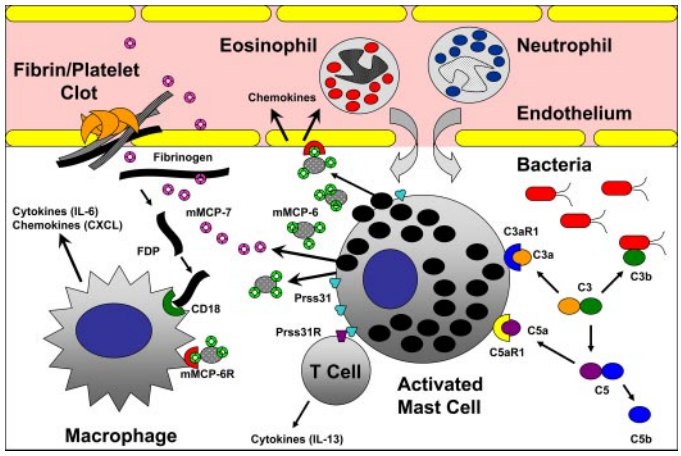

FIGURE 1. **Model of MC-restricted mouse tryptases in tissue inflammation and bacteria defense.** Bacterial infection of the peritoneal cavity or lung results in activation of the alternate complement pathway in which C3 is converted to C3a and C3b. C3b binds to the surface of the infectious organism and also converts C5 to C5a and C5b. Degranulation occurs when the anaphylatoxins C3a or C5a bind to different receptors (*e.g.* C3aR1 and C5aR1/CD88) on the surfaces of MCs. Exocytosed mMCP-7 homotetramers rapidly dissociate from their serglycin proteoglycans in the extracellular milieu, allowing the liberated tryptase to diffuse toward the endothelium-blood barrier where this protease cleaves incoming fibrinogen to limit clotting. It is likely that some of the resulting fibrinogen-degradation products (*FDP*) bind to CD18 on the surface of resident macrophages to increase their expression of cytokines and chemokines that lead to the extravasation of eosinophils into the inflammatory site. In contrast, mMCP-6 remains tightly bound to its serglycin proteoglycan outside of the activated MC. The resulting mMCP-6·proteoglycan macromolecular complexes act locally via mechanisms that involve CXCL/IL-8-like chemokines to recruit neutrophils. The mMCP-6-susceptible protein(s) needed for neutrophil recruitment remains to be identified, but it presumably resides in the extracellular matrix or on the surfaces of nearby cells such as endothelial cells and/or macrophages. Prss31 translocates from the granule to the outer leaflet of the plasma membrane following MC activation. This tryptase induces nearby T cells by an undefined mechanism to increase their expression of IL-13 and possibly other factors, which ultimately results in increased expression of mucins and secretion of mucus to trap bacteria and facilitate their clearance from the lungs.

protease inhibitors even after they dissociate from serglycin proteoglycans. Measurable amounts of human β tryptases can be found in the blood after MC activation in systemic anaphylaxis (32), and human β tryptase tetramers also are more resistant to inactivation by circulating protease inhibitors than their monomers (42).

Using a phage display peptide library screening approach, it was discovered that recombinant mMCP-7 prefers to cleave after Arg in the Ser-Leu-Ser-Ser-Arg-Gln-Ser sequence (38). The protruding C-terminal domain of the α chain of fibrinogen has a similar sequence. Even though fibrinogen is a 3-chain protein with an overall 340-kDa molecular mass, mMCP-7 is a potent anticoagulant because the susceptible sequence in the exposed α chain of fibrinogen can access the central pore of the mMCP-7 tetramer (38). In support of these mouse data, an undefined tryptase purified from human lung also can cleave fibrinogen (43). Eosinophils accumulate in the peritoneal cavities of mice when mMCP-7 is administered into that site (39) (Fig. 1). The observation that certain proteolytic fragments of fibrinogen can induce monocytes to secrete pro-inflammatory cytokines (44) raises the possibility that mMCP-7 generates biologically active factors from endogenous precursor proteins that exacerbate inflammation *in vivo*.

Screening of a phage display peptide library with mMCP-6·heparin (37) and hTryptase β1·heparin complexes (39) revealed that these proteases differ from mMCP-7 in that they prefer substrates with a sequence of Lys/Arg-Pro-*X*-Lys/Arg (where *X* can be 0–3 noncharged amino acids). Harris *et al.* (45) obtained similar data when they screened their combinatorial tetrapeptide substrate libraries with recombinant hTryptase β1·heparin and hTryptase β2·heparin complexes. Although the physiological substrates of mMCP-6 remain to be identified, this protease and its hTryptase β1 ortholog regulate neutrophil recruitment *in vivo* (37, 46), and both tryptases induce cultured endothelial cells to produce substantial amounts of the CXCL/IL-8 family of chemokines that regulate neutrophil chemotaxis (39, 47, 48).

MCs also have been implicated in fibrosis, and numerous *in vitro* studies have raised the possibility that one or more of the MC's tetramer-forming tryptases participate in the connective tissue remodeling that occurs in wound healing by promoting the proliferation of fibroblasts and the expression of type I collagen (49).

Quantitative locus analysis of airway hyper-responsiveness in A/J x C57BL/6J mice (50) linked an undefined gene at the tryptase locus to the constitutive susceptibility of the lungs to methacholine. Despite the large numbers of neutrophils that extravasate into the lungs of mMCP-6- and hTryptase β1-treated mice, surprisingly, no substantial change in airway responsiveness to methacholine was noted in these animals (15, 39). In contrast, when comparable amounts of recombinant Prss31 were instilled into a mouse trachea, a substantial increase in airway reactivity was obtained 24 h later. In this mouse model of airway hyper-responsiveness, Prss31 activated an IL-13/IL-4Rα/STAT6-dependent pathway in the lung. Because these data raise the possibility that Prss31 might play a significant adverse role in the lungs of asthma patients, a better understanding of the activation, inhibition, and role of this tryptase in MC-dependent disorders is needed.

MC Tryptases in Bacterial Infections

MCs release their preformed granule mediators when their IgE-bearing FcεRI receptors encounter the appropriate antigen. Life-threatening systemic anaphylaxis and inflammatory disorders occur when too many MCs become activated or when activation occurs at an inappropriate time. Thus, MCs have been viewed historically in a negative light, and many investigators have focused their attention on identifying the factors and mechanisms that can diminish MC numbers and/or responses in tissues. Nevertheless, the discovery of MC-like cells in lower organisms coupled with the discovery that many populations of MCs can be activated by non-IgE-dependent mechanisms has profoundly changed our perception of the beneficial value of these cells in innate immunity.

Mammalian MCs release their preformed mediators when they encounter the complement anaphylatoxins C3a and C5a (Fig. 1), and organisms that attack humans often produce exogenous factors (*e.g.* bacteria-derived ADP and mite-derived proteases) that also induce the release of the MC's granule constituents via different surface receptors. The beneficial roles of MCs and their exocytosed mediators became more apparent with the discovery that MC-deficient WBB6F$_1$-*Kit*^{W}/*Kit*$^{W\text{-}v}$ (*W*/*W*^{v}) mice have profound defects in combating bacterial infections of their lungs and peritoneal cavity (51, 52). When these CD117/c-*kit*-defective mice were repopulated with *in vitro* differentiated mMCP-6^{+} mBMMCs from wild-type mice, the ability of the reconstituted mice to combat bacterial infections was restored. Although IgE-dependent activation of MCs is important in host defense against recurrent helminth infections (53), the involvement of MCs in the initial defense against a pathogen does not involve adaptive immunity due to the need to respond rapidly to the invading organism. For example, C5a activation of the MCs in the peritoneal cavity of naive mice is needed for zymosan-mediated early accumulation of neutrophils in this tissue site (54).

Although it is likely that MCs produce a diverse array of factors that are beneficial in innate immunity, tumor necrosis factor-α (TNF-α) was initially implicated as the key MC-derived factor that mediates resistance to bacterial infection (51, 52). Nevertheless, because TNF-α-expressing macrophages greatly outnumber MCs in the peritoneal cavity and lung, it became apparent that factors more specific to the MC are needed for anti-bacterial responses. Indeed, it was subsequently shown that MC activation significantly improves host defense to infection even in TNF-α-null mice (55). Although WBB6F$_1$-*tg*/*tg* (*tg*/*tg*) mice also have reduced numbers of peritoneal MCs and impaired survival after bacterial peritonitis, the defective response in these bacteria-treated mice was not improved after receiving TNF-α (56). Because the genetic defect in *tg*/*tg* mice results in reduced expression of the microphthalmia transcription factor (MITF) (57), a MC-restricted gene in which expression is regulated by MITF likely plays a critical role in resistance to bacterial pathogens. Knight *et al.* (58) noted that expulsion of the nematode *Trichinella spiralis* is significantly delayed in mMCP-1-null mice. Even though this study was the first to show a critical role for a MC-restricted granule protease in an immunological response to an infectious organism, the MCs in the mouse's peritoneal cavity lack mMCP-1 (12), and there is no evidence that the expression of mMCP-1 is regulated by MITF.

MITF regulates transcription of the *mMCP-6* gene (59), and the ability of *W*/*W*^{v} mice to combat *Klebsiella pneumoniae* improved significantly when these MC-deficient animals were given recombinant human hTryptase β1 or mMCP-6 ~24 h before being inoculated with bacteria (39). mMCP-6-null mice also cannot combat *K. pneumoniae* infections of their peritoneal cavities efficiently (60). The accumulated data suggest that in most instances mMCP-6 and its human ortholog play beneficial roles in host defenses against bacteria and probably other common pathogens.

C57BL/6J mice are deficient in mMCP-7 (27, 28), yet these animals can combat *K. pneumoniae* efficiently (60). It therefore is unlikely that this tryptase is as important as mMCP-6 in conferring resistance to bacteria. Nevertheless, mucins are needed to help rid the lungs of bacteria and other airborne pathogens. IL-13 participates in innate immunity and regulates mucin production in the lung (for review see Ref. 61). Because Prss31 induces IL-13 expression in the lung and cultured Jurkat T cells (15) (Fig. 1), it is likely that this MC-restricted tryptase also has a beneficial role in pathogen defense, at least in the lung.

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