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Mast cell tryptases and chymases in inflammation and host defense

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Summary

Tryptases and chymases are the major proteins stored and secreted by mast cells. The types, amounts and properties of these serine peptidases vary by mast cell subtype, tissue, and mammal of origin. Membrane-anchored γ-tryptases are tryptic, prostasin-like, type I peptidases that remain membrane-attached upon release and act locally. Soluble tryptases, including their close relatives, mastins, form inhibitor-resistant oligomers that act more remotely. Befitting their greater destructive potential, chymases are quickly inhibited after release, although some gain protection by associating with proteoglycans. Most chymase-like enzymes, including mast cell cathepsin G, hydrolyze chymotryptic substrates, an uncommon capability in the proteome. Some rodent chymases, however, have mutations resulting in elastolytic activity. Secreted tryptases and chymases promote inflammation, matrix destruction, and tissue remodeling by several mechanisms, including destroying pro-coagulant, matrix, growth and differentiation factors, and activating proteinase-activated receptors, urokinase, metalloproteinases, and angiotensin. They also modulate immune responses by hydrolyzing chemokines and cytokines. At least one chymase protects mice from intestinal worms. Tryptases and chymases also can oppose inflammation by inactivating allergens and neuropeptides causing inflammation and bronchoconstriction. Thus, like mast cells themselves, mast cell serine peptidases play multiple roles in host defense and any accounting of benefit versus harm is necessarily context-specific.

Keywords

Tryptase; chymase; cathepsin G; mast cell

Introduction

The most abundant proteins stored in mast cell secretory granules are endopeptidases, which are released outside of the cell during exocytosis. The major enzymes are tryptic and chymotryptic peptidases called tryptases and chymases, respectively. All of these are serine-class peptidases of the trypsin family, but otherwise they are diverse in form, activity and in patterns of expression. Mast cells maturing in different tissue microenvironments can vary widely in the types and amounts of tryptases and chymases expressed. These differences provide a classic means of dividing mast cells into subsets, which in some cases also exhibit differences in behavior. Because mast cells can express certain of the peptidases at a very high level, immunohistochemistry and immunoassay approaches using antibodies directed against these enzymes are useful experimentally as well as clinically in assessing mast cell numbers, locations, activation, and disease-association. The numbers and types of expressed tryptases

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(see Table 1) and chymase-like enzymes (see Table 2) also differ substantially between mammals, and especially between humans and rodents. It is therefore important that these differences are kept in mind in making inferences about roles of these peptidases in humans from findings in animal models. The following primer attempts to identify the mast cell tryptases and chymases most likely to play roles in mammalian inflammation and host defense.

Tryptases

The tryptase family—Mast cell tryptases are a somewhat eclectic group of secreted, serine-class peptidases with trypsin-like target preferences, which is to say that they cleave peptide and protein substrates after lysine and arginine. In comparison with trypsin, however, they can exhibit major physical and behavioral differences, such as intracellular pre-activation prior to secretion, membrane anchorage, and formation of toroidal, proteasome-like oligomers that resist circulating anti-peptidases such as α_1-antitrypsin and α_2-macroglobulin. Although the functions of mast cell tryptases remain to be fully explained, they are hard to ignore if only because of abundance. In most human mast cells, tryptases are packed so densely into secretory granules with other biomolecular components that they form semi-crystalline structures. When mast cells degranulate in response to allergen-bound IgE or to non-immunological stimuli, tryptases are released with histamine, heparin proteoglycan and other granule constituents. Such release of soluble tryptases can be local, as in bronchi in acute asthma, or widespread, as in systemic mastocytosis or anaphylaxis. Systemic release often is followed by large increases inof blood levels of immunoreactive tryptase, which can reflect total mast burden or severity of anaphylaxis (1). Tryptase mRNA levels also are very high in human mast cells, even exceeding those of classic housekeeping genes.

Tryptases: rubric and definitions—Mast cell tryptases are not closely related to the many tryptic peptidases associated with digestion, hemostasis, clot lysis and complement activation. In one sense this is unfortunate because it hampers attempts to make conjectures about tryptase function via comparisons to peptidases of established function. On the other, it underscores their unique position in the hierarchy of trypsin-family peptidases and encourages consideration of non-classical functions. So far tryptases have been described only in mammals, in which they exhibit considerable variety of form and activity and in some cases redundancy, particularly in humans, which have four loci of expressed mast cell tryptase genes (*TPSG1, TPSB2, TPSAB1*, and *TPSD1*), plus various pseudogenes (2-4). They fall into two major groups: membrane-anchored and soluble. The sole known member of the membrane-anchored group is γ (also known as transmembrane tryptase), which is a type I tryptic enzyme tethered to the cell surface by a C-terminal transmembrane peptide following secretion (3,5). In humans, the soluble group includes α, β and mouse mast cell protease (MCP)-7-like/δ (2); in mice, it includes MCP-6 and MCP-7, expression of the latter being strain-dependent (e.g., not expressed in C57BL/6 (6)). Dogs, pigs and mice express an additional mast cell tryptase-like enzyme, mastin (in mice also known as MCP-11/Prss34) (7-10). Mastins are not expressed in humans because the gene appears to have mutated into a pseudogene (10). Most if not all soluble tryptases self-compartmentalize into inhibitor-resistant oligomers (11), which, in the case of human β are heparin-stabilized tetramers (12). Mastins may form higher-order oligomers, with some structures stabilized by disulfide links formed between catalytic subunits (9,10).

Membrane-anchored tryptases: ancestral forms?—The closest relatives of mast cell tryptases are type I membrane-anchored serine peptidases, such as prostasins, which are found in amphibians as well as mammals and thus have deeper roots (3). This relationship suggests that membrane-anchored γ-tryptases may resemble tryptases in their ancestral form and that the extant soluble tryptases evolved from membrane-anchored forms by shedding a C-terminal anchor. Although at first glance this may seem improbable, a similar conversion between

soluble and membrane-anchored forms has occurred during evolution of a related serine peptidase, pancreasin, which is soluble in humans and predicted to be membrane-anchored in mice (13). Although it is clear that γ-tryptase has inflammatory potential (14), its roles and importance vis-a-vis soluble tryptases remains to be established. Hints that it may not play a key or central role in mammalian biology include low or absent expression in some mouse strains, such as BALB/c and 129/Sv, and absence of the γ gene in current versions of some nearly complete genomes, such as those of dog and chimpanzee. Studies in C57BL/6 mice suggest that it may be expressed early in mast cell development and in a tissue-selective manner (mainly in intestines in adults) (5). In humans, immunoreactive γ is present in extracts of a mast cell line (3) and in cells resembling mast cells in sections of trachea (3) and intestine (5). However, the mast cell specificity of γ expression remains to be established; γ mRNA is present in a variety of human non-mast cell tumor lines (5), indicating the potential for expression in other cell types. Studies of recombinant human γ in engineered, soluble forms lacking the C-terminal anchor show that its preferences for cleaving small peptide-based substrates differ substantially from those of βI, as does its susceptibility to inhibition by drug-like small inhibitors (15). Of particular note, γ generally resists the types of aromatic dibasic β-tryptase inhibitors that have shown efficacy in allergic inflammation models, suggesting that the efficacy of these inhibitors is not due to bystander inhibition of γ. In recombinant soluble form, γ is inactivated by serpin-class inhibitors which β1 resists, suggesting that γ does not form the proteasome-like torus that protects β tetramers from circulating proteinaceous inhibitors. It is not known if the substrate and inhibitor profiles of the membrane-tethered form of γ are similar to those of the engineered, soluble form. Likewise, there is no direct evidence to date that γ is shed from the mast cell surface after secretion. Although some peptide anchors of Type I serine peptidases are exchanged for lipid (GPI) anchors that can be shed by a phospholipase (16), this does not appear to be the case for γ's transmembrane segment. Recent studies (16) in which the C-terminal anchor of mouse prostasin was replaced with that of mouse γ/transmembrane tryptase reveal that the γ segment does not contain a lipid anchor signal but that it can be cleaved when the chimera is expressed in epithelial cells, resulting in shedding of the attached protease.

Soluble mast cell tryptases: the α/β dichotomy—Among soluble human tryptases α, β and δ, only β seems likely to be to play important roles outside of the cell. Both α and δ have catalytic domain defects that severely reduce activity, with α having a key mutation deranging the active site, and with δ having a premature stop codon leading to deletion of much of the protein's C-terminal region, including several residues thought to be essential determinants of specificity and catalytic competence in all serine peptidases. Furthermore, both α and δ have a pro-peptide mutation that appears to impair autolytic processing of proenzyme to the point that most protein is secreted from mast cells in the inactive, zymogen form. It is not yet known if there are alternative intracellular activation pathways or an extracellular mechanism for activating these secreted pro-enzymes. However, given the catalytic domain defects, even if such a mechanism exists, the resulting protein will have little peptidolytic activity. These considerations allow us to concentrate on β-tryptases (occurring in three principal forms: I, II and III (17)), as the soluble mast cell tryptases most likely to be involved in pathogenic inflammatory events in humans. Indeed β tryptases are the major focus of drug development for therapeutic inhibition. β-Tryptases also are expressed in basophils. Dissection of the human tryptase gene locus on chromosome 16p13.3, which contains the soluble as well as membrane-anchored tryptase genes, reveals that β genes are distributed among two adjacent sites, one of which also appears capable of accepting an α gene (2-4). The δ and γ genes occur in separate sites nearby. This construction of the tryptase locus implies that the α gene is an allele at the βI site and predicts that some humans will lack an α gene, which indeed is the case in 20-25% of the population (18). The clinical implications of lacking genes encoding a largely inactive enzyme remain to be established, although one prediction is that the resulting inheritance of

extra β genes, whose translation products are active, results in greater severity of diseases associated with mast cell degranulation, such as allergic inflammation. It also is likely that products of α genes, if present and correctly processed from proenzyme forms, will weaken the inflammatory potential of β tryptases by forming α/β heterotetramers with reduced catalytic power. For example, an ααββ tetramer would operate on only two of four "cylinders", compared to all four in a ββββ tetramer. Heterotetramer formation can occur *in vitro* between mouse tryptases MCP-6 and -7 (19), which differ from each in primary structure considerably more than any human α versus β pairing. On these grounds, formation of mixed tetramers involving all subtypes of human α and β tryptases is likely, although it remains to be demonstrated *in vivo*.

Chymotryptic mast cell peptidases: Chymases and cathepsin G

Mast cells make two major types of peptidases with chymotrypsin-like activity, defined as a propensity for cleaving peptide and protein targets after aromatic amino acids, especially tyrosine and phenylalanine. These are the chymases and cathepsin G. Chymases are expressed fairly selectively by mast cells. Although there is some evidence of smooth muscle expression, only mast cells appear capable of accumulating chymases in secretory granules. On the other hand, cathepsin G is expressed in myelomonocytic cells (especially neutrophils) in addition to mast cells. In dogs and humans, mast cell chymase is the product of a single gene, *CMA1* (20,21). In rodents, however, chymases and chymase-like genes have proliferated in abundance (22-26). In humans and rodents alike, cathepsin G is the product of a single gene, *CTSG*. Compared to most chymases, cathepsin G is generally a weaker enzyme and at the same time broader in peptidase specificity. It exhibits the unusual property of cleaving peptide targets after amino acids with basic as well as aromatic side chains, and thus has tryptic as well as chymotryptic acitivity. It is similar in these respects to mast cell duodenases, which have been identified so far only in ruminant mammals (specifically, cows and sheep). In humans, mast cell expression of chymase and cathepsin G is confined largely to MC_C and MC_{TC} subsets, which tend be most abundant in the dermis of the skin. They are expressed little or not at all in MC_T cells, which are over-represented in mast cells populating wet mucosa, such as the gut. The large family of chymase-like genes expressed in mice is challenging to relate to the sole human chymase not only due to multiplicity but also because several of the rodent enzymes depart significantly from human chymase in function. Two prominent examples are mouse and rat MCP-5. Although these peptidases are the rodent chymases most closely related to human chymase in a phylogenetic sense, they have acquired a catalytic domain mutation that fundamentally changes specificity: namely, from chymotryptic to elastolytic (27,28). The mouse chymase most similar to human chymase in target preferences and tissue pattern of expresson is MCP-4 (29), even though it is much less similar in primary amino acid sequence to human chymase than the functionally dissimilar MCP-5. Thus, phylogenetic similarity turns out to be an unreliable predictor of functional similarities in this group of proteins (4). This can be attributed to the ease with which changes in one or a small number of residues near the active site produce large differences in target specificity and enzyme performance. This phenomenon has been demonstrated not only by comparing the properties of natural chymases but also via site-directed mutagenesis. For example, by changing just one amino acid in dog chymase for its human counterpart among 40 residues differing between the dog and human enzyme, Diego Muilenberg and others in my laboratory converted an angiotensin-destroying enzyme into a human–like enzyme with highly selective angiotensin-generating activity (30).

Mast cells in inflammation: General principles

Inflammation mediated by mast cell activation can be deleterious or protective

—Mast cells are traditionally considered to be inflammatory. Recently, some investigations challenge this rather entrenched view, suggesting instead that the net contribution of mast cells can be either pro- or anti-inflammatory, depending on context. Probably the best known and

most extensively studied examples of a pro-inflammatory role are allergic phenomena, which have been studied in animal models of allergic inflammation (31,32) as well as in humans. There is a vast literature on this topic, which will not be reviewed comprehensively here, providing abundant evidence that the inflammatory consequences of mast cell activation in allergic disease can be deleterious, as in bee sting anaphylaxis and severe asthma. However, there is also mounting evidence that not all mast cell-initiated inflammation is unfavorable. Indeed, it can be life-saving, as in a mouse model of septic peritonitis, in which acute release of TNFα and other factors from mast cells upon exposure to colonic bacteria appears to be critically important in recruiting neutrophils quickly enough to control the infection (33). On the other hand, in murine peritonitis induced by cecal ligation and puncture, the balance between protective and deleterious contributions of the mast cell can be subtle, for although mice without mast cells almost always die--and mice with normal mast cells sometimes die--mice with mast cells deficient in chymases and tryptases do better even than wild-type mice (34). In this instance, the serine peptidases seem to increase mortality by cleaving survival-enhancing cytokines, like IL-6. In a contrasting example, although mast cells can mediate fatal anaphylactic responses to venom proteins, they also can release peptidases to detoxify venom proteins--and this can make a difference between living or dying after envenomation (35). Thus, mast cells, along with their armory of deployable peptidases, have the potential to help as well as harm, sometimes simultaneously.

Mast cells can be *anti*-inflammatory—Although mast cells clearly can cause inflammation, which can be bad or good with respect to mortality and other endpoints, it is important to acknowledge an enlarging body of evidence that mast cells also can suppress inflammation. For example, Xu and others in my laboratory showed that mast cells help to protect mice against tracheobronchitis and pneumonia from *Mycoplasma pulmonis*, which is a natural rodent pathogen causing chronic respiratory tract infection and inflammation that can last for the lifetime of the animal (36). The features of this model showcase the antibacterial as well as the anti-inflammatory role of mast cells. Mycoplasma given to naïve, mast cell-deficient mice causes an acute tracheobronchitis and pneumonia, which is much more severe than that seen in naïve wild-type mice exposed to the same number of live organisms. Indeed, mast cell-deficient mice are much more likely than wild-type mice to die from this infection. In the acute phase of the infection, infected mast cell-deficient mice develop much higher numbers of live bacteria in the lung than in infected wild-type mice, which is one likely explanation for the more severe inflammation in mast cell-deficient animals. Intriguingly, in the chronic phase of the infection (4 weeks after first exposure to mycoplasma), the numbers of live bacteria become nearly identical in the two types of infected mice, and yet the residual inflammation is more severe in mast cell-deficient mice. This suggests that mast cells suppress inflammation in this setting. Studies from other laboratories also hint at anti-inflammatory roles for mast cells. For example, mast cell degranulation associated with mosquito bites (37) and UVB irradiation (38,39) sharply dampen antigen-specific delayed-type hypersensitivity responses in the skin. Furthermore, mast cells recruited and activated under the influence of IL-9 and other products of regulatory T-cells are required for full expression of tolerance of transplanted skin (40). The exact basis of mast cell-mediated suppression of inflammation and immune responses is not clear. It is tempting to speculate that mast cell-derived “TH2” cytokines, like IL-4, IL-13 and TNFα may antagonize some aspects of TH1-type immune responses, or may have other counter-regulatory effects on other immune cells. Additional secreted potentially anti-inflammatory products of mast cells include heparin, which prevents exercise-induced asthma when aerosolized into human airways (41), and histamine, which can suppress neutrophil infiltration in rats and TH2-type responses in mice by stimulating H2 receptors (42,43). Potential anti-inflammatory actions of secreted chymases and tryptases are considered below after reviewing their proposed roles in inflammatory conditions.

Pro-inflammatory actions of mast cell serine peptidases

Tryptases and allergic inflammation—Most approaches used to probe an *in vivo* role for tryptases in allergic inflammation have been pharmacological (for review, see (44)). My laboratory, working in collaboration with Richard Tidwell, identified the first high-potency inhibitors of β-tryptases with pharmaceutical potential (45). These were aromatic diamidines, including the most potent of our series, bis(5-amidino-2-benzimidazolyl) methane (BABIM), which later was shown to prevent late-phase bronchoconstriction and to block post-allergen challenge airway hyper-responsiveness in allergic sheep (46). Along with the peptidic compound APC-366, BABIM provided the first *in vivo* evidence that β-tryptases worsen asthma-like allergic inflammation. When BABIM's structure was solved in a complex with pancreatic trypsin, it was seen to adopt a unique and unanticipated Zn^{+2}-dependent mode of slowly reversible active site blockade (47). This Zn^{+2}-binding scaffold was then used to create selective inhibitors of other serine peptidases (48).

Some later-generation inhibitors with more drug-like properties and greater β-tryptase selectivity are related to BABIM in the sense that they are dibasic aromatic compounds. Among others (49-51), these include “rational” bifunctional inhibitors engineered to bridge two active sites across the 33-45 Å hole of the β-tryptase “donut” (52-54). They also include nafamostat (55), which is especially interesting because it is an existing drug given to humans to treat disseminated intravascular coagulation and pancreatitis. Nafamostat was not developed as a tryptase inhibitor but since its identification as such it was recognized to exhibit a variety of anti-inflammatory actions, such as inhibition of radiological contrast-induced pulmonary edema and trinitrobenzene sulfonic acid-induced colitis in rats (56). Nafamostat also reduces asthma-like inflammation in mice (57), decreasing infiltration by eosinophils and bronchial responsiveness to methacholine in a model of dust mite-induced asthma. Another example using a different inhibitor, MOL 6131, developed against human mast cell β-tryptase was studied in ovalbumin-induced allergic airway inflammation (51), revealing reduced lavage and tissue eosinophilia, goblet cell hyperplasia, mucus secretion and peribronchial edema. β-Tryptase inhibitors also show promise in human asthma (58) and allergic rhinitis (59). These findings indicate that β-like tryptases potentiate allergic inflammation and also may contribute specifically to the airway remodeling that accompanies chronic allergic inflammation.

The means by which tryptases promote allergic inflammation are not entirely clear, although several mechanisms are proposed. For example, studies in sheep lung and skin suggest that tryptase itself promotes histamine release and therefore may help to spread the degranulation signal from one group of activated mast cells to others (60,61). Theoretically, tryptases also may potentiate the appearance and persistence of local edema by acting in concert with mast cell-derived heparin to inhibit local formation of a fibrin clot (62). One contribution of β-tryptases to this phenomenon is as part of the heparin delivery package, by virtue of its physical attachment to heparin proteoglycan deposited in the vicinity of degranulating mast cells. Tryptases also can contribute by destroying pro-coagulant proteins such as high molecular weight kininogen and fibrinogen itself, as demonstrated *in vitro* (63-66). Selective cleavage by tryptase renders fibrinogen unclottable by thrombin. Tryptase also has some potential to activate kinin-generating pro-kallikreins and to release vascular permeability-enhancing kinins directly from kininogens (67-69).

Other likely mechanisms of tryptase-induced inflammation include leukocyte recruitment mediated indirectly by stimulating chemokine release from epithelial and endothelial cells. For example, β-tryptases can provoke responses from airway epithelial cells, including adhesion molecule expression and IL-8 release, thereby attracting neutrophils and other inflammatory cells to the airway (70). Indeed, this occurs when human βI (but not α) tryptase is placed into mouse airway, as shown by Stevens and colleagues (71). Neutrophilic infiltration also follows injection of mouse tryptase MCP-6 (but not MCP-7) into the peritoneal cavity (72). More

apropos to allergic-type inflammation, the Stevens group also reported that recombinant human γ-tryptase, introduced to the airway in a form engineered to be soluble, provokes production of the allergic cytokine IL-13, and bronchial irritability (14). While not establishing whether γ-tryptase in its native, membrane-anchored form possesses these capabilities, this study does suggest a potential link between γ-tryptase and physiological responses more specific to asthma.

Tryptases in airway hyperresponsiveness and remodeling—Effects of tryptase on narrowing of the bronchial lumen, “twitchiness” in response to bronchoconstricting agonists, and asthma-associated remodeling may not be related to inflammation, and so will be considered here as a potentially separate phenomenon. A series of *in vitro* and *ex vivo* experiments in my laboratory in the late 1980's suggested that tryptases could promote bronchoconstriction by cleaving bronchodilating peptides of the non-adrenergic, non-cholinergic nervous system: namely, vasoactive intestinal peptide (VIP) and related peptides. These studies established that VIP is an excellent substrate of β-like canine and human tryptases and that the resulting fragmentation ablates VIP's muscle-relaxing activity (73,74). Further, we showed that inhibitors of tryptase and other peptidases enhance VIP's bronchodilator potency in isolated human bronchi (75). These findings led us to hypothesize that airway tryptase worsens asthmatic bronchoconstriction. In related studies, Sekizawa, Nadel and I showed that purified mast cell tryptase added to isolated dog bronchi, while not contracting smooth muscle by itself, greatly enhanced responses to histamine and other bronchoconstricting agonists (76). These studies were the first to implicate tryptase in hyperresponsiveness--a poorly understood phenomenon sometimes considered to be the physiological *sine qua non* of asthma--and encouraged us to collaborate with Judy Black and others in studies of human bronchi (77), in which tryptase augmented histamine-induced muscle contraction in bronchi excised from allergic individuals. Patrick Berger, Manuel Tunon-de-Lara and colleagues later reported similar findings in non-sensitized human bronchi, in which tryptase induced migration of mast cells into the airway sub-epithelium nearer to smooth muscle (78). These studies foreshadowed the intriguing finding of Peter Bradding and colleagues that infiltration of smooth muscle bundles by mast cells—sometimes called mast cell myositis--is a consistent and perhaps unique feature of asthmatic bronchi (79).

Asthma-associated remodeling includes reticular basement membrane thickening/sub-epithelial fibrosis, increased airway smooth muscle mass, and goblet cell metaplasia. Several lines of evidence suggest that these architectural changes, which also may be associated with some of the physiological derangements afflicting asthmatics, may be influenced directly or indirectly by tryptase. Indications of direct effects come from the *in vitro* discovery that β-like tryptases provoke a variety of responses from fibroblasts and airway smooth muscle cells. The first hints that mast cell tryptases are growth factors came from investigations conducted by Steve Ruoss and Thomas Hartmann in my laboratory, who showed that tryptases upregulate DNA synthesis and proliferation in fibroblasts (80,81). These studies revealed not only that tryptase is a strong mitogen in its own right, but that it synergizes with more traditional growth factors, such as PDGF and FGF. Other investigators showed that tryptase stimulates fibroblast chemotaxis and production of collagen (82,83). Furthermore, a series of studies conducted with James Brown revealed that tryptase is an airway smooth muscle cell mitogen activating intracellular MAP kinase pathways (84-86). These effects do not seem to be mediated by PAR-2, although some tryptases have PAR-2-activating capacity (see below). Although details of the mechanism of direct tryptase-mediated mitogenesis remain to be worked out, the likelihood that they play a role in asthma-associated airway remodeling is high given the nature of the *in vitro* observations, demonstration of tryptase release in asthmatic airways (87), and effects of tryptase inhibition on airway remodeling in a mouse model of allergic inflammation (51).

Tryptases in neutrophil recruitment, itching and neurogenic inflammation— Tryptases can cause inflammation not classically allergic in nature. This includes the aforementioned neutrophilic peritonitis provoked by mouse MCP-6 (72). Not all tryptases (for example, not mouse MCP-7 or human α) exhibit this property. Among human tryptases, β-tryptases appear to have the greatest potential in this regard, for recombinant βI-tryptase instilled into mouse airway causes neutrophil influx (71). Interestingly, human βI placed into mouse airway protects against *Klebsiella* pneumonia (71). Thus, tryptase can be pro-inflammatory yet protective. Although the mechanism of protection is not known, it may have parallels with the mechanism of the proposed role of mast cell TNFα in cecal ligation and puncture, in which TNF's facilitation of neutrophil influx into peritoneum allows spilled bacteria to be controlled and contained early enough to affect mortality (33). If tryptase is protective because it recruits neutrophils to kill bacteria, then the positive contributions of tryptases will be inseparable from the inflammation they cause.

At times, tryptase-induced inflammation may do more harm than good. This may be the case in inflammatory bowel disease, e.g. ulcerative colitis, a condition in which tryptase inhibitors may be beneficial (88). Tryptase's contribution to inflammatory bowel disease may relate not only to neutrophilic inflammation but also to cleavage-activation of proteinase-activate receptor (PAR)-2. My laboratory helped Nigel Bunnett's research team establish that β-tryptases activate PAR-2 on cultured rat colonic myocytes and that PAR-2 regulates motility in explants of colon (89). β-Tryptases are not intrinsically as potent as pancreatic trypsin in stimulating PAR-2. However, this difference in potency may be offset by high concentration of tryptases in the immediate vicinity of a degranulating mast cell, expression of tryptases in tissue locations in which trypsin is not expressed, and remarkable inhibitor resistance of tryptases compared with trypsin. Further studies with the Bunnett group revealed that tryptase activates PAR-2 expressed by human keratinocytes (90). Because numbers of dermal mast cells increase in atopic dermatitis, tryptase may play a role in keratinocyte growth and pruritus associated with this condition. Indeed, tryptase and PAR-2 both are linked to itching (91-93). Some itch-promoting effects of tryptase may be mediated via PAR-2 expressed by afferent neurons, a capability suggested by more recent studies with Martin Steinhoff and Nigel Bunnett (94). The interaction of tryptases with neural PAR-2 in skin, gut and elsewhere suggest a mechanism for mast cell peptidases to promote neurogenic inflammation. However, as further discussed below, tryptases and chymases also can prevent neurogenic inflammation from getting out of hand by destroying pro-inflammatory neuropeptides. Another interesting property of β-tryptase is an ability to stimulate cultured vascular endothelial cells to form tubes (95). This behavior suggests one of several means by which mast cells could promote the growth and differentiation of new blood vessels (i.e., angiogenesis), as my group demonstrated in studies conduced with Lisa Coussens in a model of skin cancer and mast cell-dependent angiogenesis (96). β-Tryptase also induces cardiac endothelial cells to produce pro-inflammatory and pro-angiogenic chemokines, as demonstrated in a study conducted with the Frangogiannis group, who provided evidence of mast cell activation and involvement in the healing phase of myocardial infarction (97).

Chymases and allergic inflammation—In rodent models of allergic phenomena, the value of chymases as biomarkers of anaphylactic mast cell degranulation is well established. For example, the rat mucosal mast cell chymase MCP-II appears in blood (98) and translocates to gut lumen after intestinal anaphylaxis in parasitized mice challenged with worm antigen (99). Rat MCP-II also can appear in the blood after mast cell degranulation provoked by a Pavlovian response (100), thereby underscoring the connections between mast cells and the nervous system. In a mouse model of dust mite-induced allergic airway inflammation (101), intratracheal challenge with *Der f* produced a serum rise in the chymase MCP-1. Unfortunately in humans equivalent assays for assessing systemic release of chymase are not generally available. Although the rodent studies suggest that some types of chymases can achieve a wide

distribution, many if not most effects of chymases in allergic events are likely to be local rather than systemic.

In a series of experiments conducted with Sommerhoff, Nadel and others, we showed that mast cell chymase and the related mast cell/neutrophil enzyme cathepsin G are potent stimulators of secretion from cultured airway gland serous cells (102,103). Further, in a histochemical study of human bronchi, we showed that chymase-positive mast cells are locally enriched in the immediate vicinity of submucosal glands, where secreted chymase might be positioned to fulfill its potential as a secretagogue (104). Working with purified dog chymase, which is highly similar to the human enzyme in structure and function (21,105-107), we showed that chymase augments the size of skin wheals (108). Although chymase alone does not cause a wheal when injected into skin, it greatly increases the size of the wheal caused by co-injected histamine; furthermore, chymase inhibitors decrease wheals induced by allergen. Thus it appears that chymase can worsen allergic inflammation by acting in conjunction with other inflammatory products of mast cells. To date the evidence from rodent models suggests that one or more chymases released in the setting of allergic inflammation is protective. In particular, mice lacking MCP-1 chymase have difficulty expelling the intestinal nematode, *Trichinella spiralis* (109). Because mouse chymase MCP-1 has no direct counterpart in dogs or humans and is not closely related to human chymase in form, function or tissue expression, it is perhaps hazardous to speculate that human chymase plays a similar protective role. If human chymase is in fact required to protect humans from parasites, systemic pharmacological inactivation of chymase for the sake of inhibiting allergic inflammation or other properties of chymase, such as its ability to generate angiotensin II, may be unwise. Nonetheless, there are a variety of reasons that human chymase might be a good target for inhibition; indeed a variety of chymase inhibitors are under development and are being explored for their pharmaceutical potential.

Chymases in matrix destruction and turnover—Compared to β-type tryptases, most chymases have more destructive potential, given that can cleave a fairly wide variety of peptide and protein targets. Perhaps another indication of this potential is the observation that high copy number transgenic expression of human chymase in mice is lethal *in utero* (110). On the other hand, chymases are more susceptible to inhibition by circulating and extravascular anti-peptidases, including serpins and α_2-macroglobulin, and therefore may have a shorter active life after secretion. One recent and rather unexpected piece of *in vivo* evidence of a role for chymases in connective tissue turnover was supplied by the MCP-4 knockout mouse, as reported Gunnar Pejler and colleagues (111). Compared to wild-type mice, MCP-4 chymase-deficient animals have thicker ears and more connective tissue, suggesting that lack of MCP-4 produces an imbalance between extracellular matrix production and destruction favoring the former. MCP-4 is the mouse enzyme most resembling human chymase in enzyme activity and tissue expression, though not its closest relative in a phylogenetic sense.

Chymases contribute to matrix destruction directly by cleaving proteins such as fibronectin and non-helical collagens. However, their indirect contributions may be just as significant, if not more so. For example, Ken Fang, working with dog chymase, matrix metalloproteases (MMPs) and cultured mast cells in my laboratory, discovered that dog mastocytoma cells secrete and activate MMP-9/gelatinase B. This broad-specificity MMP directly cleaves a wide spectrum of matrix proteins and also activates cascades of MMP activity by activating other MMPs. In his cells, the activation of MMP-9 was coupled to mast cell degranulation (112)—specifically, to release of chymase. He went on to identify the mode, sites and mechanism of activation, which was proteolytic (113). In important further work, he showed that although pro-MMP-9 is released from mast cells (and perhaps all cells) bound to an inhibitor, TIMP-1, chymase destroys TIMP-1 during the activation process (114). This work established the principal that mast cells—unlike other MMP-9-secreting cells—are the complete package: they secrete, activate and disinhibit MMP-9, and thus can immediately get down to the business of

degrading matrix, without help from other sources. The secretion and activation of MMP-9 does not seem to be a property just of mast cell tumors, as it has been validated by non-malignant cells, including mast cells cultured from canine bone marrow (115). Chymase-mediated activation of MMP-9 is also a prominent feature of mast cell-rich hyperplastic skin in a mouse cancer model (96), in which MMP-9 contributes to tumor progression (116). As further evidence that chymases are major activators of MMP-9 in vivo, Gunnar Pejler and colleagues showed that MCP-4-deficient mice have almost exclusively inactive pro-MMP-9 in tissue extract, compared to wild type mice (111); additionally, MCP-4-deficient mice have defective thrombin regulation and FN turnover (29). It bears noting that the above actions of chymase differ from those of β-tryptases, which are not effective activators of MMP-9, although their ability to activate pro-stromelysin/MMP-3 provides a potential means of activating MMP-9 indirectly via MMP-3 (117). Interestingly, my group and others showed that tryptases, mastins and chymases, like MMP-9 itself, are strong gelatinases (10,96,118). However, the extent to which an ability to hydrolyze denatured collagens (which are the main components of gelatin) predicts *in vivo* importance in matrix destruction and remodeling remains to be resolved.

There also are hints that mast cell serine peptidases promote ischemia-reperfusion injury, particularly of muscle. For example, the Gurish group showed that this type of injury to skeletal muscle is mast cell-dependent and is less severe in mice lacking the elastolytic chymase-like enzyme MCP-5 (119). It remains to be seen whether human chymase has similar potential to harm skeletal muscle, given that the human enzyme is mainly chymotryptic rather than elastolytic in its cleavage preferences, as we showed by screening a combinatorial library of peptide substrates (120). It is possible that tryptase, if not chymase, damages tissues in ischemia-reperfusion injury of heart muscle associated with myocardial infarction, as suggested by collaborators in the Frangogiannis laboratory (97).

Chymases in hypertension, atherosclerosis, and diseases of mycocardium— The body of experimental evidence that chymases are important in cardiovascular diseases, including regulation of blood pressure (121), is large and expanding. Much of this evidence, which has been reviewed elsewhere (122-124), is beyond the scope of this review. Nonetheless, it bears emphasizing that the presumed basis of many chymase effects on cardiovascular pathology is an ability to activate angiotensin I to angiotensin II. Indeed, chymases (but not angiotensin converting enzyme) appear to be the principal source of extravascular angiotensin II in mammals. Human chymase was shown to be a potent angiotensin II-generator over a quarter of a century ago by Travis and coworkers (125). Colleagues in my laboratory showed that angiotensin II-generating activity is a property shared by a number of mammalian chymases despite substantial divergence in primary structure (22,107). These studies suggested that the major angiotensin-generating chymase in mice is MCP-4, a prediction which is supported by studies by Gunnar Pejler and colleagues on mast cells from mice lacking MCP-4 (126). As demonstrated by intravital microscopy of small vessels in hamster skeletal muscle in collaboration with Israel Rubinstein and colleagues (127), angiotensin II-generating chymases may modulate acute changes in vessel caliber in response to lipopolysaccharide and other influences. However, angiotensin II also can have chronic effects on cardiovascular smooth muscle growth and remodeling. The likelihood that chymases influence remodeling by this mechanism is suggested by studies in animal models using pharmacological inhibitors (reviewed by (124)), as well as by the phenotype of transgenic mice over-expressing chymase (110,128,129). However, some short- and long-term effects of chymase on vessels and myocardium may be mediated by other means, such as by activating endothelin (130), which plays a pathological role in pulmonary hypertension. Other effects on matrix remodeling could be mediated by activation of pro-MMP-9, as discussed above. The ability of mast cell chymases to activate MMP-9 and of β-tryptases to activate MMP cascades by cleaving pro-MMP-3 prompted speculation that these peptidases destabilize and help to rupture atherosclerotic plaques (123,131), which is thought to be a precipitating event in myocardial infarction.

Chymase also may weaken plaques by degrading apolipoproteins and matrix proteins directly (132), by inhibiting production of structural components of plaque matrix by smooth muscle cells (133), and by attacking proteins attaching endothelial cells to matrix (134).

Anti-inflammatory actions of mast cell serine peptidases

Because peptidases are so often associated with inflammation, it may be counterintuitive that mast cell peptidases can limit inflammation—and yet several lines of evidence suggest that indeed this indeed is the case, as can be true of mast cells themselves (36). One mechanism by which this can occur is by cleavage of pro-inflammatory chemokines and cytokines. For example, human β-tryptase cleaves and markedly reduces the activity of eosinophil chemotaxins eotaxin and RANTES *in vitro* (135). This suggests a potential explanation for the paucity of eosinophils in human asthmatic airway smooth muscle bundles, where mast cells congregate. Human chymase similarly affects eotaxin, without affecting RANTES and several other chemokines (135). The studies of Zhao and Schwartz showed that a variety of cytokines produced by cultured human mast cells, including IL-5, -6, -13 and TNFα, are cleaved by mast cell peptidases (primarily chymase and/or cathepsin G rather than tryptases (136)). In the absence of added peptidase inhibitors, cytokine production by human masts in culture is seriously underestimated. The extent to which destruction of human cytokines and chemokines by tryptases and chymases is important in limiting inflammatory responses *in vivo* is not yet clear. However, studies in mice conducted in collaboration with Paul Wolters and others suggest that hydrolysis of mast cell-derived IL-6 by mouse tryptase MCP-6 increases mortality in mice with septic peritonitis (34); thus, the net effect of cytokine inactivation by mast cell peptidases can be negative or even lethal.

However, when the target of mast cell peptidases is a toxic or inflammatory endogenous peptide, inactivation can be beneficial. For example, destruction of endothelin by mast cell carboxpeptidase and chymases reduces toxicity *in vivo*, as shown in mice by Galli and colleagues (137). Furthermore, Brain and Williams showed that the CGRP-mediated flare reaction (a classic feature of inflammation associated with the “triple response of Lewis”) initiated by substance P in the skin is rapidly terminated by unidentified peptidases (138)—most likely tryptases, which Tam and others in my laboratory showed to be cleaved by tryptase (74). Indeed, from a kinetic perspective, CGRP is the best natural substrate identified for human β-tryptase. Tryptases and chymases also may cleave and detoxify foreign peptides and proteins. For example, chymase cleaves and reduces allergenicity of birch profilin (139), a common aeroallergen and cause of allergic rhinitis, and may also play a role in detoxifying venoms (35). Although at least one mouse chymase, MCP-1, helps to expel intestinal parasites (109), it is not yet clear whether this involves direct attack of worm proteins or an indirect effect on intestinal inflammation or motility.

In general, although mast cells tend to bolster host defense in intestinal parasitosis, peritonitis and pneumonia, the resulting inflammation can be harmful and even fatal to the host. Based on the evidence reviewed above, mast cell tryptases and chymases embody similar dichotomies of benefit versus detriment, control versus spread of inflammation, and tissue defense versus destruction. Of course similar dichotomies and tradeoffs are inherent in the actions of other mediators of allergic inflammation, including “TH2” cytokines (140), and of immunity generally.

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References

1. Caughey GH. Tryptase genetics and anaphylaxis. J Allergy Clin Immunol 2006;117:1411–1414. [PubMed: 16751005]
2. Pallaoro M, Fejzo MS, Shayesteh L, Blount JL, Caughey GH. Characterization of genes encoding known and novel human mast cell tryptases on chromosome 16p13.3. J Biol Chem 1999;274:3355–3362. [PubMed: 9920877]
3. Caughey GH, et al. Characterization of human γ-tryptases, novel members of the chromosome 16p mast cell tryptase and prostasin gene families. J Immunol 2000;164:6566–6575. [PubMed: 10843716]
4. Caughey GH. Genetic insights into mast cell chymase and tryptase function. Clin Exp All Rev 2004;4:96–101.
5. Wong GW, et al. Identification of a new member of the tryptase family of mouse and human mast cell proteases which possesses a novel COOH-terminal hydrophobic extension. J Biol Chem 1999;274:30784–30793. [PubMed: 10521469]
6. Ghildyal N, et al. Lack of expression of the tryptase mouse mast cell protease 7 in mast cells of the C57BL/6J mouse. J Immunol 1994;153:2624–2630. [PubMed: 8077672]
7. Vanderslice P, Craik CS, Nadel JA, Caughey GH. Molecular cloning of dog mast cell tryptase and a related protease: structural evidence of a unique mode of serine protease activation. Biochemistry 1989;28:4148–4155. [PubMed: 2504277]
8. Yezzi MJ, Hsieh IE, Caughey GH. Mast cell and neutrophil expression of dog mast cell proteinase-3, a novel tryptase-related serine proteinase. J Immunol 1994;152:3064–3072. [PubMed: 8144904]
9. Raymond WW, Tam EK, Blount JL, Caughey GH. Purification and characterization of dog mast cell protease-3, an oligomeric relative of tryptases. J Biol Chem 1995;270:13164–13170. [PubMed: 7768912]
10. Raymond WW, Sommerhoff CP, Caughey GH. Mastin is a gelatinolytic mast cell peptidase resembling a mini-proteasome. Arch Biochem Biophys 2005;435:311–322. [PubMed: 15708374]
11. Caughey GH, Viro NF, Ramachandran J, Lazarus SC, Borson DB, Nadel JA. Dog mastocytoma tryptase: affinity purification, characterization and amino-terminal sequence. Arch Biochem Biophys 1987;258:555–563. [PubMed: 3118812]
12. Schwartz LB, Bradford TR. Regulation of tryptase from human lung mast cells by heparin. Stabilization of the active tetramer. J Biol Chem 1986;261:7372–7379. [PubMed: 3519608]
13. Bhagwandin VJ, Hau LW, Mallen-St Clair J, Wolters PJ, Caughey GH. Structure and activity of human pancreasin, a novel tryptic serine peptidase expressed primarily by the pancreas. J Biol Chem 2003;278:3363–3371. [PubMed: 12441343]
14. Wong GW, et al. Biochemical and functional characterization of human transmembrane tryptase (TMT)/tryptase gamma. TMT is an exocytosed mast cell protease that induces airway hyperresponsiveness in vivo via an interleukin-13/interleukin-4 receptor α/signal transducer and activator of transcription (STAT) 6-dependent pathway. J Biol Chem 2002;277:41906–41915. [PubMed: 12194977]
15. Yuan J, et al. Expression and characterization of recombinant γ-tryptase. Protein Expr Purif 2006;49:47–54. [PubMed: 16815034]
16. Verghese GM, Gutknecht F, Caughey GH. Prostasin regulates epithelial monolayer function: cell-specific Gpld1-mediated secretion and functional role for the GPI anchor. Am J Physiol Cell Physiol. 2006in press
17. Vanderslice P, Ballinger SM, Tam EK, Goldstein SM, Craik CS, Caughey GH. Human mast cell tryptase: Multiple cDNAs and genes reveal a multigene serine protease family. Proc Natl Acad Sci USA 1990;87:3811–3815. [PubMed: 2187193]
18. Soto D, Malmsten C, Blount JL, Muilenburg DJ, Caughey GH. Genetic deficiency of human mast cell α-tryptase. Clin Exp Allergy 2002;32:1000–1006. [PubMed: 12100045]
19. Huang C, et al. Formation of enzymatically active, homotypic, and heterotypic tetramers of mouse mast cell tryptases. Dependence on a conserved Trp-rich domain on the surface. J Biol Chem 2000;275:351–358. [PubMed: 10617625]

20. Caughey GH, et al. The human mast cell chymase gene (CMA1): mapping to the cathepsin G/granzyme gene cluster and lineage-restricted expression. Genomics 1993;15:614–620. [PubMed: 8468056]
21. Caughey GH, Blount JL, Koerber KL, Kitamura M, Fang KC. Cloning and expression of the dog mast cell α-chymase gene. J Immunol 1997;159:4367–4375. [PubMed: 9379034]
22. Caughey GH. New developments in the genetics and activation of mast cell proteases. Mol Immunol 2002;38:1353–1357. [PubMed: 12217407]
23. Wouters MA, Liu K, Riek P, Husain A. A despecialization step underlying evolution of a family of serine proteases. Mol Cell 2003;12:343–354. [PubMed: 14536074]
24. Puente XS, Lopez-Otin C. A genomic analysis of rat proteases and protease inhibitors. Genome Res 2004;14:609–622. [PubMed: 15060002]
25. Gallwitz M, Reimer JM, Hellman L. Expansion of the mast cell chymase locus over the past 200 million years of mammalian evolution. Immunogenetics 2006;58:655–669. [PubMed: 16807745]
26. Gallwitz M, Hellman L. Rapid lineage-specific diversification of the mast cell chymase locus during mammalian evolution. Immunogenetics 2006;58:641–654. [PubMed: 16807746]
27. Kunori Y, et al. Rodent α-chymases are elastase-like proteases. Eur J Biochem 2002;269:5921–5930. [PubMed: 12444981]
28. Karlson U, Pejler G, Tomasini-Johansson B, Hellman L. Extended substrate specificity of rat mast cell protease 5, a rodent α-chymase with elastase-like primary specificity. J Biol Chem 2003;278:39625–39631. [PubMed: 12900423]
29. Tchougounova E, Pejler G, Abrink M. The chymase, mouse mast cell protease 4, constitutes the major chymotrypsin-like activity in peritoneum and ear tissue. A role for mouse mast cell protease 4 in thrombin regulation and fibronectin turnover. J Exp Med 2003;198:423–431. [PubMed: 12900518]
30. Muilenburg DJ, Raymond WW, Wolters PJ, Caughey GH. Lys40 but not Arg143 influences selectivity of angiotensin conversion by human α-chymase. Biochim Biophys Acta 2003;1596:346–356. [PubMed: 12007614]
31. Williams CM, Galli SJ. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. J Exp Med 2000;192:455–462. [PubMed: 10934234]
32. Yu M, Tsai M, Tam SY, Jones C, Zehnder J, Galli SJ. Mast cells can promote the development of multiple features of chronic asthma in mice. J Clin Invest 2006;116:1633–1641. [PubMed: 16710480]
33. Malaviya R, Ikeda T, Ross E, Abraham SN. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-α. Nature 1996;381:77–80. [PubMed: 8609993]
34. Mallen-St Clair J, Pham CT, Villalta SA, Caughey GH, Wolters PJ. Mast cell dipeptidyl peptidase I mediates survival from sepsis. J Clin Invest 2004;113:628–634. [PubMed: 14966572]
35. Metz M, et al. Mast cells can enhance resistance to snake and honeybee venoms. Science 2006;313:526–530. [PubMed: 16873664]
36. Xu X, et al. Mast cells protect mice from mycoplasma pneumonia. Am J Respir Crit Care Med 2006;173:219–225. [PubMed: 16210667]
37. Depinay N, Hacini F, Beghdadi W, Peronet R, Mecheri S. Mast cell-dependent down-regulation of antigen-specific immune responses by mosquito bites. J Immunol 2006;176:4141–4146. [PubMed: 16547250]
38. Hart PH, Grimbaldeston MA, Swift GJ, Jaksic A, Noonan FP, Finlay-Jones JJ. Dermal mast cells determine susceptibility to ultraviolet B-induced systemic suppression of contact hypersensitivity responses in mice. J Exp Med 1998;187:2045–2053. [PubMed: 9625764]
39. Alard P, Kurimoto I, Niizeki H, Doherty JM, Streilein JW. Hapten-specific tolerance induced by acute, low-dose ultraviolet B radiation of skin requires mast cell degranulation. Eur J Immunol 2001;31:1736–1746. [PubMed: 11385618]
40. Lu LF, et al. Mast cells are essential intermediaries in regulatory T-cell tolerance. Nature 2006;442:997–1002. [PubMed: 16921386]
41. Ahmed T, Garrigo J, Danta I. Preventing bronchoconstriction in exercise-induced asthma with inhaled heparin. N Engl J Med 1993;329:90–95. [PubMed: 8510708]

42. Hirasawa N, Watanabe M, Mue S, Tsurufuji S, Ohuchi K. Downward regulation of neutrophil infiltration by endogenous histamine without affecting vascular permeability responses in air-pouch-type carrageenin inflammation in rats. Inflammation 1991;15:117–126. [PubMed: 1712757]
43. Jutel M, et al. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. Nature 2001;413:420–425. [PubMed: 11574888]
44. Fang, KC.; Caughey, GH. Mast cell proteases. In: Eissa, NT.; Huston, DP., editors. Therapeutic targets in airway inflammation. Marcel Dekker, Inc.; New York: 2003. p. 349-371.
45. Caughey GH, Raymond WW, Bacci E, Lombardy RJ, Tidwell RR. Bis(5-amidino-2-benzimidazolyl) methane and related amidines are potent, reversible inhibitors of mast cell tryptases. J Pharmacol Exp Therap 1993;264:676–682. [PubMed: 8437115]
46. Clark JM, et al. Tryptase inhibitors block allergen-induced airway and inflammatory responses in allergic sheep. Am J Resp Crit Care Med 1995;152:2076–2083. [PubMed: 8520778]
47. Katz BA, et al. Design of potent selective zinc-mediated serine protease inhibitors. Nature 1998;391:608–612. [PubMed: 9468142]
48. Janc JW, Clark JM, Warne RL, Elrod KC, Katz BA, Moore WR. A novel approach to serine protease inhibition: kinetic characterization of inhibitors whose potencies and selectivities are dramatically enhanced by Zinc(II). Biochemistry 2000;39:4792–4800. [PubMed: 10769136]
49. Ono S, Kuwahara S, Takeuchi M, Sakashita H, Naito Y, Kondo T. Syntheses and evaluation of amidinobenzofuran derivatives as tryptase inhibitors. Bioorg Med Chem Lett 1999;9:3285–3290. [PubMed: 10612586]
50. Wright CD, et al. Inhibition of allergen-induced pulmonary responses by the selective tryptase inhibitor 1,5-bis-[4-[(3-carbamimidoyl-benzenesulfonylamino)-methyl]-phenoxy]-pentane (AMG-126737). Biochem Pharmacol 1999;58:1989–1996. [PubMed: 10591155]
51. Oh SW, et al. Tryptase inhibition blocks airway inflammation in a mouse asthma model. J Immunol 2000;168:1992–2000. [PubMed: 11823536]
52. Burgess LE, et al. Potent selective nonpeptidic inhibitors of human lung tryptase. Proc Natl Acad Sci USA 1999;96:8348–8352. [PubMed: 10411878]
53. Schaschke N, Dominik A, Matschiner G, Sommerhoff CP. Bivalent inhibition of β-tryptase: distance scan of neighboring subunits by dibasic inhibitors. Bioorg Med Chem Lett 2002;12:985–988. [PubMed: 11959009]
54. Selwood T, Elrod KC, Schechter NM. Potent bivalent inhibition of human tryptase-beta by a synthetic inhibitor. Biol Chem 2003;384:1605–1611. [PubMed: 14719803]
55. Mori S, Itoh Y, Shinohata R, Sendo T, Oishi R, Nishibori M. Nafamostat mesilate is an extremely potent inhibitor of human tryptase. J Pharmacol Sci 2003;92:420–423. [PubMed: 12939527]
56. Sendo T, et al. A potent tryptase inhibitor nafamostat mesilate dramatically suppressed pulmonary dysfunction induced in rats by a radiographic contrast medium. Br J Pharmacol 2003;138:959–967. [PubMed: 12642398]
57. Chen CL, et al. Serine protease inhibitors nafamostat mesilate and gabexate mesilate attenuate allergen-induced airway inflammation and eosinophilia in a murine model of asthma. J Allergy Clin Immunol 2006;118:105–112. [PubMed: 16815145]
58. Krishna MT, et al. Inhibition of mast cell tryptase by inhaled APC 366 attenuates allergen-induced late-phase airway obstruction in asthma. J Allergy Clin Immunol 2001;107:1039–1045. [PubMed: 11398082]
59. Erin EM, et al. Effects of a reversible β-tryptase and trypsin inhibitor (RWJ-58643) on nasal allergic responses. Clin Exp Allergy 2006;36:458–464. [PubMed: 16630150]
60. Molinari JF, Moore WR, Clark J, Tanaka R, Butterfield JH, Abraham WM. Role of tryptase in immediate cutaneous responses in allergic sheep. J Appl Physiol 1995;79:1966–1970. [PubMed: 8847261]
61. Molinari JF, Scuri M, Moore WR, Clark J, Tanaka R, Abraham WM. Inhaled tryptase causes bronchoconstriction in sheep via histamine release. Am J Respir Crit Care Med 1996;154:649–653. [PubMed: 8810600]
62. Caughey GH. Building a better heparin. Am J Respir Cell Mol Biol 2003;28:129–132. [PubMed: 12540478]

63. Maier M, Spragg J, Schwartz LB. Inactivation of human high molecular weight kininogen by human mast cell tryptase. J Immunol 1983;130:2352–2356. [PubMed: 6339626]
64. Ren S, Lawson AE, Carr M, Baumgarten CM, Schwartz LB. Human tryptase fibrinogenolysis is optimal at acidic pH and generates anticoagulant fragments in the presence of the anti-tryptase monoclonal antibody B12. J Immunol 1997;159:3540–3548. [PubMed: 9317153]
65. Huang CF, et al. The tryptase, mouse mast cell protease 7, exhibits anticoagulant activity in vivo and in vitro due to its ability to degrade fibrinogen in the presence of the diverse array of protease inhibitors in plasma. J Biol Chem 1997;272:31885–31893. [PubMed: 9395536]
66. Thomas VA, Wheeless CJ, Stack MS, Johnson DA. Human mast cell tryptase fibrinogenolysis: kinetics, anticoagulation mechanism, and cell adhesion disruption. Biochemistry 1988;37:2291–2298. [PubMed: 9485375]
67. Proud D, Siekierski ED, Bailey GS. Identification of human lung mast cell kininogenase as tryptase and relevance of tryptase kininogenase activity. Biochem Pharmacol 1988;37:1473–1480. [PubMed: 3282517]
68. Imamura T, Dubin A, Moore W, Tanaka R, Travis J. Induction of vascular permeability enhancement by human tryptase: dependence on activation of prekallikrein and direct release of bradykinin from kininogens. Lab Invest 1996;74:861–870. [PubMed: 8642782]
69. Kozik A, Moore RB, Potempa J, Imamura T, Rapala-Kozik M, Travis J. A Novel Mechanism for bradykinin production at inflammatory sites. Diverse effects of a mixture of neutrophil elastase and mast cell tryptase versus tissue and plasma kallikreins on native and oxidized kininogens. J Biol Chem 1998;273:33224–33229. [PubMed: 9837892]
70. Cairns JA, Walls AF. Mast cell tryptase is a mitogen for epithelial cells: stimulation of IL-8 production and intercellular adhesion molecule-1 expression. J Immunol 1996;156:275–283. [PubMed: 8598474]
71. Huang C, et al. Evaluation of the substrate specificity of human mast cell tryptase βI and demonstration of its importance in bacterial infections of the lung. J Biol Chem 2001;276:26276–26284. [PubMed: 11335723]
72. Huang C, et al. Induction of a selective and persistent extravasation of neutrophils into the peritoneal cavity by tryptase mouse mast cell protease 6. J Immunol 1998;160:1910–1919. [PubMed: 9469453]
73. Franconi GM, Graf PD, Lazarus SC, Nadel JA, Caughey GH. Mast cell chymase and tryptase reverse airway smooth muscle relaxation induced by vasoactive intestinal peptide in the ferret. J Pharmacol Exp Ther 1989;248:947–951. [PubMed: 2495355]
74. Tam EK, Caughey GH. Degradation of airway neuropeptides by human lung tryptase. Am J Respir Cell Mol Biol 1990;3:27–32. [PubMed: 1694672]
75. Tam EK, Franconi GM, Nadel JA, Caughey GH. Protease inhibitors potentiate smooth muscle relaxation induced by vasoactive intestinal peptide in isolated human bronchi. Am J Respir Cell Mol Biol 1990;2:449–452. [PubMed: 2187492]
76. Sekizawa K, Caughey GH, Lazarus SC, Gold WM, Nadel JA. Mast cell tryptase causes airway smooth muscle hyperresponsiveness in dogs. J Clin Invest 1989;83:175–179. [PubMed: 2642918]
77. Johnson PRA, Ammit AJ, Carlin SM, Armour CL, Caughey GH, Black JL. Mast cell tryptase potentiates histamine-induced contraction in human sensitized bronchus. Eur Resp J 1997;10:38–43.
78. Berger P, et al. Mast cell tryptase as a mediator of hyperresponsiveness in human isolated bronchi. Clin Exp Allergy 1999;29:804–812. [PubMed: 10336598]
79. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. N Engl J Med 2002;346:1699–1705. [PubMed: 12037149]
80. Ruoss SJ, Hartmann T, Caughey GH. Mast cell tryptase is a mitogen for cultured fibroblasts. J Clin Invest 1991;88:493–499. [PubMed: 1864960]
81. Hartmann T, Ruoss SJ, Raymond WW, Seuwen K, Caughey GH. Human tryptase as a potent, cell-specific mitogen: role of signalling pathways in synergistic responses. Am J Physiol Lung Cell Mol Physiol 1992;262:L528–L534.
82. Gruber BL, et al. Human mast cells activate fibroblasts: tryptase is a fibrogenic factor stimulating collagen messenger ribonucleic acid synthesis and fibroblast chemotaxis. J Immunol 1997;158:2310–2317. [PubMed: 9036979]

83. Cairns JA, Walls AF. Mast cell tryptase stimulates synthesis of type I collagen in human lung fibroblasts. J Clin Invest 1997;99:1313–1321. [PubMed: 9077541]
84. Brown JK, Tyler CL, Jones CA, Ruoss SJ, Hartmann T, Caughey GH. Tryptase, the dominant secretory granular protein in humans mast cells, is a potent mitogen for cultured dog tracheal smooth muscle cells. Am J Resp Cell Mol Biol 1995;13:227–236.
85. Brown JK, Jones CA, Rooney LA, Caughey GH, Hall IP. Tryptase's potent mitogenic effects in human airway smooth muscle cells are via nonproteolytic actions. Am J Physiol Lung Cell Mol Physiol 2002;282:197–206.
86. Brown JK, Jones CA, Rooney LA, Caughey GH. Mast cell tryptase activates extracellular-regulated kinases (p44/p42) in airway smooth-muscle cells: importance of proteolytic events, time course, and role in mediating mitogenesis. Am J Respir Cell Mol Biol 2001;24:146–154. [PubMed: 11159048]
87. Wenzel SE, Fowler A, Schwartz LB. Activation of pulmonary mast cells by bronchoalveolar allergen challenge. In vivo release of histamine and tryptase in atopic subjects with and without asthma. Am Rev Respir Dis 1988;137:1002–1008. [PubMed: 2461667]
88. Tremaine WJ, et al. Treatment of mildly to moderately active ulcerative colitis with a tryptase inhibitor (APC 2059): an open-label pilot study. Aliment Pharmacol Ther 2002;16:407–413. [PubMed: 11876693]
89. Corvera CU, et al. Mast cell tryptase regulates rat colonic myocytes through proteinase-activated receptor 2. J Clin Invest 1997;100:1383–1393. [PubMed: 9294103]
90. Steinhoff M, et al. Proteinase-activated receptor-2 in human skin: tissue distribution and activation of keratinocytes by mast cell tryptase. Exp Dermatol 1999;8:282–294. [PubMed: 10439226]
91. Steinhoff M, et al. Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. J Neurosci 2003;23:6176–6180. [PubMed: 12867500]
92. Ui H, Andoh T, Lee JB, Nojima H, Kuraishi Y. Potent pruritogenic action of tryptase mediated by PAR-2 receptor and its involvement in anti-pruritic effect of nafamostat mesilate in mice. Eur J Pharmacol 2006;530:172–178. [PubMed: 16359660]
93. Dugas-Breit S, Schopf P, Dugas M, Schiffl H, Rueff F, Przybilla B. Baseline serum levels of mast cell tryptase are raised in hemodialysis patients and associated with severity of pruritus. J Dtsch Dermatol Ges 2005;3:343–347. [PubMed: 16372800]
94. Steinhoff M, et al. Agonists of proteinase-activated receptor 2 induce inflammation by a neurogenic mechanism. Nat Med 2000;6:151–158. [PubMed: 10655102]
95. Blair RJ, et al. Human mast cells stimulate vascular tube formation. Tryptase is a novel, potent angiogenic factor. J Clin Invest 1997;99:2691–2700. [PubMed: 9169499]
96. Coussens LM, et al. Inflammatory mast cells upregulate angiogenesis during squamous epithelial carcinogenesis. Genes & Development 1999;13:1382–1397. [PubMed: 10364156]
97. Somasundaram P, et al. Mast cell tryptase may modulate endothelial cell phenotype in healing myocardial infarcts. J Pathol 2005;205:102–111. [PubMed: 15586361]
98. Miller HRP, Woodbury RG, Huntley JF, Newlands G. Systemic release of mucosal mast-cell protease in primed rats challenged with *Nippostrongylus brasiliensis*. Immunology 1983;49:471–479. [PubMed: 6345346]
99. Scudamore CL, Thornton EM, McMillan L, Newlands GF, Miller HR. Release of the mucosal mast cell granule chymase, rat mast cell protease-II, during anaphylaxis is associated with the rapid development of paracellular permeability to macromolecules in rat jejunum. J Exp Med 1995;182:1871–1881. [PubMed: 7500033]
100. MacQueen G, Marshall J, Perdue M, Siegel S, Bienenstock J. Pavlovian conditioning of rat mucosal mast cells to secrete rat mast cell protease II. Science 1989;243:83–85. [PubMed: 2911721]
101. Yu CK, Chen CL. Activation of mast cells is essential for development of house dust mite *Dermatophagoides farinae*-induced allergic airway inflammation in mice. J Immunol 2003;171:3808–3815. [PubMed: 14500682]
102. Sommerhoff CP, Caughey GH, Finkbeiner WE, Lazarus SC, Basbaum CB, Nadel JA. Mast cell chymase. A potent secretagogue for airway gland serous cells. J Immunol 1989;142:2450–2456. [PubMed: 2494259]

103. Sommerhoff CP, Nadel JA, Basbaum CB, Caughey GH. Neutrophil elastase and cathepsin G stimulate secretion from cultured bovine airway gland serous cells. J Clin Invest 1990;85:682–689. [PubMed: 2107207]
104. Matin R, Tam EK, Nadel JA, Caughey GH. Distribution of chymase-containing mast cells in human bronchi. J Histochem Cytochem 1992;40:781–786. [PubMed: 1588024]
105. Caughey GH, Viro NF, Lazarus SC, Nadel JA. Purification and characterization of dog mastocytoma chymase. Identification of an octapeptide conserved in chymotryptic leukocyte proteases. Biochim Biophys Acta 1988;952:142–149. [PubMed: 3122835]
106. Caughey GH, Raymond WW, Vanderslice P. Dog mast cell chymase: molecular cloning and characterization. Biochemistry 1990;29:5166–5171. [PubMed: 2378872]
107. Caughey GH, Raymond WW, Wolters PJ. Angiotensin II generation by mast cell α- and β-chymases. Biochim Biophys Acta 2000;1480:245–257. [PubMed: 10899625]
108. Rubinstein I, Nadel JA, Graf PD, Caughey GH. Mast cell chymase potentiates histamine-induced wheal formation in the skin of ragweed-allergic dogs. J Clin Invest 1990;86:555–559. [PubMed: 2384602]
109. Knight PA, Wright SH, Lawrence CE, Paterson YY, Miller HR. Delayed expulsion of the nematode *Trichinella spiralis* in mice lacking the mucosal mast cell-specific granule chymase, mouse mast cell protease-1. J Exp Med 2000;192:1849–1856. [PubMed: 11120781]
110. Koga T, et al. Human chymase expression in a mice induces mild hypertension with left ventricular hypertrophy. Hypertens Res 2003;26:759–768. [PubMed: 14620933]
111. Tchougounova E, Lundequist A, Fajardo I, Winberg JO, Abrink M, Pejler G. A key role for mast cell chymase in the activation of pro-matrix metalloprotease-9 and pro-matrix metalloprotease-2. J Biol Chem 2005;280:9291–9296. [PubMed: 15615702]
112. Fang KC, Raymond WW, Lazarus SC, Caughey GH. Dog mastocytoma cells secrete a 92-kD gelatinase activated extracellularly by mast cell chymase. J Clin Invest 1996;97:1589–1596. [PubMed: 8601622]
113. Fang KC, Raymond WW, Blount JL, Caughey GH. Dog mast cell α-chymase activates progelatinase B by cleaving the Phe88-Phe89 and Phe91-Glu92 bonds of the catalytic domain. J Biol Chem 1997;272:25628–25635. [PubMed: 9325284]
114. Frank BT, Rossall JC, Caughey GH, Fang KC. Mast cell tissue inhibitor of metalloproteinase-1 is cleaved and inactivated extracellularly by α-chymase. J Immunol 2001;166:2783–2792. [PubMed: 11160345]
115. Lin TY, London CA. A functional comparison of canine and murine bone marrow derived cultured mast cells. Vet Immunol Immunopathol. 2006in press
116. Coussens LM, Tinkle CL, Hanahan D, Werb Z. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell 2000;103:481–490. [PubMed: 11081634]
117. Gruber BL, et al. Synovial procollagenase activation by human mast cell tryptase. Dependence upon matrix metalloproteinase 3 activation. J Clin Invest 1989;84:1657–1662. [PubMed: 2553780]
118. Fajardo I, Pejler G. Human mast cell β-tryptase is a gelatinase. J Immunol 2003;171:1493–1499. [PubMed: 12874242]
119. Abonia JP, et al. Mast cell protease 5 mediates ischemia-reperfusion injury of mouse skeletal muscle. J Immunol 2005;174:7285–7291. [PubMed: 15905575]
120. Raymond WW, Waugh Ruggles S, Craik CS, Caughey GH. Albumin is a substrate of human chymase: Prediction by combinatorial peptide screening and development of a selective inhibitor based on the albumin cleavage site. J Biol Chem 2003;278:34517–34524. [PubMed: 12815038]
121. Li M, et al. Involvement of chymase-mediated angiotensin II generation in blood pressure regulation. J Clin Invest 2004;114:112–120. [PubMed: 15232618]
122. Dell'Italia LJ, Husain A. Dissecting the role of chymase in angiotensin II formation and heart and blood vessel diseases. Curr Opin Cardiol 2002;17:374–379. [PubMed: 12151872]
123. Lindstedt KA, Kovanen PT. Mast cells in vulnerable coronary plaques: potential mechanisms linking mast cell activation to plaque erosion and rupture. Curr Opin Lipidol 2004;15:567–573. [PubMed: 15361793]
124. Miyazaki M, Takai S, Jin D, Muramatsu M. Pathological roles of angiotensin II produced by mast cell chymase and the effects of chymase inhibition in animal models. Pharmacol Ther. 2006in press

125. Reilly CF, Tewksbury DA, Schechter NB, Travis J. Rapid conversion of angiotensin I to angiotensin II by neutrophil and mast cell proteinases. J Biol Chem 1982;257:8619–8622. [PubMed: 6807977]
126. Lundequist A, Tchougounova E, Abrink M, Pejler G. Cooperation between mast cell carboxypeptidase A and the chymase mouse mast cell protease 4 in the formation and degradation of angiotensin II. J Biol Chem 2004;279:32339–32344. [PubMed: 15173164]
127. Suzuki H, Caughey GH, Gao XP, Rubinstein I. Mast cell chymase-like protease(s) modulates *Escherichia coli* lipopolysaccharide-induced vasomotor dysfunction in skeletal muscle in vivo. J Pharmacol Exp Therap 1998;284:1156–1164. [PubMed: 9495878]
128. Chen LY, et al. Transgenic study of the function of chymase in heart remodeling. J Hypertens 2002;20:2047–2055. [PubMed: 12359984]
129. Ju H, Gros R, You X, Tsang S, Husain M, Rabinovitch M. Conditional and targeted overexpression of vascular chymase causes hypertension in transgenic mice. Proc Natl Acad Sci USA 2001;98:7469–7474. [PubMed: 11416217]
130. Nagata N, Niwa Y, Nakaya Y. A novel 31-amino-acid-length endothelin, ET-1(1-31), can act as a biologically active peptide for vascular smooth muscle cells. Biochem Biophys Res Commun 2000;275:595–600. [PubMed: 10964709]
131. Johnson JL, Jackson CL, Angelini GD, George SJ. Activation of matrix-degrading metalloproteinases by mast cell proteases in atherosclerotic plaques. Arterioscler Thromb Vasc Biol 1998;18:1707–1715. [PubMed: 9812908]
132. Lindstedt KA, Kovanen PT. Proteolysis of pericellular matrix: a process linking inflammation to plaque destabilization and rupture. Arterioscler Thromb Vasc Biol 2004;24:2205–2206. [PubMed: 15576642]
133. Leskinen MJ, et al. Mast cell chymase induces smooth muscle cell apoptosis by disrupting NF-κB-mediated survival signaling. Exp Cell Res 2006;312:1289–1298. [PubMed: 16460729]
134. Mayranpaa MI, Heikkila HM, Lindstedt KA, Walls AF, Kovanen PT. Desquamation of human coronary artery endothelium by human mast cell proteases: implications for plaque erosion. Coron Artery Dis 2006;17:611–621. [PubMed: 17047445]
135. Pang L, Nie M, Corbett L, Sutcliffe A, Knox AJ. Mast cell β-tryptase selectively cleaves eotaxin and RANTES and abrogates their eosinophil chemotactic activities. J Immunol 2006;176:3788–3795. [PubMed: 16517749]
136. Zhao W, Oskeritzian CA, Pozez AL, Schwartz LB. Cytokine production by skin-derived mast cells: endogenous proteases are responsible for degradation of cytokines. J Immunol 2005;175:2635–2642. [PubMed: 16081839]
137. Maurer M, et al. Mast cells promote homeostasis by limiting endothelin-1-induced toxicity. Nature 2004;432:512–516. [PubMed: 15543132]
138. Brain SD, Williams TJ. Substance P regulates the vasodilator activity of calcitonin gene-related peptide. Nature 1988;335:73–75. [PubMed: 2457810]
139. Mellon MB, Frank BT, Fang KC. Mast cell α-chymase reduces IgE recognition of birch pollen profilin by cleaving antibody-binding epitopes. J Immunol 2002;168:290–297. [PubMed: 11751973]
140. Finkelman FD, Urban JF Jr. The other side of the coin: the protective role of the TH2 cytokines. J Allergy Clin Immunol 2001;107:772–780. [PubMed: 11344341]

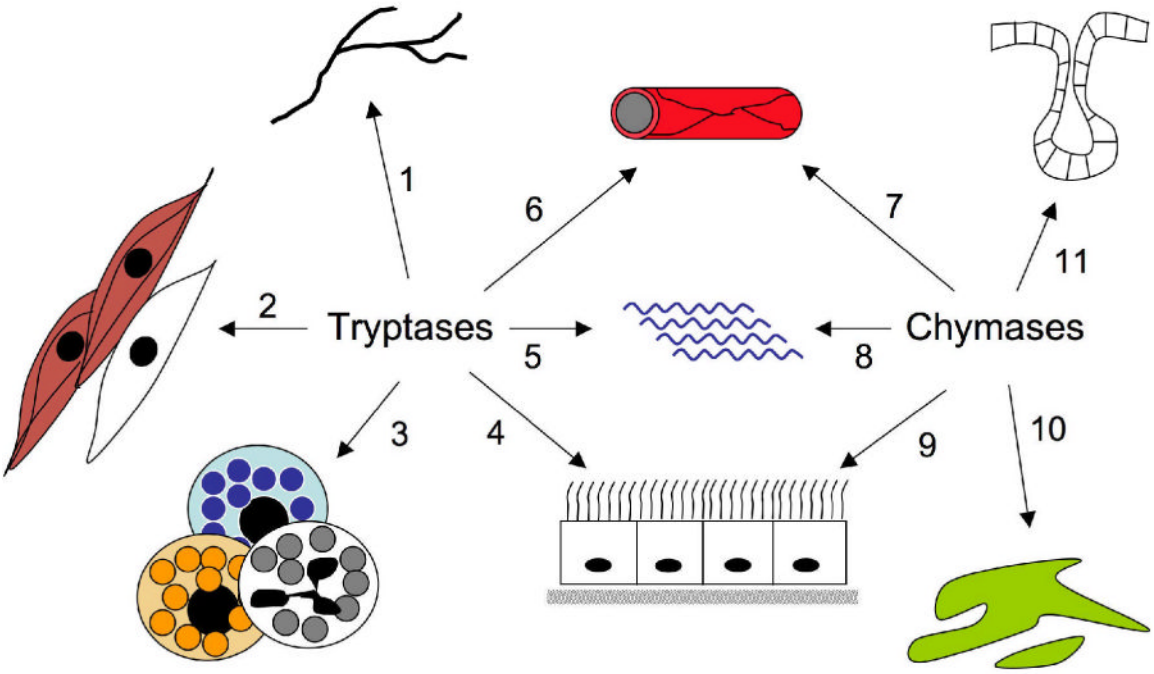

Fig. 1. Summary of targets of pro-inflammatory actions of mast cell tryptases and chymases

1) Soluble β-tryptases stimulate nerves by activating proteinase-activated receptor 2 (PAR2) and 2) affect airway smooth muscle and fibroblasts by acting as mitogens and by promoting secretion of matrix proteins. They also 3) recruit neutrophils and eosinophils and degranulate mast cells, 4) provoke airway epithelial cells to produce interleukin-8, 5) degrade extracellular matrix and fibrin clots by activating pro-stromelysin and pro-urokinase, respectively, and 6) stimulate endothelial cells to form vessels. Chymases overlap tryptases in some of their targets, but with unique effects, including the following: 7) they alter arterial caliber and intimal responses to injury by generating angiotensin II and promote new vessel growth by activating pro-MMP-9; 8) they degrade matrix proteins directly (as well as indirectly by activating pro-MMP-9); they may alter epithelial growth and repair by degrading hepatocyte growth factor; 10) they may affect the properties of mucus by degrading serous cell proteoglycans and 11) also stimulate gland cell secretion itself.

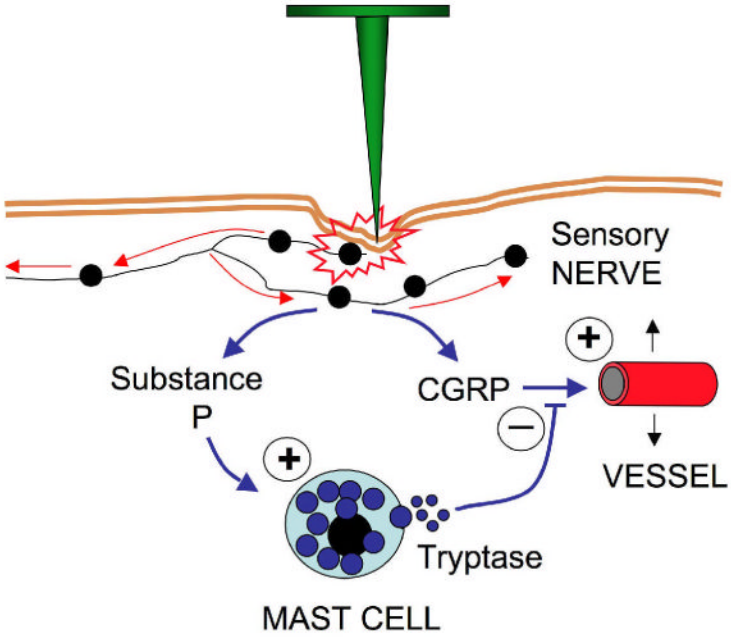

Fig. 2. Cutaneous example of an anti-inflammatory effect of mast cell serine peptidases

Skin trauma stimulates dermal sensory nerves, which propagate antegrade and retrograde impulses and liberate inflammatory neuropeptides such as substance P and calcitonin gene-related peptide (CGRP). The latter is a potent vasodilator contributing to the flare reaction surrounding sites of injury. Tryptase released from local mast cells by substance P cleaves and inactivates CGRP, thereby shortening duration of the flare.

Table 1

Expressed mast cell tryptases

Species	Enzyme	Gene	Features
Human	α-Tryptase	*TPSAB1*	Soluble; largely *in*active; not stored
	βI-Tryptase	*TPSAB1*	Soluble; active; tetrameric; stored
	βII-Tryptase	*TPSB2*	Soluble; active; stored
	βIII-Tryptase	*TPSB2*	? Soluble; active; stored
	γ-Tryptase	*TPSG1*	Membrane anchored; two-chain; active
	δ-Tryptase	*TPSD1*	Truncated; chimeric; largely *in*active
Mouse	MCP-6	*Mcpt6*	Soluble; active; tetrameric; stored
	MCP-7	*Mcpt7*	Soluble; active; tetrameric
	γ-Tryptase/TMT	*Tpsg1*	Membrane anchored; two-chain
	Mastin/MCP-11	*Prss34*	Soluble; active
Dog	Tryptase		Soluble; active; tetrameric
	Mastin	*LOC448801*	Soluble; active; multimeric

Table 2

Expressed mast cell chymases and chymase-like peptidases

Species	Enzyme	Gene	Features
Human	α-Chymase	*CMA1*	Stored; chymotryptic
	Cathepsin G	*CTSG*	Stored; broad spectrum activity (tryptic, chymotryptic, met-ase)
Mouse	α-Chymase/MCP-5	*Mcpt5*	Weakly active; elastolytic
	β-Chymase MCP-1	*Mcpt1*	Stored; chymotryptic
	β-Chymase MCP-2	*Mcpt2*	Largely inactive
	β-Chymase MCP-4	*Mcpt4*	Stored; chymotryptic
	Cathepsin G	*Ctsg*	?
Dog	α-Chymase	*CMA1*	Stored; chymotryptic
	Cathepsin G	*CTSG*	?