

Novel Insight into the *in vivo* Function of Mast Cell Chymase: Lessons from Knockouts and Inhibitors

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Abstract

Mast cells are now recognized as key players in diverse pathologies, but the mechanisms by which they contribute in such settings are only partially understood. Mast cells are packed with secretory granules, and when they undergo degranulation in response to activation the contents of the granules are expelled to the extracellular milieu. Chymases, neutral serine proteases, are the major constituents of the mast cell granules and are hence released in large amounts upon mast cell activation. Following their release, chymases can cleave one or several of a myriad of potential substrates, and the cleavage of many of these could potentially have a profound impact on the respective pathology. Indeed, chymases have recently been implicated in several pathological contexts, in particular through studies using chymase inhibitors and by the use of chymase-deficient animals. In many cases, chymase has been shown to account for mast cell-dependent detrimental effects in the respective conditions and is therefore emerging as a promising drug target. On the

other hand, chymase has been shown to have protective roles in other pathological settings. More unexpectedly, chymase has also been shown to control certain homeostatic processes. Here, these findings are reviewed.

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Introduction

Mast cells are currently emerging as key actors in many types of immune responses, having either beneficial or detrimental activities depending on the particular setting. Undoubtedly, mast cells are mostly well known for their harmful effects in allergic reactions, but detrimental activities of mast cells have also been reported in other settings, including arthritis, dermatitis, obesity, atherosclerosis, abdominal aortic aneurysms and cancer [reviewed in 1–4]. However, mast cells are also well recognized for their protective functions in the immunity towards a variety of pathogens, including bacteria, viruses and parasites [5–7].

Based on this development, it is important to investigate the mechanisms behind the contribution of mast

Table 1. Mast cell chymases in humans and mice

Chymase	Chymase family	Enzymatic activity	MC subclass	Interaction with serglycin	Knockout	Chromosomal location
<i>Human</i>						
CMA1	α	chymotrypsin-like	MC _{TC}	+	– ^a	14q12
<i>Mouse</i>						
Mcpt1 (mMCP1)	β	chymotrypsin-like	MMC	–	Wastling et al. [21]	14 C3; 14 28.19 cM
Mcpt2 (mMCP2)	β	non-active	MMC	(+)	not available	14 C3; 14 28.19 cM
Mcpt4 (mMCP4)	β	chymotrypsin-like	CTMC	+	Tchougounova et al. [23]	14 C3; 14 28.19 cM
Mcpt5 (mMCP5)	α	elastase-like	CTMC	+	Abonia et al. [22]	14 C3; 14 28.19 cM
Mcpt9 (mMCP9)	β	chymotrypsin-like	uterine-specific (CTMC)	+(predicted)	not available	14 C3; 14 28.19 cM

MC, mast cell; CMA1, MC_{TC}, subtype also expressing tryptase and carboxypeptidase A3; MMC, mucosal mast cell; CTMC, connective tissue mast cell. ^a Humans with altered chymase expression have not been identified.

cells to these diverse pathological settings, and, indeed, this is a major current focus for many laboratories worldwide. When mast cells are activated, which can be accomplished by IgE-mediated and a range of other mechanisms [4], they may respond by degranulation. This causes a massive release of the preformed mediators that are stored within the mast cell secretory granules [8]. Clearly, one mode by which mast cells could influence a given inflammatory condition is by effects attributed to these released compounds, a notion that is gaining support from recent research [8].

The preformed mast cell mediators include biogenic amines (histamine, serotonin), serglycin proteoglycan, certain preformed cytokines (e.g., tumor necrosis factor, TNF) and a number of mast cell-specific proteases, the latter encompassing chymases, tryptases and carboxypeptidase A3 [8–10]. However, activation of mast cells does not necessarily lead to degranulation, as evidenced by the ability of mast cells to release numerous inflammatory mediators under circumstances where degranulation is not evident [4]. It should also be emphasized that several of the compounds released by activated mast cells are synthesized *de novo*, rather than being released from preformed pools [4].

Chymases belong to the large family of serine proteases, and their expression is essentially unique to mast cells [11–13]. Mast cells can express high levels of chymase-encoding mRNA, previous findings having re-

vealed that up to 2.5% of the total mRNA pool in mast cells can code for chymases [13, 14]. Notably, chymase expression in mast cells is constitutive and in most cases not affected to any major extent by mast cell activation [10]. The high expression of chymase mRNA is also reflected at the protein level, where it has been calculated that chymases can account for up to 25% of the total cellular protein of mast cells [15, 16].

For many years, the knowledge of the *in vivo* function of chymase was quite rudimentary, mainly based on data from *in vitro* experiments. However, the generation of chymase-deficient animals and the development of selective chymase inhibitors have opened up possibilities for investigations of the *in vivo* function of mast cell chymase. By using these tools, important insight into the biological function of chymase has been obtained and has revealed an important role for chymase in modulating a diverse array of pathological but also homeostatic conditions. Here, these findings are reviewed.

Chymases and Chymase Knockout Strains

In humans, only one mast cell chymase gene is expressed (CMA1; classified as an α -chymase), located on chromosome 14 (Table 1). In contrast, the corresponding chymase locus on chromosome 14 in mice has un-

dergone extensive expansion, and encompasses 5 chymase genes: Mcpt1 (previously denoted mMCP1; classified as a β -chymase), Mcpt2 (mMCP2; β -chymase), Mcpt4 (mMCP4; β -chymase), Mcpt5/Cma1 (mMCP5; α -chymase) and Mcpt9 (mMCP9; β -chymase) [10]. The chymase expression profile differs among mast cell subtypes. In humans, chymase is expressed by the MC_{TC} subtype (also expressing tryptase and carboxypeptidase A3), which are predominant in skin but also found in many other locations. In the mouse, mast cells are generally divided into connective tissue- (CTMC) and mucosal (MMC)-type mast cells, of which CTMCs are found in skin and various other locations such as intestinal submucosa, tongue and trachea, whereas MMCs are predominant in the intestinal mucosa. CTMCs and MMCs differ with regard to chymase expression, with CTMCs expressing Mcpt4 and Mcpt5, whereas MMCs express Mcpt1 and Mcpt2. However, mixed phenotypes have been identified, e.g. in the lung, in which mast cells can express both CTMC and MMC chymases [17].

Mcpt4 and Mcpt5 are both strongly dependent on electrostatic interactions with sulfated (thereby negatively charged) serglycin proteoglycans for storage in mast cell granules, as shown by a major reduction in the respective proteins (but not mRNA) in serglycin^{-/-} mast cells [18]. Also, the lack of NDST2, an enzyme that is essential for the sulfation of serglycin proteoglycans, causes a strong reduction in the storage of Mcpt4 and Mcpt5 [19]. In contrast, Mcpt1 storage is independent of serglycin, whereas Mcpt2 storage is partly serglycin-dependent [20].

Based on amino acid sequence similarities, Mcpt5 may be regarded as the homologue to human chymase (both are classified as α -chymases). However, Mcpt5 and human chymase have fundamentally divergent substrate cleavage profiles (see “Enzymatic properties of chymases”). For this reason, Mcpt5 is most likely not the functional counterpart to human chymase. Of the remaining murine chymases, Mcpt4 has a similar substrate cleavage profile to human chymase (see “Enzymatic properties of chymases”), has a similar tissue distribution and also has similar proteoglycan-binding properties to human chymase. In contrast, Mcpt1, Mcpt2 and Mcpt9 all have different expression patterns as compared with human chymase. Hence, out of the murine chymases, Mcpt4 may be regarded as a close functional homologue to human chymase, and studies on Mcpt4-deficient animals may thus provide particularly important clues to the function of human chymase.

The first reported chymase knockout came from Miller’s group, who reported the targeted deletion of Mcpt1 [21]. Also Mcpt5 [22] and Mcpt4 [23] knockouts have been generated but, to date, knockouts for Mcpt2 and Mcpt9 have not been reported.

Enzymatic Properties of Chymases

Chymases are monomeric serine proteases, i.e., their active sites contain a Ser-His-Asp catalytic triad, of which the serine has a direct role in cleaving the target peptide bond by forming a covalent intermediate. Chymases are endopeptidases, i.e. have the ability to cleave proteins/peptides within the interior of the peptide chains. In terms of cleavage specificity, chymases are chymotrypsin-like and thereby show strong preference for peptide bonds with an aromatic amino acid residue (Phe, Tyr, Trp) on the N-terminal side of the scissile bond (the P1 position) [24]. With regard to the extended substrate cleavage specificities, different chymases have variable preferences. Human chymase (CMA1) has a strong preference for peptide bonds where an acidic residue (Asp, Glu) is located two amino acids C-terminal of the cleavage bond (P2’ position) and has also preference for aliphatic amino acid residues at positions P2–P4 [25]. These preferences are largely shared by Mcpt4 [26], supporting the notion that Mcpt4 represents the functional homologue to human chymase. Mcpt1, in contrast, lacks the preference for an acidic P2’ residue, prefers Phe at the P1 position, shows preference for Ser at P1’, prefers large hydrophobic residues at P2 and aliphatic residues at P3–P4 [27]. Mcpt2 is considered to be enzymatically inactive [28, 29]. Mcpt5, although being structurally more similar to CMA1 than Mcpt4 in terms of amino acid sequence homology, has evolved elastase-like (prefers aliphatic amino acid residues at the P1 position) rather than chymotrypsin-like specificity [30].

With regard to macromolecular substrates, chymase has been shown to cleave large numbers of proteins/peptides, including fibronectin [23, 31, 32], procollagenase [33], pro-MMP9 [34–36], pro-MMP2 [35, 37], IL-6 [38, 39], IL-13 [38], IL-15 [39], IL-33 [39, 40], pro-IL1 β [41], pro-IL-18 [39, 42], TNF [43], CCL6/9/15/23 [44], angiotensin I [45], thrombin [23, 46, 47], latent transforming growth factor β (TGF- β) [48–50], vasoactive intestinal peptide [51], substance P [51], HMGB1 [52], tight junction proteins [37, 53], big-endothelin-1 [54], chemerin [55] and CTAP-III [56] (Fig. 1; see also [57]). However, many of these substrates have been identified through ex-

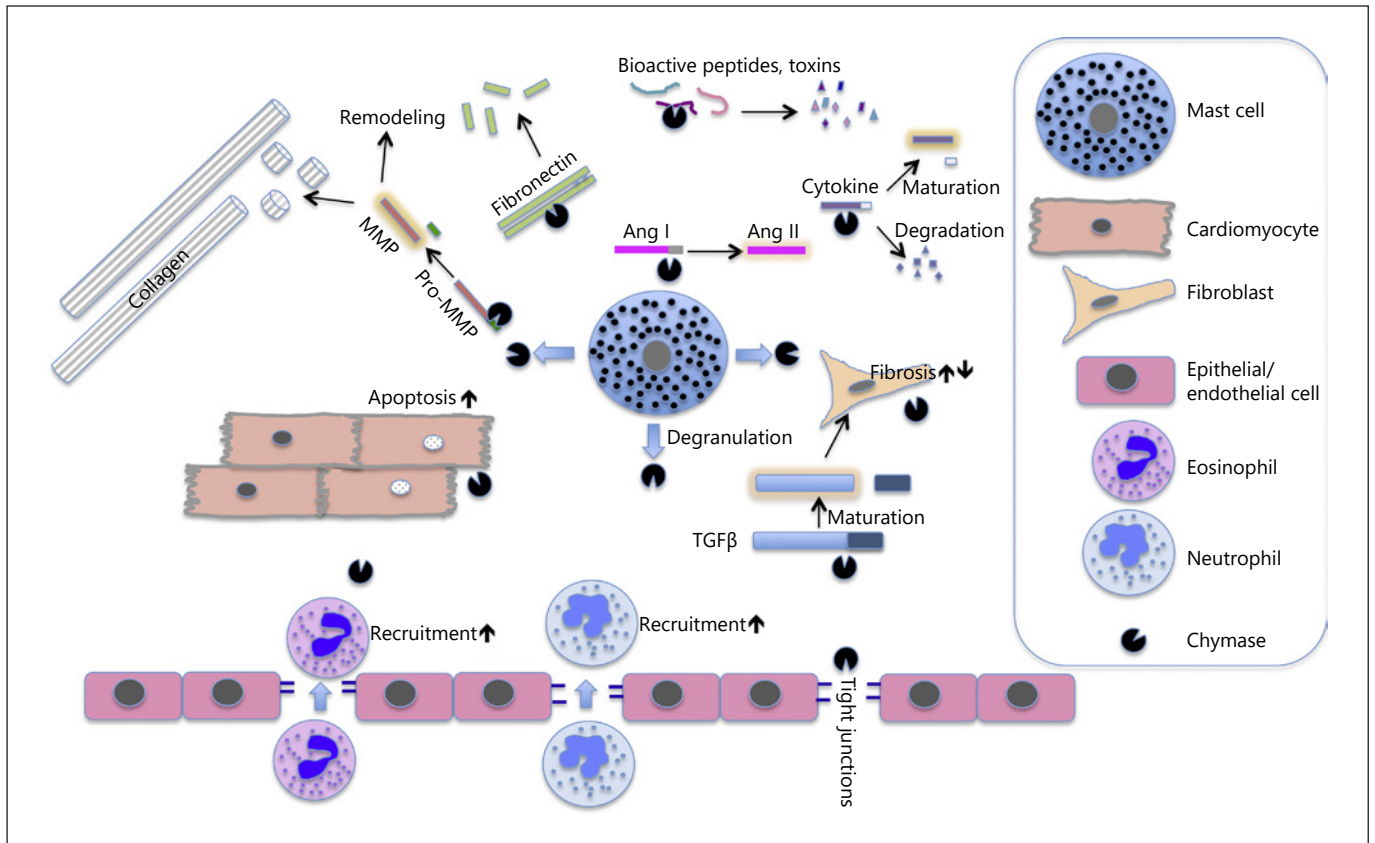


Fig. 1. Examples of mast cell chymase function. MMP, matrix metalloproteinase; TGF- β , transforming growth factor β .

periments in purified systems, and it is not certain whether all of them represent physiological targets for chymase *in vivo*.

Chymase Inhibitors

Several synthetic, selective chymase inhibitors have been developed over the last few decades (Table 2). These have been assessed for effects in various disease models (see below), and, in many cases, promising beneficial effects of such inhibitors have been seen, hence supporting a role for chymase in the respective condition. However, it is important to note that chymase is highly similar to neutrophil cathepsin G in terms of cleavage specificity, and many of the developed chymase inhibitors also target cathepsin G, at least to some extent (Table 2). For several of the used chymase inhibitors, their selectivity over cathepsin G has not been reported (Table 2). Hence, it is not clear under all circumstances whether effects of chymase inhibitors are indeed due to inhibition of chymase, as op-

posed to off-target inhibition of cathepsin G. Moreover, chymase inhibitors are usually developed to target human chymase rather than the corresponding endogenous chymase type expressed by the respective experimental animals. Hence, it is in many cases not clear whether the intended target chymase is in fact efficiently inhibited by the applied inhibitor. These considerations should be taken into account, and some caution should accordingly be taken when interpreting data derived from usage of chymase inhibitors in animal models for disease.

Biological Functions of Chymases: General Considerations

As discussed below, based on studies of chymase knockout animals and on pharmacological chymase inhibition, a role for chymase in a multitude of pathological (and non-pathological) contexts has been identified (Table 3; Fig. 1). At first sight, the range of proposed functions for chymase may seem bewildering. Moreover, it may seem contradic-

Table 2. Chymase inhibitors

Compound	IC ₅₀ /K _i (human chymase)	Selectivity over human cathepsin G, <i>n</i> -fold	Effect on nonhuman chymases	Reference
TY-51469	IC ₅₀ = 7.0 nM	n.r.	IC ₅₀ = 0.4 nM (simian)	98
TY-51184	IC ₅₀ = 37 nM	>2,700	IC ₅₀ = 11 μM (dog)	99
TY-51076	IC ₅₀ = 56 nM	>10,000	n.r.	152
NK3201	IC ₅₀ = 2.5 nM	n.r.	IC ₅₀ = 1.2 nM (dog); IC ₅₀ = 28 nM (hamster)	153
SUN-C8257	IC ₅₀ = 130 nM	18	IC ₅₀ = 890 nM (Mcpt4); IC ₅₀ = 1,200 nM (Mcpt1)	129, 154
SUN-C8077	IC ₅₀ = 360 nM	0.4	IC ₅₀ = 180 nM (Mcpt4)	154, 155
SUN13834	K _i = 160 nM	n.r.	K _i = 63 nM (Mcpt4)	133
JNJ-10311795 (RWJ-355871)	K _i = 2.3 nM	16.5	n.r.	72
BCEAB	IC ₅₀ = 5.4 nM	n.r.	n.r.	156
SPF-32629 A	IC ₅₀ = 0.25 μg/mL	20	n.r.	157
TEI-E548	K _i = 6.2 nM	n.r.	n.r.	102
TEI-F00806	K _i = 6.2 nM	n.r.	K _i = 30.6 nM (hamster)	102
TEI-E00548	n.r.	n.r.	n.r.	94
Y-40613	K _i = 22.6 nM	32	K _i = 3.68 nM (dog); K _i = 103 nM (rat), K _i = 40.4 nM (mouse)	158
ASB17061	IC ₅₀ = 20 nM	1,605	IC ₅₀ = 30 nM (Mcpt4)	112
BAY 1142524 (fulacimstat)	IC ₅₀ = 4 nM	n.r.	IC ₅₀ = 3 nM (hamster)	159
TPC-806	n.r.	n.r.	n.r.	83
RO5066852	IC ₅₀ = 11 nM	27	IC ₅₀ = 3 nM (hamster)	110
<i>Peptide based</i>				
Z-Ile-Glu-Pro-Phe-COOMe	K _i = 1 nM	n.r.	n.r.	160
Diphenyl N ^α -benzocarbonyl-L- Arg-Glu-Thr-Phe ^P -phosphonate	IC ₅₀ = 3.8 nM	2,700	n.r.	161
Suc-Val-Pro-PheP(OPh) ₂	n.r.	0.42	k _{obsd} /[I] (M ⁻¹ s ⁻¹) = 15,000 (rat chymase)	162

n.r., not reported in the scientific literature; k_{obsd}, pseudo-first order rate constant.

tory that chymase, in some contexts, can in fact have an opposing impact depending on the exact setting and on the specific tools used. However, it is important to note that chymase-expressing mast cells are widely distributed in the body and thereby are present, either as resident or recruited cells, in the context of multiple pathologies. Moreover, it is known that mast cells can undergo degranulation (and thereby release chymase) in response to a wide panel of stimulants [4], and it is also important to emphasize that chymase is a major component of mast cell granules. Consequently, large amounts of released chymase will be present in a very wide panel of pathologies. It is also important to note that chymase can cleave a large range of substrates (see “Enzymatic properties of chymases”), and the cleavage of the respective substrates can have highly variable consequences, ranging from protective functions by degrading harmful/proinflammatory substances to a detrimental impact by activating molecules that contribute to the pathology

(Fig. 1). The exact impact of chymase will thus be dependent on the availability of potential chymase substrates under the respective condition. Clearly, the levels and repertoire of chymase substrates may vary extensively between different pathological settings, and also under different phases of the respective condition. The wide array of functions for chymase may thus be a reflection of its presence under the respective settings, and the variable impact of chymase may reflect the variable repertoire of available chymase substrates in different conditions and in different phases of these.

Chymase in Host Defense

Mast cells show a preferential localization to sites in close contact with the external environment, such as skin and mucosal surfaces of the gut and lung. Based on this and on that mast cells express numerous pattern recognition recep-

Table 3. Detrimental and protective functions of chymase

Condition	Role of chymase	Supported by studies in knockout mice	Supported by chymase inhibition	Proposed impact of chymase	Reference
Abdominal aortic aneurysms	detrimental	yes	yes	MMP9 generation; monocyte recruitment; regulation of cysteine cathepsins; elastin degradation; angiogenesis; vascular cell apoptosis	111, 112
Adhesions	detrimental	no	yes	TGF- β generation	145–149
Arthritis	detrimental	yes	no	proinflammatory; promotion anti-collagen-IgG generation	78, 79
Asthma	protective detrimental	yes no	no yes	degradation of IL-33, IL-13 proinflammatory	40, 69, 70, 71
Atherosclerosis	detrimental	yes	yes	promotion of necrosis; adverse effect on plaque stability	109, 110
Atopic dermatitis	detrimental	no	yes	itch induction; proinflammatory	129, 133, 144
Bleomycin-induced lung inflammation/fibrosis	detrimental	yes	yes	profibrotic; proinflammatory	74, 75
Bullous pemphigoid	detrimental	yes	no	MMP9 generation; hemidesmosome degradation	36
Burn injury	detrimental	yes	no	degradation of tight junctions	53
Cardiac dysfunction	detrimental	yes	yes	cardiomyocyte apoptosis; adverse cardiac remodeling; MMP9 formation, TGF- β activation; cytokine/chemokine induction; macrophage recruitment; contribution to fibrosis; fibronectin loss; vascular proliferation	81, 89–92, 94, 95, 97–105, 153
Diabetes	detrimental	no	yes	albuminuria; pancreatic island damage; oxidative stress; Ang II generation	121–123
Experimental autoimmune encephalomyelitis	detrimental	yes	no	proinflammatory	80
Graft rejection	detrimental	yes	no	neutrophil recruitment	163
Inflammatory bowel disease	detrimental	no	yes	proinflammatory; negative impact on T _{reg} populations	135
Ischemic kidney injury	protective	yes	no	limiting neutrophil recruitment; modification of integrin/selectin expression	126
Lung inflammation (LPS, silicosis)	detrimental	no	yes	neutrophil influx; cytokine induction	72, 73
Nephritis (partial ureteral obstruction)	detrimental	yes	no	fibrosis; CCL2 production; α -smooth muscle actin expression	125
Nephritis (unilateral ureteral obstruction)	protective	yes	no	suppression of fibrosis/ α -smooth muscle actin/collagen deposition/CCL2/TGF- β	32

Table 3 (continued)

Condition	Role of chymase	Supported by studies in knockout mice	Supported by chymase inhibition	Proposed impact of chymase	Reference
Nephritis (immune-complex mediated)	detrimental	yes	no	formation of Ang II, collagen 1, TNF, MCP-1/CCL2	119
Posttraumatic brain inflammation	protective	yes	yes	regulation of astrogliosis and T-cell infiltration/microglia infiltration	141
Posttraumatic spinal cord damage	protective	yes	no	degradation of proinflammatory cytokines/chemokines; limiting of scar formation: modulation of scar protein expression	139, 140
Protection against toxins	protective	yes	no	degradation of toxic peptides	62
Scleroderma	detrimental	no	yes	generation of profibrotic TGF- β	143
Sepsis	protective	yes	no	degradation of TNF	43, 60
Steatohepatitis	detrimental	no	yes	contribution to fibrosis; collagen induction; Ang II generation; α -smooth muscle actin induction; MMP9 generation	114–118
Thrombin-induced skin inflammation	protective			degradation of thrombin	47
<i>Trichinella spiralis</i> infection	protective	yes	no	promotes parasite expulsion; inhibits muscle larva deposition	58

MMP, matrix metalloproteinase; TGF- β , transforming growth factor β ; Ang, angiotensin; T_{reg}, regulatory T cells; LPS, lipopolysaccharide; CCL2, CC chemokine ligand 2; TNF, tumor necrosis factor; MCP-1, monocyte chemoattractant protein 1.

tors capable of sensing a plethora of pathogen-expressed factors [5], it is generally thought that mast cells are important sentinel cells acting in the early phases of innate immune responses against pathogens [5–7]. Regarding the mechanism(s) by which mast cells confer protection against infectious agents, there is evidence that chymase can contribute to such protective functions. In an early study, Knight et al. [58] showed that mice lacking Mcpt1 display a delayed expulsion of the nematode *Trichinella spiralis* and increased deposition of larvae in tissues. In contrast, the expulsion of *Nippostrongylus brasiliensis* was not affected by the absence of Mcpt1, indicating that mast cell chymases preferentially act against selected parasite worms [58]. Chymase has also been shown to have a role in protection against various types of bacterial insults. In a model where sepsis is induced by cecal ligation and puncture, it was demonstrated that the absence of Mcpt4 resulted in markedly more severe pathology than that seen in wild-type mice [43], and data were presented to suggest that the protective

effect of Mcpt4 was due to its ability to suppress the levels of TNF [43]. In subsequent studies it was shown that Mcpt4 can additionally reduce the severity of group B streptococcus infections by degrading fibronectin in the extracellular matrix of the host, thereby reducing bacterial adherence [59]. On a different angle, it has been demonstrated that Mcpt2 can have a protective role in the cecal ligation and puncture model, and that IL-15 can constrain antibacterial defense capabilities of mast cells by downregulating Mcpt2 expression [60]. However, since Mcpt2 appears to lack enzymatic activity [28, 29], its antibacterial effect is probably due to nonenzymatic mechanisms. Mast cells have also been implicated in the host defense against various toxins produced by venomous animals [61], and there is evidence that chymase (Mcpt4) can account for some of these effects [62]. To date, the potential role of chymase in viral infections has not been evaluated. Further, the possible effects of pharmacological chymase inhibition on host defense mechanisms remain to be explored.

Chymase in Pulmonary Inflammation

There is currently a wealth of evidence supporting a detrimental role for mast cells in asthma, both from clinical studies and animal experimentation approaches [reviewed in 63–66]. Hence, it would be reasonable to assume that chymase could account, at least to some extent, for such effects. However, somewhat unexpectedly, evidence has accumulated to suggest that chymase in fact has a protective role in asthma. This is supported by evidence from clinical studies where chymase positivity has been correlated with preserved lung function [67, 68]. In addition, when evaluating Mcpt4^{-/-} mice in two models for asthma, it was revealed that the absence of Mcpt4 resulted in elevated airway reactivity and increased eosinophil infiltration [40, 69]. Mechanistically, it has been suggested that Mcpt4 alleviates airway inflammation by degrading IL-33 [40] or IL-13 [70]. Based on these findings it would be expected that pharmacological chymase inhibition would result in worsened outcome in asthma models. However, it was demonstrated that chymase inhibition in fact had a dampening effect on airway responses in rat and sheep models for allergic airway inflammation [71]. Although these findings are in seeming discrepancy, it should be noted that the chymase inhibitor used (RWJ-355871) has dual specificity for chymase and cathepsin G (Table 2). Hence, it cannot be excluded that the beneficial effect of this chymase inhibitor is due to its effects on cathepsin G rather than on chymase. To firmly establish this issue, it would be warranted to perform studies where more selective chymase inhibitors are assessed in animal models of asthma. Chymase inhibition has also been shown to dampen airway inflammation induced by bacterial lipopolysaccharide [72] and in a silicosis model [73]. In the latter study, chymase inhibition also reduced the lung fibrosis. In line with these findings, the absence of chymase was associated with less inflammatory responses in a bleomycin-induced lung fibrosis model [74], and the latter condition was also improved by pharmacological chymase inhibition [75].

Chymase in Autoimmune Settings

There is both clinical and experimental evidence from mast cell-deficient mice indicating an aggravating impact of mast cells in arthritis [76]. Intriguingly, whereas mast cells were redundant in passively induced experimental arthritis, they were shown to contribute profoundly in antigen-induced disease [77]. Mechanistically, there is

some evidence to suggest that mast cell chymases can contribute to the pathology in such settings. Magnusson et al. [78] showed that animals lacking Mcpt4 exhibited a milder course of disease in collagen-induced arthritis than did the corresponding wild-type counterparts. Moreover, it was shown more recently that also Mcpt5 can contribute to experimental arthritis, as shown in models where arthritis was induced either by transfer of K/BxN serum or by meBSA/IL-1 β [79]. However, it should be kept in mind that Mcpt5 has no known functional homologue in humans (see “Chymases and chymase knockouts”), and the bearing of this finding on human arthritis is thus not clear. Chymase has also been reported to contribute to the pathology of experimental autoimmune encephalomyelitis, a model for autoimmune multiple sclerosis [80].

Chymase in Cardiovascular Conditions

It has been recognized for a long time that mast cell chymase can have an impact on various cardiovascular conditions. A hallmark finding that boosted this area of research was the finding that heart chymase can cleave angiotensin (Ang) I to generate the potent pressor Ang II [45], i.e. having overlapping activity with that of angiotensin-converting enzyme (ACE). Based on this finding, a number of studies have been conducted where the effects of pharmacological chymase inhibition on Ang II formation have been studied. In a hallmark study it was demonstrated that chronic ACE inhibition did not repress Ang II levels in the cardiac interstitial fluid, indicating the presence of non-ACE-dependent Ang II-generating activity. Intriguingly, chymase inhibition blocked such Ang II-forming activity in mast cell-sufficient but not in mast cell-deficient mice, suggesting that chymase accounts for ACE-independent Ang II generation within the cardiac tissue [81]. In further support for this notion, the knockout of ACE resulted in abrogated Ang II formation within the circulation but failed to suppress cardiac Ang II levels [82].

Considering that chymase has the capacity to generate Ang II, it would appear likely that chymase repression can lead to reduced blood pressure. Indeed, there are studies in various animal models showing that chymase inhibitors can cause reduced blood pressure in response to various triggers [83–85]. Based on these animal studies, it is reasonable to assume that chymase inhibition could potentially have the effect to lower blood pressure in humans. However, two recent clinical studies evaluating

BAY 1142524 (fulacimstat) did not reveal any significant effects of chymase inhibition on basal blood pressure [86, 87], although the drugs used were proven to be safe and well tolerated.

In addition to its role in Ang II formation, there is evidence that chymase has an important role in generation of the potent pressor endothelin-1 (ET-1) from big ET-1. This is supported by reduced ET-1 formation and increase in blood pressure in Mcpt4^{-/-} animals in response to administration of big ET-1 (the precursor for ET-1) [54, 85]. In agreement with a crucial function for chymase in this process, ET-1 generation (from big ET-1) was blocked by the chymase inhibitors TY51469 or Suc-Val-Pro-Phe(P)(OPh)(2) [54, 88].

There is also evidence that chymase can have a profound impact on cardiac dysfunction following tissue injury, independently of effects on regulating blood pressure. In one study it was shown that the absence of Mcpt4 was associated with reduced cardiac dysfunction after myocardial infarction, and it was proposed that Mcpt4 deficiency results in increased cardiomyocyte survival [89]. These findings are in agreement with other studies where chymase Mcpt4 was shown to promote cell death and cardiac remodeling after myocardial infarction [90] and where chymase inhibition resulted in improved cardiac function following myocardial infarction in hamsters [81]. A detrimental function of chymase during heart infarction was also seen in a study where permanent myocardial infarction was induced. In this study, the absence of chymase Mcpt4 led to substantially enhanced survival and cardiac function [91]. In line with these findings, there is a massive documentation revealing protective effects of chymase inhibition in various settings of cardiovascular injury, including myocardial ischemia-reperfusion injury [92], vascular dysfunction in stroke-prone rats [93], cardiac fibrosis [94, 95], inflammation after acute myocardial ischemia/reperfusion [95], cardiomyocyte function in dogs with isolated mitral regurgitation [96], cardiac function after left ventricular repair in rats [97], progression to heart failure after autoimmune myocarditis in rats [98], as well as in many other settings of cardiac pathology [99–105]. Based on these findings, it has been proposed that chymase could represent a novel pharmacological target for treatment of cardiovascular disease [reviewed in 106, 107], and ongoing clinical trials are aiming at evaluating this concept.

There is extensive documentation suggesting that chymase can modify lipoproteins (high- and low-density lipoproteins) [108] such that atherosclerotic progression is promoted. In support of this, the genetic ablation of Mcpt4

has been associated with reduced atherosclerotic lesions in apolipoprotein E-deficient mice [109]. In addition, pharmacological inhibition of chymase with RO5066852 was shown to reduce atherosclerotic progression in apolipoprotein E-deficient mice [110]. It has also been shown that chymase promotes the generation of abdominal aortic aneurysms, as shown both by evaluating chymase (Mcpt4)-deficient animals [111] and by using the chymase inhibitors ASB17061 [112] and NK3201 [113].

Chymase in Steatohepatitis/Liver Pathology

There are several studies showing that chymase inhibition has a substantial beneficial impact on the development of steatohepatitis, as shown in animal models where the condition is induced by different pathogenic diets [114–116]. Chymase inhibition has also been shown to have beneficial effects in acute liver failure in hamsters [117] and in tetrachloride-induced liver fibrosis [118].

Chymase in Inflammatory Kidney Disease

There are several indications that chymase can affect the outcome of inflammatory kidney disease, but the impact of chymase seems to depend strongly on the exact condition. In immune complex-mediated glomerulonephritis, Mcpt4 chymase was shown to contribute to the inflammation and fibrosis, and the absence of Mcpt4 led to lower levels of various pathogenic factors, including Ang II, collagen 1, TNF and MCP-1/CCL2 [119]. In support of a role for chymase in generating Ang II in the context of kidney pathology, pharmacological chymase inhibition was shown to suppress renal Ang II formation in diabetic mice [120] and in mice treated with Ang I (precursor of Ang II) [84]. Additionally, chymase inhibition has been shown to protect against albuminuria in diabetic mice [121] and rats [122], to protect against diabetes-induced oxidative stress and renal dysfunction in hamsters [123] and to confer pancreatic islet protection in experimental diabetes [124]. In line with a detrimental impact of chymase in kidney pathology, Mcpt4 was shown to partly account for the inflammation induced by partial ureteral obstruction [125]. In that study it was observed that Mcpt4 promoted kidney hyper/hypotrophy, development of fibrosis, CCL2 secretion and α -smooth muscle actin expression [125]. In contrast, when kidney inflammation was induced by unilateral ureteral obstruction, chymase was shown to have a protective function [32]. Here, increased

fibrosis, α -smooth muscle actin, collagen deposition, CCL2 and TGF- β were seen in animals lacking Mcpt4 expression, i.e. in sharp contrast to the findings in the partial ureteral obstruction setting. The reasons behind these apparent discrepancies are not entirely clear. However, it was reasoned that these differences could be explained by the specific pathophysiological phenotypes associated with the respective types of lesions, and possibly related to different disease kinetics of the models [125]. A protective function for chymase has also been seen in ischemic kidney injury, where Mcpt4 was shown to limit neutrophil recruitment and activation [126]. It was suggested that chymase exerted this role by modifying the surface expression of CD11b integrin and P/E-selectin [126].

Chymase in Regulation of the Coagulation System

There is evidence suggesting that chymase could be involved in the regulation of certain blood coagulation parameters. It was shown in an early study that purified chymase Mcpt4 efficiently degrades thrombin, thereby abolishing its enzymatic activity [46]. This was supported by studies showing that the knockout of Mcpt4 abolished the ability of mast cells to proteolytically inactivate thrombin [23]. In further agreement with this, a more recent study demonstrated that Mcpt4 has a major function in limiting thrombin-induced skin inflammation *in vivo* [47]. It has also been shown that chymase has the capacity to regulate plasmin activity [127]. Further, it was shown in a recent study that chymase, *in vivo*, has a role in regulating FXIIIa (transglutaminase) activity [128]. Altogether, these findings suggest that chymase can regulate the coagulation cascade at various levels, potentially affecting blood coagulation. Indeed, Mcpt4 deficiency has recently been shown to cause reduced bleeding times during experimental sepsis [128]. However, considering that chymase is generally found outside of the circulation, it appears likely that chymase may predominantly affect extravascular coagulation events.

The Role of Chymase in Regulating Inflammatory Cell Recruitment

There is substantial evidence that chymase has the ability to promote inflammatory cell recruitment in various settings. This is, firstly, supported by experiments in which administration of purified/recombinant chymase in different animal models has been shown to cause the accumula-

tion of various inflammatory cell types, including eosinophils [129, 130], neutrophils [73, 130, 131], lymphocytes [130] and macrophages [130]. In support of this, various chymase inhibitors have been demonstrated to suppress the accumulation of leukocytes under different inflammatory conditions. For example, chymase inhibition has been shown to attenuate eosinophilia [132, 133], to inhibit neutrophil influx in various inflammatory settings [71–73, 95] and to reduce monocyte/macrophage recruitment [93, 134]. It has additionally been demonstrated that chymase inhibition can dampen inflammatory responses in a rat model of inflammatory bowel disease [135].

At present it is not entirely clear by what mechanism chymase causes leukocyte recruitment. However, there is evidence suggesting that chymase has a major role in degrading tight junction proteins [53, 136], and a plausible scenario could thus be that such chymase-dependent degradation of cell-cell contacts could be an important factor in promoting the migration of inflammatory cells from the circulation to sites of tissue injury. In agreement with this notion, chymase has been shown to enhance paracellular permeability of the gut epithelium, both at baseline conditions [136] and during anaphylaxis [137]. Chymase has also been shown to cause increased microvascular leakage in skin [138]. On a similar angle, chymase has shown to cleave hemidesmosome proteins in the context of autoimmune bullous pemphigoid, thereby contributing to the inflammation seen in this condition [36].

In seeming discrepancy with these findings (see discussion under “Biological functions of chymases: general considerations”), it has been shown that chymase may also exert anti-inflammatory properties, e.g. by degrading proinflammatory alarmins [52], IL-33 [40, 69] or IL-13 [70]. Further, chymase has been shown to protect from posttraumatic spinal cord damage, most likely by degrading proinflammatory cytokines [139]. Moreover, the absence of Mcpt4 led to increased scar formation under this condition [140]. Also in posttraumatic brain inflammation, chymase has been shown to have a protective role, by reducing brain inflammation [141].

Chymase in Skin Pathologies

Chymase-expressing mast cells are abundant in skin, and it is therefore reasonable to assume that chymase could have a significant impact on pathologies of this organ. Indeed, there are numerous studies in support of this notion. In a scleroderma model (tight skin mice;

Tsk), selective upregulation of chymase Mcpt4 was seen [142] and pharmacological chymase inhibition by SUN-C8257 was shown to dampen the pathology significantly [143]. It was proposed that the beneficial effect of chymase inhibition was due to blunted generation of profibrotic TGF- β . It has also been shown that chymase inhibition (by SUN-C8257 or SUN13834) improves atopic dermatitis-like skin lesions in NC/Nga mice [129, 133, 144]. Further, it was shown in a burn-induced injury model that both Mcpt4 and Mcpt5 contribute profoundly to the pathology [53]. Chymase has also been shown to be crucial for limiting thrombin-induced skin inflammation [47].

Chymase in Adhesions after Surgery

Formations of adhesions are serious adverse effects associated with surgery. Several animal experimental studies have revealed that mast cell chymase can contribute to this condition, as shown by attenuated peritoneal adhesion formation after administration of Suc-Val-Pro-PheP(OPh)₂ in rat [145], hamster [146] and canine [147] models. Peritoneal adhesion formation was also inhibited by using the TY-51184 and NK3201 chymase inhibitors in hamster models [148, 149]. However, a role for chymase in adhesion formation has not been confirmed by studies in chymase-deficient animals.

Chymase in the Regulation of Homeostasis

It is generally thought that biological effects of the mast cell granule proteases are seen under inflammatory conditions where mast cells have been activated, by IgE-mediated or other mechanisms. However, there is accumulating evidence suggesting that chymase can exert biological effects even in conditions where mast cells are not overtly activated, i.e. to affect body homeostasis. In one study it was shown that the absence of Mcpt4 caused decreased intestinal permeability at baseline conditions, and it was shown that the absence of Mcpt4 led to increased expression of the tight junction protein claudin-3 [136]. Hence, this suggests that chymase can enhance epithelial permeability under homeostatic conditions by degrading tight junctions. Notably, this is in agreement with a study showing that Mcpt4 can degrade claudin-4 during skin inflammation caused by burn injury [53], and with an earlier study where it was demonstrated that infusion of chymase to the cranial mesenteric artery caused in-

creased gut permeability in rats [150]. There is also evidence that the absence of chymase Mcpt4 results in an age-dependent increase in bone mass in female mice under baseline conditions, i.e. suggesting that Mcpt4 has a homeostatic impact on the regulation of bone metabolism [151]. Finally, there is evidence to suggest that chymase has a role in the regulation of homeostatic extracellular matrix deposition, as shown by an age-dependent, excessive accumulation of collagen and fibronectin in mice lacking Mcpt4 [35].

Concluding Remarks and Future Directions

Research conducted over the last decades has provided considerable insight into the biological function of chymase. In particular, the use of chymase knockout animals and the use of selective chymase inhibitors have made it possible to elucidate the *in vivo* impact of chymase in diverse pathological, but also homeostatic, conditions. Intriguingly, the gathered knowledge from these efforts suggests that chymase has a highly complex role in regulating pathological processes, ranging from protective functions in some cases to being detrimental in others (Table 3). To add further complexity, chymase can even have opposing functions in a given condition, depending on the methodology/tools adapted. So, can we exploit this knowledge for medical purposes? Clearly, in cases where there is a well-documented detrimental impact of chymase, chymase inhibition could be of therapeutic significance. The most notable example of the latter is cardiac dysfunction after heart injury, where a wealth of evidence from various animal experimental models and from evaluating a wide range of chymase inhibitors indicates that chymase inhibition can alleviate the pathology in a profound way. Indeed, clinical trials to assess this notion are currently under way. On the contrary, in cases where chymase has a proven beneficial role, it is conceivable that recombinant chymase could be used as a biological drug. However, since chymase carries a high positive charge, it may be anticipated that its pharmacological properties are suboptimal. Given the range of additional pathologies where chymase has been implicated (Table 3), it is likely that chymase inhibition will be assessed for beneficial effects in a range of novel clinical settings in the near future. It is also likely that upcoming research will provide a more detailed insight into the exact mechanism of chymase action under diverse pathological situations. For example, it will be important to gain more knowledge of the *in vivo* substrates for chymase under various pathological

settings, and also under different phases of a given pathological process. Most likely, such efforts will provide a more comprehensive picture of the exact biological function of chymase, knowledge that will aid in exploiting chymase for therapeutic or diagnostic purposes.

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Statement of Ethics

The author has considered ethical aspects related to this review article.

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