

CHAPTER 6

Mast Cell Proteases

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Abstract

Mast cells (MCs) are traditionally thought of as a nuisance for its host, for example, by causing many of the symptoms associated with allergic reactions. In addition, recent research has put focus on MCs for displaying harmful effects during various autoimmune disorders. On the other hand, MCs can also be beneficial for its host, for example, by contributing to the defense against insults such as bacteria, parasites, and snake venom toxins. When the MC is challenged by an external stimulus, it may respond by degranulation. In this process, a number of powerful preformed inflammatory “mediators” are released, including cytokines, histamine, serglycin proteoglycans, and several MC-specific proteases: chymases, tryptases, and carboxypeptidase A. Although the exact effector mechanism(s) by which MCs carry out their either beneficial or harmful effects *in vivo* are in large parts unknown, it is reasonable to assume that these mediators may contribute in profound ways. Among the various MC mediators, the exact biological function of the MC proteases has for a long time been relatively obscure. However, recent progress involving successful genetic targeting of several MC protease genes has generated powerful tools, which will enable us to unravel the role of the MC proteases both in normal physiology as well as in pathological settings. This chapter summarizes the current knowledge of the biology of the MC proteases.

ABBREVIATIONS

Ang	angiotensin
α_1 -AC	α_1 -antichymotrypsin
BMMC	bone marrow-derived mast cell
CGRP	calcitonin gene-related peptide
CPA	carboxypeptidase
CTAP-III	connective tissue-activating peptide III
CTMC	connective tissue type mast cell
GAG	glycosaminoglycan
HDL	high-density lipoprotein
KO	knockout
LDL	low-density lipoprotein
MC	mast cell
MMC	mucosal mast cell
mMCP	mouse mast cell protease
MMP	matrix metalloprotease
NAP-2	neutrophil-activating peptide 2
NDST	<i>N</i> -deacetylase/ <i>N</i> -sulfotransferase
PAR	protease activated receptor
PG	proteoglycan
PHM	peptide histidine-methionine
α_1 -PI	α_1 -proteinase inhibitor
rMCP	rat mast cell protease
SCF	stem cell factor
SG	serglycin
SMC	smooth muscle cell
TGF	transforming growth factor
TMT	transmembrane tryptase
VIP	vasoactive intestinal peptide

1. INTRODUCTION

Mast cells (MCs) originate from the bone marrow, circulate in the blood as immature precursors, and then migrate into various tissues in which they undergo terminal differentiation under influence of local growth factors, in particular stem cell factor (SCF) (Gurish and Boyce, 2002). MCs are widely distributed throughout the body with a particular preponderance at sites close to the exterior, for example, mucosal surfaces and skin. This anatomic distribution is clearly in line with the notion of the MC as an important player in first-line defense toward external insults. For various general aspects of MC biology, the reader is referred to excellent review articles (Galli *et al.*, 2005a,b; Metcalfe *et al.*, 1997).

When MCs mature, they acquire numerous electron dense cytoplasmic granules, which can be released following an appropriate stimulus. Out of such stimuli, cross-linking of IgE molecules bound to the high-affinity IgE receptor, FcεRI, by polyvalent antigen is the most well characterized (Blank and Rivera, 2004). It has also been known for many years that MCs can be activated in response to several other types of stimuli, including anaphylatoxins C5a and C3a, neuropeptides (e.g., substance P), endothelin 1 (ET-1), and engagement of Toll-like receptors or FcγRIII (Galli *et al.*, 2005b).

MCs are undoubtedly most well known for their harmful effects in connection with immediate hypersensitivity reactions (Yu *et al.*, 2006), but MCs are also implicated in various additional disorders such as multiple sclerosis (Secor *et al.*, 2000), arthritis (Lee *et al.*, 2002a), bullous pemphigoid (Chen *et al.*, 2001), congestive heart failure (Hara *et al.*, 2002), and squamous carcinoma (Coussens *et al.*, 1999). Although reports describing harmful effects of MCs dominate the literature, it is important to emphasize that MCs can also be beneficial for their host by contributing to the innate immune defense toward certain pathogens, including bacteria (Echtenacher *et al.*, 1996; Malaviya *et al.*, 1996), parasites (Ha *et al.*, 1983), and even toward snake venom poisoning (Metz *et al.*, 2006). Much of this knowledge is based on experiments using a mouse strain that lacks MCs (the W/W^v strain), as a result of a mutation in the SCF receptor, c-kit. By comparing the response to a certain stimulus, for example an experimentally induced disease, between wild-type and MC-deficient mice, it has been possible to specifically pinpoint an involvement of the MC in a variety of pathological settings. Importantly however, the exact mechanism by which MCs contribute to the various pathological conditions listed above has in many cases not been clarified.

When MCs degranulate, they release a number of preformed components to the exterior. MC activation may also cause *de novo* synthesis and release of additional compounds, including PGD₂, PGE₂, LTB₄, and LTC₄ as well as cytokines (Galli *et al.*, 2005a,b; Metcalfe *et al.*, 1997). Out of the preformed MC mediators, histamine is by far the most thoroughly characterized in terms of biological function. In addition, it has been known for a long time that MC granules contain proteoglycans (PGs), that is, protein “cores” to which glycosaminoglycan (GAG) side chains are attached, and it is now well established that MCs also contain preformed cytokines, for example, TNF-α (Gordon *et al.*, 1990). Finally, MC granules contain a number of MC-specific proteases: tryptases, chymases, and MC carboxypeptidase A (MC-CPA). Tryptases and chymases belong to the serine protease class, while MC-CPA is a zinc-dependent metalloprotease. The designation of these proteases relates to their substrate specificities, with tryptases having trypsin-like cleavage specificities, while chymases have chymotrypsin-like specificities. MC-CPA has acquired its designation

through its specificity for cleaving proteins/peptides from their C-terminal end. The term “MC proteases” usually refers to the chymases, tryptases, and CPA that are specifically expressed by MCs and stored within MC granules. However, in addition to these MC-specific proteases, MCs may contain and secrete other proteases, for example, matrix metalloproteases (MMPs) (Baram *et al.*, 2001), cathepsin D, C, and E (Dragonetti *et al.*, 2000; Henningsson *et al.*, 2005; Wolters *et al.*, 2000). In this chapter, the term “MC proteases” will refer to the tryptases, chymases, and CPA that are present as releasable compounds within MC secretory granules.

Mature MCs contain conspicuously large amounts of stored proteases. For example, it has been calculated that human skin MCs contain altogether $\sim 16 \mu\text{g}$ of tryptase and chymase per 10^6 cells (Schwartz *et al.*, 1987). Thus, MC degranulation will result in release of very large quantities of proteases and it is therefore not far fetched to assume that they may profoundly affect any process in which MCs are involved and in which MC degranulation is a component. Indeed, a number of potential functions for various MC proteases have been outlined during the years. Noteworthy however, only in a few cases has any suggested function of a MC protease been confirmed *in vivo*, for example, in experiments taking advantage of genetically modified animals. In this chapter, the aim has been to summarize current knowledge of the MC proteases, including novel insights into their biological function.

2. EXPRESSION OF MC PROTEASES

The specific expression patterns for the MC proteases vary considerably between species and also among different MC subclasses found within a certain species (Table 1). Moreover, the repertoire of expressed MC proteases is also a function of the degree of cellular differentiation and can be modified following, for example, the course of a disease. The phylogenetic relationships between the various MC tryptases and chymases are indicated in Figs. 1 and 2, respectively. As indicated in Fig. 1, MC chymases are classified as belonging to either the α - or β -class, based on phylogenetic relationships (Chandrasekharan *et al.*, 1996).

2.1. Human MC proteases

In humans, MCs are actually classified according to their content of MC proteases, with MCs of the “MC_T” class expressing tryptase only and the “MC_{TC}” subclass expressing both tryptase and chymase. The MC_{TC} subclass also expresses MC-CPA (Table 1). The MC_T subclass is mainly present in mucosal surfaces, for example, in lung and gut, whereas the MC_{TC} subclass dominates, for example, in skin and intestinal submucosa

TABLE 1 Expression of MC proteases by different MC subclasses in human, mouse, and rat

	Tryptase	Chymase	MC-CPA
Human			
MC _T	β , α	–	–
MC _{TC}	β , α	Chymase	MC-CPA
Cord blood-derived MCs	β , α	Chymase	?
Rat			
CTMCs	rMCP-6, -7	rMCP-1, -5	MC-CPA
MMC	–	rMCP-2, -3, -4, -8, -9, -10	–
BMMC	?	rMCP-2	–
Mouse			
CTMCs	mMCP-6, -7	mMCP-4, -5	MC-CPA
MMC	–	mMCP-1, -2	–
BMMC-IL-3 (WEHI-3B)	mMCP-6, -7	mMCP-5	MC-CPA
BMMC-IL-3/SCF	mMCP-6, -7	mMCP-4, -5	MC-CPA
BMMC-IL-3/IL-9/TGF- β 1/SCF	mMCP-6, -7	mMCP-1, -2, -4, -5	MC-CPA

(Metcalf *et al.*, 1997). Importantly, only one human chymase gene, belonging to the α -family, has been identified (Caughey *et al.*, 1991; Johnson *et al.*, 1986; Urata *et al.*, 1991), whereas two classes of secreted tryptases, α and β , are expressed by human MCs (Miller *et al.*, 1989, 1990; Pallaoro *et al.*, 1999; Schwartz *et al.*, 1981a; Smith *et al.*, 1984; Vanderslice *et al.*, 1990). Three similar β -tryptases have been identified, β I, β II, and β III, of which β II and β III may be allelic variants at one locus, and β I and α -tryptase allelic variants at a neighboring locus (Pallaoro *et al.*, 1999). Accordingly, it has been found that as much as 29% of the human population are genetically deficient in α -tryptase (Soto *et al.*, 2002). Human MCs also express a transmembrane tryptase (denoted γ -tryptase or hTMT) (Caughey *et al.*, 2000a; Wong *et al.*, 2002), which appears to get exposed at the cell surface after MC degranulation (Wong *et al.*, 2002). In addition, human MCs have been shown to express an enzyme denoted δ -tryptase, a protease of trypsin-like cleavage specificity but so far of unknown biological significance (Wang *et al.*, 2002). Like in all other species investigated, only one MC-CPA gene has been identified in humans (Goldstein *et al.*, 1989; Reynolds *et al.*, 1989a, 1992). It has also been reported that

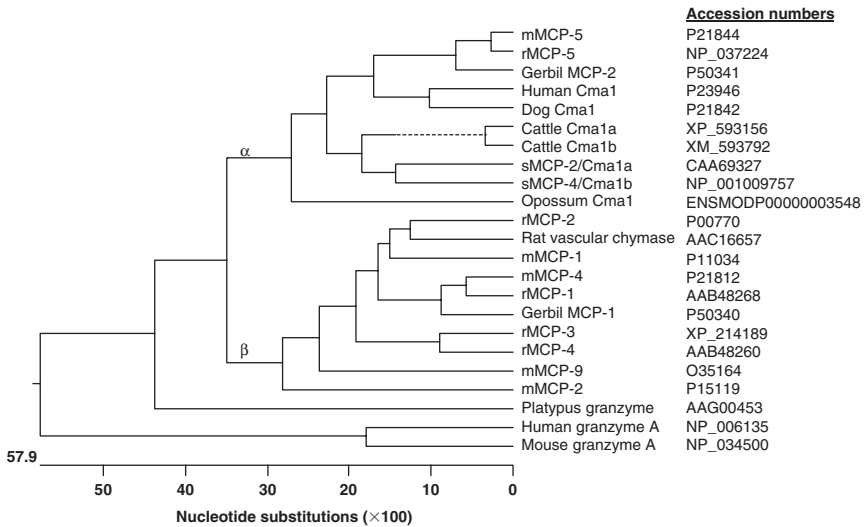


FIGURE 1 Dendrogram of mammalian α - and β -chymases. Phylogenetic analyses of the amino acid sequence of mature proteins were performed, using the CLUSTAL W algorithm implemented in the DNASTar™ software. The length of each branch is proportional to the number of mismatched residues between groups or pairs of sequences. Granzyme A serves as an out-group. Accession numbers from the protein database of NCBI (or Ensembl for Opossum) are listed to the right. m, murine; r, rat; s, sheep; MCP, mast cell protease; and CMA, α -chymase.

human MCs contain and express neutrophil cathepsin G, a serine protease with chymotrypsin-like cleavage specificity. This finding was originally based on immunohistochemical evidence (Schechter *et al.*, 1990). However, immunoblot analysis revealed that the MC cathepsin G antigen had a higher molecular weight than that of reference cathepsin G. In a subsequent study, cathepsin G mRNA was identified by RT-PCR analysis of skin tissue from patients with urticaria pigmentosa (Schechter *et al.*, 1994). However, the MCs were not purified, and it is therefore difficult to completely rule out that the cathepsin G mRNA could be derived from other cell types found within the skin. To fully establish cathepsin G as a MC protease, it would thus be important to identify cathepsin G mRNA expression in purified MCs.

2.2. Murine MC proteases

In rodents, MCs are usually divided into two major subclasses: mucosal MCs (MMCs) and connective tissue type MCs (CTMCs). As this designation indicates, MMCs are preferentially located in mucosal surfaces, for example, in gut, whereas CTMCs are located in, for example, skin, peritoneum, and intestinal submucosa (Metcalf *et al.*, 1997).

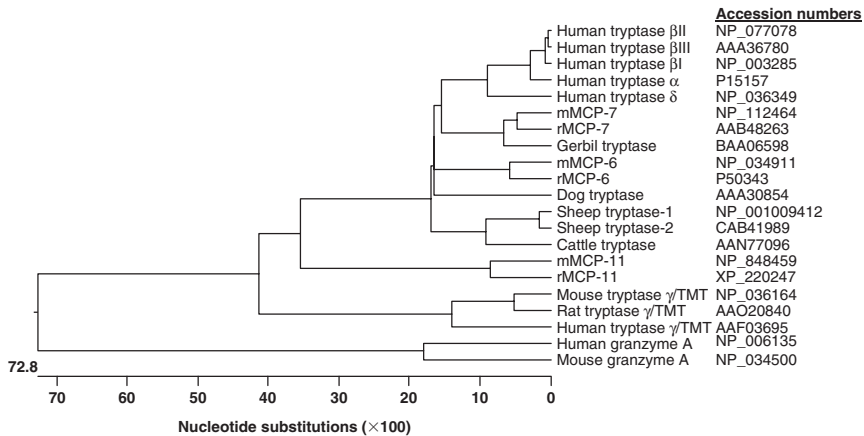


FIGURE 2 Dendrogram of mammalian tryptases. Phylogenetic analyses of the amino acid sequence of mature proteins were performed, using the CLUSTAL W algorithm implemented in the DNASTarTM software. The length of each branch is proportional to the number of mismatched residues between groups or pairs of sequences. Granzyme A serves as an out-group. Accession numbers from the protein database of NCBI are listed to the right. m, murine; r, rat; s, sheep; MCP, mast cell protease; and TMT, transmembrane tryptase.

In mouse, MMCs mainly express two β -chymases, mouse MC protease 1 (mMCP-1) and mMCP-2 (Chu *et al.*, 1992; Reynolds *et al.*, 1990; Serafin *et al.*, 1990; Trong *et al.*, 1989), but do not express tryptase or MC-CPA. CTMCs also express chymases, but instead of mMCP-1 and -2 they predominantly express mMCP-4 (β) and one α -chymase, mMCP-5 (Huang and Hellman, 1991; Reynolds *et al.*, 1990). Yet another chymase, mMCP-9, has been identified and shown to be specifically expressed by MCs in the uterus (Hunt *et al.*, 1997). In addition to chymases, mouse CTMCs express two tryptases: mMCP-6 and -7 (Huang and Hellman, 1991; McNeil *et al.*, 1992; Reynolds *et al.*, 1990, 1991), and also MC-CPA (Reynolds *et al.*, 1989b). Similar to human MCs, mouse MCs express a transmembrane form of tryptase, designated mTMT (Wong *et al.*, 1999). More recently, it was found that murine MCs express an additional trypsin-like enzyme, mMCP-11. However, similar to mMCP-7, mMCP-11 was mostly expressed at very early stages of MC differentiation (Wong *et al.*, 2004).

2.3. Rat MC proteases

Rat MCs display a protease expression pattern highly similar to that of murine MCs. However, for historical reasons, the designation of rat MC proteases is not in perfect analogy with the nomenclature used in mice. Rat

MMCs express predominantly the β -chymase rMCP-2, which is structurally most similar to mMCP-1 in mice, as well as two additional β -chymases: rMCP-3 and -4 (Lützel Schwab *et al.*, 1997, 1998). In addition, rat MMCs express several members of the rMCP-8 family (rMCP-8, -9, and -10), a protease family of unknown substrate specificity and biological function (Lützel Schwab *et al.*, 1997, 1998). One member of this protease family, mMCP-8, is expressed in mouse, but it appears that mMCP-8 is expressed by basophils rather than MCs (Poorafshar *et al.*, 2000b). Similar to the situation in mouse, rat MMCs do not express tryptases or MC-CPA. Rat CTMCs express two chymases, rMCP-1 (β) and rMCP-5 (α), which are homologues to mMCP-4 and -5, respectively (Le Trong *et al.*, 1987b; Lützel Schwab *et al.*, 1997). Thus, despite their discrepancy in nomenclature, rMCP-1 and mMCP-4 are the corresponding mouse and rat β -chymases. In addition, rat CTMCs express MC-CPA (Cole *et al.*, 1991; Lützel Schwab *et al.*, 1997) and two tryptases, rMCP-6 and -7 (Lützel Schwab *et al.*, 1997), with the latter being the corresponding homologues to mMCP-6 and -7. No detectable mRNA expression of cathepsin G was found in a cDNA library derived from purified rat peritoneal MCs (Lützel Schwab *et al.*, 1997).

2.4. MC proteases from other species

In sheep, two tryptases have been identified, tryptase-1 and -2 (Pemberton *et al.*, 2000), and two other MC proteases have also been characterized, sMCP-1 and -3. Interestingly, sMCP-1 has dual trypsin- and chymotrypsin-like cleavage specificity (McAleese *et al.*, 1998). An MC protease of such dual cleavage specificity has also been isolated from parasite-infected goat jejunum (Macaldowie *et al.*, 1998). Canine MCs express one chymase, belonging to the α -family (Caughey *et al.*, 1990). In addition, a dog MC tryptase has been characterized (Vanderslice *et al.*, 1989). Two similar tryptases have been isolated and characterized from bovine sources (Gambacurta *et al.*, 2003). Porcine MC tryptase with properties similar to those of human β -tryptase has also been isolated (Chen *et al.*, 2000). In gerbil MCs, tryptase activity has been identified and two different chymases have been characterized: a β -chymase homologous to mMCP-4 and an α -chymase homologous to mMCP-5 (Itoh *et al.*, 1996).

2.5. MC protease expression profiles as MC markers

The specific expression pattern for MC proteases is a useful way to determine the subclass of MCs. In addition, cells can actually be identified as MCs based on their expression of MC proteases. The reason for this is that MC proteases are highly specific for MCs. However, in some cases, proteases classified as "MC proteases" can be expressed in other cell

types. One example is the basophil lineage, which can express α -tryptase, β -tryptase, and chymase, although at much lower levels than in MCs (Jogie-Brahim *et al.*, 2004; Li *et al.*, 1998). Another example is the reported expression of both α - and β -tryptases by human cell lines of monoblast-like characteristics (Huang *et al.*, 1993). Moreover, a chymase highly similar to rMCP-2 has been reported to be expressed by rat smooth muscle cells (SMCs), and has consequently been named SMC chymase (Guo *et al.*, 2001; Ju *et al.*, 2001).

3. GENETIC ORGANIZATION AND REGULATION OF TRANSCRIPTION

3.1. The chymase locus

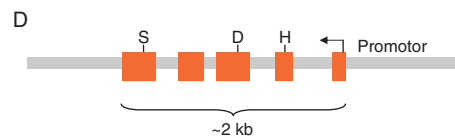
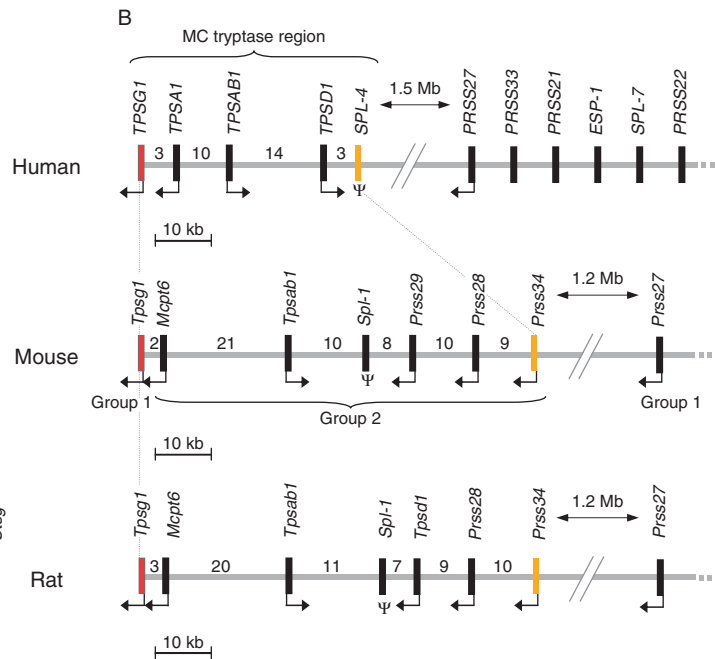
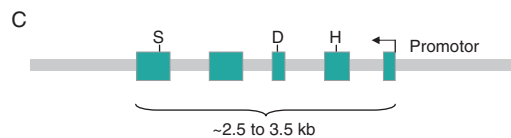
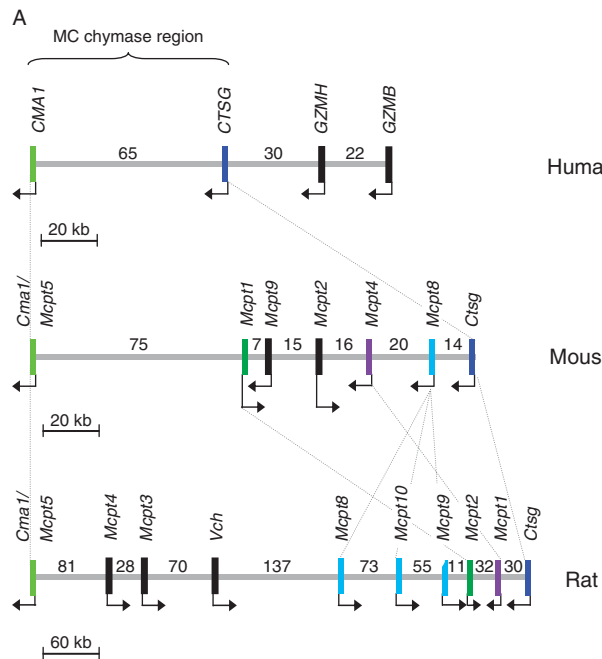
The human MC α -chymase gene (*CMA1*) is located on chromosome 14q11.2 at one end of a small cluster of four genes, covering ~130 kb (Caughey *et al.*, 1993b). This cluster contains the neutrophil-specific cathepsin G gene (*CTSG*) 65 kb upstream of *CMA1* and the T-cell-specific granzyme H and B genes (*GZMH* and *GZMB*) located 30 and 55 kb upstream of *CTSG* (Table 2; Fig. 3A). The transcriptional orientations of these genes are the same, and their overall genetic organization is conserved in rodents, dog, and cattle (Gallwitz and Hellman, 2006; Gallwitz *et al.*, 2006). However, species-specific expansion of the locus has occurred. The locus can be divided into a chymase and a granzyme region, and particularly in rodents, a massive expansion of the chymase region is evident. The exon/intron organization of a MC chymase gene is depicted in Fig. 3C.

In rat, one α -chymase and eight β -chymase genes are clustered on chromosome 15p12. The complete locus has expanded to 1.1 Mb of which the chymase region covers ~550 kb. The genetic organization and transcriptional orientations of *Ctsg*, the rMCP-5 (*Cma1/Mcpt5*), and rMCP-1 (*Mcpt1*) genes are the same as the orientation of the genes in the human chymase locus but, interestingly, all other chymase genes, that is, *Mcpt2*, -3, -4, -8, -9, and -10, transcribe in the opposite orientation (Fig. 3A). *Mcpt8*, -9, and -10 are found in rat but not in human and only one member (*Mcpt8*) is found in mouse.

In mouse, the MC chymase genes are closely clustered on chromosome 14C3, covering ~160 kb of the complete 325-kb locus (Fig. 3A). Again, the transcriptional orientations of the mMCP-5 gene (*Cma1/Mcpt5*) and *Ctsg* are the same as in humans and also the mMCP-4, -8, and -9 genes (*Mcpt4*, -8, and -9) are transcribed in the same direction. In contrast, the mMCP-1 and -2 genes (*Mcpt1* and -2) transcribe in opposite orientation. Possibly, the opposite transcriptional direction of mouse *Mcpt1/2* versus *Mcpt4/5* may be related to their preferential expression in MMCs and CTMCs, respectively.

TABLE 2 Gene designation, chromosomal location, and homologues of human, mouse, and rat MC proteases

MC proteases	Gene designation	Chromosomal location	Homologues
MC chymases			
Chymase 1	<i>CMA1</i>	14q11.2	mMCP-5, rMCP-5
rMCP-1	<i>Mcpt1</i>	15p12/13	mMCP-4
rMCP-2	<i>Mcpt2</i>		mMCP-2
rMCP-3	<i>Mcpt3</i>		mMCP-9
rMCP-4	<i>Mcpt4</i>		mMCP-1
rMCP-5	<i>Cma1/ Mcpt5</i>		mMCP-5
rMCP-8	<i>Mcpt8</i>		mMCP-8
rMCP-9	<i>Mcpt9</i>		
rMCP-10	<i>Mcpt10</i>		
mMCP-1	<i>Mcpt1</i>	14C1/2	
mMCP-2	<i>Mcpt2</i>		
mMCP-4	<i>Mcpt4</i>		
mMCP-5	<i>Cma1/ Mcpt5</i>		
mMCP-9	<i>Mcpt9</i>		
MC tryptases			
hTMT/Tryptase γ	<i>TPSG1</i>	16p13.3	mTMT, rTMT
hTryptase α I/ β I	<i>TPSA1</i>		mMCP-6, rMCP-6
hTryptase β II/ β III	<i>TPSAB1</i>		mMCP-6, rMCP-6
hTryptase δ	<i>TPSD1</i>		mMCP-7, rMCP-7
	<i>hSPL-4</i>		mMCP-11, rMCP-11
rTMT	<i>Tpsg1</i>	10q12	mTMT
rMCP-6	<i>Mcpt6</i>		mMCP-6
rMCP-7	<i>Tpsab1</i>		mMCP-7
rMCP-11	<i>Prss34</i>		mMCP-11
mTMT	<i>Tpsg1</i>	17A3.3	
mMCP-6	<i>Mcpt6</i>		
mMCP-7	<i>Tpsab1</i>		
mMCP-11	<i>Prss34</i>		
MC-CPA			
hMC-CPA	<i>CPA3</i>	3q24	rMC-CPA, mMC-CPA
rMC-CPA	<i>Cpa3</i>	2q24	
mMC-CPA	<i>Cpa3</i>	3A3	



In support for such a notion, the rat MMC and CTMC chymase genes are also transcribed in opposite directions (Fig. 3A).

3.2. The tryptase locus

The human tryptase locus resides on chromosome 16p13.3 and spans ~1.6 Mb. This locus harbors at least 11 genes (Fig. 3B). In mouse, the tryptase locus covers 1.5 Mb, harbors 14 genes, and is situated on chromosome 17A3.3 (Fig. 3B; note that the genes beyond *mPancreasin* are not depicted in the figure). It contains 13 functional tryptic genes and 1 non-functional gene, *mSpl*. Both the human and rodent tryptase loci are organized into two separate clusters spaced by ~1.2–1.5 Mb (Fig. 3B). The genes have been divided into group 1 and group 2 tryptases due to conserved amino acid motifs in their propeptides (Wong *et al.*, 2004). Group 1 tryptases share, in their propeptide, a conserved upstream Cys-Gly sequence and a conserved Arg/Lys at position –1. Group 2 genes share a conserved Gly residue at position –1. In human and mouse, the γ -tryptase/TMT gene (*TPSG1/Tpsg1*), a group 1 gene, defines one border of the locus, whereas human ϵ -tryptase (*TPSE1*) or mouse *mT6* (not shown in the figure) defines the other end. The group 2 genes are clustered close to *TPSG1/Tpsg1* and have average sizes of only 2 kb. In contrast to the chymase locus, the tryptase locus shows a fairly high degree of conservation as regards MC-specific transcripts, without any evident species-specific expansion of homologous genes [Ensembl, March 2007 (Wong *et al.*, 2004)]. Both the mouse and rat tryptase loci contain four MC-specific genes (Table 2): a gene for γ -tryptase/TMT (a group 1 tryptase; *Tpsg1*) and genes for three group 2 tryptases: mMCP-6/rMCP-6 (*Mcpt6*), mMCP-7/rMCP-7 (*Tpsab1*), and mMCP-11/rMCP-11 (*Prss34*). The exon/intron organization of an MC tryptase gene is depicted in Fig. 3D.

3.3. The MC-CPA locus

The MC-CPA gene organization is well conserved in human (*CPA3*), mouse, and rat (*Cpa3*). In human and mouse, *CPA3/Cpa3* is situated on chromosome 3q24 and 3A3 and contains 11 exons that span over 32 and

FIGURE 3 Genetic organization of MC proteases. An overall organization comparison of the chymase (A) and tryptase (B) locus in human, mouse, and rat. Note the expansion of chymase genes in rodents. Numbers indicate the distances in kilobases (kb) between genes. Arrows at standing bars show the transcriptional orientation of respective gene. Ψ indicates a nonfunctional gene. The scale is indicated by a bar and a number (kb); note that the rat chymase locus is displayed at a size 1:3 to the human and mouse locus. The human, mouse, and rat tryptase locus scale is 10 kb. The overall exon/intron structures of chymase and tryptase genes are depicted in (C) and (D), respectively. The positions of the serine protease catalytic triad residues, His, Asp, Ser, are indicated.

27 kb, respectively. In rat, *Cpa3* is situated on chromosome 2q24 and is built from 12 exons that span over 34 kb (Ensembl, March 2007).

3.4. Transcriptional regulation

During differentiation, MCs depend on different cytokines for proliferation, maturation, and survival (Gurish and Boyce, 2002). To study various aspects of MC differentiation, including regulation of MC protease expression, many studies have used protocols in which bone marrow cells are differentiated *in vitro* into MC phenotype, so-called bone marrow-derived MCs (BMMCs). In a hallmark study, Gurish *et al.* (1992) developed murine BMMCs in the presence of conditioned medium from WEHI-3B cells (a murine macrophage-like myelomonocytic cell line) and showed that *Mcpt5*, -6, and *Cpa3* were upregulated within the first week of culture. WEHI-3B-conditioned medium thus contains factors that drive expression of MC proteases. Two of these factors were identified as IL-3 and SCF, of which IL-3 seems to promote expression of *Mcpt5* and *Cpa3*. *Cpa3* is actually one of the first MC protease genes to be expressed and detected in MCs differentiating from bone marrow progenitors (Rodewald *et al.*, 1996). SCF induced similar protease expression as IL-3 but also promoted expression of *Mcpt4*, which is considered to be a marker of terminally differentiated CTMCs. Interestingly, when IL-3 and SCF were combined, *Mcpt4* was not expressed, indicating that IL-3 arrests the MCs in a more immature state that SCF cannot override (Gurish *et al.*, 1992). Other cytokines that influence the transcriptional activity of MC proteases include TGF- β 1, activin A, IL-4, IL-9, and IL-10. TGF- β 1 and IL-9 seem to promote the *in vitro* differentiation of bone marrow cells into more mucosal-like MCs, enhancing the expression of the characteristic MMC chymase genes, *Mcpt1* and -2 (Miller *et al.*, 1999). Whereas *Mcpt4* expression appears to be a late differentiation marker for CTMCs, *Mcpt2* expression has been suggested to be a late differentiation marker for MMCs (Serafin *et al.*, 1990). A combination of IL-9 or IL-10 and SCF induces expression of *Mcpt1*, -2, and -4, an induction that was counteracted when IL-4 was added to the cultures (Eklund *et al.*, 1993). So, how is the transcription and the protein levels of the MC proteases controlled? Is it dictated by specific transcription factors or by posttranscriptional events? In one study, it was reported that IL-10 induces expression of *Mcpt1* and -2 in BMMCs (Xia *et al.*, 1996). However, when using a nuclear run-on analysis, transcripts for *Mcpt1* and -2 were found even in the absence of IL-10, indicating that *Mcpt1* and -2 are constitutively transcribed by the MCs. Based on these findings, it was suggested that IL-10 stimulation results in higher levels of *Mcpt1* and -2 transcripts by increasing the mRNA stability (Xia *et al.*, 1996). In contrast, glucocorticoids seem to inhibit IL-10-induced stabilization of *Mcpt1* and -2 transcripts by

promoting mRNA degradation. The mRNA levels for *Mcpt6* and -5 were not affected to a major extent by glucocorticoids but, strikingly, transcriptional levels for the serglycin (SG) gene and *Cpa3* were upregulated (Eklund *et al.*, 1997).

In mouse, the 5 exon/4 intron organization of the chymase genes is well conserved, and also the promotor/enhancer regions display strong conservation (Huang and Hellman, 1994). In fact, the upstream regions (up to -1200) of *Mcpt1*, -2, -4, and -9 all show very strong sequence conservation, pointing to a common theme in their transcriptional regulation. In contrast, the upstream region of *Mcpt5* displays only partial homology to the rest of the chymase locus. The upstream region of *Mcpt8* shows no homology to the rest of the chymase locus (Lunderius and Hellman, 2001; Tchougounova *et al.*, 2003).

In a series of elegant studies, Kitamura and coworkers identified that the mi transcription factor (MITF), a basic helix-loop-helix-leucine zipper transcription factor, controls and enhances the transcriptional activity of most of the MC protease genes. MITF regulates transcription through specific E-boxes (CANNTG motifs) located -100 to -1200 upstream of the respective gene. These studies took advantage of a mouse strain with a mutant form of MITF which is a poor transactivator of the MC protease genes (Ge *et al.*, 2001; Jippo *et al.*, 1999; Morii *et al.*, 1994, 1996, 1997). It was reported that the leucine zipper (ZIP) domain of MITF is important for normal MC development and that overexpression of normal MITF rescued expression of the MC protease genes (Morii *et al.*, 2001a). Of the chymase genes, *Mcpt4* was shown to be regulated directly by MITF as a targeted deletion of the E-box, at position -383 to -378, abrogated the transcriptional activity (Jippo *et al.*, 1999). In contrast, *Mcpt5* was indirectly regulated by MITF as MITF did not bind to the promotor region of *Mcpt5*. Here, addition of SCF rescued the transcriptional activity of *Mcpt5* in mi mutant MCs (Morii *et al.*, 1997). Also *Mcpt2* and -9 are regulated by MITF (Ge *et al.*, 2001), whereas, in contrast, *Cpa3* transcription appears to be MITF independent (Morii *et al.*, 1996). Finally, a striking observation in the mi mutant mice was that the mRNA levels for *Ctsg* and *Gzmb* were also diminished. Therefore, it has been suggested that one or several locus control regions, guided by MITF, controls the transcriptional activity of the chymase locus in mice (Jippo *et al.*, 1999).

Also MC tryptase expression is regulated by MITF. Kitamura and coworkers demonstrated that the expression of *Mcpt6* was directly regulated by MITF (Morii *et al.*, 1994, 1996). On interaction of MITF with polyomavirus enhancer-binding protein 2/core-binding factor (PEBP2/CBF), a synergistic effect was seen on the level of *Mcpt6* transcription. The interaction between PEBP2/CBF and MITF was achieved through the N-terminal region of MITF (Ogihara *et al.*, 1999). In another study, the myc-associated zinc finger protein-related factor (MAZR) was found to interact with the ZIP

domain of MITF and synergistically activate *Mcpt6*. The zinc finger domain of MAZR was crucial for the interaction to MITF (Morii *et al.*, 2002). In addition, the transcription factor c-jun has been implicated in regulation of *Mcpt6* through interaction with MITF (Kim and Lee, 2004). In contrast, *Tpsab1* (the gene for mMCP-7) does not seem to be positively regulated by MITF (Ogihara *et al.*, 2001). The levels of *Tpsab1* transcript increase when MCs are activated with TGF- β 1 and activin A and decrease in the presence of MITF. The role of MITF in this system may be to inhibit Smad3-mediated transcription (Funaba *et al.*, 2003). Finally, MITF regulates TMT gene expression by binding to two E-boxes located at position -1708 and -1805, respectively (Morii *et al.*, 2001b). In summary, MITF may control MC protease gene regulation either by making direct contact with promotor elements or by interacting with other transcription factors.

Considering that MC proteases may be important components of inflammatory pathways (Section 10), it would be likely that their expression is induced by inflammatory stimuli, for example, MC-activating agents. However, there is to date no firm evidence supporting such a notion. Instead, it appears that the expression of the MC proteases is constitutive. As an example, MCs from various sources (e.g., BMMCs, peritoneal MCs) contain high steady state levels of transcript coding for the various MC proteases. Further, there is no striking evidence that MC activation, for example, by Fc ϵ RI cross-linking, results in increased transcription of the MC protease genes (Xia *et al.*, 1997). In many cases, however, it has been shown that the mRNA levels for MC proteases are markedly increased in a tissue during the course of a disease. For example, the infection of rats with *Nippostrongylus brasiliensis* induces upregulation of MMC protease expression (Table 1; Lützelshwab *et al.*, 1997). However, the parasite infection causes a dramatic influx of MMCs into the infected tissue. Hence, it appears likely that the increased mRNA levels arise from the increased numbers of MCs present, rather than upregulated mRNA levels within individual MCs.

4. EVOLUTION OF MC PROTEASES

4.1. MCs in evolution

MCs or MC-like cells have been observed in essentially all vertebrates, as well as in some invertebrates, for example, clams (Ulrich and Boon, 2001) and tunicates (Cavalcante *et al.*, 2002). The widespread presence of MCs indicates that they have indeed played an important beneficial role, also in early evolution. However, most research on MC-derived proteases has been done in mammals. Therefore, it is still an open question whether MC proteases have an ancient origin or if they are restricted to mammalian species.

4.2. MC proteases in early evolution

It has not been clearly established whether MC proteases are present in MCs of primitive vertebrates or invertebrates. A recent study searching specifically for chymase-related genes in bony fish (teleosts) revealed that the closest related group of proteases in teleosts differs significantly in primary structure from their mammalian counterparts (Wernersson *et al.*, 2006). Thus, there are no direct structural homologues in teleosts to MC chymase or to any of the other proteases encoded by the chymase locus, that is, to granzyme B-H, rodent mMCP-8/rMCP-8 family, cattle duodenases, or cathepsin G. In contrast, the T-cell granzyme A (and K) can clearly be traced back to early vertebrate evolution, as demonstrated by the identification of single genes with close sequence homologies in several species of teleosts (Praveen *et al.*, 2004; Wernersson *et al.*, 2006), together with data supporting functional homology (Praveen *et al.*, 2006). The lack of primary structure homologues to MC chymase in teleosts does not exclude the possibility that functional homologues may exist. Indeed, a previous report suggests that chymotrypsin-like activity is conferred by granzyme-like proteases in teleosts (Praveen *et al.*, 2006). Certainly, in order to determine the origin and evolutionary importance of the MC proteases, it will be of great interest to identify proteases with chymotrypsin-like, trypsin-like, or CPA activity in MC-like cells of primitive life-forms.

4.3. MC chymase in mammalian evolution

The phylogenetic relationships of MC proteases in mammals have been more thoroughly investigated (Caughey, 2001; Huang and Hellman, 1994; Lützelshwab *et al.*, 1997). One or two α -chymase genes seem to be present in every mammalian species studied so far, whereas β -chymases are restricted to rodents. Although the rodent α -chymases, mMCP-5 and rMCP-5, cluster together with the single human α -chymase in phylogenetic analyses (Fig. 1), they are most likely not the closest functional homologues to human chymase since they have no chymase activity. Instead they have acquired elastase activity (Karlson *et al.*, 2003; Kunori *et al.*, 2002), presumably as a result of a reduced selective pressure on α -chymases in rodents due to the presence of β -chymases. It is possible that β -chymases have emerged exclusively in rodents. On the other hand, the identification of a dog chymase-like gene, *Cma2*, related to both α - and β -chymases favors the alternative hypothesis that an ancestral β -chymase gene was present, at least before the divergence of carnivores and rodents, which subsequently was lost in primates and ruminants (Gallwitz and Hellman, 2006).

Interestingly, the chymase locus can be dated back to at least 200 Myr ago, that is to early mammalian evolution. This was demonstrated by the

presence of a single chymase-like gene in platypus and two related genes in opossum (Gallwitz *et al.*, 2006; Poorafshar *et al.*, 2000a). In fact, of all the genes within the chymase locus, the α -chymase gene seems to have emerged first since one of the chymase-related genes in opossum clustered firmly within the α -chymase group in phylogenetic analyses, whereas the other gene related nearly equally well to any of the other genes of the chymase locus (Gallwitz *et al.*, 2006). The chymase locus has undergone diverse changes during mammalian evolution, characterized by multiple species-specific gene duplications, deletions, neofunctionalization, or loss of function (Gallwitz *et al.*, 2006). In this context, rodents stand out as their chymase loci have expanded remarkably in relation to that in other mammals. For instance, mice and rats hold at least four and eight expressed chymase genes, respectively, as compared to the single chymase gene in humans. The reason for this difference is not known, but one may speculate that a larger pathogenic exposure in rodent lifestyle has fueled the expansion and diversification of the chymase gene family in mice and rats.

4.4. MC tryptase in mammalian evolution

The MC tryptase locus has undergone extensive gene duplications, similar to that seen in the rodent chymase locus. However, these duplications have most likely occurred already before the separation between humans and rodents, as demonstrated by the presence of similar numbers of corresponding genes in humans, mice, and rats (Wong *et al.*, 2004). It is difficult to trace the origin of the tryptase locus since most investigations have studied the genes only of placental mammals. Yet, the high degree of diversification and homology between different species implies an ancient origin. For example, the closely related γ -tryptase genes in mice, rats, and humans suggests that an ancestor gene must have separated from the other tryptase genes long before the separation of rodents and primates (Caughey, 2001; Fig. 2). The recently completed genome databases from many distantly related species will be rich sources of information in tracking the evolutionary events forming the tryptase locus.

5. PROTEIN ORGANIZATION AND PROCESSING

The MC proteases are all synthesized as preproenzymes, that is, with an N-terminal signal peptide directing them into the ER lumen. Cleavage of the signal peptide then results in generation of proforms of the respective proteases, and subsequent cleavage of the propeptide results in formation of mature enzyme. In no case has a proform of any MC protease been isolated from natural sources to allow N-terminal sequencing and

determination of the exact site for cleavage of the signal peptide *in vivo*. However, cleavage sites in the signal peptides have been predicted based on the rules of [von Heijne \(1984\)](#). Hence, mMCP-6 and -7 have been predicted to have 10-amino acid-long propeptides ([McNeil et al., 1992](#); [Reynolds et al., 1991](#)), whereas human β -tryptase has been proposed to have a slightly longer signal peptide, 12 amino acids ([Sakai et al., 1996a](#)). In chymases, it has been postulated that the preproenzyme is cleaved at the -2 position, leaving a 2-amino acid activation pro/activation peptide ([Caughey et al., 1991](#); [Huang et al., 1991](#); [Serafin et al., 1991](#); [Urata et al., 1991](#)). This is in analogy with a number of other serine proteases of hematopoietic origin, for example, granzymes, elastase, and cathepsin G, which all are known to possess 2-amino acid activation peptides. The preproenzymes of both mouse and human MC-CPA have been predicted to have 15-amino acid-long signal peptides, followed by 94 amino acid pro/activation peptides ([Reynolds et al., 1989a, 1991](#)).

An unusual feature of the MC proteases is that they are all stored as fully processed enzymes, that is, with the propeptide removed. This is in sharp contrast with, for example, the pancreatic digestive proteases, which are stored as proenzymes and are activated after release into the gut lumen. The storage of such massive amounts of fully active proteases within MC granules probably imposes a major challenge for the host cell, considering that escape of the granular proteases into the cytosol may cause serious damage. Most likely, the MC needs protective systems in form of protease inhibitors that neutralize proteases that escape into the cytosol. The existence of such protective mechanisms has not been investigated to any major extent. A likely possibility is that protection against MC proteases leaking into the cytoplasm is conferred by one or several of the relatively recently identified intracellular protease inhibitors of the serpin class ([Silverman et al., 2004](#)). In line with such a notion, [Strik et al. \(2004\)](#) showed that an intracellular serpin, PI6, is present in human MCs and forms complexes with monomeric β -tryptase.

The fact that MC proteases are stored in fully active form may have an important impact on the understanding of their biological function. Indeed, the storage of the proteases in active form and in such large amounts strongly suggests that the ability to rapidly release preactivated proteases is functionally important for the MCs. The most obvious implication of this is that the MC proteases are aimed to function in the initial phase during a response toward pathogens.

The exact mechanism and enzymes responsible for propeptide processing, leading to mature MC proteases, are only partly understood and are best characterized for the chymase family. [Dikov et al. \(1994\)](#) showed that inhibitors of dipeptidyl peptidase I (DPPI; synonym for cathepsin C), a cysteine protease with specificity for cleaving dipeptides from the N-terminus, prevented prochymase activation in the KiSV-M9 cell line,

suggesting that DPPI could be involved in processing of the N-terminal dipeptide of prochymases. Support for this notion came when it was demonstrated in a purified system that DPPI can process the N-terminal dipeptide of recombinant human prochymase and that this cleavage resulted in active enzyme (Murakami *et al.*, 1995). An important implication of this finding is that the N-terminal processing of the propeptide is itself sufficient for gaining enzymatic activity, which appears to be in contrast to the tryptases (see below). The ability of DPPI to cleave off the propeptide in human prochymase has also been confirmed by other laboratories (McEuen *et al.*, 1998). Further strong support for a role of DPPI in prochymase processing came through the knockout (KO) of the DPPI gene in mice, and the demonstration that mice lacking DPPI fail to generate active CTMC chymases *in vivo*. This was shown both by *in situ* enzymatic determinations and by that mMCP-5 was exclusively present in its proform in DPPI^{-/-} BMNCs (Wolters *et al.*, 2001). Hence, DPPI is essential for activation of the CTMC chymases and is presumably also involved in the propeptide processing of the MMC chymases, mMCP-1 and -2. Moreover, it is likely that DPPI has the corresponding function in processing of human prochymase, although this notion remains to be proven *in vivo*. An interesting finding in this context is a loss-of-function mutation in the DPPI gene in the Papillon-Lefevre syndrome, a rare disorder characterized by increased susceptibility to early onset periodontitis (Toomes *et al.*, 1999). By using material from patients suffering from this disorder, it would be possible to investigate if DPPI indeed activates also the human prochymase.

The N-terminal processing mechanisms involved in tryptase activation are much less characterized than those of the chymases. In one report, it was suggested that, similar to the chymases, also protryptases were N-terminally processed by DPPI (Sakai *et al.*, 1996b). It was proposed that protryptase was capable of autocatalytic processing at the Arg(-3)-Val(-2) bond and that DPPI proceeded by cleaving off the remaining dipeptide. Further support for a role of DPPI in tryptase processing came from studies on the DPPI KO strain in which it was demonstrated that the levels of mature tryptase were somewhat lower in DPPI^{-/-} MCs than in wild-type counterparts (Wolters *et al.*, 2001). However, in contrast to the effect on chymases, there was no corresponding accumulation of protryptase in DPPI^{-/-} cells and it is thus not possible to firmly establish a role for DPPI in protryptase processing.

The processing steps leading to formation of mature MC-CPA are not fully understood. In an early report, it was shown that addition of a cysteine proteases inhibitor (E-64d) to KiSV-MC14 cells, a cell line in which both pro- and mature MC-CPA is detected, resulted in the accumulation of pro-MC-CPA (Springman *et al.*, 1995). However, pro-MC-CPA processing was not affected by a DPPI inhibitor, indicating that the processing is catalyzed

by a cysteine protease other than DPPI. An interesting observation was that the pro-MC-CPA processing occurred within the secretory granule rather than in ER or Golgi (Springman *et al.*, 1995), a finding that is supported by an independent study (Rath-Wolfson, 2001). More recently, we sought to identify the proteases involved in processing of pro-MC-CPA by making use of mouse strains lacking individual cathepsins. In line with the previous observations, we did not see any accumulation of pro-MC-CPA in BMMCs lacking DPPI/cathepsin C (Henningsson *et al.*, 2003). Instead, the KO of DPPI caused an accumulation of mature MC-CPA, suggesting that DPPI may be involved in regulating the amount of stored mature MC-CPA within the MC secretory granule (Henningsson *et al.*, 2003). Further, no accumulation of pro-MC-CPA was detected in MCs from animals deficient in either cathepsin B, -L, or -S (Henningsson *et al.*, 2003, 2005). In contrast, when analyzing MCs from mice lacking cathepsin E, an aspartic protease, we noticed a clear accumulation of pro-MC-CPA (Henningsson *et al.*, 2005). However, mature MC-CPA was present to some extent even in the absence of cathepsin E, indicating that cathepsin E is not essential for pro-MC-CPA processing. Absence of a related aspartic acid protease, cathepsin D, did not alter the levels of pro-MC-CPA. Thus, the ability to process pro-MC-CPA is not a general property of aspartic acid proteases (Henningsson *et al.*, 2005).

6. THREE-DIMENSIONAL STRUCTURE

6.1. Chymase

The crystal structures have been solved both for rMCP-2 (Remington *et al.*, 1988) and human chymase (McGrath *et al.*, 1997; Pereira *et al.*, 1999; Fig. 4A). In addition, the structure of human prochymase was solved (Reiling *et al.*, 2003). The three-dimensional structure of human MC chymase revealed a high degree of similarity with pancreatic chymotrypsin, rMCP-2, and cathepsin G. A striking feature of human chymase, but not of rMCP-2, is the presence of a large number of basic amino acid residues (Lys/Arg) in two distinct clusters on the molecular surface (Fig. 4B). Most likely, these clustered basic residues are involved in the interaction of chymase with anionic GAGs (Section 8). By solving the crystal structure of human prochymase, important clues were given as to the molecular events taking place during the chymase activation process. It was shown that two loops, the autolysis loop (Gly142-Thr154) and the 180 s loop (Pro185A-Asp194), were positioned in a way that may prevent enzymatic activity in the proform, and that rearrangement of these structural elements after propeptide processing unleashes the chymase proteolytic activity (Reiling *et al.*, 2003).

Additional insights into the three-dimensional structures of the chymases have been derived from molecular modeling studies. In one study,

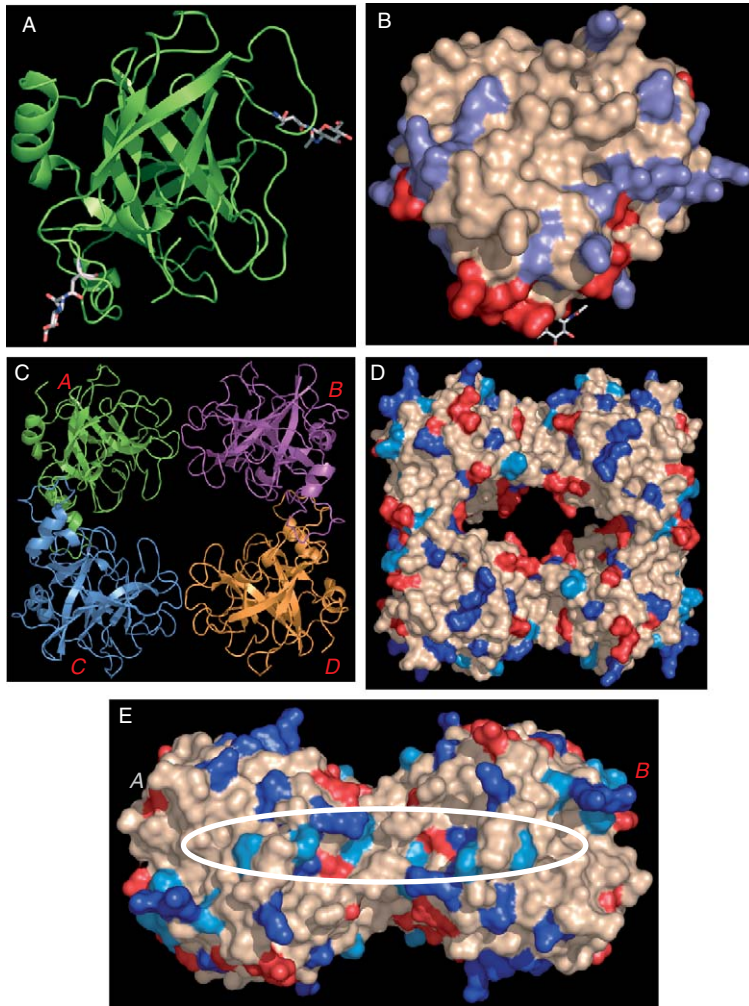


FIGURE 4 (A) Structure of human chymase in ribbon representation. Two N-linked *N*-acetyl-glucosamine units are shown as stick models. (B) Surface representation of human chymase. Positively charged residues (Arg, Lys, His) are indicated in blue and negative residues (Glu, Asp) in red. Note the defined patches of positively charged residues. One N-linked *N*-acetyl-glucosamine unit (Asn 72) is visible (sticks). (C) Structure of tetrameric human β -tryptase in ribbon representation. The four monomer units (A–D; red coloring) are represented in different coloring. (D) Surface representation of human β -tryptase viewed down the central cavity. Positively charged residues are indicated in blue, with His residues in light blue and Arg/Lys in dark blue; negative residues in red. (E) Surface representation of tryptase tetramer rotated 90° around the horizontal axis, showing the A–B interface. Note the cluster of His residues (indicated by a white oval) spanning over the A–B interface.

the four major murine MC chymases, mMCP-1, -2, -4, and -5, were modeled (Sali *et al.*, 1993). An interesting finding in that study was that the murine chymases differed markedly with regard to their electrical charge distribution on the molecular surface. Both mMCP-4 and -5 were, similar to the human chymase (Fig. 4B), predicted to contain two distinct patches of positive charge (Lys/Arg residues), and it was proposed that these regions mediate tight interactions with the highly negatively charged heparin PGs that are present in mMCP-4/5-expressing MCs (i.e., CTMCs). In contrast, mMCP-1 and -2 lacked one of these positive patches, and it was discussed that the lower exposure of positive charge on the mMCP-1/2 surface is related to the lower charge of the PGs resident in mMCP-1/2-expressing MCs (MMCs) than of the CTMC PGs (Enerback *et al.*, 1985).

6.2. Trypsase

In contrast to the chymases, which are active in monomeric form, it was early discovered that the active form of MC trypsin is a tetramer (Schwartz, 1981a). It is now known that MC trypsins from other mammalian species share the tetrameric organization. Further, it was shown that disruption of the trypsin tetramer into monomers caused inactivation of the trypsin (Schwartz and Bradford, 1986). Thus, it was inferred that the tetramerization procedure is necessary for gaining enzymatic activity. Another remarkable feature of MC trypsin is its complete resistance to endogenous macromolecular protease inhibitors (Smith *et al.*, 1984), although the enzyme is readily inhibited by synthetic low molecular weight inhibitors. The latter findings indicated that the active sites of trypsin have limited access to the exterior, but the explanation for these findings remained obscure for a long time. These issues were all resolved when human β -trypsin was crystallized and its three-dimensional structure solved (Pereira *et al.*, 1998). It was found that the trypsin tetramer was organized as a flat rectangular frame with one trypsin monomer in each of its corners (Fig. 4C). A central pore is formed at the interior of the tetramer and, remarkably, all of the active sites were facing this central pore (Fig. 4C and D). Since the entry into the central pore is quite narrow ($\sim 40 \times 15$ Å), most macromolecular compounds would not be able to enter, thus providing a perfect explanation for the resistance of tetrameric trypsin to macromolecular protease inhibitors. Further, this structural anatomy implies that many macromolecular potential substrates are excluded from access to the active sites. Indeed, many of the identified trypsin substrates are low molecular compounds (Table 6). In addition to β -trypsin, also the structure of human α -trypsin has been solved. α -Trypsin displayed a similar tetrameric organization and general structural features as β -trypsin (Marquardt *et al.*, 2002).

Although it is clear that active tryptase predominantly occurs in a tetrameric form, it has been suggested that tryptase also can be active in a monomeric state (Addington and Johnson, 1996). Further evidence for this came when Hallgren *et al.* (2001b) showed that recombinant mMCP-6, when activated by low molecular weight heparin, gained enzymatic activity without concomitant tetramerization (Fig. 5A). Importantly, it was demonstrated that active monomeric mMCP-6, in contrast to its tetrameric counterpart, was readily inhibited by a macromolecular protease inhibitor. Further, monomeric mMCP-6, in contrast to the tetrameric form, degraded fibronectin (Hallgren *et al.*, 2005), a protein that is predicted to be too large to enter the central pore of a tryptase tetramer (Sommerhoff *et al.*, 2000). In a subsequent study, it was demonstrated that tetrameric human β -tryptase, under conditions mimicking those that tryptase is exposed to after its exocytosis *in vivo*, can dissociate to functionally distinct active monomers (Fajardo and Pejler, 2003a). The notion that active monomers can be generated from tetrameric human β -tryptase has also been confirmed independently (Fukuoka and Schwartz, 2004). Moreover, similar to mMCP-6, it has been reported that activation of human recombinant β -tryptase with low molecular weight heparin resulted in generation of active tryptase monomers with properties distinct from those of tetrameric tryptase (Hallgren *et al.*, 2005).

6.3. MC-CPA

To date, no MC-CPA has been crystallized. Thus, insights into the three-dimensional structure of MC-CPA are so far derived from predictions made by modeling of MC-CPA based on the known structure of other carboxypeptidases. Using such strategy, it was predicted that murine pro-MC-CPA displays high structural similarity to porcine pancreatic pro-CPA and -B (Springman *et al.*, 1995). Moreover, it was found that large areas of positive charges were present on the molecular surface, and it was suggested that these areas most likely are involved in interactions with anionic PGs (Springman *et al.*, 1995).

7. CLEAVAGE SPECIFICITY

The key to understanding the function of any protease is to identify its *in vivo* substrate(s). Important clues to this issue may come from determining the fine cleavage specificity of a protease, and then search for matching peptide sequences in potential target proteins/peptides. Information regarding the exact cleavage specificity of a protease can also be useful when constructing inhibitors of the protease. In the following, cleavage sites are designated by using the nomenclature of Schechter and Berger (1967),

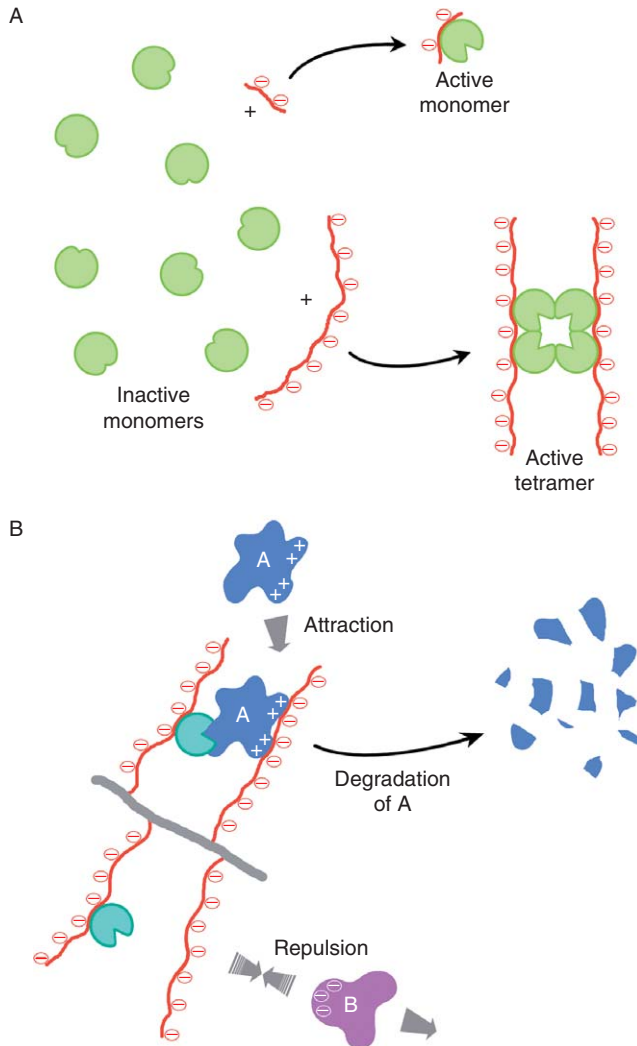


FIGURE 5 Different effects of SG PG on MC tryptase and chymase activity. (A) Model showing cross-linking of inactive tryptase monomers by GAGs, leading to tetramerization and enzymatic activation. GAGs that are of insufficient chain length to accommodate two monomers simultaneously can induce activation but not tetramerization, that is, active monomers. (B) Model for the effect of SG PG on chymase activity. *In vivo*, many MC chymases occur in complexes with SG PG, containing anionic GAG side chains. Proteins/peptides with affinity for GAGs will interact with SG PG, and are thereby presented to the chymase. This results in efficient degradation of GAG-binding compounds, whereas proteins that do not interact with GAGs may be electrostatically repelled and protected from degradation.

that is, where the P1 position corresponds to the residue at the N-terminal side of the cleavage site and the P1' residue is on the C-terminal side. Hence, the P1–P1' bond constitutes the cleavage site (scissile bond), P2, P3, and so on, residues are located further to the N-terminal end, and P2', P3', and so on, are to the C-terminal end of the cleavage site (Table 3).

Mainly two types of approaches have been employed for determining the substrate specificities of the MC proteases. The first type of approach utilizes chromogenic substrates where a chromophore (usually 4-nitroanilide; pNA) is replacing the P1' residue and where the P1, P2, and so on, residues are varied. A disadvantage with this methodology is that no information is obtained with regard to the contribution of the P1', P2', and so on, residues to the cleavage specificity. Another inherent limitation of this methodology is that a full covering of all possible variants at, for example, the P1–P4 positions would require chemical synthesis of an enormous amount of different chromogenic substrates. Another approach to determine cleavage specificity is to use peptide phage display technique. An advantage of this method is that information is also gained regarding the influence of the P1', P2' residues to the cleavage specificity. Further, a peptide phage library will contain far more combinations of sequences than what is practically feasible using a pNA substrate-based approach.

7.1. Chymase

In an early study, Powers *et al.* (1985) investigated the substrate specificities of rMCP-1, -2, human chymase, and dog chymase by using a chromogenic substrate-based approach. They found that all of these chymases showed a preference for Phe at the P1 position, but Tyr, Trp, and Leu were also well tolerated. By varying the P2–P4 positions, a slight preference for hydrophobic residues at the P2 and P3 positions was noted (Powers *et al.*, 1985). Importantly, the substrate specificities of all tested chymases were relatively similar. Karlson *et al.* (2002) employed a peptide phage display-based approach to study the cleavage specificity of the rat chymase, rMCP-4. In accordance with its predicted chymotrypsin-like specificity, rMCP-4 preferred Tyr and Phe at the P1 position. Further, there was a striking preference for bulky/aromatic residues at the P2 position, for Val at P3, for Leu at the P4 position, for residues with small side chains at the P2' position, and a slight preference for Arg at the P1' position. Based on the findings, a consensus sequence of Leu-Val-Trp-Phe-Arg-Gly was generated, and the corresponding synthetic peptide was cleaved by rMCP-4 exclusively at the Phe–Arg bond. However, it remains to be shown whether this consensus sequence is the *in vivo* target for rMCP-4. In a follow-up study, Karlson *et al.* (2003) studied the cleavage specificity of rMCP-5 by peptide phage display technique. Prior to the study, it had been predicted that rMCP-5 (and its mouse homologue, mMCP-5)

TABLE 3 Identified cleavage sites for MC chymases

Chymase		References
	↓	
Human chymase	P4-P3-P2-P1-P1'-P2'-P3'-P4'	
Ang I	I H P F H L	Reilly <i>et al.</i> , 1982
Fibronectin	S G P F T D V R	Okumura <i>et al.</i> , 2004
-''-	Q M I F E E H G	Okumura <i>et al.</i> , 2004
Serum albumin	R E T Y G E M A	Raymond <i>et al.</i> , 2003
C1-inhibitor	K M L F V E P I	Schoenberger <i>et al.</i> , 1989
Procollagenase	Q F V L T E G N	Saarinén <i>et al.</i> , 1994
α_2 -macroglobulin	R V G F Y E S D	Walter <i>et al.</i> , 1999
IL-1 β	N E A Y V H D A	Mizutani <i>et al.</i> , 1991
Procollagen 1 α	L K S L S Q Q I	Kofford <i>et al.</i> , 1997
SCF	T K P F M L P P	Longley <i>et al.</i> , 1997
ET-1	V V P Y G L G S	Nakano <i>et al.</i> , 1997
Hepatocyte growth factor	I V N L D H P V	Raymond <i>et al.</i> , 2006
apoA-I	A T V Y V D V L	Lee <i>et al.</i> , 2003a
apoA-I	V S Q F E G S A	Lee <i>et al.</i> , 2003a
proIL-18	Q V L F L D Q G	Omoto <i>et al.</i> , 2006
rMCP-1		
Ang I	D R V Y I H P F	Le Trong <i>et al.</i> , 1987a
Fibronectin	V E V F I T E T	Tchougounova <i>et al.</i> , 2001
-''-	T T P F S P L V	Tchougounova <i>et al.</i> , 2001
-''-	I T I Y A V E E	Tchougounova <i>et al.</i> , 2001
Thrombin	E K T F G A G E	Pejler <i>et al.</i> , 1995
-''-	R E T W T T S V	Pejler <i>et al.</i> , 1995
-''-	L S D Y I H P V	Pejler <i>et al.</i> , 1995
Dog chymase		
Pro-MMP-9	L G K F Q T F E	Fang <i>et al.</i> , 1997
-''-	F Q T F E G D L	Fang <i>et al.</i> , 1997
mMCP-4		
Ang I	D R V Y I H P F	Caughey <i>et al.</i> , 2000b; Lundequist <i>et al.</i> , 2004
Ang I	I H P F H L	Caughey <i>et al.</i> , 2000b; Lundequist <i>et al.</i> , 2004
Hepatocyte growth factor	I V N L D H P V	Raymond <i>et al.</i> , 2006

Cleavage sites are indicated using the nomenclature of Schechter and Berger (1967). The scissile bond is indicated by an arrow.

actually may display elastase-like cleavage specificity, that is, cleave after small aliphatic residues rather than after aromatic residues. The basis for this prediction is the presence of a Val (Val199; corresponding to position 216 in chymotrypsin) residue at the primary specificity pocket (S1 subsite), a property that normally confers elastase-like cleavage specificity. In chymotrypsin-like serine proteases, the corresponding residue is occupied by a Gly residue (Solivan *et al.*, 2002). Indeed, the peptide phage display analysis, along with data using chromogenic substrates, clearly indicated that rMCP-5 has elastase-like enzymatic properties with a preference for Ile, Val, or Ala at the P1 position (Karlson *et al.*, 2003). A further examination of the residues flanking the scissile bond revealed a slight preference for hydrophobic residues at the P2 and P3 positions, that is, similarly to other studied chymases (Powers *et al.*, 1985). Further, there was a marked preference for an acidic residue at the P2' position. The elastase-like properties of rMCP-5 (and also mMCP-5) are also supported by another study (Kunori *et al.*, 2002).

An interesting feature of the human chymase structure is the cationic properties of the S2' subsite, that is, the binding site for the P2' residue (Table 3), due to the Lys40 residue (not present in pancreatic chymotrypsin). The cationic nature of S2' is clearly compatible with accommodation of negatively charged residues at the substrate P2' position. This, in turn, may explain the productive binding of angiotensin I (Ang I; Asp1-Arg2-Val3-Tyr4-Ile5-His6-Pro7-Phe8-His9-Leu10) to the active site of human chymase, with the carboxyl group of the C-terminal Leu residue of Ang I interacting with the S2' subsite. Clearly, these findings are well in line with the notion that human chymase preferentially cleaves the Phe8-His9 bond of Ang I (rather than the Tyr4-Ile5 bond which results in Ang I degradation), leading to activation (Section 9.1.1.).

An additional approach to address the cleavage specificity of a protease is to identify the cleavage sites after incubation of the protease with a substrate protein/peptide. Alignment of identified cleavage sites may then reveal consensus sequences for cleavage. Examples of identified cleavage sites for MC chymases in various proteins are displayed in Table 3. An examination of such identified cleavage sites confirms the strict requirements for aromatic residues or Leu at the P1 position. An interesting observation is that Pro is often found at the P2 position of sequences recognized by human chymase, in agreement with that chromogenic substrates with a P2 Pro are good substrates for human chymase (Powers *et al.*, 1985). Aliphatic residues are also frequently found at P2. Moreover, it is clear that the P3-P4 positions show a high degree of variability. Another finding in line with the phage display data reported for rMCP-5 (see above) and the predictions based on the crystal structure of human chymase (see above) is that acidic residues (Asp/Glu) often occupy the P2' position of sites cleaved by human chymase.

Together, the available data on the fine substrate specificities of chymases suggest that they are relatively promiscuous, except for the strict requirements at the P1 position and the preference for an acidic P2' residue. It may thus not be possible to determine the physiological targets for the chymases, solely based on identification of preferred cleavage sites. Rather, selection of physiological targets will most likely depend on other factors such as availability of potential substrates at sites of MC degranulation and concentration of substrate. In addition, it is important to note that several of the MC chymases, for example human chymase, mMCP-4, and -5, most likely occur *in vivo* in macromolecular complexes with heparin/chondroitin sulfate (CS) PGs, and there is much evidence suggesting that such association with PGs may profoundly influence the selection of substrates and affect the rate of substrate degradation (Section 8). The actual site of cleavage may also differ depending on the absence or presence of heparin (Le Trong *et al.*, 1987a).

7.2. Tryptase

Important knowledge concerning the fine substrate specificity of tryptases has been generated using similar phage display-based systems as those employed for chymases. In one study, Stevens *et al.* examined the specificity of mMCP-7. They found that mMCP-7 showed a remarkable preference for cleaving a consensus sequence of Leu-Ser-Ser-Arg-Gln-Ser (cleavage after the Arg residue), a sequence found within one of the identified tryptase substrates, fibrinogen (Huang *et al.*, 1997). Later, the same group examined the cleavage specificity of mMCP-6 using similar techniques. The results indicated a rather broad extended cleavage specificity, with a large degree of variability at the P2–P4 as well as P1'–P3' positions (Huang *et al.*, 1998). Using phage display technique, Harris *et al.* found that β I- and β II-tryptase both displayed a strict requirement for a P1 Arg or Lys. Further, both β I- and β II-tryptase showed a slight preference for Gln at the P2 position, preference for Arg/Lys at P3 and some preference for Pro at P4 (Harris *et al.*, 2001). In a parallel study, Huang *et al.* (2001) also found that Pro residues were common components of preferred sequences for human β I-tryptase cleavage. However, Pro residues dominated at P3 rather than P4 (see above), although Pro was also found at P2 and P4. In addition, they found that all of the cleaved sequences had a Lys residue N-terminal of the cleavage site, in particular at the P6 position (Huang *et al.*, 2001).

7.3. MC-CPA

The available data on the cleavage properties of MC-CPA have mainly been generated by HPLC analysis of products obtained after cleavage of defined peptides by purified MC-CPA. Using such an approach, it was

shown that human MC carboxypeptidase was able to hydrolyze the C-terminal Leu of Ang I (Table 3), but not the C-terminal His of the generated des-Leu10 Ang I to form Ang II. Further, MC carboxypeptidase was, in contrast to pancreatic CPB, unable to cleave the C-terminal Arg in bradykinin. It was also shown that the MC enzyme hydrolyzed a synthetic dipeptide substrate with a terminal Phe but not the corresponding substrate with a C-terminal Arg (Goldstein *et al.*, 1989). These results suggested that MC carboxypeptidase has cleavage properties similar to that of pancreatic CPA and therefore that the MC carboxypeptidase is a CPA. Also murine MC-CPA has been demonstrated to cleave Ang I, generating des-Leu10 Ang I (Serafin *et al.*, 1987). More recently, it was shown in an *ex vivo* culture of peritoneal MCs that murine MC-CPA in fact may hydrolyze the C-terminal His of des-Leu10 Ang I to form Ang II, and also can hydrolyze the Phe8 C-terminal of Ang II. However, no further hydrolysis was seen, indicating that the Pro residue which is positioned at the C-terminal after cleavage of Phe8 (Table 3) is a poor substrate for MC-CPA (Lundequist *et al.*, 2004). Also in an earlier report, it was noted that Pro, at the penultimate position of dipeptide substrates, prevents hydrolysis by MC-CPA (Everitt and Neurath, 1980).

8. INTERACTION OF MC PROTEASES WITH PGs: IMPLICATIONS FOR STORAGE, ACTIVITY, AND PROCESSING

8.1. Storage

Based on the colocalization of MC proteases with anionic heparin/CS PGs within granules, it was early suggested that these compounds are interacting (Schwartz and Austen, 1980). Evidence for such an interaction between PGs and MC proteases came when it was shown that degranulation of BMMCs caused release of complexes of PGs and serine proteases (Serafin *et al.*, 1986) as well as MC-CPA/PG complexes (Serafin *et al.*, 1987). High concentrations of NaCl disrupted these complexes, demonstrating that the interaction, as expected from the anionic and cationic properties of the MC PGs and proteases, respectively, was of electrostatic nature.

In 1999, two groups simultaneously presented strong evidence supporting an interaction of MC proteases and heparin PG *in vivo* (Forsberg *et al.*, 1999; Humphries *et al.*, 1999). The strategy in both studies was to genetically target the gene for *N*-deacetylase/*N*-sulfotransferase 2 (NDST-2), an enzyme that is required for sulfation of heparin (Forsberg and Kjellen, 2001), but has no role in CS synthesis. NDST-2 appears to be ubiquitously expressed in various tissues and cell types, and is in most tissues coexpressed with an isozyme, NDST-1 (Kusche-Gullberg *et al.*, 1998). However,

CTMCs appear to predominantly express the NDST-2 isoform (Kusche-Gullberg *et al.*, 1998), and with no other compensating NDST isoform present in NDST-2^{-/-} CTMCs, sulfation of heparin is expected to be defective. Accordingly, it was shown that the KO of NDST-2 resulted in profound reductions in the content of sulfated GAGs in CTMCs (Forsberg *et al.*, 1999; Humphries *et al.*, 1999). A striking finding in both studies was that the KO of NDST-2 resulted in an essentially complete absence of stored CTMC proteases, that is, mMCP-4, -5, -6, and MC-CPA (Forsberg *et al.*, 1999; Humphries *et al.*, 1999). Interestingly though, the mRNA levels for the CTMC proteases were unaltered, indicating that the absence of NDST-2 affected the proteases at the level of storage rather than synthesis. Storage of mMCP-6 in BMMCs, in contrast to mMCP-6 storage in CTMCs, was virtually unaffected by the absence of NDST-2 (Henningsson *et al.*, 2002; Humphries *et al.*, 1999). This is presumably due to the presence of CS in these cells (Razin *et al.*, 1982), and that CS can compensate for the lack of heparin in promoting mMCP-6 storage. However, despite the presence of CS, BMMCs were unable to store mMCP-5 and mature MC-CPA (Henningsson *et al.*, 2002; Humphries *et al.*, 1999). Apparently, the latter MC proteases are strictly dependent on GAGs of heparin type for proper storage. In contrast to CTMCs, MMCs were morphologically unaffected by the lack of NDST-2 (Forsberg *et al.*, 1999), a finding that is in good agreement with the predominant presence of CS rather than heparin in MMCs (Enerback *et al.*, 1985).

The NDST-2 KO thus provides strong evidence for a critical role of GAGs of heparin type in storage of several CTMC proteases. However, it does not provide information relating to the core protein that the GAGs are attached to, nor are the NDST-2^{-/-} mice relevant tools for studying the potential role of PGs for granule storage in MMCs. It has been believed for a long time that GAGs in MC granule, irrespective of MC subclass (e.g., CTMC/MMC) or GAG type (heparin/CS), are attached to the same protein core, SG (Kjellén *et al.*, 1989). However, since there are a number of different PG core proteins, it has not been possible to exclude the contribution of GAGs attached to non-SG core proteins to the total pool of PGs stored within MC granules. Therefore, to specifically study the role of serglycin proteoglycan (SG PG) in MC granule homeostasis, the SG core protein gene was targeted (Åbrink *et al.*, 2004). The results revealed, in agreement with the results from the NDST-2 KO, that mMCP-4, -5, -6, and MC-CPA were all critically dependent on SG PG for storage in CTMCs, but not for mRNA expression. Moreover, it was shown that the same proteases, when expressed by BMMCs, were strongly SG-dependent for storage. In contrast, it was noticed both *in vivo* and in BMMCs differentiated into an MMC-like phenotype that mMCP-1 was independent on SG for storage and that mMCP-2 was only partly dependent on SG for storage (Braga *et al.*, 2007). Further, it was

shown that mMCP-7 storage was not affected by the KO of SG (Braga *et al.*, 2007). The latter, together with previous findings (Humphries *et al.*, 1999), supports the notion that mMCP-7 is not dependent on SG PG for storage within MC granules.

Together, the findings derived from the NDST-2 and SG KOs indicate that certain MC proteases are strictly dependent on SG PG for storage, whereas others are non- or only partly SG dependent (Table 4). Although we cannot with certainty explain the differential SG dependence for storage, the most likely explanation lies within the basic charge exposure and corresponding GAG-binding properties of the respective proteases. As displayed in Table 4, mMCP-4, -5, and MC-CPA are all strongly basic proteins and expose defined patches of positive charge on their molecular surface (Section 6). Thus, their strong dependence on SG is not surprising. Also mMCP-6, despite a negative net charge, has been shown to expose defined clusters of positively charged amino acid residues at the molecular surface (most notably His residues; Fig. 4E; Hallgren *et al.*, 2004) and interacts strongly with heparin and other anionic polysaccharides (Hallgren *et al.*, 2001b). mMCP-1, on the other hand, has a much lower net positive charge than mMCP-4/5, well explaining its lack of SG dependence for storage (Braga *et al.*, 2007). Further, the intermediate positive charge of mMCP-2 is well in line with its only partial dependence on SG (Braga *et al.*, 2007). The lack of SG dependence for mMCP-7 storage is in agreement with its independence of NDST-2 (Humphries *et al.*, 1999). On the other hand, it has been shown that recombinant mMCP-7 binds, at low salt conditions, to heparin-Sepharose at acidic pH (Matsumoto *et al.*, 1995).

Although the findings generated through the KO of NDST-2 and the SG core protein firmly establish the importance of SG PGs for storage of CTMC protease, the underlying mechanisms are not fully understood.

TABLE 4 Net charge of MC proteases and their dependence on SG for storage

Protease	Net charge ^a (pH 7.0)	SG dependence for storage	References
mMCP-1	+4.7	—	Braga <i>et al.</i> , 2007
mMCP-2	+7.2	(+)	Braga <i>et al.</i> , 2007
mMCP-4	+16.7	+	Åbrink <i>et al.</i> , 2004
mMCP-5	+13.9	+	Åbrink <i>et al.</i> , 2004
mMCP-6	−2.2	+	Åbrink <i>et al.</i> , 2004
mMCP-7	−6.2	—	Braga <i>et al.</i> , 2007
MC-CPA	+18.5	+	Åbrink <i>et al.</i> , 2004

^a Net charges were calculated for mature proteins, that is, without activation peptide.

An attractive hypothesis would be that SG is necessary for intracellular targeting (sorting) of the SG-dependent proteases into the granule, that is, that SG is sorted to the pathway of regulated secretion and that the various proteases are accompanying SG PG into the granule as cargo. According to such a mechanism, lack of SG would be expected to result in failure to sort MC proteases into granule, accompanied by missorting of MC proteases into the constitutive pathway of secretion. However, the evidence available so far indicate that MC proteases are correctly targeted into MC granule also in SG^{-/-} MCs, and that missorting into the constitutive pathway of secretion is only a partial explanation for the lack of granule-associated MC proteases in SG^{-/-} MCs (Henningsson *et al.*, 2006).

Considering that the interactions between many of the MC proteases and GAGs are of very high affinity, it is likely that the proteases will remain attached to SG PG following MC degranulation. Indeed, such an association has been demonstrated directly by the isolation of MC protease/PG complexes in supernatants obtained from degranulated MCs (Serafin *et al.*, 1986). Such an association may involve a large number of protease units associated with each PG, and there is additionally a potential of cross-linking several PG/protease complexes. For these reasons, high molecular weight aggregates may form with a possible restriction in their ability to diffuse away from the site of MC degranulation. In line with such a scenario, it is known that chymases tend to remain associated with the MC surface after degranulation (Schwartz *et al.*, 1981b; Tchougounova and Pejler, 2001). In fact, chymase activity is present on the surface of MCs even without induction of degranulation (Gervasoni *et al.*, 1986), suggesting that chymase is to some extent constitutively released. In further support, mMCP-6 was reported to remain bound to the extracellular matrix (ECM) following passive systemic anaphylaxis, whereas, in contrast, mMCP-7 is rapidly transported to the blood (Ghildyal *et al.*, 1996). A plausible explanation for these findings is that mMCP-6 remains associated with SG PG, whereas mMCP-7 rapidly diffuses away from the MC surface due to its low affinity for SG PG (see above). Interestingly, it has been shown that mMCP-1 is constitutively released by BMMCs of MMC-like phenotype (Brown *et al.*, 2003), in good agreement with an inability of mMCP-1 to interact with SG, which could result in decreased retention in MMC granules.

The exact organization of the SG PG/MC protease complexes within MC secretory granules is not known. In one potential scenario, individual PG molecules may bind to different proteases, resulting in macromolecular complexes that accommodate both tryptase, chymase, and MC-CPA, with the alternative scenario being that the individual proteases occur in separate complexes with SG PGs. These possibilities have not been addressed so far in rodent systems. However, there is evidence indicating that human MCs actually secrete MC-CPA and chymase in complexes

with PGs which are separate from PG/tryptase complexes (Goldstein *et al.*, 1992). The underlying mechanism behind such a subdivision of protease/PG complexes in the human MC is intriguing, but has not been investigated further. Indeed, it is difficult to envisage how certain PG molecules preferentially “select” tryptase over MC-CPA/chymase and vice versa, considering that the interaction between all of the MC proteases and GAGs appears to be of unspecific, electrostatic nature (see below). On a speculative angle, one possibility could be that MCs synthesize tryptase and MC-CPA/chymase with different timing, that is, tryptase/PG complexes are formed during a time frame when tryptase is preferentially synthesized over MC-CPA/chymase and the other way around. Such a mechanism would be in some analogy with the mechanisms behind the formation of granules of discrete contents in neutrophils (Faurschou and Borregaard, 2003). In further support for a separation of tryptase/PG complexes and complexes of PGs with MC-CPA/chymase, it has been shown by ultrastructural analysis that chymase accumulated in electron dense areas of human MC granule, whereas tryptase is found in more electron translucent, crystalline parts of the granule (Whitaker-Menezes *et al.*, 1995). However, such a compartmentalization of granules has not been described for rodent MCs.

It is clear from the above that PGs have a profound impact on the storage of proteases in MC granules. However, it is reasonable to assume that MC granule homeostasis is a dynamic process where each of the granule constituents will affect storage of other granule components. In line with such a notion, it has been shown that lack of mMCP-1 disturbs the crystal intragranular structures in MMCs (Wastling *et al.*, 1998). Further, it was found that lack of MC-CPA affects the solubility of heparin PG in peritoneal MCs (Feyerabend *et al.*, 2005). Another interesting finding is that the KO of MC-CPA caused an impaired ability of CTMCs to store mMCP-5 (Feyerabend *et al.*, 2005). Conversely, it was previously reported that the KO of mMCP-5 resulted in an inability of CTMCs to store MC-CPA (Stevens *et al.*, 1996). These findings, along with a report showing that an increase in MC-CPA storage in cathepsin C^{-/-} BMMCs was accompanied by increased mMCP-5 storage (Henningsson *et al.*, 2003), strongly suggest that mMCP-5 and MC-CPA are dependent not only on SG PG but also on each other for proper storage. Clearly, a plausible explanation would be that MC-CPA and mMCP-5 engage in physical contacts and that such interactions are required for proper sorting into granules or for protection toward proteolytic degradation. In contrast, mMCP-4 and -6 storage was not affected by the KO of MC-CPA (Feyerabend *et al.*, 2005), and the increased storage of MC-CPA in cathepsin C^{-/-} BMMCs was not accompanied by increased mMCP-6 storage (Henningsson *et al.*, 2003), demonstrating that interdependence of MC-CPA and mMCP-5 exhibits a large degree of specificity.

Further support for an interdependence of various granule compounds comes from a study in which the gene for histidine decarboxylase, that is, the enzyme that converts histidine to histamine, was targeted (Ohtsu *et al.*, 2001). It was found that lack of histamine in peritoneal MCs caused reduced metachromatic staining, indicative of a reduced storage of SG PG. Further, it was demonstrated that storage of mMCP-4, -5, -6, and MC-CPA, that is, the SG-dependent CTMC proteases, was defective. A likely explanation for these findings could be that histamine is required for balancing the negative charge of SG PGs in the granules and that the absence of histamine thereby causes defects in PG storage, that in turn may lead to a decrease in the levels of stored proteases.

8.2. Effect of PGs on MC protease activity/activation and processing

Although a major function of SG PG is to promote protease storage, much evidence points to a regulative role of SG PG for MC protease function at various other levels. It has been known for a long time that tryptase tetramers require heparin (or other structurally related polysaccharides) for stability (Schwartz and Bradford, 1986). Further, it has been demonstrated that both mMCP-6 and human β -tryptase are dependent on heparin-like saccharides for tetramerization and enzymatic activation (Hallgren *et al.*, 2001b, 2005; Fig. 5A). Along this line, heparin antagonists have been proven to be potent inhibitors of tryptases (Hallgren *et al.*, 2001a; Lundequist *et al.*, 2003), and one study has suggested that heparin is also needed for processing of protryptase to active enzyme (Sakai *et al.*, 1996b). For a more detailed discussion of various functional aspects of the interaction of tryptase with heparin, see Hallgren and Pejler (2006).

In contrast to the tryptases, chymases do not require GAGs for gaining or maintaining enzymatic activity, but the association of chymase with SG PG may profoundly modulate chymase activity. In early reports, heparin was shown to promote degradation of C3a by rat chymase (Gervasoni *et al.*, 1986) and to enhance the degradation of thrombin by both mMCP-4 (Pejler and Karlstrom, 1993) and rMCP-1 (Pejler *et al.*, 1994). Analysis of the underlying mechanism showed that thrombin, a heparin-binding protein (Sheehan and Sadler, 1994), was attracted to the heparin moiety of the chymase/heparin complex, thus providing an effective presentation of the substrate (thrombin) to the chymase (Pejler and Sadler, 1999; Fig. 5B). As evidence for such a mechanism, it was demonstrated that thrombin in which the heparin-binding region was mutated was degraded to a much lower extent than wild-type thrombin (Pejler and Sadler, 1999). In further support for the proposed mechanism, it has been reported that heparin antagonists efficiently prevent the chymase-dependent degradation of heparin-binding substrates (Tchougounova and Pejler, 2001;

Tchougounova *et al.*, 2001). Together, these findings indicate that the association of chymase with SG PG will favor cleavage of substrates with affinity for heparin. Indeed, several of the known chymase substrates are GAG-binding proteins. However, it should be noted that heparin-binding properties are not absolute prerequisites for being substrates for chymase, as evident by the many nonheparin-binding peptides and proteins (e.g., albumin and Ang I) that are favorable chymase substrates. In fact, it has been shown that cleavage of some substrates, for example, Ang I (Murakami *et al.*, 1995), is actually decreased in the presence of heparin. It should also be stressed that the proposed mechanism is, for obvious reasons, only operative for chymases that interact with anionic GAGs. An important question is if this mechanism is also operative *in vivo*. Certainly, since many chymases most likely remain tightly associated with SG PG also after their release, it is plausible that SG PG will influence the selection of chymase substrates also *in vivo*. In support for this, one of the likely *in vivo* substrates for mMCP-4, fibronectin, is a GAG-binding protein (Maccarana and Lindahl, 1993).

In addition to influencing the selection of substrates, it has been shown that heparin PG offers protection of chymases toward a range of endogenous macromolecular protease inhibitors, including α_1 -antichymotrypsin (α_1 -AC), α_2 -macroglobulin, and α_1 -protease inhibitor (α_1 -PI) (Lindstedt *et al.*, 2001b; Pejler, 1996; Pejler and Berg, 1995). Thus, SG PG may modulate the activity of GAG-binding chymases at several levels: by promoting selection of certain substrates, by restricting chymase mobility, and by increasing the life span of chymases after their exocytosis.

There are currently no reports describing effects of GAGs/PGs on the enzymatic activities of MC-CPA. However, there is evidence indicating that the processing of pro-MC-CPA into active protease is dependent on PGs. First, it was shown that the processing of pro-MC-CPA was defective in BMMCs lacking NDST-2 (Henningsson *et al.*, 2002) and it has also been demonstrated that BMMCs lacking SG show defects in pro-MC-CPA processing (Henningsson *et al.*, 2006). The underlying mechanism behind these findings is not clear, but it may be speculated that the pro-MC-CPA-processing protease(s) is dependent on SG PG or that the association of pro-MC-CPA with SG PG is required for proper presentation of the cleavage site to the pro-MC-CPA-processing enzyme(s).

8.3. Structural basis for GAG: MC protease interaction

The structural basis for the interaction between GAGs and chymases has been the subject of investigation. It was found that the binding of GAGs to both rMCP-1 (Pejler and Maccarana, 1994) and human chymase (Sayama *et al.*, 1987) was of high affinity and of unspecific electrostatic nature, that is, no specific structural element in the GAG was required for binding.

Moreover, it was determined that the minimal size for binding of heparin to rMCP-1 was a 14-mer oligosaccharide (Pejler and Maccarana, 1994); thus, the minimal binding saccharide was relatively large as compared with minimal oligosaccharides required for binding to other heparin-binding proteins such as antithrombin (Baglin *et al.*, 2002). Also the interaction between MC tryptase and GAGs have been shown to be of unspecific electrostatic nature (Alter *et al.*, 1987; Hallgren *et al.*, 2001b, 2005); see also a review on this subject (Hallgren and Pejler, 2006). To date, there are no reports in which the nature of the interaction between GAGs and MC-CPA is described.

9. SUBSTRATES FOR MC PROTEASES

Given that the MC proteases, with certain exceptions, have a relatively broad cleavage specificity (Section 7), it would be expected that a large number of proteins/peptides could be potential substrates. Indeed, a multitude of proteins and peptides are known to be cleaved by MC proteases (Tables 5–7). Many of these have been identified through incubation of the MC protease with the protein/peptide in a purified system. In other cases, substrates have been identified in cell culture systems to which a MC protease has been added; alternatively, the use of MC protease inhibitors has implicated certain proteins/peptides as substrates. In only a few cases, there is *in vivo* evidence linking an MC protease to degradation of a certain substrate (Table 8).

In order to understand the *in vivo* function of the MC proteases, both in normal physiology as well as in pathological settings, it is imperative to identify their true *in vivo* substrates. Certainly, identification of a given compound as a substrate for an MC protease *in vitro* can give important clues as to this, but one should be careful when attempting to extrapolate such findings into an *in vivo* setting. Most likely, the “selection” of substrates by the MC proteases *in vivo* is dependent on a variety of factors. Obvious factors are the availability of potential MC protease substrates at the site of MC degranulation and, importantly, the concentration of a potential substrate in relation to the $k_{\text{cat}}/K_{\text{m}}$ ratio of the MC protease for that particular compound. If indeed a potential substrate is present at the site of MC degranulation, and if its concentration is favorable, it is likely that cleavage will occur *in vivo*. It is also very likely that the “selection” of substrates by a given MC protease may differ during different phases of a biological process. For example, immediately following the release of a MC protease, for example, in early phases of an inflammatory reaction, only resident potential substrates have the chance of being degraded by the MC protease. Such resident compounds may include various ECM components. In the next phase, compounds that are newly synthesized

TABLE 5 Substrates for MC chymases

	References
Procollagen	Kofford <i>et al.</i> , 1997
Procollagenase	Saarinen <i>et al.</i> , 1994
Pro-MMP-9	Chen <i>et al.</i> , 2002; Fang <i>et al.</i> , 1996; Tchougounova <i>et al.</i> , 2005
Fibronectin	Banovac and De Forteza, 1992; Lazaar <i>et al.</i> , 2002; Tchougounova <i>et al.</i> , 2003; Vartio <i>et al.</i> , 1981
Vitronectin	Banovac <i>et al.</i> , 1993
TIMP-1	Frank <i>et al.</i> , 2001
Substance P	Caughey <i>et al.</i> , 1988
VIP	Caughey <i>et al.</i> , 1988
Bradykinin	Reilly <i>et al.</i> , 1985
Kallidin	Reilly <i>et al.</i> , 1985
Big-endothelin 1/2	Kido <i>et al.</i> , 1998; Nakano <i>et al.</i> , 1997; Takai <i>et al.</i> , 1998; Wypij <i>et al.</i> , 1992
Neurotensin	Goldstein <i>et al.</i> , 1991
Hepatocyte growth factor	Raymond <i>et al.</i> , 2006
CTAP-III	Schiemann <i>et al.</i> , 2006
Pro-IL-18	Omoto <i>et al.</i> , 2006
IL-6, IL-13	Zhao <i>et al.</i> , 2005
TGF- β 1	Taipale <i>et al.</i> , 1995
IL-1 β -precursor	Mizutani <i>et al.</i> , 1991
SCF	de Paulis <i>et al.</i> , 1999; Longley <i>et al.</i> , 1997
apoE, apoA-I, apoA-II	Lee <i>et al.</i> , 2002b, 2003a; Lindstedt <i>et al.</i> , 1996
apoB	Kokkonen <i>et al.</i> , 1986
Phospholipid transfer protein	Lee <i>et al.</i> , 2003b
PAR-1	Schechter <i>et al.</i> , 1998
C3a	Gervasoni <i>et al.</i> , 1986; Kajita and Hugli, 1991
Albumin	Raymond <i>et al.</i> , 2003
Occludin	Scudamore <i>et al.</i> , 1998
C1 inhibitor	Schoenberger <i>et al.</i> , 1989
Ang I	Urata <i>et al.</i> , 1990
Thrombin	Pejler and Karlstrom, 1993

and secreted into the tissue as a consequence of the onset of an inflammatory response, for example, cytokines and chemokines, may be targets for MC proteases. Somewhat later, plasma components that have escaped

TABLE 6 Substrates for MC tryptases

	References
Kininogen	Imamura <i>et al.</i> , 1996
Prekallikrein	Imamura <i>et al.</i> , 1996
Fibrinogen	Huang <i>et al.</i> , 1997; Schwartz <i>et al.</i> , 1985
Gelatin	Fajardo and Pejler, 2003b; Raymond <i>et al.</i> , 2005
VIP	Caughey <i>et al.</i> , 1988
PHM	Tam and Caughey, 1990
CGRP	Tam and Caughey, 1990
Pro-uPA	Stack and Johnson, 1994
Fibronectin	Fajardo and Pejler, 2003a; Hallgren <i>et al.</i> , 2001a; Kaminska <i>et al.</i> , 1999; Lohi <i>et al.</i> , 1992
HDL	Lee <i>et al.</i> , 2002c
pro-MMP-3	Gruber <i>et al.</i> , 1989; Lees <i>et al.</i> , 1994
PAR-2	Cenac <i>et al.</i> , 2002; Corvera <i>et al.</i> , 1997; Mirza <i>et al.</i> , 1997; Molino <i>et al.</i> , 1997
Type VI collagen	Kielty <i>et al.</i> , 1993
Pre-elafin	Guyot <i>et al.</i> , 2005
RANTES	Pang <i>et al.</i> , 2006
Eotaxin	Pang <i>et al.</i> , 2006

TABLE 7 Substrates for MC-CPA

	References
ET-1	Metsarinne <i>et al.</i> , 2002
Sarafotoxin	Metz <i>et al.</i> , 2006
Ang I	Goldstein <i>et al.</i> , 1989; Lundquist <i>et al.</i> , 2004; Serafin <i>et al.</i> , 1987
apoB-100	Kokkonen <i>et al.</i> , 1986
Neurotensin	Goldstein <i>et al.</i> , 1991

into the tissue as a consequence of increased capillary permeability may be cleaved. During this phase of the reaction, it is also likely that protease inhibitors derived from extravasated blood plasma will downregulate the activity of the MC proteases. In a following healing phase of the inflammatory reaction, yet other substrates could be targets for MC proteases. Alternatively, if the acute inflammation proceeds to a chronic phase, additional substrates could be cleaved.

An important consideration regards the consequences of the cleavage of a certain compound by a given MC protease. In many cases, the result

TABLE 8 MC protease KO strains and KO strains in which MC proteases are affected

Knockout	Phenotype	References
mMCP-1	Altered granular organization; delayed expulsion of <i>T. spiralis</i>	Knight <i>et al.</i>, 2000; Wastling <i>et al.</i>, 1997
mMCP-4	Defective pro-MMP-9 processing; skin fibrosis	Tchougounova <i>et al.</i>, 2003, 2005
mMCP-5	Defective MC-CPA storage; protection in ischemia reperfusion injury	Abonia <i>et al.</i>, 2005
MC-CPA	Altered granular staining; defective mMCP-5 storage	Feyerabend <i>et al.</i>, 2005
SG	Severely defective MC protease storage CTMCs, MMCs and BMMCs affected	Åbrink <i>et al.</i>, 2004; Braga <i>et al.</i>, 2007
NDST-2	Severely defective MC protease storage CTMCs affected	Forsberg <i>et al.</i>, 1999; Humphries <i>et al.</i>, 1999
Histidine decarboxylase	Reduced MC PG and protease content	Ohtsu <i>et al.</i>, 2001

could be degradation of the compound and thereby loss of functional properties. In fact, given that the MC proteases generally have quite broad cleavage specificities, they are indeed well suited to carry out such general degradation processes ([Fig. 6](#)). In other situations, the cleavage of a certain compound can lead to its activation, as exemplified by the activation of several proenzymes by different MC proteases ([Table 5](#)). It is important to stress that these modes of action are not necessarily mutually exclusive. For example, an initial activating cleavage in a proenzyme may be followed by additional inactivating cleavages.

9.1. Chymase

9.1.1. Angiotensin I

A multitude of substrates has been identified for different MC chymases ([Table 5](#)). Among these, Ang I (Asp1-Arg2-Val3-Tyr4-Ile5-His6-Pro7-Phe8-His9-Leu10) has by far attracted the largest attention. Early reports

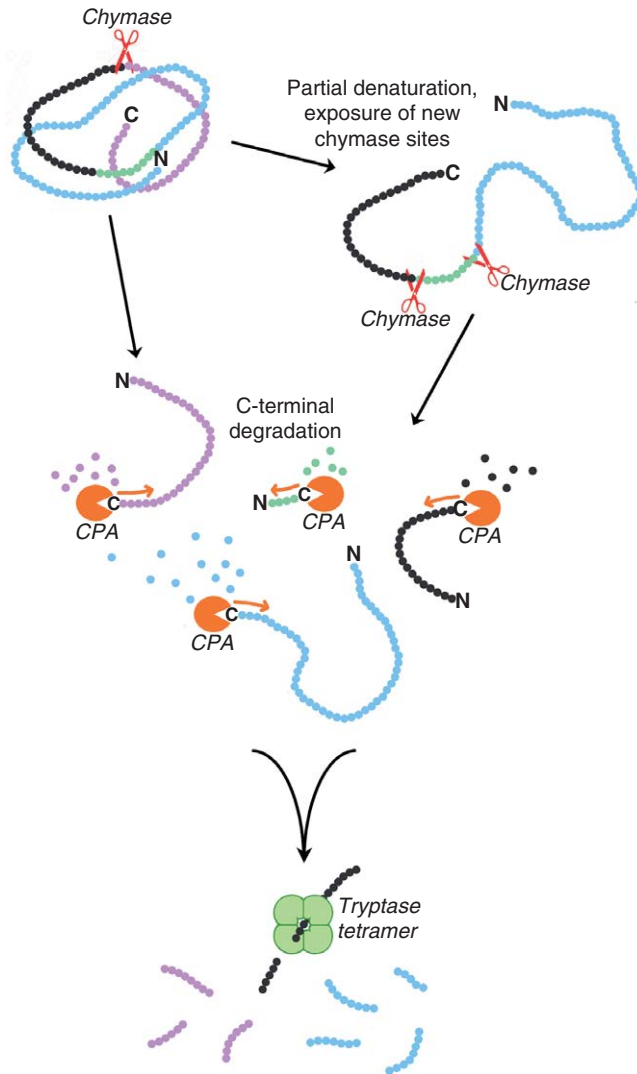


FIGURE 6 Hypothetical model for concerted action of MC proteases in substrate degradation. Initial internal cleavages by chymases expose new C-terminal ends that may be substrates for MC-CPA. Chymase-catalyzed internal cleavages may also lead to partial denaturation and exposure of new chymase cleavage sites. Partially degraded substrates may reach a size compatible with entry into the central pore of tetrameric tryptase, for further degradation.

showed that human chymase can cleave Ang I at the Phe8–His9 bond (Table 3), thereby forming Ang II, a potent vasoconstrictor (Reilly *et al.*, 1982; Urata *et al.*, 1990). It was also demonstrated that whereas human

chymase predominantly cleaves the Phe8–His9 bond, rat chymase (rMCP-1) preferentially cleaves at the Tyr4–Ile5 bond, resulting in degradation of the peptide and destroyed biological activity (Sanker *et al.*, 1997). These findings indicated that the mode of Ang I cleavage, that is, at the Phe8–His9 or Tyr4–Ile5 bond, is a functional parameter that discriminates the α - from the β -chymases, with α -chymases preferentially catalyzing the activation cleavage and β -chymases predominantly causing Ang I degradation. However, it was later shown that certain β -chymases, for example, mMCP-4, in addition to degrading Ang I also can cleave at the Phe8–His9 bond, yielding Ang II (Caughey *et al.*, 2000b). Thus, the ability to activate or degrade Ang I may not be a property that strictly belongs to either the α - or β -chymase family. The possibility that chymase may have a role in the generation of Ang II *in vivo* has been studied intensely (reviewed in Dell'Italia and Husain, 2002). This notion has, for example, been prompted by the findings that Ang-converting enzyme (ACE) inhibitors only produce partial inhibition of Ang II formation (Padmanabhan *et al.*, 1999; Wolny *et al.*, 1997). Further, the KO of ACE only partially prevented Ang II formation (Wei *et al.*, 2002), suggesting that other Ang II-generating mechanisms may be operative, such as chymase catalyzed. Indeed, *in vivo* studies, for example, by using isolated human mammary arteries have shown that chymase accounts for a substantial part of the total Ang II-generating capacity (Richard *et al.*, 2001). Moreover, transgenic overexpression of human chymase in mice indicated a correlation between chymase expression and cardiac content of Ang II (Chen *et al.*, 2002). In further support for a role of MCs in Ang II formation, it was shown that mice lacking MCs (W/W^v mice) showed an impaired ability to generate Ang II (Li *et al.*, 2004). In the latter study, it was suggested that mMCP-5 is a major player in MC-dependent Ang II formation. However, this notion is difficult to reconcile with the elastase-like substrate specificity of mMCP-5 (Section 7), which is not compatible with cleavage of the Phe8–His9 bond in Ang I. To firmly establish a role of MC chymase in Ang II generation *in vivo*, it will be important to investigate Ang II generation in mice lacking appropriate MC chymases. However, this remains to be done. Interestingly, recent evidence suggests that a chymase-dependent pathway for Ang II production, induced by advanced glycation end products (AGEs), contributes to complications in diabetes (Koka *et al.*, 2006).

9.1.2. ECM components

An examination of the identified chymase substrates reveals that many of them are components of the ECM or are involved in regulation of ECM turnover (Table 5). Indeed, the location of MCs within connective tissues and, hence, the release of chymase into connective tissues in association with MC degranulation is well in line with a role for chymase in regulating

ECM composition. It was early shown, in a purified system, that fibronectin is a substrate for human chymase (Vartio *et al.*, 1981). Later it was demonstrated that human chymase released fibronectin fragments from the ECM of cultured airway SMCs (Lazaar *et al.*, 2002) and that fibronectin is also a substrate for murine MC chymases (Tchougounova *et al.*, 2001). More recently, it was reported that the KO of mMCP-4 caused marked accumulation of fibronectin, both in *in vitro*-cultured peritoneal exudate cells and *in vivo* (Tchougounova *et al.*, 2003, 2005). Thus, chymase is likely to be involved in fibronectin turnover by catalyzing its degradation. Also vitronectin, another prominent ECM component, has been shown to be a substrate for MC chymase (Banovac *et al.*, 1993).

Chymase may also regulate ECM composition indirectly, by cleaving and thereby activating compounds that, in turn, can modulate ECM composition. For example, chymase has been found to release latent TGF- β 1 bound to ECM, unleashing its stimulating activity on fibroblast ECM production (Taipale *et al.*, 1995). Moreover, rat chymase may activate latent TGF- β 1 present within the MC secretory granule (Lindstedt *et al.*, 2001a). Such effects on TGF- β 1 would thus promote ECM deposition. Another indirect activity of chymase that would lead to increased ECM deposition, if operative *in vivo*, is the cleavage of procollagen leading to fibril formation (Kofford *et al.*, 1997). Chymase has also been reported to have activities that indirectly can cause ECM degradation. One example is the activating cleavage of procollagenase by human chymase (Saarinen *et al.*, 1994). Another, and well-studied, example is the activation of pro-MMP-9. This activity was first demonstrated for dog chymase, which was shown to cleave pro-MMP-9 released by a mastocytoma cell line (Fang *et al.*, 1996, 1997). Thus, MCs could have the capability of both generating pro-MMP-9 and activating it without involving accessory cells. It has also been shown that mMCP-4 can activate pro-MMP-9 in a purified system (Coussens *et al.*, 1999) and strong evidence for a physiological role of chymase in pro-MMP-9 activation came when it was demonstrated that mice lacking mMCP-4 failed to activate pro-MMP-9 *in vivo* (Tchougounova *et al.*, 2005). Moreover, the lack of mMCP-4 caused exaggerated deposition of collagen in skin, supporting a role for chymase in regulating connective tissue homeostasis *in vivo* (Tchougounova *et al.*, 2005). In further support for a role of chymases in promoting MMP activities, it has been reported that chymase degrades tissue inhibitor of matrix metalloproteases-1 (TIMP-1), an inhibitor of various MMPs (Frank *et al.*, 2001), and that MC chymase can activate pro-MMP-2 (Lundequist *et al.*, 2006).

9.1.3. Lipoproteins

A number of studies from Kovanen and coworkers point to a potential role for chymase in regulating various lipoprotein components. Rat chymase (rMCP-1) was found to degrade lipoproteins containing apolipoprotein

A-I (apoA-I), apoA-II (Lee *et al.*, 2003a; Lindstedt *et al.*, 1996), and apoB (Kokkonen *et al.*, 1986). Moreover, human chymase can degrade apoA-I, -II (Lee *et al.*, 2003a) as well as apoE (Lee *et al.*, 2002b), and phospholipid transfer protein (Lee *et al.*, 2003b). The action of chymase on these apolipoproteins was shown to reduce the transfer of cholesterol from macrophages to lipoprotein particles, thereby promoting foam cell formation. It has therefore been suggested that chymase accordingly may have a role in the formation of atherosclerotic plaques (reviewed in Lee-Rueckert and Kovanen, 2006). However, this notion remains to be proven experimentally *in vivo*, for example, by using animals lacking the appropriate chymase.

9.1.4. Inflammatory substances/bioactive peptides

MC chymases have been implicated in regulating the levels of diverse inflammatory substances and bioactive peptides. One important example is the activation of the IL-1 β precursor by human chymase (Mizutani *et al.*, 1991). Other examples include the activation of big-ET-1 and -2 into ET-1 and -2 by human, rat, and monkey chymase (Kido *et al.*, 1998; Nakano *et al.*, 1997; Takai *et al.*, 1998; Wypij *et al.*, 1992); the ability of human chymase to activate connective tissue-activating peptide III (CTAP-III) into neutrophil-activating peptide 2 (NAP-2/CXCL7) (Schiemann *et al.*, 2006); the degradation of TNF by soybean trypsin inhibitor-sensitive proteases (presumably rMCP-1/5) released from rat peritoneal MCs (Alshurafa *et al.*, 2004), and the recently discovered ability of human chymase to activate the IL-18 precursor in a purified system (Omoto *et al.*, 2006). Interestingly, platelet factor 4 (PF-4), a heparin antagonist (Lane *et al.*, 1986), inhibited CTAP-III processing without being cleaved itself. Although not proven, we may speculate that PF-4 acts by binding to the SG PG to which chymase is attached (Section 8; Fig. 5B), thereby affecting chymase activity toward CTAP-III. Chymases have also been shown to degrade substance P (Caughey *et al.*, 1988), vasoactive intestinal peptide (VIP) (Caughey *et al.*, 1988), kallidin (Reilly *et al.*, 1985), bradykinin (Reilly *et al.*, 1985), and C3a (Gervasoni *et al.*, 1986; Kajita and Hugli, 1991). Hence, chymase may play proinflammatory roles by activating certain substances but may also have a dampening effect by degrading others.

9.2. Trypsase

The substrate profile for trypsin exhibits several similarities to that of chymase. First, trypsin has also been reported to act on various ECM compounds or components related to ECM turnover. For example, trypsin can degrade fibronectin (Kaminska *et al.*, 1999; Lohi *et al.*, 1992), although evidence indicates that only the monomeric form of trypsin has this capacity (Fajardo and Pejler, 2003a; Hallgren *et al.*, 2005). Further,

trypsinases can degrade denatured collagen (gelatin) (Fajardo and Pejler, 2003b) and type VI collagen (Kielty *et al.*, 1993), as well as activate pro-MMP-3, a protease that may regulate ECM composition by subsequently activating pro-MMP-1 (Lees *et al.*, 1994). Second, trypsinase has, similar to chymase, been demonstrated to degrade a number of bioactive peptides including VIP, peptide histidine-methionine (PHM), and calcitonin gene-related peptide (CGRP) (Caughey *et al.*, 1988; Tam and Caughey, 1990). Third, also trypsinase has been implicated in lipoprotein degradation, through its ability to cleave the apolipoprotein components of high-density lipoprotein (HDL) (Lee *et al.*, 2002c).

9.2.1. Fibrinogen

One of the best-characterized substrates for trypsinase is fibrinogen. Schwartz *et al.* (1985) showed that fibrinogen was degraded by human lung trypsinase and that fibrinogen thereby lost its clotting activity. Later, Stevens *et al.* expressed recombinant mMCP-7 and when examining its cleavage specificity by phage display technique, they found that mMCP-7 preferentially cleaves a sequence found in fibrinogen (Huang *et al.*, 1997; Section 7). They also reported that mMCP-7 prolonged the clotting time when added to plasma (Huang *et al.*, 1997), suggesting a role in regulation of blood coagulation. On the other hand, there is to our knowledge no report suggesting that mice deficient in mMCP-7 (the C57BL/6 strain) suffer from clotting disorders. Trypsinase may also have the potential of influencing the blood clotting system at another level, by proteolytically activating prourokinase plasminogen activator (Stack and Johnson, 1994). Noteworthy is that also chymase has been reported to have activities of relevance for regulating blood clotting/fibrinolysis, through its ability to degrade both thrombin (Pejler and Karlstrom, 1993) and plasmin (Tchougounova and Pejler, 2001).

9.2.2. Protease-activated receptor 2

Out of the implicated trypsinase substrates, protease-activated receptor 2 (PAR-2) has by far attracted the largest attention. PAR-2 belongs to a group of cell surface receptors (PAR-1–4) that all expose a “tethered” ligand which after cleavage binds to its receptor, thereby inducing signaling events that can be recorded by influx of calcium (reviewed in Ossovskaya and Bunnett, 2004). A number of *in vitro* studies have shown that trypsinase has the ability to cleave and activate PAR-2 (Corvera *et al.*, 1997; Mirza *et al.*, 1997; Molino *et al.*, 1997), thereby inducing various signaling events such as the MAP kinase pathway (Temkin *et al.*, 2002; Weidinger *et al.*, 2005a; Yoshii *et al.*, 2005), MEK (Yoshii *et al.*, 2005), phosphatidylinositol 3-kinase (Brown *et al.*, 2005), and ERK1/2 (Brown *et al.*, 2005). Lately, evidence has been obtained that PAR-2 may in fact be an *in vivo* substrate for trypsinase. Kelso *et al.* demonstrated that injection of human trypsinase into mice caused an

arthritis-like joint inflammation. Interestingly, this response was abrogated in animals lacking PAR-2 expression, thus strongly indicating tryptase to cause joint inflammation through activation of PAR-2 (Kelso *et al.*, 2005). Additional evidence for an *in vivo* relevance of tryptase-catalyzed PAR-2 cleavage comes from a study in which tryptase-induced intestinal inflammation was suppressed in PAR-2^{-/-} mice (Cenac *et al.*, 2002).

9.2.3. Other substrates

Tryptase was shown to degrade RANTES and eotaxin, thereby abolishing their chemotactic activities on eosinophils (Pang *et al.*, 2006). Further, a recent study indicated that tryptase may have a role in processing of pre-elafin into active elafin, a serpin protease inhibitor that has been linked to asthma (Guyot *et al.*, 2005).

9.2.4. Effect of the tetrameric organization on substrate cleavage

An important issue when considering possible physiological substrates for tryptase is its tetrameric organization, with the active sites facing a central pore. The narrow entrance of the central pore will most likely cause steric hindrance for entry, and it is therefore difficult to envisage how large proteins, such as fibronectin, fibrinogen, and so on (Table 6), gain access to the tryptase active sites [as discussed by Sommerhoff *et al.* (2000)]. One possibility is that a part of the substrate protein is “extracted,” by unknown mechanisms, allowing entry into the central pore and subsequent proteolysis (Sommerhoff *et al.*, 2000). Another possibility is that the tryptase tetramer may dissociate into active monomers, and that monomeric tryptase cleaves at least some of the known tryptase substrates. Indeed, it has been demonstrated that fibronectin is completely resistant to degradation by tetrameric tryptase, but is efficiently cleaved by the respective active monomers (Fajardo and Pejler, 2003a; Hallgren *et al.*, 2005). On the other hand, it is clear that fibrinogen, despite its large size, is degraded equally well by tetrameric and monomeric β -tryptase (Fajardo and Pejler, 2003a). Thus, the mode of tryptase-catalyzed degradation may vary for different protein substrates. We may also speculate that partly unfolded proteins enter the central pore of tryptase more readily than the respective folded counterpart (Fig. 6). In line with the latter notion, it has been shown that denatured collagen (gelatin) is a substrate for tetrameric β -tryptase (Fajardo and Pejler, 2003b) and mastin, a trypsin-like protease secreted by canine MCs (Raymond *et al.*, 2005), whereas intact collagen appears to be resistant (Fajardo and Pejler, 2003b). Clearly, the geometry of the central pore would not impose any constraints for cleavage of smaller peptides. Accordingly, several of the identified tryptase substrates are low molecular weight peptides such as VIP, PHM, and CGRP (Table 6).

9.3. MC-CPA

In comparison with chymase and tryptase, MC-CPA is much less characterized in terms of potential physiological substrates. It has been observed that purified human MC-CPA is able to hydrolyze Ang I to des-Leu10 Ang I (Goldstein *et al.*, 1989). Moreover, it was shown in an *ex vivo* system of peritoneal exudates cells that MC-CPA in fact can convert Ang I into Ang II (Lundequist *et al.*, 2004). Interestingly, when MC-CPA was inhibited, mMCP-4 was shown to compensate for MC-CPA in Ang I conversion. However, when both mMCP-4 and MC-CPA were inhibited, Ang II formation was completely abrogated. Further, not only were MC-CPA and mMCP-4 important players in Ang II formation, both proteases were also shown to be key players in subsequent degradation of generated Ang II (Lundequist *et al.*, 2004). MC-CPA has also been reported to cleave other bioactive peptides, including Leu5-enkephalin, neurotensin, kinstensin (Goldstein *et al.*, 1991), and snake venom sarafotoxin (Metz *et al.*, 2006; Section 10).

Similar to both tryptase and chymase, MC-CPA has been implicated in regulation of lipoproteins, by degrading the apoB component of LDL (Kokkonen *et al.*, 1986). Interestingly, it was suggested that chymase and MC-CPA cooperate in degradation of apoB.

9.4. Concerted action of the MC proteases

Considering that chymases and MC-CPA have been shown to cooperate in the cleavage of several substrates, for example Ang I and LDL, and, furthermore, their possible interdependence in terms of storage (Feyerabend *et al.*, 2005; Stevens *et al.*, 1996; Section 8), it is likely that cooperation between these enzymes is a common mechanism. In fact, we suggest that cooperation between the various MC protease classes can represent a general mechanism leading to efficient substrate degradation (Fig. 6). In this context, it is noteworthy that the MC proteases collectively, at least in rodents, encompass a panel of different proteolytic activities, mimicking those of the pancreatic digestive proteases: trypsin-like (mMCP-6/7), chymotrypsin-like (mMCP-4), elastase-like (mMCP-5), and carboxypeptidase (MC-CPA) activities. A possible scenario is that chymase first performs cleavages at various interior sites in the target protein, thus exposing new C-terminal ends which may be further processed by MC-CPA. Peptides that are released by this process could be small enough to enter the tryptase central pore and be further degraded. This, in turn, may expose new C-terminal targets for MC-CPA and can expose new sites for cleavage by chymase. In addition, the concerted action of chymase and MC-CPA on a given protein target can cause partial denaturation, with the partially denaturated protein being able to enter the

tryptase central pore and become degraded further. According to this suggested scenario, protein targets may thus become extensively degraded (Fig. 6).

10. *IN VIVO* FUNCTION

A multitude of potential biological functions have been ascribed to the various MC proteases (Tables 9 and 10). These functions have been inferred by different approaches. In many cases, the protease has been added to a cell culture system followed by recording of a response. In other approaches, purified protease has been administered into an experimental animal, followed by assessment of, for example, inflammatory parameters. MC protease functions have also been indicated by the correlation between the presence of an MC protease in a tissue and a certain disease. More recently, a number of studies have utilized more or less selective MC protease inhibitors in different *in vivo* models for disease. Finally, functions of MC proteases have in a few cases been inferred using mice lacking individual MC proteases (Table 8) or lacking potential MC protease substrates.

10.1. Chymase

10.1.1. Inflammation

A number of studies have reported various proinflammatory activities of chymases (Table 9). In one pioneering study, injection of purified human chymase into guinea pig skin caused influx of both neutrophils and eosinophils (He and Walls, 1998a). In support of a role for chymase in inflammatory cell recruitment, it has been reported that human chymase is chemotactic *in vitro* for neutrophils and monocytes (Tani *et al.*, 2000; Tomimori *et al.*, 2002a). It has also been demonstrated that intradermal injection of human chymase in mice caused inflammatory cell influx, including eosinophils (Tomimori *et al.*, 2002a,b; Watanabe *et al.*, 2002b). Moreover, it was shown that chymase inhibitors reduced eosinophilic inflammation in various models: (1) a model for atopic dermatitis based on use of the NC/Nga mouse strain (Watanabe *et al.*, 2002b), (2) a model in which contact dermatitis is induced by dinitrofluorobenzene (Tomimori *et al.*, 2002a), and (3) in ascaris extract-induced skin reactions (Tomimori *et al.*, 2002b). A further link between chymase and eosinophil function was provided by the finding that human chymase induces ERK1/2 phosphorylation (Terakawa *et al.*, 2005) and IL-8 production (Terakawa *et al.*, 2006) in eosinophil-like cells. Additional support for a role of chymase in the pathogenesis of allergic skin reactions comes from studies in which presence of chymase correlates with disease progression

TABLE 9 Biological processes in which MC chymase has been implicated

	References
Eosinophil/neutrophil/ monocyte recruitment	He and Walls, 1998a; Watanabe <i>et al.</i> , 2002a
Induction of eosinophil migration	Terakawa <i>et al.</i> , 2005
Induction of allergic skin reactions	Tomimori <i>et al.</i> , 2002b
Degradation of dermal– epidermal junctions	Briggaman <i>et al.</i> , 1984
Ischemia reperfusion injury	Abonia <i>et al.</i> , 2005
Mucosal permeability	Scudamore <i>et al.</i> , 1995, 1998
Decreased barrier function in epithelium	Ebihara <i>et al.</i> , 2005a
Induction of vascular permeability	He and Walls, 1998b
Defense toward parasites	Knight <i>et al.</i> , 2000
IL-8 induction in eosinophil- like cells	Terakawa <i>et al.</i> , 2006
Inhibition of SMC proliferation	Wang <i>et al.</i> , 2001
Reversal of VIP-induced SMC relaxation	Franconi <i>et al.</i> , 1989
Induction of SMC apoptosis	Leskinen <i>et al.</i> , 2001, 2006
Inhibition of SMC collagen synthesis	Wang <i>et al.</i> , 2001
Mitogen for fibroblasts	Algermissen <i>et al.</i> , 1999; Maruichi <i>et al.</i> , 2004; Pemberton <i>et al.</i> , 1997
Fibrosis	Jones <i>et al.</i> , 2004; Kakizoe <i>et al.</i> , 2001; Nakajima and Naya, 2002; Sakaguchi <i>et al.</i> , 2004; Satomura <i>et al.</i> , 2003; Takai <i>et al.</i> , 2003a; Tomimori <i>et al.</i> , 2003
Induction of MC degranulation	Kido <i>et al.</i> , 1985; Schick and Austen, 1986; Schick <i>et al.</i> , 1984
Secretagogue for airway gland serous cells	Sommerhoff <i>et al.</i> , 1989
Potentiation of histamine release	Hultsch <i>et al.</i> , 1988; Rubinstein <i>et al.</i> , 1990
Atopic eczema	Mao <i>et al.</i> , 1996, 1998; Tanaka <i>et al.</i> , 1999
Atopic dermatitis and nummular eczema	Jarvikallio <i>et al.</i> , 1997

(continued)

TABLE 9 (*continued*)

	References
Dermatitis	Badertscher <i>et al.</i> , 2005; Imada <i>et al.</i> , 2002; Watanabe <i>et al.</i> , 2002b
Protective role in lung function in severe asthma	Balzar <i>et al.</i> , 2005
Stimulation of IgE and IgG1 synthesis	Yoshikawa <i>et al.</i> , 2001
Peritoneal adhesion formation	Okamoto <i>et al.</i> , 2002b, 2004a,b; Soga <i>et al.</i> , 2004
Regulation of blood pressure	Li <i>et al.</i> , 2004
Involvement in aneurysm formation	Tsunemi <i>et al.</i> , 2002, 2004
Myocardial infarction	Jin <i>et al.</i> , 2003; Hoshino <i>et al.</i> , 2003
Bypass degeneration	Ortlepp <i>et al.</i> , 2001
Congenital heart disease	Hamada <i>et al.</i> , 1999
Angiogenesis	Kondo <i>et al.</i> , 2006; Muramatsu <i>et al.</i> , 2000, 2002; Russo <i>et al.</i> , 2005
Wound healing	Nishikori <i>et al.</i> , 1998
Lung adenocarcinoma	Nagata <i>et al.</i> , 2003
Aortic lipid deposition	Uehara <i>et al.</i> , 2002
Regulation of HDL levels	Fukuda <i>et al.</i> , 2002
Psoriasis	Harvima <i>et al.</i> , 1990
Chronic leg ulcers	Huttunen and Harvima, 2005
Conjunctival epithelial cell apoptosis	Ebihara <i>et al.</i> , 2005b
Scleroderma	Shiota <i>et al.</i> , 2005
Arteriovenous fistula formation	Jin <i>et al.</i> , 2005
Glycogen-induced peritonitis	de Garavilla <i>et al.</i> , 2005
Helicobacter pylori-associated gastritis	Matsuo <i>et al.</i> , 2003
Intimal hyperplasia after balloon injury	Takai <i>et al.</i> , 2003b

(Badertscher *et al.*, 2005) and from studies in which chymase polymorphisms have been correlated with atopic skin disorders (Mao *et al.*, 1996; Tanaka *et al.*, 1999; Weidinger *et al.*, 2005b). On the other hand, studies demonstrating a negative correlation between chymase presence and

TABLE 10 Biological processes in which MC tryptase has been implicated

	References
Airway hyperresponsiveness/inflammation	Berger <i>et al.</i> , 1999; Clark <i>et al.</i> , 1995; Jarjour <i>et al.</i> , 1991; Molinari <i>et al.</i> , 1996; Oh <i>et al.</i> , 2002; Rice <i>et al.</i> , 2000b; Sylvén <i>et al.</i> , 2002; Wright <i>et al.</i> , 1999
Neutrophil recruitment	Hallgren <i>et al.</i> , 2000; He <i>et al.</i> , 1997; Huang <i>et al.</i> , 1998
Eosinophil recruitment	He <i>et al.</i> , 1997
Vascular permeability increase	He and Walls, 1997
Fibrosis	Kondo <i>et al.</i> , 2001
Sepsis	Mallen-St Clair <i>et al.</i> , 2004
Ulcerative colitis	Tremaine <i>et al.</i> , 2002
Angiogenesis	Blair <i>et al.</i> , 1997; Nico <i>et al.</i> , 2004
Arthritis	Buckley <i>et al.</i> , 1997, 1998; Tetlow and Woolley, 1995
Multiple sclerosis	Pedotti <i>et al.</i> , 2003a; Rozniecki <i>et al.</i> , 1995
Sudden infant death syndrome	Buckley <i>et al.</i> , 2001
Duchenne muscular dystrophy	Nico <i>et al.</i> , 2004
Psoriasis	Namazi, 2005; Naukkarinen <i>et al.</i> , 1994
Joint inflammation	Kelso <i>et al.</i> , 2005
Intestinal inflammation	Cenac <i>et al.</i> , 2002
Atopic dermatitis	Jarvikallio <i>et al.</i> , 1997
Itching	Ui <i>et al.</i> , 2006
Tumor cell proliferation	Yoshii <i>et al.</i> , 2005
Reversal of VIP-induced SMC relaxation	Franconi <i>et al.</i> , 1989

atopic dermatitis/nummular eczema have suggested a protective role for chymase in allergic skin disorders (Jarvikallio *et al.*, 1997). Interestingly, a study demonstrated that presence of chymase in small airways showed a positive correlation with preserved lung function in asthmatic patients, suggesting a protective role for chymase in asthma (Balzar *et al.*, 2005). In another correlative study, Harvima *et al.* (1990) found that the number of chymase-positive MCs was clearly decreased in the upper dermis of psoriatic lesions. Although it is not certain if, and how, chymase influences disease progression, it is striking to note that chymase has been linked to such a variety of skin disorders. Other examples in which chymase has been implicated in skin disease include a study where scleroderma in

tight-skin mice was suppressed by a chymase inhibitor (Shiota *et al.*, 2005) and a study suggesting an association of chymase with the pathogenesis of chronic leg ulcers (Huttunen and Harvima, 2005). Chymase presence has also been correlated with other inflammatory disorders, such as *Helicobacter pylori*-associated gastritis (Matsuo *et al.*, 2003).

The mechanism by which chymase may participate in an inflammatory response is intriguing. In several early studies, it was reported that purified chymase may act in an “autocrine” fashion by inducing MC degranulation accompanied by histamine release (Schick *et al.*, 1984; Sommerhoff *et al.*, 1989). Moreover, it has been shown that chymase inhibition may dampen histamine release from MCs (Kido *et al.*, 1985). However, the mechanism by which chymase induces MC degranulation has not been outlined. Another mode of action for chymase could be to degrade epithelial cell–cell or cell–basement membrane contacts, thereby increasing capillary permeability. Indeed, several reports support such a notion. In a hallmark study, Miller *et al.* found that rMCP-2 release strongly correlated with increased mucosal permeability in rats infected with *N. brasiliensis* antigen. Moreover, infusion of purified rMCP-2 induced marked mucosal permeability (Scudamore *et al.*, 1995). Later, the same group reported that the effect of rMCP-2 on epithelial cell integrity may be due to degradation of the tight junction proteins occludin and ZO-1 (Scudamore *et al.*, 1998). The possibility that chymase may act on tight junction proteins is also supported by a study in which human chymase was demonstrated to induce decreased barrier function of human corneal epithelium, an activity that was ascribed to degradation of occludin and fibronectin (Ebihara *et al.*, 2005a). Additional support for a role of chymase in increasing capillary permeability comes from a study in which purified human chymase induced microvascular permeability in guinea pigs (He and Walls, 1998b). In a more recent report, it was shown that MCs were required for increased mucosal permeability during infection with *Trichinella spiralis*, and that this effect could be related to cleavage of occludin by mMCP-1 (McDermott *et al.*, 2003). The collected evidence from these studies strongly suggest that one major function of chymase, in an inflammatory context, may be to promote microvascular permeability by degrading components involved in epithelial cell–cell contacts.

10.1.2. Effect on SMCs

Several reports have implicated MC chymase in regulation of SMC growth and function. Kovanen and coworkers have shown that rat chymase inhibits rat aortic SMC proliferation (Wang *et al.*, 2001). The same group also found that chymase caused apoptosis of SMCs, an effect which was ascribed to cleavage of fibronectin present in the ECM deposited by the SMCs, and proapoptotic activities of released fibronectin fragments (Leskinen *et al.*, 2003). In a follow up study, it was suggested that the SMC

apoptosis induced by chymase was caused by disrupting NF- κ B-mediated survival signaling (Leskinen *et al.*, 2006). Of note is that chymase in addition has been demonstrated to be apoptotic for conjunctival epithelial cells, also through a mechanism involving degradation of fibronectin (Ebihara *et al.*, 2005b). Certainly, these findings are well in line with the multitude of evidence suggesting fibronectin to be an *in vivo* substrate for chymase. In another study, human chymase was found to inhibit the proliferative response of airway SMCs to epidermal growth factor (Lazaar *et al.*, 2002). Moreover, chymase has been shown to inhibit collagen synthesis in SMCs (Wang *et al.*, 2001) and to reverse the effect of VIP on SMC relaxation (Franconi *et al.*, 1989). The possibility that chymase may influence SMC function is also supported by the colocalization of MCs and SMCs in human airways (Matin *et al.*, 1992). Considering the strong association between the presence of MCs within the SMC layer and the pathogenesis of asthma (Brightling *et al.*, 2002), it is clear that effects of chymase on SMC function can profoundly influence the pathogenesis. However, whether chymase in this context is harmful or protective (or an “innocent bystander”) remains to be demonstrated.

10.1.3. Cardiac disorders

Given the discovery that chymase can convert Ang I into Ang II, and the huge potential for this activity in terms of cardiac function, it is not surprising that numerous studies have addressed the role of chymase in heart disease and in models thereof. Thus, correlative evidence has linked chymase to aneurysm formation in humans (Tsunemi *et al.*, 2002) and to congenital heart disease (Hamada *et al.*, 1999), and a chymase polymorphism has been associated with bypass degeneration with implications for atherosclerosis (Ortlepp *et al.*, 2001). Further, studies employing various chymase inhibitors in animal models have supported a role for chymase in formation of abdominal aortic aneurysms (Tsunemi *et al.*, 2004), myocardial infarction (Hoshino *et al.*, 2003; Jin *et al.*, 2003), and intimal hyperplasia after balloon injury (Takai *et al.*, 2003b). The potential of using chymase inhibitors in treatment of heart disease has been reviewed (Takai *et al.*, 2004a). In line with a role for chymase in heart disease, it has also been shown that chymase inhibitors may reduce aortic lipid deposition (Uehara *et al.*, 2002) and that a chymase polymorphism correlates with HDL levels (Fukuda *et al.*, 2002).

10.1.4. Fibrosis

Several lines of evidence point toward a role for chymase in fibrotic conditions. For instance, chymase presence has been correlated with fibrosis in experimental diabetes (Jones *et al.*, 2004) and in autoimmune liver fibrosis (Satomura *et al.*, 2003). Fibrosis progression in sclerotic skin of tight-skin mice has been correlated with a selective upregulation of

mMCP-4, but not mMCP-5, expression (Kakizoe *et al.*, 2001). Further, chymase inhibitors can reduce fibrosis in animal models (Sakaguchi *et al.*, 2004; Takai *et al.*, 2003a; Tomimori *et al.*, 2003). How chymase may contribute to fibrotic conditions is not known but a number of reports indicate that chymase is mitogenic for different types of fibroblasts (Algermissen *et al.*, 1999; Maruichi *et al.*, 2004; Pemberton *et al.*, 1997). On a different angle, the ability of chymase to release ECM-bound TGF- β 1 (Taipale *et al.*, 1995) and to activate latent TGF- β 1 (Lindstedt *et al.*, 2001a) may contribute to chymase-dependent fibrosis.

10.1.5. Angiogenesis

MCs have been implicated in angiogenesis and, although the exact role of MCs in this process is not clear, several reports suggest a possible involvement of chymase. For example, hamster sponge granuloma models have been used to show inhibition of angiogenesis by a chymase inhibitor (BCEAB) (Muramatsu *et al.*, 2002) and by chymostatin, a broad spectrum inhibitor of chymotrypsin-like proteases (Muramatsu *et al.*, 2000). An effect of chymase on angiogenesis could potentially affect both physiological and pathological processes. Indeed, chymase positivity has been correlated with both wound healing (Nishikori *et al.*, 1998) and tumor angiogenesis (Kondo *et al.*, 2006). Interestingly, the latter report indicated that chymase presence was associated with poor prognosis in gastric cancer. Along the same line, chymase was associated with poor prognosis in lung adenocarcinoma (Nagata *et al.*, 2003). Additional support for a role of chymase in angiogenesis has been obtained in a study in which inhibition of mMCP-5 expression, by antisense nucleotide technology, reduced granuloma-associated angiogenesis in a rat model (Russo *et al.*, 2005). Although the mechanism by which chymase could influence angiogenesis is not known, it may be speculated that chymase can promote angiogenesis by, for example, degrading ECM to facilitate vessel growth, activate proangiogenic pro-MMPs such as pro-MMP-9, or activate latent growth factors.

10.1.6. Peritoneal adhesion

Postoperative intraperitoneal adhesions are common problems during surgery. Several reports have shown that various chymase inhibitors (BCEAB, NK3201, TY-51184; Table 11) reduce the degree of peritoneal adhesion in experimental models, suggesting that chymase inhibitors have the potential of ameliorating this pathological condition (Okamoto *et al.*, 2002a,b, 2004a,b; Soga *et al.*, 2004).

10.1.7. Regulation of immunoglobulin levels

Although it is likely that chymase exerts many of its actions during the acute phase of an inflammatory response, as exemplified by effects on mucosal permeability and histamine release (see above), it cannot be

TABLE 11 Low molecular weight MC chymase inhibitors

	K_i/IC_{50}	Selectivity over cathepsin G	References
Nonpeptide			
NK3201	$IC_{50} = 2.5 \text{ nM}$	–	Takai <i>et al.</i> , 2001a
SUN-C8257	$IC_{50} = 0.31 \text{ }\mu\text{M}$	18	Fukami <i>et al.</i> , 2000
SUN-C8077	$IC_{50} = 2.5 \text{ }\mu\text{M}$	0.4	Fukami <i>et al.</i> , 2000
JNJ-10311795	$K_i = 2.3 \text{ nM}$	16.5	de Garavilla <i>et al.</i> , 2005
Cpd 41	$IC_{50} = 22 \text{ nM}$	>4500	Niwata <i>et al.</i> , 1997
BCEAB	$IC_{50} = 5.4 \text{ nM}$	ND	Takai <i>et al.</i> , 2001b
TY-51184	$IC_{50} = 37 \text{ nM}$	>2700	Takai <i>et al.</i> , 2004b
SPF-32629 A	$IC_{50} = 0.25 \text{ }\mu\text{g/ml}$	20	Shimatani <i>et al.</i> , 2006
TY-51076	$IC_{50} = 56 \text{ nM}$	>400	Masaki <i>et al.</i> , 2003
TEI-E548	$K_i = 6.2 \text{ nM}$	–	Hoshino <i>et al.</i> , 2003
Y-40613	$K_i = 22.6 \text{ nM}$	32	Imada <i>et al.</i> , 2002
Peptide based			
Z-Ile-Glu-Pro-Phe-COOMe	$K_i = 1 \text{ nM}$	–	Bastos <i>et al.</i> , 1995
Diphenyl N^α -benzocarbonyl-l-Arg-Glu-Thr-Phe ^P -phosphonate	$IC_{50} = 3.8 \text{ nM}$	2700	Raymond <i>et al.</i> , 2003

excluded that chymase also acts at other levels. For example, there is some evidence that chymase (rMCP-1) stimulates IgE and IgG synthesis *in vitro* (Yoshikawa *et al.*, 2001) and, along the same line, there is limited evidence suggesting that a chymase polymorphism correlates with IgE levels in patients suffering from atopic dermatitis (Iwanaga *et al.*, 2004). Clearly, the function of chymase in connection with inflammation may be highly complex and much future work will be needed to fully understand its role in such settings.

10.1.8. Chymase functions implicated from gene KO studies

Only in a few cases have *in vivo* functions of chymases been suggested from studies involving mice deficient in specific chymases (Table 8). In one important study, Miller and coworkers demonstrated that mice lacking mMCP-1 were less effective in clearance of *T. spiralis* than wild-type controls (Knight *et al.*, 2000). This finding is well in line with previous

studies showing that animals lacking MCs altogether have a reduced capacity in *T. spiralis* clearance (Ha *et al.*, 1983), and suggests that the effect of MCs in defense against parasites, at least partly, is dependent on their content of chymase. However, it is not clear to what extent these findings can be extrapolated to humans, considering that mMCP-1 has no obvious functional human homologue. In another study, Gurish and coworkers demonstrated that mice lacking mMCP-5 are protected in an ischemia reperfusion injury model (Abonia *et al.*, 2005). It should be noted that mMCP-5^{-/-} animals additionally lack MC-CPA (Stevens *et al.*, 1996), and it is thus uncertain whether the protection is due to lack of mMCP-5 or due to the secondary defect in MC-CPA storage.

10.1.9. Human chymase polymorphisms

Several studies have been performed in search for correlations between different pathological states and certain polymorphisms in the human chymase gene. The majority of reports focus on two polymorphisms: one located in the promotor region and one in intron 2. It should be noted that both of these polymorphisms are named in various ways in different publications. The promotor polymorphism is presented as, for example, BstXI, G3255A, -1905G/A, rs1800875, or CMA/B. The intron 2 polymorphism is usually referred to as A1625G or CMA/A. As described above, chymase polymorphisms have been correlated with atopic skin disorders, atherosclerosis, and HDL levels. In addition, chymase polymorphisms have been connected to diabetic chronic renal insufficiency (Prasad *et al.*, 2006) and an elevated risk of developing gastric ulcer and gastric cancer (Sugimoto *et al.*, 2006), the latter in specific allele combinations with an ACE polymorphism. Some data also support a possible additive effect of chymase and ACE polymorphisms on hypertrophic cardiomyopathy (Gumprecht *et al.*, 2002; Pfeufer *et al.*, 1996).

10.2. Trypsase

10.2.1. Inflammation

MC tryptase has been linked to a variety of disorders, mainly of inflammatory type (Table 10), but the *in vivo* significances of these findings have in most cases not been verified. Similarly to chymase, a number of studies have reported proinflammatory activities of tryptase. He *et al.* (1997) showed that injection of human tryptase into guinea pig skin induced influx of neutrophils and eosinophils. Using the same model, they also demonstrated that the proinflammatory action of tryptase was associated with increased microvascular leakage (He and Walls, 1997). Subsequently, two independent studies showed that recombinant mMCP-6, when injected intraperitoneally in mice, induced an influx of neutrophils (Hallgren *et al.*, 2000; Huang *et al.*, 1998). Interestingly, when mice instead

were injected with mMCP-7, an influx of eosinophils rather than neutrophils was observed (Huang *et al.*, 2001). Further, it has been reported that instillation of human recombinant β -tryptase into trachea of mice provokes a neutrophilic inflammation (Huang *et al.*, 2001). Using sheep as a model organism, it has been shown that human tryptase induces cutaneous inflammation and that a tryptase inhibitor reduces cutaneous inflammation in allergic animals (Molinari *et al.*, 1995). The mechanisms behind these proinflammatory activities are not known, but it has been suggested that tryptase can stimulate IL-8 and ICAM expression in endothelial cells (Compton *et al.*, 1998; Huang *et al.*, 1998). Similar to chymase, tryptase may induce MC degranulation, and it was reported that tryptase inhibitors can suppress MC degranulation in response to calcium ionophore or IgE receptor cross-linking (He *et al.*, 1998). In addition, tryptase has been reported to provoke degranulation also of eosinophils (Vliagoftis *et al.*, 2004).

10.2.2. Fibrosis

Similarly to chymase, tryptase has been implicated in fibrosis. Several reports have shown that tryptase is a mitogen for fibroblasts and can stimulate collagen synthesis (Abe *et al.*, 1998; Akers *et al.*, 2000; Frungieri *et al.*, 2002; Gruber *et al.*, 1997; Hartmann *et al.*, 1992; Ruoss *et al.*, 1991). Tryptase is also mitogenic for SMCs (Brown *et al.*, 1995, 2002) and myocytes (Corvera *et al.*, 1997). The possibility that tryptase accordingly may have a role in fibrotic conditions, for example in allergic asthma, has been discussed in a previous review (Levi-Schaffer and Piliponsky, 2003).

10.2.3. Airway inflammation

A number of reports suggest a role for MC tryptase in allergic airway inflammation. Strong support for this notion was given when it was demonstrated that two different tryptase inhibitors, APC-366 and BABIM, suppressed inflammatory responses and airway hyperreactivity in allergic sheep provoked by antigen (Clark *et al.*, 1995). APC-366 was later reported to have beneficial effects also in allergic pigs (Sylvén *et al.*, 2002). Further, it was shown that instillation of human tryptase into the trachea of sheep induced bronchoconstriction mediated by histamine release, and that the airway response was suppressed by APC-366 (Molinari *et al.*, 1996). Additional support for a role of MC tryptase in airway responses comes from studies in which human tryptase was found to induce airway reactivity and histamine release in isolated human (Berger *et al.*, 1999) and guinea pig (Barrios *et al.*, 1998) bronchi.

In light of the observed beneficial effects of APC-366 in animal models for allergic airway responses, it subsequently underwent a clinical trial for treatment of asthma (Krishna *et al.*, 2001). However, the outcome of this trial was relatively disappointing, with only a minor beneficial effect

observed. This may thus argue against a major role for MC tryptase in allergic airway responses. On the other hand, APC-366 is a very slow-acting inhibitor, with several hours required for optimal tryptase inhibition (Rice *et al.*, 1998) and, moreover, it is nonselective (Table 12). It can therefore not be ruled out that the poor outcome of the clinical trial is related to these properties and that the contribution of tryptase to airway responses would be more apparent if better tryptase inhibitors would be used. Indeed, more selective and potent tryptase inhibitors have been developed (Table 12), and several of these, including AMG-126737 (Wright *et al.*, 1999) and MOL-6131 (Oh *et al.*, 2002), suppress airway

TABLE 12 Low molecular weight MC tryptase inhibitors

	K_i/IC_{50}	Selectivity over trypsin	References
APC-366	$K_i = 0.33\text{--}450\text{ }\mu\text{M}$	0.5	Clark <i>et al.</i> , 1995; Hallgren <i>et al.</i> , 2001a; Rice <i>et al.</i> , 1998
Gabexate mesylate	$K_i = 3.4\text{ nM}$	500	Erbaa <i>et al.</i> , 2001
Nafamostat mesilate	$IC_{50} = 0.016\text{ nM}$	–	Sendo <i>et al.</i> , 2003
BABIM	$K_i = 5\text{ nM}$	18	Caughey <i>et al.</i> , 1993a; Katz <i>et al.</i> , 1998
RWJ-56423	$K_i = 10\text{ nM}$	0.8	Costanzo <i>et al.</i> , 2003
Cyclotheonamide E4	$IC_{50} = 5.1\text{ nM}$	1.2	Murakami <i>et al.</i> , 2002
AMG-126737	$K_i = 90\text{ nM}$	28	Wright <i>et al.</i> , 1999
MOL-6131	$K_i = 45\text{ nM}$	24	Oh <i>et al.</i> , 2002
BMS-262084	$IC_{50} = 4\text{ nM}$	18	Sutton <i>et al.</i> , 2002
BMS-354326	$IC_{50} = 1.8\text{ nM}$	5,600	Bisacchi <i>et al.</i> , 2004
BMS-363131	$IC_{50} < 1.7\text{ nM}$	>3,000	Slusarchyk <i>et al.</i> , 2002
Compound 27	$K_i = 0.07\text{ nM}$	560,000	Rice <i>et al.</i> , 2000a
APC-2059	$K_i = 0.1\text{ nM}$	150,000	Rice <i>et al.</i> , 2000b
Diketopiperazine- based	$K_i = 10\text{--}2400\text{ nM}$	–	Schaschke <i>et al.</i> , 2002
Compound 11b	$K_i < 0.01\text{ nM}$	650,000	Burgess <i>et al.</i> , 1999
Benzamidine based	$K_i = 1\text{ nM}$	46,000	Dener <i>et al.</i> , 2001

responses in animal models. In many cases, a high selectivity for MC tryptase is obtained by developing dibasic inhibitors, that is, inhibitors that simultaneously interact with two of the active sites in the tryptase tetramer. Examples of such inhibitors include AMG-126737 (Wright *et al.*, 1999), MOL-6131 (Oh *et al.*, 2002), and APC-2059 (Rice *et al.*, 2000b; Tremaine *et al.*, 2002).

10.2.4. Angiogenesis

Tryptase has been shown to promote capillary growth and to be a mitogen for endothelial cells, indicating a potential role for tryptase in angiogenesis (Blair *et al.*, 1997). This is supported by the ability of tryptase to induce proangiogenic, but not antiangiogenic cytokines (Somasundaram *et al.*, 2005). Moreover, a correlation between tryptase-positive neurons and extent of angiogenesis has been reported in a mouse model for Duchenne muscular dystrophy (Nico *et al.*, 2004).

10.2.5. Correlative studies

Correlative studies have indicated potential links between MC tryptase and a variety of disorders, including multiple sclerosis (Pedotti *et al.*, 2003b; Rozniecki *et al.*, 1995), arthritis (Tetlow and Woolley, 1995), sudden infant death syndrome (Buckley *et al.*, 2001), psoriasis (Naukkarinen *et al.*, 1994), fibrosis (Kondo *et al.*, 2001), and atopic dermatitis (Jarvikallio *et al.*, 1997).

10.3. MC-CPA

Until recently, very little information regarding the *in vivo* function of MC-CPA has been available. Few reports have investigated the effect of MC-CPA in cell culture-based systems, although one report suggests that MC-CPA may be a mitogen for human dermal fibroblasts (Abe *et al.*, 2000). Importantly, a mouse strain lacking MC-CPA expression has been generated (Feyerabend *et al.*, 2005), an accomplishment that will greatly facilitate investigations of its *in vivo* function. In the initial report describing the MC-CPA^{-/-} strain, it was demonstrated that MC-CPA did not contribute to passive cutaneous anaphylaxis. Interestingly, secretory granules of MC-CPA^{-/-} MCs showed defective staining with cationic dyes, indicating alterations in storage of PGs, with the latter notion also being supported by an increased solubility of ³⁵S-sulfate labeled PGs in MC-CPA^{-/-} peritoneal MCs (Feyerabend *et al.*, 2005). In an alternative approach, strong evidence for an *in vivo* relevance of MC-CPA-catalyzed degradation of snake venom sarafotoxin was obtained. Using the W/W^v strain, Metz *et al.* demonstrated elegantly that MCs have a key role in defense toward sarafotoxin-mediated lethality. Reconstitution of MC-deficient mice with wild-type MCs restored protection toward sarafotoxin

while, strikingly, reconstitution with MCs in which MC-CPA expression was suppressed by RNA interference failed to give protection (Metz *et al.*, 2006). Clearly, these recent and important findings have put MC-CPA on the map of innate immunity, and we anticipate that new and important functions of MC-CPA will be revealed in the near future.

11. MC PROTEASE INHIBITORS

11.1. Synthetic inhibitors

As a consequence of the multitude of pathological settings in which MC proteases have been implicated, much effort has been invested in generating potent and selective MC protease inhibitors. Some of these inhibitors are peptide based, as exemplified by the potent chymase inhibitor Z-Ile-Glu-Pro-Phe-COOMe (Bastos *et al.*, 1995). However, due to a requirement for orally available compounds with favorable pharmacokinetics, recently developed MC protease inhibitors are mainly nonpeptide based and of low molecular weight (Tables 11 and 12). Such inhibitors have been assessed in a variety of animal models for disease and have in many cases proved to be effective in ameliorating the respective condition. Thus, MC proteases are now considered as promising drug targets. However, the recent progress within this area should be taken with some caution. First, several of the used inhibitors show a moderate degree of selectivity for the respective MC protease or, alternatively, the selectivity has to our knowledge not been characterized (Tables 11 and 12). For example, two of the most frequently used MC protease inhibitors, APC-366 and SUN-C8077, do not show any selectivity toward trypsin and cathepsin G, respectively (Tables 11 and 12). Thus, the reported effect in a disease model may not only be a consequence of inhibiting the target MC protease but also to inhibition of related proteases of non-MC origin. Second, most of the inhibitors have been developed for their ability to inhibit human proteases, that is, human chymase or β -tryptase, but are used for targeting an animal counterpart protease. Therefore, it cannot be ruled out that the potency for inhibition of the relevant animal MC protease may differ considerably. For example, we found that APC-366, a potent inhibitor of human β -tryptase (Table 12), was a very weak inhibitor ($IC_{50} \approx 1$ mM) of mMCP-6 (Hallgren *et al.*, 2001a). Hence, in order to fully evaluate the relevance of inhibiting MC proteases in different disease models, it would be useful to develop and assess selective inhibitors of the relevant endogenous MC protease. Nevertheless, studies using various MC protease inhibitors have led to the implication of MC proteases in several novel settings (Section 10).

11.2. *In vivo* regulation

Most likely, the MC proteases are under strict control after their release from degranulated MCs in order to prevent the unleashing of excessive protease activity that could cause damage to the host tissue. However, the *in vivo* mechanisms that control MC protease activities have not been extensively studied. For example, with the exception of the identification of tryptase monomer in complex with the intracellular protease inhibitor PI6 (Strik *et al.*, 2004), *in vivo*-generated complexes of MC proteases and endogenous protease inhibitors have not been isolated to date.

Several studies have shown that α_1 -AC, an endogenous protease inhibitor of serpin type, is an efficient chymase inhibitor (Pejler, 1996; Pejler and Berg, 1995; Schechter *et al.*, 1989). Since skin MCs, at least under certain pathological conditions, are positive for α_1 -AC (Huttunen and Harvima, 2005), it is likely that α_1 -AC indeed is an endogenous inhibitor of chymase. In support for a role of serpins in chymase inhibition, Pemberton *et al.* (2006) showed that mMCP-1, but not mMCP-2, was recovered in complexes with serpins after the protease had been injected into the circulation of mice. Most likely, since α_1 -AC is a plasma protein, chymase inhibition by α_1 -AC would predominantly occur during inflammatory conditions in which α_1 -AC has been extravasated into the tissue. Further, it is of note that chymase, when present in its physiological form, that is, in complex with heparin PG (Section 8), is largely resistant to inhibition by α_1 -AC (Pejler and Berg, 1995). Also other plasma protease inhibitors, for example, α_2 -macroglobulin (Walter *et al.*, 1999) and α_1 -PI (Schechter *et al.*, 1989), have been reported to inhibit chymases, although it has been demonstrated that chymase shows resistance also to these inhibitors when present in complex with heparin PG (Pejler and Berg, 1995). A likely explanation for the protective effect of heparin PG toward plasma protease inhibitors is that these inhibitors do not show affinity for heparin. As a consequence, they may be repelled by the SG PG to which chymase is attached, according to the scheme outlined in Fig. 5B. Another chymase inhibitor of potential physiological significance is secretory leukocyte proteinase inhibitor (SLPI), a protease inhibitor found in mucus secretions. Interestingly, chymase inhibition by SLPI was more effective in the presence of heparin PG, thus in contrast to inhibition by plasma protease inhibitors (Walter *et al.*, 1996). A likely explanation for this finding is that SLPI is a GAG-binding protein (Fath *et al.*, 1998) and would thus be attracted by the SG PG to which chymase is bound (Fig. 5B). Another potential endogenous chymase inhibitor is squamous cell carcinoma antigen 2 (Schick *et al.*, 1997), a serpin that serves as a serological marker for advanced squamous cell tumors. In this context, it is interesting to note that chymase (mMCP-4) has been implicated in squamous carcinoma through its abilities to activate pro-MMP-9 and to induce hyperplastic skin to become angiogenic (Coussens *et al.*, 1999).

The *in vivo* regulation of tryptase is an intriguing issue. To date, no endogenous inhibitor capable of inhibiting tetrameric tryptase has been identified. Therefore, the most likely scenario is that tryptase regulation *in vivo* occurs by other means. A plausible mechanism is that the tryptase tetramer, after exocytosis, dissociates into monomers that rapidly lose activity. This notion is largely based on the known instability of tryptase tetramers in conditions that prevail after its release into the extracellular space, that is, neutral pH and body temperature (Schwartz and Bradford, 1986). However, it should be noted that tryptase monomers generated during such conditions may be active, at least for a short time (Fajardo and Pejler, 2003a). Hence, the active tryptase monomers may become targets for endogenous protease inhibitors that fail to inhibit tetrameric tryptase. In an alternative scenario, released tryptase tetramers may be confronted to various polycationic proteins, for example, neutrophil-derived lactoferrin and myeloperoxidase. Such polycationic compounds will compete with tryptase for binding to tryptase-stabilizing GAGs, leading to tryptase dissociation and inactivation (Cregar *et al.*, 1999; Elrod *et al.*, 1997; Hallgren *et al.*, 2001a).

Very little is known about the control of MC-CPA activity. In one study, Uratani *et al.* (2000) reported that an MC-CPA inhibitory protein-denoted latexin is present intracellularly within rat peritoneal MCs, in granules distinct from those containing MC-CPA and histamine. Latexin was not released following exposure of the MCs to either calcium ionophore or compound 48/80, and it thus remains to be shown whether latexin plays a role in the extracellular control of MC-CPA activity. Another endogenous inhibitor of MC-CPA, denoted tissue carboxypeptidase inhibitor (TCI), was characterized by Normant *et al.* (1995). TCI is expressed in various MC-containing tissues, for example, lung and intestine, and may thus have the potential to regulate MC-CPA *in vivo*.

Clearly, the *in vivo* mechanisms that regulate MC proteases are likely to be of great importance in fine-tuning various MC protease-dependent conditions. Additional insight into this issue is therefore welcome and will hopefully be generated in future research.

12. SUMMARY AND FUTURE PERSPECTIVES

As is evident from this chapter, past research on various MC proteases has gathered large amounts of information regarding their structure, processing mechanisms, expression profiles, cleavage specificity, and potential *in vivo* substrates and functions. However, the critical questions remain in large parts unanswered, that is, what is their true biological function? Since the MC proteases are released during various inflammatory conditions, it is likely that they at least under certain circumstances

may contribute to the pathological condition in which the MC participates, that is, being harmful. On the other hand, it cannot be ruled out that the MC proteases in some cases may actually have a role in dampening the inflammatory response by, for example, degrading inflammatory substances. In fact, we may even envisage a scenario where they may play different roles during various phases of a given response, for example aiding in recruitment of inflammatory cells in the early inflammatory phase and by limiting a too extensive response by degrading inflammatory molecules in subsequent stages of the reaction. Moreover, we cannot rule out that MC proteases also can contribute to tissue homeostasis under normal conditions, that is, in the absence of MC degranulation. To answer these critical questions, it will be imperative to genetically target the various MC protease genes and assess the outcome in different disease models, in particular those in which MCs have been shown to contribute to the pathogenesis. We are now at a stage where KO strains for many of the major MC proteases are available, that is, animals lacking mMCP-1, -4, -5, and MC-CPA (Table 8). With help of these KO strains, and by assessing animals with combined deletions of the respective MC proteases, we anticipate that the next few years will reveal a number of *in vivo* settings in which MC proteases play a role. The next challenge will then be to determine the exact mechanism behind a given finding, with the key question being to determine which substrate that is the *in vivo* target for the protease.

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REFERENCES

- Abe, M., Kurosawa, M., Ishikawa, O., Miyachi, Y., and Kido, H. (1998). Mast cell tryptase stimulates both human dermal fibroblast proliferation and type I collagen production. *Clin. Exp. Allergy* **28**, 1509–1517.
- Abe, M., Kurosawa, M., Ishikawa, O., and Miyachi, Y. (2000). Effect of mast cell-derived mediators and mast cell-related neutral proteases on human dermal fibroblast proliferation and type I collagen production. *J. Allergy Clin. Immunol.* **106**, S78–S84.
- Abonia, J. P., Friend, D. S., Austen, W. G., Jr., Moore, F. D., Jr., Carroll, M. C., Chan, R., Afnan, J., Humbles, A., Gerard, C., Knight, P., Kanaoka, Y., Yasuda, S., *et al.* (2005). Mast cell protease 5 mediates ischemia-reperfusion injury of mouse skeletal muscle. *J. Immunol.* **174**, 7285–7291.
- Åbrink, M., Grujic, M., and Pejler, G. (2004). Serglycin is essential for maturation of mast cell secretory granule. *J. Biol. Chem.* **279**, 40897–40905.

- Addington, A. K., and Johnson, D. A. (1996). Inactivation of human lung tryptase: Evidence for a re-activatable tetrameric intermediate and active monomers. *Biochemistry* **35**, 13511–13518.
- Akers, I. A., Parsons, M., Hill, M. R., Hollenberg, M. D., Sanjar, S., Laurent, G. J., and McAnulty, R. J. (2000). Mast cell tryptase stimulates human lung fibroblast proliferation via protease-activated receptor-2. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **278**, L193–L201.
- Algermissen, B., Hermes, B., Feldmann-Boeddeker, I., Bauer, F., and Henz, B. M. (1999). Mast cell chymase and tryptase during tissue turnover: Analysis on *in vitro* mitogenesis of fibroblasts and keratinocytes and alterations in cutaneous scars. *Exp. Dermatol.* **8**, 193–198.
- Alshurafa, H. N., Stenton, G. R., Wallace, J. L., Hollenberg, M. D., Befus, A. D., and Vliagoftis, H. (2004). A protease activated receptor-2 (PAR-2) activating peptide, tc-LIGRLO-NH₂, induces protease release from mast cells: Role in TNF degradation. *BMC Pharmacol.* **4**, 12.
- Alter, S. C., Metcalfe, D. D., Bradford, T. R., and Schwartz, L. B. (1987). Regulation of human mast cell tryptase. Effects of enzyme concentration, ionic strength and the structure and negative charge density of polysaccharides. *Biochem. J.* **248**, 821–827.
- Badertscher, K., Bronnimann, M., Karlen, S., Braathen, L. R., and Yawalkar, N. (2005). Mast cell chymase is increased in chronic atopic dermatitis but not in psoriasis. *Arch. Dermatol. Res.* **296**, 503–506.
- Baglin, T. P., Carrell, R. W., Church, F. C., Esmon, C. T., and Huntington, J. A. (2002). Crystal structures of native and thrombin-complexed heparin cofactor II reveal a multistep allosteric mechanism. *Proc. Natl. Acad. Sci. USA* **99**, 11079–11084.
- Balzar, S., Chu, H. W., Strand, M., and Wenzel, S. (2005). Relationship of small airway chymase-positive mast cells and lung function in severe asthma. *Am. J. Respir. Crit. Care Med.* **171**, 431–439.
- Banovac, K., and De Forteza, R. (1992). The effect of mast cell chymase on extracellular matrix: Studies in autoimmune thyroiditis and in cultured thyroid cells. *Int. Arch. Allergy Immunol.* **99**, 141–149.
- Banovac, K., Banovac, F., Yang, J., and Koren, E. (1993). Interaction of osteoblasts with extracellular matrix: Effect of mast cell chymase. *Proc. Soc. Exp. Biol. Med.* **203**, 221–235.
- Baram, D., Vaday, G. G., Salamon, P., Drucker, I., Hershkovich, R., and Mekori, Y. A. (2001). Human mast cells release metalloproteinase-9 on contact with activated T cells: Juxta-crine regulation by TNF- α . *J. Immunol.* **167**, 4008–4016.
- Barrios, V. E., Middleton, S. C., Kashem, M. A., Havill, A. M., Toombs, C. F., and Wright, C. D. (1998). Tryptase mediates hyperresponsiveness in isolated guinea pig bronchi. *Life Sci.* **63**, 2295–2303.
- Bastos, M., Maeji, N. J., and Abeles, R. H. (1995). Inhibitors of human heart chymase based on a peptide library. *Proc. Natl. Acad. Sci. USA* **92**, 6738–6742.
- Berger, P., Compton, S. J., Molimard, M., Walls, A. F., N'Guyen, C., Marthan, R., and Tunon-De-Lara, J. M. (1999). Mast cell tryptase as a mediator of hyperresponsiveness in human isolated bronchi. *Clin. Exp. Allergy* **29**, 804–812.
- Bisacchi, G. S., Slusarchyk, W. A., Bolton, S. A., Hartl, K. S., Jacobs, G., Mathur, A., Meng, W., Ogletree, M. L., Pi, Z., Sutton, J. C., Treuner, U., Zahler, R., *et al.* (2004). Synthesis of potent and highly selective nonguanidine azetidinone inhibitors of human tryptase. *Bioorg. Med. Chem. Lett.* **14**, 2227–2231.
- Blair, R. J., Meng, H., Marchese, M. J., Ren, S., Schwartz, L. B., Tonnesen, M. G., and Gruber, B. L. (1997). Human mast cells stimulate vascular tube formation. Tryptase is a novel, potent angiogenic factor. *J. Clin. Invest.* **99**, 2691–2700.
- Blank, U., and Rivera, J. (2004). The ins and outs of IgE-dependent mast-cell exocytosis. *Trends Immunol.* **25**, 266–273.
- Braga, T., Grujic, M., Lukinius, A., Hellman, L., Abrink, M., and Pejler, G. (2007). Serglycin proteoglycan is required for secretory granule integrity in mucosal mast cells. *Biochem. J.* **403**, 49–57.

- Briggaman, R. A., Schechter, N. M., Fraki, J., and Lazarus, G. S. (1984). Degradation of the epidermal-dermal junction by proteolytic enzymes from human skin and human polymorphonuclear leukocytes. *J. Exp. Med.* **160**, 1027–1042.
- Brightling, C. E., Bradding, P., Symon, F. A., Holgate, S. T., Wardlaw, A. J., and Pavord, I. D. (2002). Mast-cell infiltration of airway smooth muscle in asthma. *N. Engl. J. Med.* **346**, 1699–1705.
- Brown, J. K., Tyler, C. L., Jones, C. A., Ruoss, S. J., Hartmann, T., and Caughey, G. H. (1995). Tryptase, the dominant secretory granular protein in human mast cells, is a potent mitogen for cultured dog tracheal smooth muscle cells. *Am. J. Respir. Cell Mol. Biol.* **13**, 227–236.
- Brown, J. K., Jones, C. A., Rooney, L. A., Caughey, G. H., and Hall, I. P. (2002). Tryptase's potent mitogenic effects in human airway smooth muscle cells are via nonproteolytic actions. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **282**, L197–L206.
- Brown, J. K., Knight, P. A., Wright, S. H., Thornton, E. M., and Miller, H. R. (2003). Constitutive secretion of the granule chymase mouse mast cell protease-1 and the chemokine, CCL2, by mucosal mast cell homologues. *Clin. Exp. Allergy* **33**, 132–146.
- Brown, J. K., Hollenberg, M. D., and Jones, C. A. (2005). Tryptase activates phosphatidylinositol 3-kinases proteolytically independent from proteinase activated receptor-2 in cultured dog airway smooth muscle cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **290**, L259–L269.
- Buckley, M. G., Walters, C., Wong, W. M., Cawley, M. I., Ren, S., Schwartz, L. B., and Walls, A. F. (1997). Mast cell activation in arthritis: Detection of alpha- and beta-tryptase, histamine and eosinophil cationic protein in synovial fluid. *Clin. Sci. (Lond.)* **93**, 363–370.
- Buckley, M. G., Gallagher, P. J., and Walls, A. F. (1998). Mast cell subpopulations in the synovial tissue of patients with osteoarthritis: Selective increase in numbers of tryptase-positive, chymase-negative mast cells. *J. Pathol.* **186**, 67–74.
- Buckley, M. G., Variend, S., and Walls, A. F. (2001). Elevated serum concentrations of beta-tryptase, but not alpha-tryptase, in Sudden Infant Death Syndrome (SIDS). An investigation of anaphylactic mechanisms. *Clin. Exp. Allergy* **31**, 1696–1704.
- Burgess, L. E., Newhouse, B. J., Ibrahim, P., Rizzi, J., Kashem, M. A., Hartman, A., Brandhuber, B. J., Wright, C. D., Thomson, D. S., Vigers, G. P., and Koch, K. (1999). Potent selective nonpeptidic inhibitors of human lung tryptase. *Proc. Natl. Acad. Sci. USA* **96**, 8348–8352.
- Caughey, G. (2001). New developments in the genetics and activation of mast cell proteases. *Mol. Immunol.* **38**, 1353–1357.
- Caughey, G. H., Leidig, F., Viro, N. F., and Nadel, J. A. (1988). Substance P and vasoactive intestinal peptide degradation by mast cell tryptase and chymase. *J. Pharmacol. Exp. Ther.* **244**, 133–137.
- Caughey, G. H., Raymond, W. W., and Vanderslice, P. (1990). Dog mast cell chymase: Molecular cloning and characterization. *Biochemistry* **29**, 5166–5171.
- Caughey, G. H., Zerweck, E. H., and Vanderslice, P. (1991). Structure, chromosomal assignment, and deduced amino acid sequence of a human gene for mast cell chymase. *J. Biol. Chem.* **266**, 12956–12963.
- Caughey, G. H., Raymond, W. W., Bacci, E., Lombardy, R. J., and Tidwell, R. R. (1993a). Bis (5- amidino-2-benzimidazolyl)methane and related amidines are potent, reversible inhibitors of mast cell tryptases. *J. Pharmacol. Exp. Ther.* **264**, 676–682.
- Caughey, G. H., Schaumberg, T. H., Zerweck, E. H., Butterfield, J. H., Hanson, R. D., Silverman, G. A., and Ley, T. J. (1993b). The human mast cell chymase gene (CMA1): Mapping to the cathepsin G/granzyme gene cluster and lineage-restricted expression. *Genomics* **15**, 614–620.
- Caughey, G. H., Raymond, W. W., Blount, J. L., Hau, L. W., Pallaoro, M., Wolters, P. J., and Vergheze, G. M. (2000a). Characterization of human gamma-tryptases, novel members of

- the chromosome 16p mast cell tryptase and prostasin gene families. *J. Immunol.* **164**, 6566–6575.
- Caughey, G. H., Raymond, W. W., and Wolters, P. J. (2000b). Angiotensin II generation by mast cell alpha- and beta-chymases. *Biochim. Biophys. Acta* **1480**, 245–257.
- Cavalcante, M. C., de Andrade, L. R., Du Bocage Santos-Pinto, C., Straus, A. H., Takahashi, H. K., Allodi, S., and Pavao, M. S. (2002). Colocalization of heparin and histamine in the intracellular granules of test cells from the invertebrate *Styela plicata* (Chordata-Tunicata). *J. Struct. Biol.* **137**, 313–321.
- Cenac, N., Coelho, A. M., Nguyen, C., Compton, S., Andrade-Gordon, P., MacNaughton, W. K., Wallace, J. L., Hollenberg, M. D., Bunnett, N. W., Garcia-Villar, R., Bueno, L., Vergnolle, N., *et al.* (2002). Induction of intestinal inflammation in mouse by activation of proteinase-activated receptor-2. *Am. J. Pathol.* **161**, 1903–1915.
- Chandrasekharan, U. M., Sanker, S., Glynias, M. J., Karnik, S. S., and Husain, A. (1996). Angiotensin II-forming activity in a reconstructed ancestral chymase. *Science* **271**, 502–505.
- Chen, L. Y., Li, P., He, Q., Jiang, L. Q., Cui, C. J., Xu, L., and Liu, L. S. (2002). Transgenic study of the function of chymase in heart remodeling. *J. Hypertens.* **20**, 2047–2055.
- Chen, R., Ning, G., Zhao, M. L., Fleming, M. G., Diaz, L. A., Werb, Z., and Liu, Z. (2001). Mast cells play a key role in neutrophil recruitment in experimental bullous pemphigoid. *J. Clin. Invest.* **108**, 1151–1158.
- Chen, Y., Shiota, M., Ohuchi, M., Towatari, T., Tashiro, J., Murakami, M., Yano, M., Yang, B., and Kido, H. (2000). Mast cell tryptase from pig lungs triggers infection by pneumotropic Sendai and influenza A viruses. Purification and characterization. *Eur. J. Biochem.* **267**, 3189–3197.
- Chu, W., Johnson, D. A., and Musich, P. R. (1992). Molecular cloning and characterization of mouse mast cell chymases. *Biochim. Biophys. Acta* **1121**, 83–87.
- Clark, J. M., Abraham, W. M., Fishman, C. E., Forteza, R., Ahmed, A., Cortes, A., Warne, R. L., Moore, W. R., and Tanaka, R. D. (1995). Tryptase inhibitors block allergen-induced airway and inflammatory responses in allergic sheep. *Am. J. Respir. Crit. Care Med.* **152**, 2076–2083.
- Cole, K. R., Kumar, S., Trong, H. L., Woodbury, R. G., Walsh, K. A., and Neurath, H. (1991). Rat mast cell carboxypeptidase: Amino acid sequence and evidence of enzyme activity within mast cell granules. *Biochemistry* **30**, 648–655.
- Compton, S. J., Cairns, J. A., Holgate, S. T., and Walls, A. F. (1998). The role of mast cell tryptase in regulating endothelial cell proliferation, cytokine release, and adhesion molecule expression: Tryptase induces expression of mRNA for IL-1 beta and IL-8 and stimulates the selective release of IL-8 from human umbilical vein endothelial cells. *J. Immunol.* **161**, 1939–1946.
- Corvera, C. U., Dery, O., McConalogue, K., Bohm, S. K., Khitin, L. M., Caughey, G. H., Payan, D. G., and Bunnett, N. W. (1997). Mast cell tryptase regulates rat colonic myocytes through proteinase-activated receptor 2. *J. Clin. Invest.* **100**, 1383–1393.
- Costanzo, M. J., Yabut, S. C., Almond, H. R., Jr., Andrade-Gordon, P., Corcoran, T. W., De Garavilla, L., Kauffman, J. A., Abraham, W. M., Recacha, R., Chattopadhyay, D., and Maryanoff, B. E. (2003). Potent, small-molecule inhibitors of human mast cell tryptase. Antiasthmatic action of a dipeptide-based transition-state analogue containing a benzothiazole ketone. *J. Med. Chem.* **46**, 3865–3876.
- Coussens, L. M., Raymond, W. W., Bergers, G., Laig-Webster, M., Behrendtsen, O., Werb, Z., Caughey, G. H., and Hanahan, D. (1999). Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev.* **13**, 1382–1397.
- Cregar, L., Elrod, K. C., Putnam, D., and Moore, W. R. (1999). Neutrophil myeloperoxidase is a potent and selective inhibitor of mast cell tryptase. *Arch. Biochem. Biophys.* **366**, 125–130.

- de Garavilla, L., Greco, M. N., Sukumar, N., Chen, Z. W., Pineda, A. O., Mathews, F. S., Di Cera, E., Giardino, E. C., Wells, G. I., Haertlein, B. J., Kauffman, J. A., Corcoran, T. W., *et al.* (2005). A novel, potent dual inhibitor of the leukocyte proteases cathepsin G and chymase: Molecular mechanisms and anti-inflammatory activity *in vivo*. *J. Biol. Chem.* **280**, 18001–18007.
- de Paulis, A., Minopoli, G., Dal Piaz, F., Pucci, P., Russo, T., and Marone, G. (1999). Novel autocrine and paracrine loops of the stem cell factor/chymase network. *Int. Arch. Allergy Immunol.* **118**, 422–425.
- Dell'Italia, L. J., and Husain, A. (2002). Dissecting the role of chymase in angiotensin II formation and heart and blood vessel diseases. *Curr. Opin. Cardiol.* **17**, 374–379.
- Dener, J. M., Rice, K. D., Newcomb, W. S., Wang, V. R., Young, W. B., Gangloff, A. R., Kuo, E. Y., Cregar, L., Putnam, D., and Wong, M. (2001). Dibasic inhibitors of human mast cell tryptase. Part 3: Identification of a series of potent and selective inhibitors containing the benzamidine functionality. *Bioorg. Med. Chem. Lett.* **11**, 1629–1633.
- Dikov, M. M., Springman, E. B., Yeola, S., and Serafin, W. E. (1994). Processing of procarboxypeptidase A and other zymogens in murine mast cells. *J. Biol. Chem.* **269**, 25897–25904.
- Dragonetti, A., Baldassarre, M., Castino, R., Demoz, M., Luini, A., Buccione, R., and Isidoro, C. (2000). The lysosomal protease cathepsin D is efficiently sorted to and secreted from regulated secretory compartments in the rat basophilic/mast cell line RBL. *J. Cell Sci.* **113**(Pt. 18), 3289–3298.
- Ebihara, N., Funaki, T., Murakami, A., Takai, S., and Miyazaki, M. (2005a). Mast cell chymase decreases the barrier function and inhibits the migration of corneal epithelial cells. *Curr. Eye Res.* **30**, 1061–1069.
- Ebihara, N., Takai, S., Miyazaki, M., and Murakami, A. (2005b). Mast cell chymase induces conjunctival epithelial cell apoptosis by a mechanism involving degradation of fibronectin. *Curr. Eye Res.* **30**, 429–435.
- Echtenacher, B., Mannel, D. N., and Hultner, L. (1996). Critical protective role of mast cells in a model of acute septic peritonitis. *Nature* **381**, 75–77.
- Eklund, K. K., Ghildyal, N., Austen, K. F., and Stevens, R. L. (1993). Induction by IL-9 and suppression by IL-3 and IL-4 of the levels of chromosome 14-derived transcripts that encode late-expressed mouse mast cell proteases. *J. Immunol.* **151**, 4266–4273.
- Eklund, K. K., Humphries, D. E., Xia, Z., Ghildyal, N., Friend, D. S., Gross, V., and Stevens, R. L. (1997). Glucocorticoids inhibit the cytokine-induced proliferation of mast cells, the high affinity IgE receptor-mediated expression of TNF-alpha, and the IL-10-induced expression of chymases. *J. Immunol.* **158**, 4373–4380.
- Elrod, K. C., Moore, W. R., Abraham, W. M., and Tanaka, R. D. (1997). Lactoferrin, a potent tryptase inhibitor, abolishes late-phase airway responses in allergic sheep. *Am. J. Respir. Crit. Care Med.* **156**, 375–381.
- Enerback, L., Kolset, S. O., Kusche, M., Hjerpe, A., and Lindahl, U. (1985). Glycosaminoglycans in rat mucosal mast cells. *Biochem. J.* **227**, 661–668.
- Erbaa, F., Fiorucci, L., Pascarella, S., Menegatti, E., Ascenzi, P., and Ascoli, F. (2001). Selective inhibition of human mast cell tryptase by gabexate mesylate, an antiproteinase drug. *Biochem. Pharmacol.* **61**, 271–276.
- Everitt, M. T., and Neurath, H. (1980). Rat peritoneal mast cell carboxypeptidase: Localization, purification, and enzymatic properties. *FEBS Lett.* **110**, 292–296.
- Fajardo, I., and Pejler, G. (2003a). Formation of active monomers from tetrameric human beta-tryptase. *Biochem. J.* **369**, 603–610.
- Fajardo, I., and Pejler, G. (2003b). Human mast cell beta-tryptase is a gelatinase. *J. Immunol.* **171**, 1493–1499.
- Fang, K. C., Raymond, W. W., Lazarus, S. C., and Caughey, G. H. (1996). Dog mastocytoma cells secrete a 92-kD gelatinase activated extracellularly by mast cell chymase. *J. Clin. Invest.* **97**, 1589–1596.

- Fang, K. C., Raymond, W. W., Blount, J. L., and Caughey, G. H. (1997). Dog mast cell alpha-chymase activates progelatinase B by cleaving the Phe88-Gln89 and Phe91-Glu92 bonds of the catalytic domain. *J. Biol. Chem.* **272**, 25628–25635.
- Fath, M. A., Wu, X., Hileman, R. E., Linhardt, R. J., Kashem, M. A., Nelson, R. M., Wright, C. D., and Abraham, W. M. (1998). Interaction of secretory leukocyte protease inhibitor with heparin inhibits proteases involved in asthma. *J. Biol. Chem.* **273**, 13563–13569.
- Faurschou, M., and Borregaard, N. (2003). Neutrophil granules and secretory vesicles in inflammation. *Microbes Infect.* **5**, 1317–1327.
- Feyerabend, T. B., Hausser, H., Tietz, A., Blum, C., Hellman, L., Straus, A. H., Takahashi, H. K., Morgan, E. S., Dvorak, A. M., Fehling, H. J., and Rodewald, H. R. (2005). Loss of histochemical identity in mast cells lacking carboxypeptidase A. *Mol. Cell. Biol.* **25**, 6199–6210.
- Forsberg, E., and Kjellen, L. (2001). Heparan sulfate: Lessons from knockout mice. *J. Clin. Invest.* **108**, 175–180.
- Forsberg, E., Pejler, G., Ringvall, M., Lunderius, C., Tomasini-Johansson, B., Kusche-Gullberg, M., Eriksson, I., Ledin, J., Hellman, L., and Kjellen, L. (1999). Abnormal mast cells in mice deficient in a heparin-synthesizing enzyme [see comments]. *Nature* **400**, 773–776.
- Franconi, G. M., Graf, P. D., Lazarus, S. C., Nadel, J. A., and Caughey, G. H. (1989). Mast cell tryptase and chymase reverse airway smooth muscle relaxation induced by vasoactive intestinal peptide in the ferret. *J. Pharmacol. Exp. Ther.* **248**, 947–951.
- Frank, B. T., Rossall, J. C., Caughey, G. H., and Fang, K. C. (2001). Mast cell tissue inhibitor of metalloproteinase-1 is cleaved and inactivated extracellularly by alpha-chymase. *J. Immunol.* **166**, 2783–2792.
- Frungeri, M. B., Weidinger, S., Meineke, V., Kohn, F. M., and Mayerhofer, A. (2002). Proliferative action of mast-cell tryptase is mediated by PAR2, COX2, prostaglandins, and PPARGamma: Possible relevance to human fibrotic disorders. *Proc. Natl. Acad. Sci. USA* **99**, 15072–15077.
- Fukami, H., Imajo, S., Ito, A., Kakutani, S., Shibata, H., Sumida, M., Tanaka, T., Niwata, S., Saitoh, M., Kiso, Y., Miyazaki, M., Okunishi, H., *et al.* (2000). Substituted 3-phenylsulfonylquinazoline-2,4-dione derivatives as novel nonpeptide inhibitors of human heart chymase. *Drug Des. Discov.* **17**, 69–84.
- Fukuda, M., Ohkubo, T., Katsuya, T., Hozawa, A., Asai, T., Matsubara, M., Kitaoka, H., Tsuji, I., Araki, T., Satoh, H., Higaki, J., Hisamichi, S., *et al.* (2002). Association of a mast cell chymase gene variant with HDL cholesterol, but not with blood pressure in the Ohasama study. *Hypertens. Res.* **25**, 179–184.
- Fukuoka, Y., and Schwartz, L. B. (2004). Human beta-tryptase: Detection and characterization of the active monomer and prevention of tetramer reconstitution by protease inhibitors. *Biochemistry* **43**, 10757–10764.
- Funaba, M., Ikeda, T., Murakami, M., Ogawa, K., Tsuchida, K., Sugino, H., and Abe, M. (2003). Transcriptional activation of mouse mast cell Protease-7 by activin and transforming growth factor-beta is inhibited by microphthalmia-associated transcription factor. *J. Biol. Chem.* **278**, 52032–52041.
- Galli, S. J., Kalesnikoff, J., Grimbaldeston, M. A., Piliponsky, A. M., Williams, C. M., and Tsai, M. (2005a). Mast cells as “tunable” effector and immunoregulatory cells: Recent advances. *Annu. Rev. Immunol.* **23**, 749–786.
- Galli, S. J., Nakae, S., and Tsai, M. (2005b). Mast cells in the development of adaptive immune responses. *Nat. Immunol.* **6**, 135–142.
- Gallwitz, M., and Hellman, L. (2006). Rapid lineage-specific diversification of the mast cell chymase locus during mammalian evolution. *Immunogenetics* **58**, 641–654.
- Gallwitz, M., Reimer, J. M., and Hellman, L. (2006). Expansion of the mast cell chymase locus over the past 200 million years of mammalian evolution. *Immunogenetics* **58**, 655–669.

- Gambacurta, A., Fiorucci, L., Basili, P., Erba, F., Amoresano, A., and Ascoli, F. (2003). Bovine tryptases. *Eur. J. Biochem.* **270**, 507–517.
- Ge, Y., Jippo, T., Lee, Y. M., Adachi, S., and Kitamura, Y. (2001). Independent influence of strain difference and mi transcription factor on the expression of mouse mast cell chymases. *Am. J. Pathol.* **158**, 281–292.
- Gervasoni, J. E., Jr., Conrad, D. H., Hugli, T. E., Schwartz, L. B., and Ruddy, S. (1986). Degradation of human anaphylatoxin C3a by rat peritoneal mast cells: A role for the secretory granule enzyme chymase and heparin proteoglycan. *J. Immunol.* **136**, 285–292.
- Ghildyal, N., Friend, D. S., Stevens, R. L., Austen, K. F., Huang, C., Penrose, J. F., Sali, A., and Gurish, M. F. (1996). Fate of two mast cell tryptases in V3 mastocytosis and normal BALB/c mice undergoing passive systemic anaphylaxis: Prolonged retention of exocytosed mMCP-6 in connective tissues, and rapid accumulation of enzymatically active mMCP-7 in the blood. *J. Exp. Med.* **184**, 1061–1073.
- Goldstein, S. M., Kaempfer, C. E., Kealey, J. T., and Wintroub, B. U. (1989). Human mast cell carboxypeptidase. Purification and characterization. *J. Clin. Invest.* **83**, 1630–1636.
- Goldstein, S. M., Leong, J., and Bunnett, N. W. (1991). Human mast cell proteases hydrolyze neurotensin, kinetensin and Leu5-enkephalin. *Peptides* **12**, 995–1000.
- Goldstein, S. M., Leong, J., Schwartz, L. B., and Cooke, D. (1992). Protease composition of exocytosed human skin mast cell protease- proteoglycan complexes. Tryptase resides in a complex distinct from chymase and carboxypeptidase. *J. Immunol.* **148**, 2475–2482.
- Gordon, J. R., Burd, P. R., and Galli, S. J. (1990). Mast cells as a source of multifunctional cytokines. *Immunol. Today* **11**, 458–464.
- Gruber, B. L., Marchese, M. J., Suzuki, K., Schwartz, L. B., Okada, Y., Nagase, H., and Ramamurthy, N. S. (1989). Synovial procollagenase activation by human mast cell tryptase dependence upon matrix metalloproteinase 3 activation. *J. Clin. Invest.* **84**, 1657–1662.
- Gruber, B. L., Kew, R. R., Jelaska, A., Marchese, M. J., Garlick, J., Ren, S., Schwartz, L. B., and Korn, J. H. (1997). Human mast cells activate fibroblasts: Tryptase is a fibrogenic factor stimulating collagen messenger ribonucleic acid synthesis and fibroblast chemotaxis. *J. Immunol.* **158**, 2310–2317.
- Gumprecht, J., Zychma, M., Grzeszczak, W., Lacka, B., Burak, W., Mosur, M., Kaczmarek, J., Otulski, I., Stoklosa, T., and Czank, P. (2002). Angiotensin I-converting enzyme and chymase gene polymorphisms—relationship to left ventricular mass in type 2 diabetes patients. *Med. Sci. Monit.* **8**, CR603–CR606.
- Guo, C., Ju, H., Leung, D., Massaeli, H., Shi, M., and Rabinovitch, M. (2001). A novel vascular smooth muscle chymase is upregulated in hypertensive rats. *J. Clin. Invest.* **107**, 703–715.
- Gurish, M. F., and Boyce, J. A. (2002). Mast cell growth, differentiation, and death. *Clin. Rev. Allergy Immunol.* **22**, 107–118.
- Gurish, M. F., Ghildyal, N., McNeil, H. P., Austen, K. F., Gillis, S., and Stevens, R. L. (1992). Differential expression of secretory granule proteases in mouse mast cells exposed to interleukin 3 and c-kit ligand. *J. Exp. Med.* **175**, 1003–1012.
- Guyot, N., Zani, M. L., Berger, P., Dallet-Choisy, S., and Moreau, T. (2005). Proteolytic susceptibility of the serine protease inhibitor trappin-2 (pre-elafin): Evidence for tryptase-mediated generation of elafin. *Biol. Chem.* **386**, 391–399.
- Ha, T. Y., Reed, N. D., and Crowle, P. K. (1983). Delayed expulsion of adult *Trichinella spiralis* by mast cell-deficient W/W^v mice. *Infect. Immun.* **41**, 445–447.
- Hallgren, J., and Pejler, G. (2006). Biology of mast cell tryptase. An inflammatory mediator. *FEBS J.* **273**, 1871–1895.
- Hallgren, J., Karlson, U., Poorafshar, M., Hellman, L., and Pejler, G. (2000). Mechanism for activation of mouse mast cell tryptase: Dependence on heparin and acidic pH for formation of active tetramers of mouse mast cell protease 6. *Biochemistry* **39**, 13068–13077.
- Hallgren, J., Estrada, S., Karlson, U., Alving, K., and Pejler, G. (2001a). Heparin antagonists are potent inhibitors of mast cell tryptase. *Biochemistry* **40**, 7342–7349.

- Hallgren, J., Spillmann, D., and Pejler, G. (2001b). Structural requirements and mechanism for heparin-induced activation of a recombinant mouse mast cell tryptase, mouse mast cell protease-6: Formation of active tryptase monomers in the presence of low molecular weight heparin. *J. Biol. Chem.* **276**, 42774–42781.
- Hallgren, J., Backstrom, S., Estrada, S., Thuveson, M., and Pejler, G. (2004). Histidines are critical for heparin-dependent activation of mast cell tryptase. *J. Immunol.* **173**, 1868–1875.
- Hallgren, J., Lindahl, S., and Pejler, G. (2005). Structural requirements and mechanism for heparin-dependent activation and tetramerization of human betaI- and betaII-tryptase. *J. Mol. Biol.* **345**, 129–139.
- Hamada, H., Terai, M., Kimura, H., Hirano, K., Oana, S., and Niimi, H. (1999). Increased expression of mast cell chymase in the lungs of patients with congenital heart disease associated with early pulmonary vascular disease. *Am. J. Respir. Crit. Care Med.* **160**, 1303–1308.
- Hara, M., Ono, K., Hwang, M. W., Iwasaki, A., Okada, M., Nakatani, K., Sasayama, S., and Matsumori, A. (2002). Evidence for a role of mast cells in the evolution to congestive heart failure. *J. Exp. Med.* **195**, 375–381.
- Harris, J. L., Niles, A., Burdick, K., Maffitt, M., Backes, B. J., Ellman, J. A., Kuntz, I., Haak-Frendscho, M., and Craik, C. S. (2001). Definition of the extended substrate specificity determinants for beta-tryptases I and II. *J. Biol. Chem.* **276**, 34941–34947.
- Hartmann, T., Ruoss, S. J., Raymond, W. W., Seuwen, K., and Caughey, G. H. (1992). Human tryptase as a potent, cell-specific mitogen: Role of signaling pathways in synergistic responses. *Am. J. Physiol.* **262**, L528–L534.
- Harvima, I. T., Naukkarinen, A., Harvima, R. J., Aalto, M. L., Neittaanmaki, H., and Horsmanheimo, M. (1990). Quantitative enzyme-histochemical analysis of tryptase- and chymase-containing mast cells in psoriatic skin. *Arch. Dermatol. Res.* **282**, 428–433.
- He, S., and Walls, A. F. (1997). Human mast cell tryptase: A stimulus of microvascular leakage and mast cell activation. *Eur. J. Pharmacol.* **328**, 89–97.
- He, S., and Walls, A. F. (1998a). Human mast cell chymase induces the accumulation of neutrophils, eosinophils and other inflammatory cells *in vivo*. *Br. J. Pharmacol.* **125**, 1491–1500.
- He, S., and Walls, A. F. (1998b). The induction of a prolonged increase in microvascular permeability by human mast cell chymase. *Eur. J. Pharmacol.* **352**, 91–98.
- He, S., Peng, Q., and Walls, A. F. (1997). Potent induction of a neutrophil and eosinophil-rich infiltrate *in vivo* by human mast cell tryptase: Selective enhancement of eosinophil recruitment by histamine. *J. Immunol.* **159**, 6216–6225.
- He, S., Gaca, M. D., and Walls, A. F. (1998). A role for tryptase in the activation of human mast cells: Modulation of histamine release by tryptase and inhibitors of tryptase. *J. Pharmacol. Exp. Ther.* **286**, 289–297.
- Henningsson, F., Ledin, J., Lunderius, C., Wilen, M., Hellman, L., and Pejler, G. (2002). Altered storage of proteases in mast cells from mice lacking heparin: A possible role for heparin in carboxypeptidase A processing. *Biol. Chem.* **383**, 793–801.
- Henningsson, F., Wolters, P., Chapman, H. A., Caughey, G. H., and Pejler, G. (2003). Mast cell cathepsins C and S control levels of carboxypeptidase A and the chymase, mouse mast cell protease 5. *Biol. Chem.* **384**, 1527–1531.
- Henningsson, F., Yamamoto, K., Saftig, P., Reinheckel, T., Peters, C., Knight, S. D., and Pejler, G. (2005). A role for cathepsin E in the processing of mast-cell carboxypeptidase A. *J. Cell Sci.* **118**, 2035–2042.
- Henningsson, F., Hergeth, S., Cortelius, R., Åbrink, M., and Pejler, G. (2006). A role for serglycin proteoglycan in granular retention and processing of mast cell secretory granule components. *FEBS J.* **273**, 4901–4912.

- Hoshino, F., Urata, H., Inoue, Y., Saito, Y., Yahiro, E., Ideishi, M., Arakawa, K., and Saku, K. (2003). Chymase inhibitor improves survival in hamsters with myocardial infarction. *J. Cardiovasc. Pharmacol.* **41**(Suppl. 1), S11–S18.
- Huang, C., Wong, G. W., Ghildyal, N., Gurish, M. F., Sali, A., Matsumoto, R., Qiu, W. T., and Stevens, R. L. (1997). 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.* **272**, 31885–31893.
- Huang, C., Friend, D. S., Qiu, W. T., Wong, G. W., Morales, G., Hunt, J., and Stevens, R. L. (1998). Induction of a selective and persistent extravasation of neutrophils into the peritoneal cavity by tryptase mouse mast cell protease 6. *J. Immunol.* **160**, 1910–1919.
- Huang, C., De Sanctis, G. T., O'Brien, P. J., Mizgerd, J. P., Friend, D. S., Drazen, J. M., Brass, L. F., and Stevens, R. L. (2001). Evaluation of the substrate specificity of human mast cell tryptase beta I and demonstration of its importance in bacterial infections of the lung. *J. Biol. Chem.* **276**, 26276–26284.
- Huang, R., and Hellman, L. (1991). Cloning and structural analysis of a novel mouse mast cell specific serine protease, the MMCP-6: A connective tissue mast cell specific tryptase. In "Proceedings of XIV International Congress of Allergology and Clinical Immunology" (A. L. de Wek, Ed.), p. 122. Hoerefe & Huber, Kyoto, Japan.
- Huang, R., and Hellman, L. (1994). Genes for mast-cell serine protease and their molecular evolution. *Immunogenetics* **40**, 397–414.
- Huang, R. Y., Blom, T., and Hellman, L. (1991). Cloning and structural analysis of MMCP-1, MMCP-4 and MMCP-5, three mouse mast cell-specific serine proteases. *Eur. J. Immunol.* **21**, 1611–1621.
- Huang, R., Abrink, M., Gobl, A. E., Nilsson, G., Aveskogh, M., Larsson, L. G., Nilsson, K., and Hellman, L. (1993). Expression of a mast cell tryptase in the human monocytic cell lines U-937 and Mono Mac 6. *Scand. J. Immunol.* **38**, 359–367.
- Hultsch, T., Ennis, M., and Heidtmann, H. H. (1988). The role of chymase in ionophore-induced histamine release from human pulmonary mast cells. *Adv. Exp. Med. Biol.* **240**, 133–136.
- Humphries, D. E., Wong, G. W., Friend, D. S., Gurish, M. F., Qiu, W. T., Huang, C., Sharpe, A. H., and Stevens, R. L. (1999). Heparin is essential for the storage of specific granule proteases in mast cells [see comments]. *Nature* **400**, 769–772.
- Hunt, J. E., Friend, D. S., Gurish, M. F., Feyfant, E., Sali, A., Huang, C., Ghildyal, N., Stechschulte, S., Austen, K. F., and Stevens, R. L. (1997). Mouse mast cell protease 9, a novel member of the chromosome 14 family of serine proteases that is selectively expressed in uterine mast cells. *J. Biol. Chem.* **272**, 29158–29166.
- Huttunen, M., and Harvima, I. T. (2005). Mast cell tryptase and chymase in chronic leg ulcers: Chymase is potentially destructive to epithelium and is controlled by proteinase inhibitors. *Br. J. Dermatol.* **152**, 1149–1160.
- Imada, T., Komorita, N., Kobayashi, F., Naito, K., Yoshikawa, T., Miyazaki, M., Nakamura, N., and Kondo, T. (2002). Therapeutic potential of a specific chymase inhibitor in atopic dermatitis. *Jpn. J. Pharmacol.* **90**, 214–217.
- Imamura, T., Dubin, A., Moore, W., Tanaka, R., and Travis, J. (1996). Induction of vascular permeability enhancement by human tryptase: Dependence on activation of prekallikrein and direct release of bradykinin from kininogens. *Lab. Invest.* **74**, 861–870.
- Itoh, H., Murakumo, Y., Tomita, M., Ide, H., Kobayashi, T., Maruyama, H., Horii, Y., and Nawa, Y. (1996). Cloning of the cDNAs for mast-cell chymases from the jejunum of Mongolian gerbils, *Meriones unguiculatus*, and their sequence similarities with chymases expressed in the connective-tissue mast cells of mice and rats. *Biochem. J.* **314**, 923–929.

- Iwanaga, T., McEuen, A., Walls, A. F., Clough, J. B., Keith, T. P., Rorke, S., Barton, S. J., Holgate, S. T., and Holloway, J. W. (2004). Polymorphism of the mast cell chymase gene (CMA1) promoter region: Lack of association with asthma but association with serum total immunoglobulin E levels in adult atopic dermatitis. *Clin. Exp. Allergy* **34**, 1037–1042.
- Jarjour, N. N., Calhoun, W. J., Schwartz, L. B., and Busse, W. W. (1991). Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with increased airway obstruction. *Am. Rev. Respir. Dis.* **144**, 83–87.
- Jarvikallio, A., Naukkarinen, A., Harvima, I. T., Aalto, M. L., and Horsmanheimo, M. (1997). Quantitative analysis of tryptase- and chymase-containing mast cells in atopic dermatitis and nummular eczema. *Br. J. Dermatol.* **136**, 871–877.
- Jin, D., Takai, S., Yamada, M., Sakaguchi, M., Kamoshita, K., Ishida, K., Sukenaga, Y., and Miyazaki, M. (2003). Impact of chymase inhibitor on cardiac function and survival after myocardial infarction. *Cardiovasc. Res.* **60**, 413–420.
- Jin, D., Ueda, H., Takai, S., Okamoto, Y., Muramatsu, M., Sakaguchi, M., Shibahara, N., Katsuoka, Y., and Miyazaki, M. (2005). Effect of chymase inhibition on the arteriovenous fistula stenosis in dogs. *J. Am. Soc. Nephrol.* **16**, 1024–1034.
- Jippo, T., Lee, Y. M., Katsu, Y., Tsujino, K., Morii, E., Kim, D. K., Kim, H. M., and Kitamura, Y. (1999). Deficient transcription of mouse mast cell protease 4 gene in mutant mice of mi/mi genotype. *Blood* **93**, 1942–1950.
- Jogie-Brahim, S., Min, H. K., Fukuoka, Y., Xia, H. Z., and Schwartz, L. B. (2004). Expression of alpha-tryptase and beta-tryptase by human basophils. *J. Allergy Clin. Immunol.* **113**, 1086–1092.
- Johnson, L. A., Moon, K. E., and Eisenberg, M. (1986). Purification to homogeneity of the human skin chymotryptic proteinase "chymase." *Anal. Biochem.* **155**, 358–364.
- Jones, S. E., Gilbert, R. E., and Kelly, D. J. (2004). Tranilast reduces mesenteric vascular collagen deposition and chymase-positive mast cells in experimental diabetes. *J. Diabetes Complications* **18**, 309–315.
- Ju, H., Gros, R., You, X., Tsang, S., Husain, M., and Rabinovitch, M. (2001). Conditional and targeted overexpression of vascular chymase causes hypertension in transgenic mice. *Proc. Natl. Acad. Sci. USA* **98**, 7469–7474.
- Kajita, T., and Hugli, T. E. (1991). Evidence for *in vivo* degradation of C3a anaphylatoxin by mast cell chymase. I. Nonspecific activation of rat peritoneal mast cells by C3ades Arg. *Am. J. Pathol.* **138**, 1359–1369.
- Kakizoe, E., Shiota, N., Tanabe, Y., Shimoura, K., Kobayashi, Y., and Okunishi, H. (2001). Isoform-selective upregulation of mast cell chymase in the development of skin fibrosis in scleroderma model mice. *J. Invest. Dermatol.* **116**, 118–123.
- Kaminska, R., Helisalmi, P., Harvima, R. J., Naukkarinen, A., Horsmanheimo, M., and Harvima, I. T. (1999). Focal dermal-epidermal separation and fibronectin cleavage in basement membrane by human mast cell tryptase. *J. Invest. Dermatol.* **113**, 567–573.
- Karlson, U., Pejler, G., Froman, G., and Hellman, L. (2002). Rat mast cell protease 4 is a beta-chymase with unusually stringent substrate recognition profile. *J. Biol. Chem.* **277**, 18579–18585.
- Karlson, U., Pejler, G., Tomasini-Johansson, B., and Hellman, L. (2003). Extended substrate specificity of rat mast cell protease 5, a rodent alpha-chymase with elastase-like primary specificity. *J. Biol. Chem.* **278**, 39625–39631.
- Katz, B. A., Clark, J. M., Finer-Moore, J. S., Jenkins, T. E., Johnson, C. R., Ross, M. J., Luong, C., Moore, W. R., and Stroud, R. M. (1998). Design of potent selective zinc-mediated serine protease inhibitors. *Nature* **391**, 608–612.
- Kelso, E. B., Lockhart, J. C., Hembrough, T., Dunning, L., Plevin, R., Hollenberg, M. D., Sommerhoff, C. P., McLean, J. S., and Ferrell, W. R. (2005). Therapeutic Promise of Par2 Antagonism in Joint Inflammation. *J. Pharmacol. Exp. Ther.* **316**(3), 1017–1024.

- Kido, H., Fukusen, N., and Katunuma, N. (1985). Antibody and inhibitor of chymase inhibit histamine release in immunoglobulin E-activated mast cells. *Biochem. Int.* **10**, 863–871.
- Kido, H., Nakano, A., Okishima, N., Wakabayashi, H., Kishi, F., Nakaya, Y., Yoshizumi, M., and Tamaki, T. (1998). Human chymase, an enzyme forming novel bioactive 31-amino acid length endothelins. *Biol. Chem.* **379**, 885–891.
- Kielty, C. M., Lees, M., Shuttleworth, C. A., and Woolley, D. (1993). Catabolism of intact type VI collagen microfibrils: Susceptibility to degradation by serine proteinases. *Biochem. Biophys. Res. Commun.* **191**, 1230–1236.
- Kim, D. K., and Lee, Y. M. (2004). Requirement of c-jun transcription factor on the mouse mast cell protease-6 expression in the mast cells. *Arch. Biochem. Biophys.* **431**, 71–78.
- Kjellén, L., Pettersson, I., Lillhager, P., Steen, M. L., Pettersson, U., Lehtonen, P., Karlsson, T., Ruoslahti, E., and Hellman, L. (1989). Primary structure of a mouse mastocytoma proteoglycan core protein. *Biochem. J.* **263**, 105–113.
- Knight, P. A., Wright, S. H., Lawrence, C. E., Paterson, Y. Y., and Miller, H. R. (2000). 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.* **192**, 1849–1856.
- Kofford, M. W., Schwartz, L. B., Schechter, N. M., Yager, D. R., Diegelmann, R. F., and Graham, M. F. (1997). Cleavage of type I procollagen by human mast cell chymase initiates collagen fibril formation and generates a unique carboxyl-terminal propeptide. *J. Biol. Chem.* **272**, 7127–7131.
- Koka, V., Wang, W., Huang, X. R., Kim-Mitsuyama, S., Truong, L. D., and Lan, H. Y. (2006). Advanced glycation end products activate a chymase-dependent angiotensin II-generating pathway in diabetic complications. *Circulation* **113**, 1353–1360.
- Kokkonen, J. O., Vartiainen, M., and Kovanen, P. T. (1986). Low density lipoprotein degradation by secretory granules of rat mast cells. Sequential degradation of apolipoprotein B by granule chymase and carboxypeptidase A. *J. Biol. Chem.* **261**, 16067–16072.
- Kondo, K., Muramatsu, M., Okamoto, Y., Jin, D., Takai, S., Tanigawa, N., and Miyazaki, M. (2006). Expression of chymase-positive cells in gastric cancer and its correlation with the angiogenesis. *J. Surg. Oncol.* **93**, 36–42; discussion 42–33.
- Kondo, S., Kagami, S., Kido, H., Strutz, F., Muller, G. A., and Kuroda, Y. (2001). Role of mast cell tryptase in renal interstitial fibrosis. *J. Am. Soc. Nephrol.* **12**, 1668–1676.
- Krishna, M. T., Chauhan, A., Little, L., Sampson, K., Hawksworth, R., Mant, T., Djukanovic, R., Lee, T., and Holgate, S. (2001). Inhibition of mast cell tryptase by inhaled APC 366 attenuates allergen-induced late-phase airway obstruction in asthma. *J. Allergy Clin. Immunol.* **107**, 1039–1045.
- Kunori, Y., Koizumi, M., Masegi, T., Kasai, H., Kawabata, H., Yamazaki, Y., and Fukamizu, A. (2002). Rodent alpha-chymases are elastase-like proteases. *Eur. J. Biochem.* **269**, 5921–5930.
- Kusche-Gullberg, M., Eriksson, I., Pikas, D. S., and Kjellén, L. (1998). Identification and expression in mouse of two heparan sulfate glucosaminyl N-deacetylase/N-sulfotransferase genes. *J. Biol. Chem.* **273**, 11902–11907.
- Lane, D. A., Pejler, G., Flynn, A. M., Thompson, E. A., and Lindahl, U. (1986). Neutralization of heparin-related saccharides by histidine-rich glycoprotein and platelet factor 4 [published erratum appears in *J. Biol. Chem.* (1986 Oct 5) **261**(28), 13387]. *J. Biol. Chem.* **261**, 3980–3986.
- Lazaar, A. L., Plotnick, M. I., Kucich, U., Crichton, I., Lotfi, S., Das, S. K., Kane, S., Rosenbloom, J., Panettieri, R. A., Jr., Schechter, N. M., and Pure, E. (2002). Mast cell chymase modifies cell-matrix interactions and inhibits mitogen-induced proliferation of human airway smooth muscle cells. *J. Immunol.* **169**, 1014–1020.
- Le Trong, H., Neurath, H., and Woodbury, R. G. (1987a). Substrate specificity of the chymotrypsin-like protease in secretory granules isolated from rat mast cells [published

- erratum appears in *Proc. Natl. Acad. Sci. USA* (1987 Apr) **84**(8), 2266]. *Proc. Natl. Acad. Sci. USA* **84**, 364–367.
- Le Trong, H., Parmelee, D. C., Walsh, K. A., Neurath, H., and Woodbury, R. G. (1987b). Amino acid sequence of rat mast cell protease I (chymase). *Biochemistry* **26**, 6988–6994.
- Lee, D. M., Friend, D. S., Gurish, M. F., Benoist, C., Mathis, D., and Brenner, M. B. (2002a). Mast cells: A cellular link between autoantibodies and inflammatory arthritis. *Science* **297**, 1689–1692.
- Lee, M., Calabresi, L., Chiesa, G., Franceschini, G., and Kovanen, P. T. (2002b). Mast cell chymase degrades apoE and apoA-II in apoA-I-knockout mouse plasma and reduces its ability to promote cellular cholesterol efflux. *Arterioscler. Thromb. Vasc. Biol.* **22**, 1475–1481.
- Lee, M., Sommerhoff, C. P., von Eckardstein, A., Zettl, F., Fritz, H., and Kovanen, P. T. (2002c). Mast cell tryptase degrades HDL and blocks its function as an acceptor of cellular cholesterol. *Arterioscler. Thromb. Vasc. Biol.* **22**, 2086–2091.
- Lee, M., Kovanen, P. T., Tedeschi, G., Oungre, E., Franceschini, G., and Calabresi, L. (2003a). Apolipoprotein composition and particle size affect HDL degradation by chymase: Effect on cellular cholesterol efflux. *J. Lipid Res.* **44**, 539–546.
- Lee, M., Metso, J., Jauhainen, M., and Kovanen, P. T. (2003b). Degradation of phospholipid transfer protein (PLTP) and PLTP-generated pre-beta-high density lipoprotein by mast cell chymase impairs high affinity efflux of cholesterol from macrophage foam cells. *J. Biol. Chem.* **278**, 13539–13545.
- Lee-Rueckert, M., and Kovanen, P. T. (2006). Mast cell proteases: Physiological tools to study functional significance of high density lipoproteins in the initiation of reverse cholesterol transport. *Atherosclerosis* **189**, 8–18.
- Lees, M., Taylor, D. J., and Woolley, D. E. (1994). Mast cell proteinases activate precursor forms of collagenase and stromelysin, but not of gelatinases A and B. *Eur. J. Biochem.* **223**, 171–177.
- Leskinen, M., Wang, Y., Leszczynski, D., Lindstedt, K. A., and Kovanen, P. T. (2001). Mast cell chymase induces apoptosis of vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* **21**, 516–522.
- Leskinen, M. J., Lindstedt, K. A., Wang, Y., and Kovanen, P. T. (2003). Mast cell chymase induces smooth muscle cell apoptosis by a mechanism involving fibronectin degradation and disruption of focal adhesions. *Arterioscler. Thromb. Vasc. Biol.* **23**, 238–243.
- Leskinen, M. J., Heikkilä, H. M., Speer, M. Y., Hakala, J. K., Laine, M., Kovanen, P. T., and Lindstedt, K. A. (2006). Mast cell chymase induces smooth muscle cell apoptosis by disrupting NF-kappaB-mediated survival signaling. *Exp. Cell Res.* **312**, 1289–1298.
- Levi-Schaffer, F., and Piliponsky, A. M. (2003). Tryptase, a novel link between allergic inflammation and fibrosis. *Trends Immunol.* **24**, 158–161.
- Li, L., Li, Y., Reddel, S. W., Cherrian, M., Friend, D. S., Stevens, R. L., and Krilis, S. A. (1998). Identification of basophilic cells that express mast cell granule proteases in the peripheral blood of asthma, allergy, and drug-reactive patients. *J. Immunol.* **161**, 5079–5086.
- Li, M., Liu, K., Michalick, J., Angus, J. A., Hunt, J. E., Dell'Italia, L. J., Feneley, M. P., Graham, R. M., and Husain, A. (2004). Involvement of chymase-mediated angiotensin II generation in blood pressure regulation. *J. Clin. Invest.* **114**, 112–120.
- Lindstedt, K. A., Wang, Y., Shiota, N., Saarinen, J., Hyttiainen, M., Kokkonen, J. O., Keski-Oja, J., and Kovanen, P. T. (2001a). Activation of paracrine TGF-beta1 signaling upon stimulation and degranulation of rat serosal mast cells: A novel function for chymase. *FASEB J.* **15**, 1377–1388.
- Lindstedt, L., Lee, M., Castro, G. R., Fruchart, J. C., and Kovanen, P. T. (1996). Chymase in exocytosed rat mast cell granules effectively proteolyzes apolipoprotein AI-containing lipoproteins, so reducing the cholesterol efflux-inducing ability of serum and aortic intimal fluid. *J. Clin. Invest.* **97**, 2174–2182.

- Lindstedt, L., Lee, M., and Kovanen, P. T. (2001b). Chymase bound to heparin is resistant to its natural inhibitors and capable of proteolyzing high density lipoproteins in aortic intimal fluid. *Atherosclerosis* **155**, 87–97.
- Lohi, J., Harvima, I., and Keski-Oja, J. (1992). Pericellular substrates of human mast cell tryptase: 72,000 dalton gelatinase and fibronectin. *J. Cell. Biochem.* **50**, 337–349.
- Longley, B. J., Tyrrell, L., Ma, Y., Williams, D. A., Halaban, R., Langley, K., Lu, H. S., and Schechter, N. M. (1997). Chymase cleavage of stem cell factor yields a bioactive, soluble product. *Proc. Natl. Acad. Sci. USA* **94**, 9017–9021.
- Lundequist, A., Juliano, M. A., Juliano, L., and Pejler, G. (2003). Polycationic peptides as inhibitors of mast cell serine proteases. *Biochem. Pharmacol.* **65**, 1171–1180.
- Lundequist, A., Tchougounova, E., Åbrink, M., and Pejler, G. (2004). 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.* **279**, 32339–32344.
- Lundequist, A., Åbrink, M., and Pejler, G. (2006). Mast cell-dependent activation of pro matrix metalloprotease 2: A role for serglycin proteoglycan-dependent mast cell proteases. *Biol. Chem.* **387**, 1513–1519.
- Lunderius, C., and Hellman, L. (2001). Characterization of the gene encoding mouse mast cell protease 8 (mMCP-8), and a comparative analysis of hematopoietic serine protease genes. *Immunogenetics* **53**, 225–232.
- Lützelshwab, C., Pejler, G., Aveskogh, M., and Hellman, L. (1997). Secretory granule proteases in rat mast cells. Cloning of 10 different serine proteases and a carboxypeptidase A from various rat mast cell populations. *J. Exp. Med.* **185**, 13–29.
- Lützelshwab, C., Lunderius, C., Enerback, L., and Hellman, L. (1998). A kinetic analysis of the expression of mast cell protease mRNA in the intestines of Nippostrongylus brasiliensis-infected rats. *Eur. J. Immunol.* **28**, 3730–3737.
- Macaldowie, C. N., Mackellar, A., and Huntley, J. F. (1998). The isolation and purification of a dual specific mast cell-derived protease from parasitised caprine jejunal tissue. *Res. Vet. Sci.* **64**, 17–24.
- Maccarana, M., and Lindahl, U. (1993). Mode of interaction between platelet factor 4 and heparin. *Glycobiology* **3**, 271–277.
- Malaviya, R., Ikeda, T., Ross, E., and Abraham, S. N. (1996). Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha [see comments]. *Nature* **381**, 77–80.
- Mallen-St Clair, J., Pham, C. T., Villalta, S. A., Caughey, G. H., and Wolters, P. J. (2004). Mast cell dipeptidyl peptidase I mediates survival from sepsis. *J. Clin. Invest.* **113**, 628–634.
- Mao, X. Q., Shirakawa, T., Yoshikawa, T., Yoshikawa, K., Kawai, M., Sasaki, S., Enomoto, T., Hashimoto, T., Furuyama, J., Hopkin, J. M., and Morimoto, K. (1996). Association between genetic variants of mast-cell chymase and eczema [see comments] [published erratum appears in *Lancet* (1997 Jan 4) **349**(9044), 64]. *Lancet* **348**, 581–583.
- Mao, X. Q., Shirakawa, T., Enomoto, T., Shimazu, S., Dake, Y., Kitano, H., Hagihara, A., and Hopkin, J. M. (1998). Association between variants of mast cell chymase gene and serum IgE levels in eczema [published erratum appears in *Hum. Hered.* (1998 Mar–Apr) **48**(2), 91]. *Hum. Hered.* **48**, 38–41.
- Marquardt, U., Zettl, F., Huber, R., Bode, W., and Sommerhoff, C. (2002). The crystal structure of human alpha1-tryptase reveals a blocked substrate-binding region. *J. Mol. Biol.* **321**, 491–502.
- Maruichi, M., Takai, S., Sugiyama, T., Ueki, M., Oku, H., Sakaguchi, M., Okamoto, Y., Muramatsu, M., Ikeda, T., and Miyazaki, M. (2004). Role of chymase on growth of cultured canine Tenon's capsule fibroblasts and scarring in a canine conjunctival flap model. *Exp. Eye Res.* **79**, 111–118.

- Masaki, H., Mizuno, Y., Tatui, A., Murakami, A., Koide, Y., Satoh, S., and Takahashi, A. (2003). Structure-activity relationship of benzo[b]thiophene-2-sulfonamide derivatives as novel human chymase inhibitors. *Bioorg. Med. Chem. Lett.* **13**, 4085–4088.
- Matin, R., Tam, E. K., Nadel, J. A., and Caughey, G. H. (1992). Distribution of chymase-containing mast cells in human bronchi. *J. Histochem. Cytochem.* **40**, 781–786.
- Matsumoto, R., Sali, A., Ghildyal, N., Karplus, M., and Stevens, R. L. (1995). Packaging of proteases and proteoglycans in the granules of mast cells and other hematopoietic cells. A cluster of histidines on mouse mast cell protease 7 regulates its binding to heparin serglycin proteoglycans. *J. Biol. Chem.* **270**, 19524–19531.
- Matsuo, T., Ikura, Y., Ohsawa, M., Ogami, M., Kayo, S., Yoshimi, N., Hai, E., Naruko, T., Ohishi, M., Higuchi, K., Arakawa, T., and Ueda, M. (2003). Mast cell chymase expression in *Helicobacter pylori*-associated gastritis. *Histopathology* **43**, 538–549.
- McAleese, S. M., Pemberton, A. D., McGrath, M. E., Huntley, J. F., and Miller, H. R. (1998). Sheep mast-cell proteinases-1 and -3: cDNA cloning, primary structure and molecular modelling of the enzymes and further studies on substrate specificity. *Biochem. J.* **333**, 801–809.
- McDermott, J. R., Bartram, R. E., Knight, P. A., Miller, H. R., Garrod, D. R., and Grecis, R. K. (2003). Mast cells disrupt epithelial barrier function during enteric nematode infection. *Proc. Natl. Acad. Sci. USA* **100**, 7761–7766.
- McEuen, A. R., Ashworth, D. M., and Walls, A. F. (1998). The conversion of recombinant human mast cell prochymase to enzymatically active chymase by dipeptidyl peptidase I is inhibited by heparin and histamine. *Eur. J. Biochem.* **253**, 300–308.
- McGrath, M. E., Mirzadegan, T., and Schmidt, B. F. (1997). Crystal structure of phenylmethanesulfonyl fluoride-treated human chymase at 1.9 Å. *Biochemistry* **36**, 14318–14324.
- McNeil, H. P., Reynolds, D. S., Schiller, V., Ghildyal, N., Gurley, D. S., Austen, K. F., and Stevens, R. L. (1992). Isolation, characterization, and transcription of the gene encoding mouse mast cell protease 7. *Proc. Natl. Acad. Sci. USA* **89**, 11174–11178.
- Metcalf, D. D., Baram, D., and Mekori, Y. A. (1997). Mast cells. *Physiol. Rev.* **77**, 1033–1079.
- Metsarinne, K. P., Vehmaan-Kreula, P., Kovanen, P. T., Saijonmaa, O., Baumann, M., Wang, Y., Nyman, T., Fyhrquist, F. Y., and Eklund, K. K. (2002). Activated mast cells increase the level of endothelin-1 mRNA in cocultured endothelial cells and degrade the secreted peptide. *Arterioscler. Thromb. Vasc. Biol.* **22**, 268–273.
- Metz, M., Piliponsky, A. M., Chen, C. C., Lammel, V., Abrink, M., Pejler, G., Tsai, M., and Galli, S. J. (2006). Mast cells can enhance resistance to snake and honeybee venoms. *Science* **313**, 526–530.
- Miller, H. R., Wright, S. H., Knight, P. A., and Thornton, E. M. (1999). A novel function for transforming growth factor-beta1: Upregulation of the expression and the IgE-independent extracellular release of a mucosal mast cell granule-specific beta-chymase, mouse mast cell protease-1. *Blood* **93**, 3473–3486.
- Miller, J. S., Westin, E. H., and Schwartz, L. B. (1989). Cloning and characterization of complementary DNA for human tryptase. *J. Clin. Invest.* **84**, 1188–1195.
- Miller, J. S., Moxley, G., and Schwartz, L. B. (1990). Cloning and characterization of a second complementary DNA for human tryptase. *J. Clin. Invest.* **86**, 864–870.
- Mirza, H., Schmidt, V. A., Derian, C. K., Jesty, J., and Bahou, W. F. (1997). Mitogenic responses mediated through the proteinase-activated receptor-2 are induced by expressed forms of mast cell alpha- or beta-tryptases. *Blood* **90**, 3914–3922.
- Mizutani, H., Schechter, N., Lazarus, G., Black, R. A., and Kupper, T. S. (1991). Rapid and specific conversion of precursor interleukin 1 beta (IL-1 beta) to an active IL-1 species by human mast cell chymase. *J. Exp. Med.* **174**, 821–825.
- Molinari, J. F., Moore, W. R., Clark, J., Tanaka, R., Butterfield, J. H., and Abraham, W. M. (1995). Role of tryptase in immediate cutaneous responses in allergic sheep. *J. Appl. Physiol.* **79**, 1966–1970.

- Molinari, J. F., Scuri, M., Moore, W. R., Clark, J., Tanaka, R., and Abraham, W. M. (1996). Inhaled tryptase causes bronchoconstriction in sheep via histamine release. *Am. J. Respir. Crit. Care Med.* **154**, 649–653.
- Molino, M., Barnathan, E. S., Numerof, R., Clark, J., Dreyer, M., Cumashi, A., Hoxie, J. A., Schechter, N., Woolkalis, M., and Brass, L. F. (1997). Interactions of mast cell tryptase with thrombin receptors and PAR-2. *J. Biol. Chem.* **272**, 4043–4049.
- Morii, E., Takebayashi, K., Motohashi, H., Yamamoto, M., Nomura, S., and Kitamura, Y. (1994). Loss of DNA binding ability of the transcription factor encoded by the mutant mi locus. *Biochem. Biophys. Res. Commun.* **205**, 1299–1304.
- Morii, E., Tsujimura, T., Jippo, T., Hashimoto, K., Takebayashi, K., Tsujino, K., Nomura, S., Yamamoto, M., and Kitamura, Y. (1996). Regulation of mouse mast cell protease 6 gene expression by transcription factor encoded by the mi locus. *Blood* **88**, 2488–2494.
- Morii, E., Jippo, T., Tsujimura, T., Hashimoto, K., Kim, D. K., Lee, Y. M., Ogihara, H., Tsujino, K., Kim, H. M., and Kitamura, Y. (1997). Abnormal expression of mouse mast cell protease 5 gene in cultured mast cells derived from mutant mi/mi mice. *Blood* **90**, 3057–3066.
- Morii, E., Ogihara, H., Kim, D. K., Ito, A., Oboki, K., Lee, Y. M., Jippo, T., Nomura, S., Maeyama, K., Lamoreux, M. L., and Kitamura, Y. (2001a). Importance of leucine zipper domain of mi transcription factor (MITF) for differentiation of mast cells demonstrated using mi(ce)/mi(ce) mutant mice of which MITF lacks the zipper domain. *Blood* **97**, 2038–2044.
- Morii, E., Ogihara, H., Oboki, K., Kataoka, T. R., Jippo, T., and Kitamura, Y. (2001b). Effect of MITF on transcription of transmembrane tryptase gene in cultured mast cells of mice. *Biochem. Biophys. Res. Commun.* **289**, 1243–1246.
- Morii, E., Oboki, K., Kataoka, T. R., Igarashi, K., and Kitamura, Y. (2002). Interaction and cooperation of mi transcription factor (MITF) and myc-associated zinc-finger protein-related factor (MAZR) for transcription of mouse mast cell protease 6 gene. *J. Biol. Chem.* **277**, 8566–8571.
- Murakami, M., Karnik, S. S., and Husain, A. (1995). Human prochymase activation. A novel role for heparin in zymogen processing. *J. Biol. Chem.* **270**, 2218–2223.
- Murakami, Y., Takei, M., Shindo, K., Kitazume, C., Tanaka, J., Higa, T., and Fukamachi, H. (2002). Cyclotheonamide E4 and E5, new potent tryptase inhibitors from an *Ircinia* species of sponge. *J. Nat. Prod.* **65**, 259–261.
- Muramatsu, M., Katada, J., Hattori, M., Hayashi, I., and Majima, M. (2000). Chymase mediates mast cell-induced angiogenesis in hamster sponge granulomas. *Eur. J. Pharmacol.* **402**, 181–191.
- Muramatsu, M., Yamada, M., Takai, S., and Miyazaki, M. (2002). Suppression of basic fibroblast growth factor-induced angiogenesis by a specific chymase inhibitor, BCEAB, through the chymase-angiotensin-dependent pathway in hamster sponge granulomas. *Br. J. Pharmacol.* **137**, 554–560.
- Nagata, M., Shijubo, N., Walls, A. F., Ichimiya, S., Abe, S., and Sato, N. (2003). Chymase-positive mast cells in small sized adenocarcinoma of the lung. *Virchows Arch.* **443**, 565–573.
- Nakajima, M., and Naya, N. (2002). Development of a chymase inhibitor: Pharmacological characterization of a chymase inhibitor in inflamed tissue remodeling and fibrosis. *Jpn. J. Pharmacol.* **90**, 206–209.
- Nakano, A., Kishi, F., Minami, K., Wakabayashi, H., Nakaya, Y., and Kido, H. (1997). Selective conversion of big endothelins to tracheal smooth muscle- constricting 31-amino acid-length endothelins by chymase from human mast cells. *J. Immunol.* **159**, 1987–1992.
- Namazi, M. R. (2005). Possible molecular mechanisms to account for the involvement of tryptase in the pathogenesis of psoriasis. *Autoimmunity* **38**, 449–452.

- Naukkarinen, A., Harvima, I. T., Aalto, M. L., and Horsmanheimo, M. (1994). Mast cell tryptase and chymase are potential regulators of neurogenic inflammation in psoriatic skin. *Int. J. Dermatol.* **33**, 361–366.
- Nico, B., Marzullo, A., Corsi, P., Vacca, A., Roncali, L., and Ribatti, D. (2004). A possible role of tryptase in angiogenesis in the brain of mdx mouse, a model of Duchenne muscular dystrophy. *Neuroscience* **123**, 585–588.
- Nishikori, Y., Kakizoe, E., Kobayashi, Y., Shimoura, K., Okunishi, H., and Dekio, S. (1998). Skin mast cell promotion of matrix remodeling in burn wound healing in mice: Relevance of chymase. *Arch. Dermatol. Res.* **290**, 553–560.
- Niwata, S., Fukami, H., Sumida, M., Ito, A., Kakutani, S., Saitoh, M., Suzuki, K., Imoto, M., Shibata, H., Imajo, S., Kiso, Y., Tanaka, T., *et al.* (1997). Substituted 3-(phenylsulfonyl)-1-phenylimidazolidine-2,4-dione derivatives as novel nonpeptide inhibitors of human heart chymase. *J. Med. Chem.* **40**, 2156–2163.
- Normant, E., Martres, M. P., Schwartz, J. C., and Gros, C. (1995). Purification, cDNA cloning, functional expression, and characterization of a 26-kDa endogenous mammalian carboxypeptidase inhibitor. *Proc. Natl. Acad. Sci. USA* **92**, 12225–12229.
- Ogihara, H., Kanno, T., Morii, E., Kim, D. K., Lee, Y. M., Sato, M., Kim, W. Y., Nomura, S., Ito, Y., and Kitamura, Y. (1999). Synergy of PEBP2/CBF with mi transcription factor (MITF) for transactivation of mouse mast cell protease 6 gene. *Oncogene* **18**, 4632–4639.
- Ogihara, H., Morii, E., Kim, D. K., Oboki, K., and Kitamura, Y. (2001). Inhibitory effect of the transcription factor encoded by the mutant mi microphthalmia allele on transactivation of mouse mast cell protease 7 gene. *Blood* **97**, 645–651.
- Oh, S. W., Pae, C. I., Lee, D. K., Jones, F., Chiang, G. K., Kim, H. O., Moon, S. H., Cao, B., Ogbu, C., Jeong, K. W., Kozu, G., Nakanishi, H., *et al.* (2002). Tryptase inhibition blocks airway inflammation in a mouse asthma model. *J. Immunol.* **168**, 1992–2000.
- Ohtsu, H., Tanaka, S., Terui, T., Hori, Y., Makabe-Kobayashi, Y., Pejler, G., Tchougounova, E., Hellman, L., Gertsenstein, M., Hirasawa, N., Sakurai, E., Buzas, E., *et al.* (2001). Mice lacking histidine decarboxylase exhibit abnormal mast cells. *FEBS Lett.* **502**, 53–56.
- Okamoto, Y., Takai, S., and Miyazaki, M. (2002a). Chymase inhibitor, BCEAB, suppressed peritoneal adhesion formation in hamster. *J. Surg. Res.* **107**, 219–222.
- Okamoto, Y., Takai, S., and Miyazaki, M. (2002b). Oral administration of a novel chymase inhibitor, NK3201, prevents peritoneal adhesion formation in hamsters. *Jpn. J. Pharmacol.* **90**, 94–96.
- Okamoto, Y., Takai, S., and Miyazaki, M. (2004a). Effect of chymase-dependent transforming growth factor beta on peritoneal adhesion formation in a rat model. *Surg. Today* **34**, 865–867.
- Okamoto, Y., Takai, S., and Miyazaki, M. (2004b). Significance of chymase inhibition for prevention of adhesion formation. *Eur. J. Pharmacol.* **484**, 357–359.
- Okumura, K., Takai, S., Muramatsu, M., Katayama, S., Sakaguchi, M., Kishi, K., Jin, D., and Miyazaki, M. (2004). Human chymase degrades human fibronectin. *Clin. Chim. Acta* **347**, 223–225.
- Omoto, Y., Tokime, K., Yamanaka, K., Habe, K., Morioka, T., Kurokawa, I., Tsutsui, H., Yamanishi, K., Nakanishi, K., and Mizutani, H. (2006). Human mast cell chymase cleaves pro-IL-18 and generates a novel and biologically active IL-18 fragment. *J. Immunol.* **177**, 8315–8319.
- Ortlepp, J. R., Janssens, U., Bleckmann, F., Lauscher, J., Merkelbach-Bruse, S., Hanrath, P., and Hoffmann, R. (2001). A chymase gene variant is associated with atherosclerosis in venous coronary artery bypass grafts. *Coron. Artery Dis.* **12**, 493–497.
- Ossovskaya, V. S., and Bunnett, N. W. (2004). Protease-activated receptors: Contribution to physiology and disease. *Physiol. Rev.* **84**, 579–621.

- Padmanabhan, N., Jardine, A. G., McGrath, J. C., and Connell, J. M. (1999). Angiotensin-converting enzyme-independent contraction to angiotensin I in human resistance arteries. *Circulation* **99**, 2914–2920.
- Pallaoro, M., Fejzo, M. S., Shayesteh, L., Blount, J. L., and Caughey, G. H. (1999). Characterization of genes encoding known and novel human mast cell tryptases on chromosome 16p13.3. *J. Biol. Chem.* **274**, 3355–3362.
- Pang, L., Nie, M., Corbett, L., Sutcliffe, A., and Knox, A. J. (2006). Mast cell beta-tryptase selectively cleaves eotaxin and RANTES and abrogates their eosinophil chemotactic activities. *J. Immunol.* **176**, 3788–3795.
- Pedotti, R., De Voss, J. J., Steinman, L., and Galli, S. J. (2003a). Involvement of both 'allergic' and 'autoimmune' mechanisms in EAE, MS and other autoimmune diseases. *Trends Immunol.* **24**, 479–484.
- Pedotti, R., DeVoss, J. J., Youssef, S., Mitchell, D., Wedemeyer, J., Madanat, R., Garren, H., Fontoura, P., Tsai, M., Galli, S. J., Sobel, R. A., Steinman, L., et al. (2003b). Multiple elements of the allergic arm of the immune response modulate autoimmune demyelination. *Proc. Natl. Acad. Sci. USA* **100**, 1867–1872.
- Pejler, G. (1996). Mast cell chymase in complex with heparin proteoglycan is regulated by protamine. *FEBS Lett.* **383**, 170–174.
- Pejler, G., and Berg, L. (1995). Regulation of rat mast cell protease 1 activity. Protease inhibition is prevented by heparin proteoglycan. *Eur. J. Biochem.* **233**, 192–199.
- Pejler, G., and Karlstrom, A. (1993). Thrombin is inactivated by mast cell secretory granule chymase. *J. Biol. Chem.* **268**, 11817–11822.
- Pejler, G., and Maccarana, M. (1994). Interaction of heparin with rat mast cell protease 1. *J. Biol. Chem.* **269**, 14451–14456.
- Pejler, G., and Sadler, J. E. (1999). Mechanism by which heparin proteoglycan modulates mast cell chymase activity. *Biochemistry* **38**, 12187–12195.
- Pejler, G., Soderstrom, K., and Karlstrom, A. (1994). Inactivation of thrombin by a complex between rat mast-cell protease 1 and heparin proteoglycan. *Biochem. J.* **299**(Pt. 2), 507–513.
- Pejler, G., Karlström, A. R., and Berg, L. (1995). Identification of the proteolytic thrombin fragments formed after cleavage with rat mast cell protease 1. *Eur. J. Biochem.* **227**, 102–107.
- Pemberton, A. D., Belham, C. M., Huntley, J. F., Plevin, R., and Miller, H. R. (1997). Sheep mast cell proteinase-1, a serine proteinase with both tryptase- and chymase-like properties, is inhibited by plasma proteinase inhibitors and is mitogenic for bovine pulmonary artery fibroblasts. *Biochem. J.* **323**, 719–725.
- Pemberton, A. D., McAleese, S. M., Huntley, J. F., Collie, D. D., Scudamore, C. L., McEuen, A. R., Walls, A. F., and Miller, H. R. (2000). cDNA sequence of two sheep mast cell tryptases and the differential expression of tryptase and sheep mast cell proteinase-1 in lung, dermis and gastrointestinal tract. *Clin. Exp. Allergy* **30**, 818–832.
- Pemberton, A. D., Wright, S. H., Knight, P. A., and Miller, H. R. (2006). Anaphylactic release of mucosal mast cell granule proteases: Role of serpins in the differential clearance of mouse mast cell proteases-1 and -2. *J. Immunol.* **176**, 899–904.
- Pereira, P. J., Bergner, A., Macedo-Ribeiro, S., Huber, R., Matschiner, G., Fritz, H., Sommerhoff, C. P., and Bode, W. (1998). Human beta-tryptase is a ring-like tetramer with active sites facing a central pore. *Nature* **392**, 306–311.
- Pereira, P. J. B., Wang, Z. M., Rubin, H., Huber, R., Bode, W., Schechter, N. M., and Strobl, S. (1999). The 2.2 Å crystal structure of human chymase in complex with succinyl-Ala-Ala-Pro-Phe-chloromethylketone: Structural explanation for its dipeptidyl carboxypeptidase specificity. *J. Mol. Biol.* **286**, 163–173.

- Pfeuffer, A., Osterziel, K. J., Urata, H., Borck, G., Schuster, H., Wienker, T., Dietz, R., and Luft, F. C. (1996). Angiotensin-converting enzyme and heart chymase gene polymorphisms in hypertrophic cardiomyopathy. *Am. J. Cardiol.* **78**, 362–364.
- Poorafshar, M., Aveskogh, M., Munday, B., and Hellman, L. (2000a). Identification and structural analysis of four serine proteases in a monotreme, the platypus, *Ornithorhynchus anatinus*. *Immunogenetics* **52**, 19–28.
- Poorafshar, M., Helmby, H., Troye-Blomberg, M., and Hellman, L. (2000b). MMCP-8, the first lineage-specific differentiation marker for mouse basophils. Elevated numbers of potent IL-4-producing and MMCP-8-positive cells in spleens of malaria-infected mice. *Eur. J. Immunol.* **30**, 2660–2668.
- Powers, J. C., Tanaka, T., Harper, J. W., Minematsu, Y., Barker, L., Lincoln, D., Crumley, K. V., Fraki, J. E., Schechter, N. M., Lazarus, G. G., Nakajima, K., Nakashino, K., *et al.* (1985). Mammalian chymotrypsin-like enzymes. Comparative reactivities of rat mast cell proteases, human and dog skin chymases, and human cathepsin G with peptide 4-nitroanilide substrates and with peptide chloromethyl ketone and sulfonyl fluoride inhibitors. *Biochemistry* **24**, 2048–2058.
- Prasad, P., Tiwari, A. K., Kumar, K. M., Ammini, A. C., Gupta, A., Gupta, R., Sharma, A. K., Rao, A. R., Nagendra, R., Chandra, T. S., Tiwari, S. C., Rastogi, P., *et al.* (2006). Chronic renal insufficiency among Asian Indians with type 2 diabetes: I. Role of RAAS gene polymorphisms. *BMC Med. Genet.* **7**, 42.
- Praveen, K., Evans, D. L., and Jaso-Friedmann, L. (2004). Evidence for the existence of granzyme-like serine proteases in teleost cytotoxic cells. *J. Mol. Evol.* **58**, 449–459.
- Praveen, K., Leary, J. H., III, Evans, D. L., and Jaso-Friedmann, L. (2006). Nonspecific cytotoxic cells of teleosts are armed with multiple granzymes and other components of the granule exocytosis pathway. *Mol. Immunol.* **43**, 1152–1162.
- Rath-Wolfson, L. (2001). An immunocytochemical approach to the demonstration of intracellular processing of mast cell carboxypeptidase. *Appl. Immunohistochem. Mol. Morphol.* **9**, 81–85.
- Raymond, W. W., Ruggles, S. W., Craik, C. S., and Caughey, G. H. (2003). 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.* **278**, 34517–34524.
- Raymond, W. W., Sommerhoff, C. P., and Caughey, G. H. (2005). Mastin is a gelatinolytic mast cell peptidase resembling a mini-proteasome. *Arch. Biochem. Biophys.* **435**, 311–322.
- Raymond, W. W., Cruz, A. C., and Caughey, G. H. (2006). Mast cell and neutrophil peptidases attack an inactivation segment in hepatocyte growth factor to generate NK4-like antagonists. *J. Biol. Chem.* **281**, 1489–1494.
- Razin, E., Stevens, R. L., Akiyama, F., Schmid, K., and Austen, K. F. (1982). Culture from mouse bone marrow of a subclass of mast cells possessing a distinct chondroitin sulfate proteoglycan with glycosaminoglycans rich in N-acetylgalactosamine-4,6-disulfate. *J. Biol. Chem.* **257**, 7229–7236.
- Reiling, K. K., Krucinski, J., Miercke, L. J., Raymond, W. W., Caughey, G. H., and Stroud, R. M. (2003). Structure of human pro-chymase: A model for the activating transition of granule-associated proteases. *Biochemistry* **42**, 2616–2624.
- Reilly, C. F., Tewksbury, D. A., Schechter, N. M., and Travis, J. (1982). Rapid conversion of angiotensin I to angiotensin II by neutrophil and mast cell proteinases. *J. Biol. Chem.* **257**, 8619–8622.
- Reilly, C. F., Schechter, N. B., and Travis, J. (1985). Inactivation of bradykinin and kallidin by cathepsin G and mast cell chymase. *Biochem. Biophys. Res. Commun.* **127**, 443–449.
- Remington, S. J., Woodbury, R. G., Reynolds, R. A., Matthews, B. W., and Neurath, H. (1988). The structure of rat mast cell protease II at 1.9-Å resolution. *Biochemistry* **27**, 8097–8105.

- Reynolds, D. S., Gurley, D. S., Stevens, R. L., Sugarbaker, D. J., Austen, K. F., and Serafin, W. E. (1989a). Cloning of cDNAs that encode human mast cell carboxypeptidase A, and comparison of the protein with mouse mast cell carboxypeptidase A and rat pancreatic carboxypeptidases. *Proc. Natl. Acad. Sci. USA* **86**, 9480–9484.
- Reynolds, D. S., Stevens, R. L., Gurley, D. S., Lane, W. S., Austen, K. F., and Serafin, W. E. (1989b). Isolation and molecular cloning of mast cell carboxypeptidase A. A novel member of the carboxypeptidase gene family. *J. Biol. Chem.* **264**, 20094–20099.
- Reynolds, D. S., Stevens, R. L., Lane, W. S., Carr, M. H., Austen, K. F., and Serafin, W. E. (1990). Different mouse mast cell populations express various combinations of at least six distinct mast cell serine proteases. *Proc. Natl. Acad. Sci. USA* **87**, 3230–3234.
- Reynolds, D. S., Gurley, D. S., Austen, K. F., and Serafin, W. E. (1991). Cloning of the cDNA and gene of mouse mast cell protease-6. Transcription by progenitor mast cells and mast cells of the connective tissue subclass. *J. Biol. Chem.* **266**, 3847–3853.
- Reynolds, D. S., Gurley, D. S., and Austen, K. F. (1992). Cloning and characterization of the novel gene for mast cell carboxypeptidase A. *J. Clin. Invest.* **89**, 273–282.
- Rice, K. D., Tanaka, R. D., Katz, B. A., Numerof, R. P., and Moore, W. R. (1998). Inhibitors of tryptase for the treatment of mast cell-mediated diseases. *Curr. Pharm. Des.* **4**, 381–396.
- Rice, K. D., Gangloff, A. R., Kuo, E. Y., Dener, J. M., Wang, V. R., Lum, R., Newcomb, W. S., Havel, C., Putnam, D., Cregar, L., Wong, M., and Warne, R. L. (2000a). Dibasic inhibitors of human mast cell tryptase. Part 1: Synthesis and optimization of a novel class of inhibitors. *Bioorg. Med. Chem. Lett.* **10**, 2357–2360.
- Rice, K. D., Wang, V. R., Gangloff, A. R., Kuo, E. Y., Dener, J. M., Newcomb, W. S., Young, W. B., Putnam, D., Cregar, L., Wong, M., and Simpson, P. J. (2000b). Dibasic inhibitors of human mast cell tryptase. Part 2: Structure-activity relationships and requirements for potent activity. *Bioorg. Med. Chem. Lett.* **10**, 2361–2366.
- Richard, V., Hurel-Merle, S., Scalbert, E., Ferry, G., Lallemand, F., Bessou, J. P., and Thuillez, C. (2001). Functional evidence for a role of vascular chymase in the production of angiotensin II in isolated human arteries. *Circulation* **104**, 750–752.
- Rodewald, H. R., Dessing, M., Dvorak, A. M., and Galli, S. J. (1996). Identification of a committed precursor for the mast cell lineage. *Science* **271**, 818–822.
- Rozniecki, J. J., Hauser, S. L., Stein, M., Lincoln, R., and Theoharides, T. C. (1995). Elevated mast cell tryptase in cerebrospinal fluid of multiple sclerosis patients. *Ann. Neurol.* **37**, 63–66.
- Rubinstein, I., Nadel, J. A., Graf, P. D., and Caughey, G. H. (1990). Mast cell chymase potentiates histamine-induced wheal formation in the skin of ragweed-allergic dogs. *J. Clin. Invest.* **86**, 555–559.
- Ruoss, S. J., Hartmann, T., and Caughey, G. H. (1991). Mast cell tryptase is a mitogen for cultured fibroblasts. *J. Clin. Invest.* **88**, 493–499.
- Russo, A., Russo, G., Peticca, M., Pietropaolo, C., Di Rosa, M., and Iuvone, T. (2005). Inhibition of granuloma-associated angiogenesis by controlling mast cell mediator release: Role of mast cell protease-5. *Br. J. Pharmacol.* **145**, 24–33.
- Saarinen, J., Kalkkinen, N., Welgus, H. G., and Kovanen, P. T. (1994). Activation of human interstitial procollagenase through direct cleavage of the Leu83-Thr84 bond by mast cell chymase. *J. Biol. Chem.* **269**, 18134–18140.
- Sakaguchi, M., Takai, S., Jin, D., Okamoto, Y., Muramatsu, M., Kim, S., and Miyazaki, M. (2004). A specific chymase inhibitor, NK3201, suppresses bleomycin-induced pulmonary fibrosis in hamsters. *Eur. J. Pharmacol.* **493**, 173–176.
- Sakai, K., Long, S. D., Pettit, D. A., Cabral, G. A., and Schwartz, L. B. (1996a). Expression and purification of recombinant human tryptase in a baculovirus system. *Protein Expr. Purif.* **7**, 67–73.
- Sakai, K., Ren, S., and Schwartz, L. B. (1996b). A novel heparin-dependent processing pathway for human tryptase. Autocatalysis followed by activation with dipeptidyl peptidase I [see comments]. *J. Clin. Invest.* **97**, 988–995.

- Sali, A., Matsumoto, R., McNeil, H. P., Karplus, M., and Stevens, R. L. (1993). Three-dimensional models of four mouse mast cell chymases. Identification of proteoglycan binding regions and protease-specific antigenic epitopes. *J. Biol. Chem.* **268**, 9023–9034.
- Sanker, S., Chandrasekharan, U. M., Wilk, D., Glynias, M. J., Karnik, S. S., and Husain, A. (1997). Distinct multisite synergistic interactions determine substrate specificities of human chymase and rat chymase-1 for angiotensin II formation and degradation. *J. Biol. Chem.* **272**, 2963–2968.
- Satomura, K., Yin, M., Shimizu, S., Kato, Y., Nagano, T., Komeichi, H., Ohsuga, M., Katsuta, Y., Aramaki, T., and Omoto, Y. (2003). Increased chymase in livers with autoimmune disease: Colocalization with fibrosis. *J. Nippon Med. Sch.* **70**, 490–495.
- Sayama, S., Iozzo, R. V., Lazarus, G. S., and Schechter, N. M. (1987). Human skin chymotrypsin-like proteinase chymase. Subcellular localization to mast cell granules and interaction with heparin and other glycosaminoglycans. *J. Biol. Chem.* **262**, 6808–6815.
- Schaschke, N., Dominik, A., Matschiner, G., and Sommerhoff, C. P. (2002). Bivalent inhibition of beta-tryptase: Distance scan of neighboring subunits by dibasic inhibitors. *Bioorg. Med. Chem. Lett.* **12**, 985–988.
- Schechter, I., and Berger, A. (1967). On the size of the active site in proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **27**, 157–162.
- Schechter, N. M., Sprows, J. L., Schoenberger, O. L., Lazarus, G. S., Cooperman, B. S., and Rubin, H. (1989). Reaction of human skin chymotrypsin-like proteinase chymase with plasma proteinase inhibitors. *J. Biol. Chem.* **264**, 21308–21315.
- Schechter, N. M., Irani, A. M., Sprows, J. L., Abernethy, J., Wintroub, B., and Schwartz, L. B. (1990). Identification of a cathepsin G-like proteinase in the MCTC type of human mast cell. *J. Immunol.* **145**, 2652–2661.
- Schechter, N. M., Wang, Z. M., Blacher, R. W., Lessin, S. R., Lazarus, G. S., and Rubin, H. (1994). Determination of the primary structures of human skin chymase and cathepsin G from cutaneous mast cells of urticaria pigmentosa lesions. *J. Immunol.* **152**, 4062–4069.
- Schechter, N. M., Brass, L. F., Lavker, R. M., and Jensen, P. J. (1998). Reaction of mast cell proteases tryptase and chymase with protease activated receptors (PARs) on keratinocytes and fibroblasts. *J. Cell. Physiol.* **176**, 365–373.
- Schick, B., and Austen, K. F. (1986). Rat serosal mast cell degranulation mediated by chymase, an endogenous secretory granule protease: Active site-dependent initiation at 1 degree C. *J. Immunol.* **136**, 3812–3818.
- Schick, B., Austen, K. F., and Schwartz, L. B. (1984). Activation of rat serosal mast cells by chymase, an endogenous secretory granule protease. *J. Immunol.* **132**, 2571–2577.
- Schick, C., Kamachi, Y., Bartuski, A. J., Cataltepe, S., Schechter, N. M., Pemberton, P. A., and Silverman, G. A. (1997). Squamous cell carcinoma antigen 2 is a novel serpin that inhibits the chymotrypsin-like proteinases cathepsin G and mast cell chymase. *J. Biol. Chem.* **272**, 1849–1855.
- Schiemann, F., Grimm, T. A., Hoch, J., Gross, R., Lindner, B., Petersen, F., Bulfone-Paus, S., and Brandt, E. (2006). Mast cells and neutrophils proteolytically activate chemokine precursor CTAP-III and are subject to counterregulation by PF-4 through inhibition of chymase and cathepsin G. *Blood* **107**, 2234–2242.
- Schoenberger, O. L., Sprows, J. L., Schechter, N. M., Cooperman, B. S., and Rubin, H. (1989). Limited proteolysis of C1-inhibitor by chymotrypsin-like proteinases. *FEBS Lett.* **259**, 165–167.
- Schwartz, L. B., and Austen, K. F. (1980). Enzymes of the mast cell granule. *J. Invest. Dermatol.* **74**, 349–353.
- Schwartz, L. B., and Bradford, T. R. (1986). Regulation of tryptase from human lung mast cells by heparin. Stabilization of the active tetramer. *J. Biol. Chem.* **261**, 7372–7379.

- Schwartz, L. B., Lewis, R. A., and Austen, K. F. (1981a). Trypsinase from human pulmonary mast cells. Purification and characterization. *J. Biol. Chem.* **256**, 11939–11943.
- Schwartz, L. B., Riedel, C., Caulfield, J. P., Wasserman, S. I., and Austen, K. F. (1981b). Cell association of complexes of chymase, heparin proteoglycan, and protein after degranulation by rat mast cells. *J. Immunol.* **126**, 2071–2078.
- Schwartz, L. B., Bradford, T. R., Littman, B. H., and Wintroub, B. U. (1985). The fibrinogenolytic activity of purified trypsinase from human lung mast cells. *J. Immunol.* **135**, 2762–2767.
- Schwartz, L. B., Irani, A. M., Roller, K., Castells, M. C., and Schechter, N. M. (1987). Quantitation of histamine, trypsinase, and chymase in dispersed human T and TC mast cells. *J. Immunol.* **138**, 2611–2615.
- Scudamore, C. L., Thornton, E. M., McMillan, L., Newlands, G. F., and Miller, H. R. (1995). 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.* **182**, 1871–1881.
- Scudamore, C. L., Jepson, M. A., Hirst, B. H., and Miller, H. R. (1998). The rat mucosal mast cell chymase, RMCP-II, alters epithelial cell monolayer permeability in association with altered distribution of the tight junction proteins ZO-1 and occludin. *Eur. J. Cell Biol.* **75**, 321–330.
- Secor, V. H., Secor, W. E., Gutekunst, C. A., and Brown, M. A. (2000). Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. *J. Exp. Med.* **191**, 813–822.
- Sendo, T., Itoh, Y., Goromaru, T., Sumimura, T., Saito, M., Aki, K., Yano, T., and Oishi, R. (2003). A potent trypsinase inhibitor nafamostat mesilate dramatically suppressed pulmonary dysfunction induced in rats by a radiographic contrast medium. *Br. J. Pharmacol.* **138**, 959–967.
- Serafin, W. E., Katz, H. R., Austen, K. F., and Stevens, R. L. (1986). Complexes of heparin proteoglycans, chondroitin sulfate E proteoglycans, and [3H]diisopropyl fluorophosphate-binding proteins are exocytosed from activated mouse bone marrow-derived mast cells. *J. Biol. Chem.* **261**, 15017–15021.
- Serafin, W. E., Dayton, E. T., Gravalles, P. M., Austen, K. F., and Stevens, R. L. (1987). Carboxypeptidase A in mouse mast cells. Identification, characterization, and use as a differentiation marker. *J. Immunol.* **139**, 3771–3776.
- Serafin, W. E., Reynolds, D. S., Rogelj, S., Lane, W. S., Conder, G. A., Johnson, S. S., Austen, K. F., and Stevens, R. L. (1990). Identification and molecular cloning of a novel mouse mucosal mast cell serine protease. *J. Biol. Chem.* **265**, 423–429.
- Serafin, W. E., Sullivan, T. P., Conder, G. A., Ebrahimi, A., Marcham, P., Johnson, S. S., Austen, K. F., and Reynolds, D. S. (1991). Cloning of the cDNA and gene for mouse mast cell protease 4. Demonstration of its late transcription in mast cell subclasses and analysis of its homology to subclass-specific neutral proteases of the mouse and rat. *J. Biol. Chem.* **266**, 1934–1941.
- Sheehan, J. P., and Sadler, J. E. (1994). Molecular mapping of the heparin-binding exosite of thrombin. *Proc. Natl. Acad. Sci. USA* **91**, 5518–5522.
- Shimatani, T., Hosotani, N., Ohnishi, M., Kumagai, K., and Saji, I. (2006). SPF-32629 A and B, novel human chymase inhibitors produced by *Penicillium* sp. *J. Antibiot. (Tokyo)* **59**, 29–34.
- Shiota, N., Kakizoe, E., Shimoura, K., Tanaka, T., and Okunishi, H. (2005). Effect of mast cell chymase inhibitor on the development of scleroderma in tight-skin mice. *Br. J. Pharmacol.* **145**, 424–431.
- Silverman, G. A., Whisstock, J. C., Askew, D. J., Pak, S. C., Luke, C. J., Cataltepe, S., Irving, J. A., and Bird, P. I. (2004). Human clade B serpins (ov-serpins) belong to a cohort of evolutionarily dispersed intracellular proteinase inhibitor clades that protect cells from promiscuous proteolysis. *Cell. Mol. Life Sci.* **61**, 301–325.

- Slusarchyk, W. A., Bolton, S. A., Hartl, K. S., Huang, M. H., Jacobs, G., Meng, W., Ogletree, M. L., Pi, Z., Schumacher, W. A., Seiler, S. M., Sutton, J. C., Treuner, U., *et al.* (2002). Synthesis of potent and highly selective inhibitors of human tryptase. *Bioorg. Med. Chem. Lett.* **12**, 3235–3238.
- Smith, T. J., Hougland, M. W., and Johnson, D. A. (1984). Human lung tryptase. Purification and characterization. *J. Biol. Chem.* **259**, 11046–11051.
- Soga, Y., Takai, S., Koyama, T., Okamoto, Y., Ikeda, T., Nishimura, K., Miyazaki, M., and Komeda, M. (2004). Attenuation of adhesion formation after cardiac surgery with a chymase inhibitor in a hamster model. *J. Thorac. Cardiovasc. Surg.* **127**, 72–78.
- Solivan, S., Selwood, T., Wang, Z. M., and Schechter, N. M. (2002). Evidence for diversity of substrate specificity among members of the chymase family of serine proteases. *FEBS Lett.* **512**, 133–138.
- Somasundaram, P., Ren, G., Nagar, H., Kraemer, D., Mendoza, L., Michael, L. H., Caughey, G. H., Entman, M. L., and Frangogiannis, N. G. (2005). Mast cell tryptase may modulate endothelial cell phenotype in healing myocardial infarcts. *J. Pathol.* **205**, 102–111.
- Sommerhoff, C. P., Caughey, G. H., Finkbeiner, W. E., Lazarus, S. C., Basbaum, C. B., and Nadel, J. A. (1989). Mast cell chymase. A potent secretagogue for airway gland serous cells. *J. Immunol.* **142**, 2450–2456.
- Sommerhoff, C. P., Bode, W., Matschiner, G., Bergner, A., and Fritz, H. (2000). The human mast cell tryptase tetramer: A fascinating riddle solved by structure. *Biochim. Biophys. Acta* **1477**, 75–89.
- Soto, D., Malmsten, C., Blount, J. L., Muilenburg, D. J., and Caughey, G. H. (2002). Genetic deficiency of human mast cell alpha-tryptase. *Clin. Exp. Allergy* **32**, 1000–1006.
- Springman, E. B., Dikov, M. M., and Serafin, W. E. (1995). Mast cell procarboxypeptidase A. Molecular modeling and biochemical characterization of its processing within secretory granules. *J. Biol. Chem.* **270**, 1300–1307.
- Stack, M. S., and Johnson, D. A. (1994). Human mast cell tryptase activates single-chain urinary-type plasminogen activator (pro-urokinase). *J. Biol. Chem.* **269**, 9416–9419.
- Stevens, R. L., Qui, D., McNeil, H. P., Friend, D. S., Hunt, J. E., Austen, K. F., and Zhang, J. (1996). Transgenic mice that possess a disrupted mast cell protease 5 (mMCP-5) gene cannot store carboxypeptidase A (mMC-CPA) protein in their granules. *FASEB J.* **10**, 17772.
- Strik, M. C., Wolbink, A., Wouters, D., Bladergroen, B. A., Verlaan, A. R., van Houdt, I. S., Hijlkema, S., Hack, C. E., and Kummer, J. A. (2004). Intracellular serpin SERPINB6 (PI6) is abundantly expressed by human mast cells and forms complexes with beta-tryptase monomers. *Blood* **103**, 2710–2717.
- Sugimoto, M., Furuta, T., Shirai, N., Ikuma, M., Sugimura, H., and Hishida, A. (2006). Influences of chymase and angiotensin I-converting enzyme gene polymorphisms on gastric cancer risks in Japan. *Cancer Epidemiol. Biomarkers Prev.* **15**, 1929–1934.
- Sutton, J. C., Bolton, S. A., Hartl, K. S., Huang, M. H., Jacobs, G., Meng, W., Ogletree, M. L., Pi, Z., Schumacher, W. A., Seiler, S. M., Slusarchyk, W. A., Treuner, U., *et al.* (2002). Synthesis and SAR of 4-carboxy-2-azetidinone mechanism-based tryptase inhibitors. *Bioorg. Med. Chem. Lett.* **12**, 3229–3233.
- Sylvén, H., Dahlback, M., Van Der Ploeg, I., and Alving, K. (2002). The tryptase inhibitor APC-366 reduces the acute airway response to allergen in pigs sensitized to *Ascaris suum*. *Clin. Exp. Allergy* **32**, 967–971.
- Taipale, J., Lohi, J., Saarinen, J., Kovanen, P. T., and Keski-Oja, J. (1995). Human mast cell chymase and leukocyte elastase release latent transforming growth factor-beta 1 from the extracellular matrix of cultured human epithelial and endothelial cells. *J. Biol. Chem.* **270**, 4689–4696.

- Takai, S., Shiota, N., Jin, D., and Miyazaki, M. (1998). Chymase processes big-endothelin-2 to endothelin-2-(1-31) that induces contractile responses in the isolated monkey trachea. *Eur. J. Pharmacol.* **358**, 229–233.
- Takai, S., Jin, D., Nishimoto, M., Yuda, A., Sakaguchi, M., Kamoshita, K., Ishida, K., Sukenaga, Y., Sasaki, S., and Miyazaki, M. (2001a). Oral administration of a specific chymase inhibitor, NK3201, inhibits vascular proliferation in grafted vein. *Life Sci.* **69**, 1725–1732.
- Takai, S., Jin, D., Sakaguchi, M., Kirimura, K., and Miyazaki, M. (2001b). An orally active chymase inhibitor, BCEAB, suppresses heart chymase activity in the hamster. *Jpn. J. Pharmacol.* **86**, 124–126.
- Takai, S., Jin, D., Sakaguchi, M., Katayama, S., Muramatsu, M., Matsumura, E., Kim, S., and Miyazaki, M. (2003a). A novel chymase inhibitor, 4-[1-([bis-(4-methyl-phenyl)-methyl]-carbamoyl)3-(2-ethoxy-benzyl)-4-oxo-a zetidine-2-yloxy]-benzoic acid (BCEAB), suppressed cardiac fibrosis in cardiomyopathic hamsters. *J. Pharmacol. Exp. Ther.* **305**, 17–23.
- Takai, S., Sakonjo, H., Fukuda, K., Jin, D., Sakaguchi, M., Kamoshita, K., Ishida, K., Sukenaga, Y., and Miyazaki, M. (2003b). A novel chymase inhibitor, 2-(5-formylamino-6-oxo-2-phenyl-1,6-dihydropyrimidine-1-yl)-N-[[4-dioxo-1 -phenyl-7-(2-pyridyloxy)]2-heptyl]acetamide (NK3201), suppressed intimal hyperplasia after balloon injury. *J. Pharmacol. Exp. Ther.* **304**, 841–844.
- Takai, S., Jin, D., Muramatsu, M., Okamoto, Y., and Miyazaki, M. (2004a). Therapeutic applications of chymase inhibitors in cardiovascular diseases and fibrosis. *Eur. J. Pharmacol.* **501**, 1–8.
- Takai, S., Jin, D., Sakaguchi, M., and Miyazaki, M. (2004b). A single treatment with a specific chymase inhibitor, TY-51184, prevents vascular proliferation in canine grafted veins. *J. Pharmacol. Sci.* **94**, 443–448.
- Tam, E. K., and Caughey, G. H. (1990). Degradation of airway neuropeptides by human lung tryptase. *Am. J. Respir. Cell Mol. Biol.* **3**, 27–32.
- Tanaka, K., Sugiura, H., Uehara, M., Sato, H., Hashimoto-Tamaoki, T., and Furuyama, J. (1999). Association between mast cell chymase genotype and atopic eczema: Comparison between patients with atopic eczema alone and those with atopic eczema and atopic respiratory disease. *Clin. Exp. Allergy* **29**, 800–803.
- Tani, K., Ogushi, F., Kido, H., Kawano, T., Kunori, Y., Kamimura, T., Cui, P., and Sone, S. (2000). Chymase is a potent chemoattractant for human monocytes and neutrophils. *J. Leukoc. Biol.* **67**, 585–589.
- Tchougounova, E., and Pejler, G. (2001). Regulation of extravascular coagulation and fibrinolysis by heparin-dependent mast cell chymase. *FASEB J.* **15**, 2763–2765.
- Tchougounova, E., Forsberg, E., Angelborg, G., Kjellen, L., and Pejler, G. (2001). Altered processing of fibronectin in mice lacking heparin. a role for heparin-dependent mast cell chymase in fibronectin degradation. *J. Biol. Chem.* **276**, 3772–3777.
- Tchougounova, E., Pejler, G., and Åbrink, M. (2003). 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.* **198**, 423–431.
- Tchougounova, E., Lundequist, A., Fajardo, I., Winberg, J. O., Åbrink, M., and Pejler, G. (2005). A key role for mast cell chymase in the activation of pro-matrix metalloprotease-9 and pro-matrix metalloprotease-2. *J. Biol. Chem.* **280**, 9291–9296.
- Temkin, V., Kantor, B., Weg, V., Hartman, M. L., and Levi-Schaffer, F. (2002). Tryptase activates the mitogen-activated protein kinase/activator protein-1 pathway in human peripheral blood eosinophils, causing cytokine production and release. *J. Immunol.* **169**, 2662–2669.

- Terakawa, M., Tomimori, Y., Goto, M., Hayashi, Y., Oikawa, S., and Fukuda, Y. (2005). Eosinophil migration induced by mast cell chymase is mediated by extracellular signal-regulated kinase pathway. *Biochem. Biophys. Res. Commun.* **332**, 969–975.
- Terakawa, M., Tomimori, Y., Goto, M., and Fukuda, Y. (2006). Mast cell chymase induces expression of chemokines for neutrophils in eosinophilic EoL-1 cells and mouse peritonitis eosinophils. *Eur. J. Pharmacol.* **538**, 175–181.
- Tetlow, L. C., and Woolley, D. E. (1995). Distribution, activation and tryptase/chymase phenotype of mast cells in the rheumatoid lesion. *Ann. Rheum. Dis.* **54**, 549–555.
- Tomimori, Y., Muto, T., Fukami, H., Saito, K., Horikawa, C., Tsuruoka, N., Saito, M., Sugiura, N., Yamashiro, K., Sumida, M., Kakutani, S., and Fukuda, Y. (2002a). Chymase participates in chronic dermatitis by inducing eosinophil infiltration. *Lab. Invest.* **82**, 789–794.
- Tomimori, Y., Tsuruoka, N., Fukami, H., Saito, K., Horikawa, C., Saito, M., Muto, T., Sugiura, N., Yamashiro, K., Sumida, M., Kakutani, S., and Fukuda, Y. (2002b). Role of mast cell chymase in allergen-induced biphasic skin reaction. *Biochem. Pharmacol.* **64**, 1187.
- Tomimori, Y., Muto, T., Saito, K., Tanaka, T., Maruoka, H., Sumida, M., Fukami, H., and Fukuda, Y. (2003). Involvement of mast cell chymase in bleomycin-induced pulmonary fibrosis in mice. *Eur. J. Pharmacol.* **478**, 179–185.
- Toomes, C., James, J., Wood, A. J., Wu, C. L., McCormick, D., Lench, N., Hewitt, C., Moynihan, L., Roberts, E., Woods, C. G., Markham, A., Wong, M., *et al.* (1999). Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. *Nat. Genet.* **23**, 421–424.
- Tremaine, W. J., Brzezinski, A., Katz, J. A., Wolf, D. C., Fleming, T. J., Mordenti, J., Strenkoski-Nix, L. C., and Kurth, M. C. (2002). Treatment of mildly to moderately active ulcerative colitis with a tryptase inhibitor (APC 2059): An open-label pilot study. *Aliment. Pharmacol. Ther.* **16**, 407–413.
- Trong, H. L., Newlands, G. F., Miller, H. R., Charbonneau, H., Neurath, H., and Woodbury, R. G. (1989). Amino acid sequence of a mouse mucosal mast cell protease. *Biochemistry* **28**, 391–395.
- Tsunemi, K., Takai, S., Nishimoto, M., Yuda, A., Hasegawa, S., Sawada, Y., Fukumoto, H., Sasaki, S., and Miyazaki, M. (2002). Possible roles of angiotensin II-forming enzymes, angiotensin converting enzyme and chymase-like enzyme, in the human aneurysmal aorta. *Hypertens. Res.* **25**, 817–822.
- Tsunemi, K., Takai, S., Nishimoto, M., Jin, D., Sakaguchi, M., Muramatsu, M., Yuda, A., Sasaki, S., and Miyazaki, M. (2004). A specific chymase inhibitor, 2-(5-formylamino-6-oxo-2-phenyl-1,6-dihydropyrimidine-1-yl)-N-[[3,4-dioxo-1-phenyl-7-(2-pyridyloxy)]-2-heptyl]acetamide (NK3201), suppresses development of abdominal aortic aneurysm in hamsters. *J. Pharmacol. Exp. Ther.* **309**, 879–883.
- Uehara, Y., Urata, H., Ideishi, M., Arakawa, K., and Saku, K. (2002). Chymase inhibition suppresses high-cholesterol diet-induced lipid accumulation in the hamster aorta. *Cardiovasc. Res.* **55**, 870–876.
- Ui, H., Andoh, T., Lee, J. B., Nojima, H., and Kuraishi, Y. (2006). 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.* **530**, 172–178.
- Ulrich, P. N., and Boon, J. K. (2001). The histological localization of heparin in the northern quahog clam, *Mercenaria mercenaria*. *J. Invertebr. Pathol.* **78**, 155–159.
- Urata, H., Kinoshita, A., Misono, K. S., Bumpus, F. M., and Husain, A. (1990). Identification of a highly specific chymase as the major angiotensin II-forming enzyme in the human heart [published erratum appears in *J. Biol. Chem.* (1991 Jun 25) **266**(18), 12114]. *J. Biol. Chem.* **265**, 22348–22357.

- Urata, H., Kinoshita, A., Perez, D. M., Misono, K. S., Bumpus, F. M., Graham, R. M., and Husain, A. (1991). Cloning of the gene and cDNA for human heart chymase. *J. Biol. Chem.* **266**, 17173–17179.
- Uratani, Y., Takiguchi-Hayashi, K., Miyasaka, N., Sato, M., Jin, M., and Arimatsu, Y. (2000). Latexin, a carboxypeptidase A inhibitor, is expressed in rat peritoneal mast cells and is associated with granular structures distinct from secretory granules and lysosomes. *Biochem. J.* **346**(Pt. 3), 817–826.
- Vanderslice, P., Craik, C. S., Nadel, J. A., and Caughey, G. H. (1989). Molecular cloning of dog mast cell tryptase and a related protease: Structural evidence of a unique mode of serine protease activation. *Biochemistry* **28**, 4148–4155.
- Vanderslice, P., Ballinger, S. M., Tam, E. K., Goldstein, S. M., Craik, C. S., and Caughey, G. H. (1990). Human mast cell tryptase: Multiple cDNAs and genes reveal a multigene serine protease family. *Proc. Natl. Acad. Sci. USA* **87**, 3811–3815.
- Vartio, T., Seppa, H., and Vaheri, A. (1981). Susceptibility of soluble and matrix fibronectins to degradation by tissue proteinases, mast cell chymase and cathepsin G. *J. Biol. Chem.* **256**, 471–477.
- Vliagoftis, H., Lacy, P., Luy, B., Adamko, D., Hollenberg, M., Befus, D., and Moqbel, R. (2004). Mast cell tryptase activates peripheral blood eosinophils to release granule-associated enzymes. *Int. Arch. Allergy Immunol.* **135**, 196–204.
- von Heijne, G. (1984). How signal sequences maintain cleavage specificity. *J. Mol. Biol.* **173**, 243–251.
- Walter, M., Plotnick, M., and Schechter, N. M. (1996). Inhibition of human mast cell chymase by secretory leukocyte proteinase inhibitor: Enhancement of the interaction by heparin. *Arch. Biochem. Biophys.* **327**, 81–88.
- Walter, M., Sutton, R. M., and Schechter, N. M. (1999). Highly Efficient Inhibition of Human Chymase by alpha(2)-Macroglobulin. *Arch. Biochem. Biophys.* **368**, 276–284.
- Wang, H. W., McNeil, H. P., Husain, A., Liu, K., Tedla, N., Thomas, P. S., Raftery, M., King, G. C., Cai, Z. Y., and Hunt, J. E. (2002). Delta tryptase is expressed in multiple human tissues, and a recombinant form has proteolytic activity. *J. Immunol.* **169**, 5145–5152.
- Wang, Y., Shiota, N., Leskinen, M. J., Lindstedt, K. A., and Kovanen, P. T. (2001). Mast cell chymase inhibits smooth muscle cell growth and collagen expression *in vitro*: Transforming growth factor-beta1-dependent and -independent effects. *Arterioscler. Thromb. Vasc. Biol.* **21**, 1928–1933.
- Wastling, J. M., Scudamore, C. L., Thornton, E. M., Newlands, G. F., and Miller, H. R. (1997). Constitutive expression of mouse mast cell protease-1 in normal BALB/c mice and its up-regulation during intestinal nematode infection. *Immunology* **90**, 308–313.
- Wastling, J. M., Knight, P., Ure, J., Wright, S., Thornton, E. M., Scudamore, C. L., Mason, J., Smith, A., and Miller, H. R. (1998). Histochemical and ultrastructural modification of mucosal mast cell granules in parasitized mice lacking the beta-chymase, mouse mast cell protease-1. *Am. J. Pathol.* **153**, 491–504.
- Watanabe, N., Miura, K., and Fukuda, Y. (2002a). Chymase inhibitor ameliorates eosinophilia in mice infected with *Nippostrongylus brasiliensis*. *Int. Arch. Allergy Immunol.* **128**, 235–239.
- Watanabe, N., Tomimori, Y., Saito, K., Miura, K., Wada, A., Tsudzuki, M., and Fukuda, Y. (2002b). Chymase inhibitor improves dermatitis in NC/Nga mice. *Int. Arch. Allergy Immunol.* **128**, 229–234.
- Wei, C. C., Tian, B., Perry, G., Meng, Q. C., Chen, Y. F., Oparil, S., and Dell'Italia, L. J. (2002). Differential ANG II generation in plasma and tissue of mice with decreased expression of the ACE gene. *Am. J. Physiol. Heart Circ. Physiol.* **282**, H2254–H2258.

- Weidinger, S., Mayerhofer, A., Kunz, L., Albrecht, M., Sbornik, M., Wunn, E., Hollweck, R., Ring, J., and Kohn, F. M. (2005a). Trypsin inhibits motility of human spermatozoa mainly by activation of the mitogen-activated protein kinase pathway. *Hum. Reprod.* **20**, 456–461.
- Weidinger, S., Rummeler, L., Klopp, N., Wagenpfeil, S., Baurecht, H. J., Fischer, G., Holle, R., Gauger, A., Schafer, T., Jakob, T., Ollert, M., Behrendt, H., *et al.* (2005b). Association study of mast cell chymase polymorphisms with atopy. *Allergy* **60**, 1256–1261.
- Wernersson, S., Reimer, J. M., Poorafshar, M., Karlson, U., Wermenstam, N., Bengten, E., Wilson, M., Pilstrom, L., and Hellman, L. (2006). Granzyme-like sequences in bony fish shed light on the emergence of hematopoietic serine proteases during vertebrate evolution. *Dev. Comp. Immunol.* **30**, 901–918.
- Whitaker-Menezes, D., Schechter, N. M., and Murphy, G. F. (1995). Serine proteinases are regionally segregated within mast cell granules. *Lab. Invest.* **72**, 34–41.
- Wolny, A., Clozel, J. P., Rein, J., Mory, P., Vogt, P., Turino, M., Kiowski, W., and Fischli, W. (1997). Functional and biochemical analysis of angiotensin II-forming pathways in the human heart. *Circ. Res.* **80**, 219–227.
- Wolters, P. J., Laig-Webster, M., and Caughey, G. H. (2000). Dipeptidyl peptidase I cleaves matrix-associated proteins and is expressed mainly by mast cells in normal dog airways. *Am. J. Respir. Cell Mol. Biol.* **22**, 183–190.
- Wolters, P. J., Pham, C. T., Muilenburg, D. J., Ley, T. J., and Caughey, G. H. (2001). Dipeptidyl peptidase I is essential for activation of mast cell chymases, but not tryptases, in mice. *J. Biol. Chem.* **276**, 18551–18556.
- Wong, G. W., Tang, Y., Feyfant, E., Sali, A., Li, L., Li, Y., Huang, C., Friend, D. S., Krilis, S. A., and Stevens, R. L. (1999). 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.* **274**, 30784–30793.
- Wong, G. W., Foster, P. S., Yasuda, S., Qi, J. C., Mahalingam, S., Mellor, E. A., Katsoulotos, G., Li, L., Boyce, J. A., Krilis, S. A., and Stevens, R. L. (2002). 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 alpha/signal transducer and activator of transcription (STAT) 6-dependent pathway. *J. Biol. Chem.* **277**, 41906–41915.
- Wong, G. W., Yasuda, S., Morokawa, N., Li, L., and Stevens, R. L. (2004). Mouse chromosome 17A3.3 contains 13 genes that encode functional tryptic-like serine proteases with distinct tissue and cell expression patterns. *J. Biol. Chem.* **279**, 2438–2452.
- Wright, C. D., Havill, A. M., Middleton, S. C., Kashem, M. A., Dripps, D. J., Abraham, W. M., Thomson, D. S., and Burgess, L. E. (1999). 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.* **58**, 1989–1996.
- Wypij, D. M., Nichols, J. S., Novak, P. J., Stacy, D. L., Berman, J., and Wiseman, J. S. (1992). Role of mast cell chymase in the extracellular processing of big- endothelin-1 to endothelin-1 in the perfused rat lung. *Biochem. Pharmacol.* **43**, 845–853.
- Xia, H. Z., Zweiman, B., and Schwartz, L. B. (1997). Levels of tryptase, chymase, and Fc epsilon RI alpha messenger RNA in human skin are unchanged after IgE-dependent stimulation of cutaneous mast cells *in vivo*. *J. Allergy Clin. Immunol.* **99**, 224–226.
- Xia, Z., Ghildyal, N., Austen, K. F., and Stevens, R. L. (1996). Post-transcriptional regulation of chymase expression in mast cells. A cytokine-dependent mechanism for controlling the expression of granule neutral proteases of hematopoietic cells. *J. Biol. Chem.* **271**, 8747–8753.

- Yoshii, M., Jikuhara, A., Mori, S., Iwagaki, H., Takahashi, H. K., Nishibori, M., and Tanaka, N. (2005). Mast cell tryptase stimulates DLD-1 carcinoma through prostaglandin- and MAP kinase-dependent manners. *J. Pharmacol. Sci.* **98**, 450–458.
- Yoshikawa, T., Imada, T., Nakakubo, H., Nakamura, N., and Naito, K. (2001). Rat mast cell protease-I enhances immunoglobulin E production by mouse B cells stimulated with interleukin-4. *Immunology* **104**, 333–340.
- Yu, M., Tsai, M., Tam, S. Y., Jones, C., Zehnder, J., and Galli, S. J. (2006). Mast cells can promote the development of multiple features of chronic asthma in mice. *J. Clin. Invest.* **116**, 1633–1641.
- Zhao, W., Oskeritzian, C. A., Pozez, A. L., and Schwartz, L. B. (2005). Cytokine production by skin-derived mast cells: Endogenous proteases are responsible for degradation of cytokines. *J. Immunol.* **175**, 2635–2642.