The emerging role of mast cell proteases in asthma

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Mast cells express large amounts of proteases, including tryptase, chymase and carboxypeptidase A3. An extensive review of how these proteases impact on asthma shows that they can have both protective and detrimental functions. http://bit.ly/2Gu1Qp2

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ABSTRACT It is now well established that mast cells (MCs) play a crucial role in asthma. This is supported by multiple lines of evidence, including both clinical studies and studies on MC-deficient mice. However, there is still only limited knowledge of the exact effector mechanism(s) by which MCs influence asthma pathology. MCs contain large amounts of secretory granules, which are filled with a variety of bioactive compounds including histamine, cytokines, lysosomal hydrolases, serglycin proteoglycans and a number of MC-restricted proteases. When MCs are activated, *e.g.* in response to IgE receptor cross-linking, the contents of their granules are released to the exterior and can cause a massive inflammatory reaction. The MC-restricted proteases include tryptases, chymases and carboxypeptidase A3, and these are expressed and stored at remarkably high levels. There is now emerging evidence supporting a prominent role of these enzymes in the pathology of asthma. Interestingly, however, the role of the MC-restricted proteases is multifaceted, encompassing both protective and detrimental activities. Here, the current knowledge of how the MC-restricted proteases impact on asthma is reviewed.

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Introduction

Mast cells (MCs) are immune cells derived from myeloid precursors in the bone marrow [1]. A hallmark feature of MCs is their high content of lysosome-like secretory granules, which occupy a major fraction of the MC cytoplasm and account for their classical strong metachromatic staining [2]. The granules contain large amounts of various pre-formed inflammatory mediators such as histamine, cytokines, growth factors, serglycin proteoglycans, lysosomal hydrolases and large amounts of various MC-restricted proteases (in the following denoted the "MC proteases") [2]. The latter encompass serine proteases of the tryptase and chymase type as well as a zinc-containing metalloprotease denoted carboxypeptidase A3 (CPA3) (figure 1) [2–5].

When MCs are activated, *e.g.* by antigen-mediated cross-linking of IgE bound to their high-affinity cell surface receptors (FcεR1), they respond by degranulation, whereby the pre-formed granule contents are released. MC activation also leads to *de novo* synthesis and release of numerous additional inflammatory compounds [6].

MCs are found in most tissues of the body, but are particularly abundant at sites close to the external milieu, including the skin, tongue, gut and lung. Due to this location, it is thought that MCs can act in the first-line innate defence against foreign intruders such as bacteria and various parasites [7, 8], and it is also established that MCs can have a major function in the clearance of various toxins [9]. However, in addition to these beneficial activities, MCs are notorious for their detrimental impact on a number of pathological settings, including asthma and other allergic conditions [10].

An impact of MCs on asthma is supported by a large amount of documentation from both clinical and experimental studies (reviewed in [11–14]). For example, the presence of MCs and extent of MC degranulation within the airway smooth muscle cell (SMC) layer shows a strong correlation with asthma [15, 16] and there is also evidence that uncontrolled asthma is associated with infiltration of MCs into the lung parenchyma [17]. MCs appear to be of particular importance in the T-helper cell type 2 (Th2)-high endotype of asthma and it is noteworthy that anti-IgE therapy in mild to moderate asthmatic subjects (targeting in particular MCs and basophils) has a profound impact on type 2 markers [18]. A detrimental role of MCs in asthma is also supported by experimental approaches where MC-deficient mice have been evaluated in models of asthma, although certain features of asthma can also develop in the absence of MCs [19–21]. However, although MCs are now widely recognised as major players in asthma, it is not fully understood to what extent the different compounds secreted by MCs contribute to the pathology. Given that the MC proteases are highly expressed in MCs and are released in large quantities following MC activation [2–4], it is reasonable to assume that they account, at least partly, for the effects of MCs in asthma. Indeed, there is now a growing awareness that the MC proteases have a major impact on various features of asthma. These issues are discussed in this review.

MC proteases in humans and mice

Humans express one chymase (*CMA1*), which is classified as an α-chymase (table 1). Similar to humans, mice express one α-chymase, designated Mcpt5, but also five β-chymases: Mcpt1, Mcpt2, Mcpt4, Mcpt9 and Mcpt10 [22]. Out of the mouse chymases, Mcpt5 is the closest homologue to CMA1 in terms of amino acid sequence homology but, importantly, Mcpt4 represents the functional homologue to human chymase [23–25]. Humans express two tetrameric tryptases, α and β, of which β-tryptase is enzymatically active, whereas α-tryptase lacks enzymatic activity [26, 27]. The β-tryptases are subdivided into β1, β2 and β3; interestingly, β2- and β3-tryptase are alleles at one locus (*TPSB2*), whereas α- and β1-tryptase are alleles at a neighbouring gene (*TPSAB1*) [28, 29]. In addition to the tetrameric tryptases, human MCs express a monomeric transmembrane tryptase denoted γ-tryptase (*TPSG1*) [30]. Similar to humans, mouse MCs

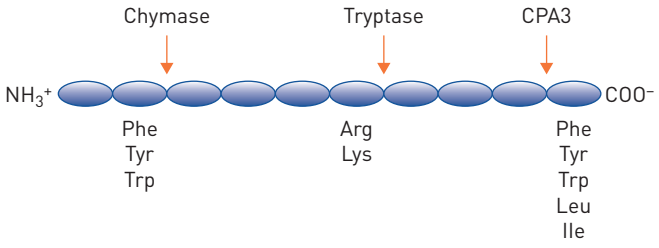

FIGURE 1 Cleavage specificities of the mast cell (MC)-restricted proteases. The figure depicts that chymase and tryptase are endopeptidases, whereas carboxypeptidase A3 (CPA3) is a C-terminal exopeptidase; chymase and CPA3 are monomeric proteases, whereas tryptase is tetrameric. The figure also depicts the substrate cleavage properties of the respective proteases, with the indicated amino acid residues representing the preferred N/C-terminal residues of the cleaved peptide bond.

TABLE 1 Characteristics of the major human and mouse mast cell (MC) proteases

Protease	Gene	Enzymatic activity	Expression in MC subtype
Human			
α-tryptase	*TPSAB1*	Serine protease; non-active	MC_T+MC_{TC}
β_1-tryptase	*TPSAB1*	Serine protease; trypsin-like	MC_T+MC_{TC}
β_2-tryptase	*TPSB2*	Serine protease; trypsin-like	MC_T+MC_{TC}
β_3-tryptase	*TPSB2*	Serine protease; trypsin-like	MC_T+MC_{TC}
γ-tryptase	*TPSG1*	Serine protease; trypsin-like	Not established
Chymase (α-chymase)	*CMA1*	Serine protease; chymotrypsin-like	MC_{TC}
Carboxypeptidase A3	*CPA3*	Metalloprotease; C-terminal exopeptidase	MC_{TC}
Mouse			
Mcpt6; mMCP6 (tryptase)	*Mcpt6*	Serine protease; trypsin-like	CTMC
Mcpt7; mMCP7[#] (tryptase)	*Mcpt7*	Serine protease; trypsin-like	CTMC
γ-tryptase	*Tpsg1*	Serine protease; trypsin-like	Not established
Mcpt1; mMCP1 (β-chymase)	*Mcpt1*	Serine protease; chymotrypsin-like	MMC
Mcpt2; mMCP2 (β-chymase)	*Mcpt2*	Serine protease; non-active	MMC
Mcpt4; mMCP4 (β-chymase)	*Mcpt4*	Serine protease; chymotrypsin-like	CTMC
Mcpt5; mMCP5 (α-chymase)	*Mcpt5/Cma1*	Serine protease; elastase-like	CTMC
Carboxypeptidase A3	*Cpa3*	Metalloprotease; C-terminal exopeptidase	CTMC

MC_T: MCs expressing tryptase only; MC_{TC}: MCs expressing tryptase, chymase and carboxypeptidase A3; mMCP: mouse MC protease (alternative nomenclature for the mouse MC proteases); CTMC: connective tissue type MC; MMC: mucosal type MC. [#]: the C57BL/6 mouse strain lacks Mcpt7 expression due to a point mutation.

express two tetrameric tryptases, Mcpt6 and Mcpt7, of which Mcpt6 is thought to be the closest homologue to human β-tryptase, as well as a transmembrane γ-tryptase (*Tpsg1*) [5]. Notably, the C57BL/6 strain lacks Mcpt7 expression due to a point mutation. In both humans and mice, one CPA3 gene is expressed [4].

MC protease phenotype in asthma

A number of studies have characterised the protease phenotype of airway MCs in various manifestations of asthma. In healthy subjects, MCs expressing tryptase only (MC_T) dominate over MCs expressing tryptase, chymase and CPA3 (MC_{TC}) (table 1) [31]. However, there is substantial evidence that airway MCs undergo a switch in their protease phenotype in association with asthmatic disease. In particular, there is an expansion of the MC_{TC} subtype under asthmatic conditions. This has been observed in the bronchi and airway submucosa of asthmatic subjects *versus* healthy controls [17, 32–34]. Furthermore, it has been shown that there is a profound increase in the population of MC_{TC} *versus* MC_T in the small airways of severe asthmatic subjects [35]. Studies have also revealed an increase in the MC_{TC} subtype in the airway submucosa and epithelium of severe, but not mild, asthmatic subjects [34]. In addition, it is notable that the majority of the MCs infiltrating the SMC layer in asthma are of the MC_{TC} subtype [15] and an increased ratio of MC_{TC} over MC_T is also supported by studies in animal models of asthma [36, 37].

As stated earlier, asthmatic disorders are strongly correlated with the appearance and/or expansion of chymase-positive MC populations of the lung, which would suggest that chymase may contribute to the pathology of asthma. However, when correlating chymase positivity with lung function parameters, studies have in fact identified a positive correlation between chymase positivity and preserved lung function. This was originally described in a study by Balzar *et al.* [35], in which a positive correlation was seen between preserved lung function and presence of chymase-positive MCs in the small airways of severe asthmatic subjects. Similarly, Zanini *et al.* [33] reported that the density of chymase-positive MCs in bronchial biopsies from patients with mild to moderate asthma correlated positively with preserved lung function. These findings thus suggest that chymase, contrary to the overall negative impact of MCs, may serve a protective function in asthma, a notion that is also supported by studies in animal models (see the later section on "MC proteases in animal models for asthma").

Most previous investigations of MC phenotype in asthma have not included staining for CPA3 and the presence of this protease in airway MCs of healthy *versus* asthmatic individuals has therefore been uncertain. However, insight into this issue came from a study where CPA3 protein positivity and *CPA3* gene expression were assessed in the lungs of healthy subjects and in patients with asthma stratified into

Th2-high and Th2-low subgroups [38]. Intriguingly, it was demonstrated that MCs with a unique tryptase^{+}chymase^{-}CPA3^{+} phenotype were prominent in the epithelium of Th2-high asthmatic subjects [38] and upregulated expression of *CPA3* gene expression in epithelial MCs of asthmatic subjects has been confirmed in other studies [34, 39, 40]. This finding is thereby in some seeming contradiction with the studies showing an increase in chymase-positive MCs in asthma [15, 17, 32–34]. However, the latter studies did not specifically assess for MC protease positivity in the airway epithelium. Moreover, it is noteworthy that most of the studies on this subject have been based on the use of immunohistochemistry, and it would thus be important to confirm key findings by independent methods such as mRNA analysis and proteomic approaches.

MC proteases as asthma biomarkers

Considering the strong implication of MCs in asthma, it would appear reasonable that the MC proteases could serve as useful biomarkers to monitor asthmatic disease. This possibility has been explored in several studies focusing on measurements of serum tryptase. In one study it was shown that children with mild and moderate to severe asthma had higher serum tryptase levels in comparison with children with mild intermittent asthma and healthy controls [41]. Moreover, serum tryptase levels could be used to predict disease severity [41]. It has also been reported that serum tryptase levels in adult subjects are associated with asthma [42–44]. In contrast, Rao *et al.* [45] found no correlation between serum tryptase and airway responses in children with moderate to severe asthma. Furthermore, no correlation between serum tryptase and either atopy, bronchial hyperresponsiveness or symptoms of allergic respiratory disease was seen in a study on adult subjects [46], and a lack of association between serum tryptase and asthma is supported by a number of additional studies [46–50]. Collectively, there is thus some discrepancy with the regard to the association of serum tryptase with asthma. A likely explanation for this is that MC degranulation under asthmatic conditions occurs locally in the lung. Hence, tryptase levels are most likely elevated in the local environment of the lung, but not to the same extent in the circulation (as discussed in [48]). An alterative strategy could therefore be to monitor the local release of tryptase, by assessing the sputum or bronchoalveolar lavage fluid (BALF) of asthmatic subjects, and there is indeed evidence that the levels of tryptase in sputum and BALF are increased both at baseline and after airway provocation in asthmatic subjects [51–59], although contradictory findings have been reported [60, 61]. However, for practical reasons, routine analysis of tryptase in sputum/BALF is not likely to become a clinically useful method to monitor asthma. In this context it is of interest to note that interference with Th2 responses caused a decrease in the sputum levels of tryptase [62].

Another possible strategy to monitor the MC contribution in asthma could be to evaluate chymase and/or CPA3 for biomarker purposes, especially considering that asthma appears to be associated with a profound increase in MC populations positive for these proteases (see the earlier section on "MC protease phenotype in asthma"), whereas tryptase-positive populations generally do not increase. Moreover, whereas tryptase in addition to being expressed by MCs can also be expressed to some extent by other cell lineages (*e.g.* basophils), chymase expression appears to be strongly confined to MCs [63, 64]. A rise in chymase levels may thus reflect more closely the extent of MC degranulation than corresponding rises in tryptase levels. Monitoring of chymase and/or CPA3 could therefore have the potential to be developed as a biomarker for MC involvement in asthma. However, to date, neither chymase nor CPA3 have been evaluated for this purpose.

Furthermore, assessment of the gene expression profiles in sputum taken from asthmatic patients has identified a signature of six highly expressed genes, including *CPA3*, that correlates positively with eosinophilic asthma [65], and can be used to predict both responsiveness to corticosteroids and future exacerbations [66, 67]. Increased expression of CPA3 in sputum cells recovered from asthmatic subjects has been confirmed in other studies [68, 69]. Altogether, there is thus strong correlative evidence implicating CPA3 in the manifestations of asthma, although this notion has not yet received support from experimental studies making use of selective CPA3 inhibitors or CPA3-deficient animals.

MC protease gene polymorphisms in asthma

There is so far only limited evidence suggesting a link between asthma and polymorphisms of the MC protease genes. With regard to chymase (*CMA1*), there is evidence that a short tandem repeat polymorphism downstream of the *CMA1* gene is linked to atopic asthma [70–72]. Interestingly, no link to non-atopic asthma was found [72]. In contrast, a single nucleotide polymorphism (−1903 G/A) in the *CMA1* promoter did not associate with asthma [70, 73]. To date, associations between polymorphisms in any of the tryptase genes with asthma have not been reported. However, it is of interest to note that ~10–40% of humans lack expression of α-tryptase, which is predicted since α- and β1-tryptase alleles compete at the *TPSAB1* locus (see the earlier section on "MC proteases in humans and mice") [28, 74]. Moreover, it has been shown that a high copy number of α-tryptase is related to a higher atopy score

and worsened bronchial function in comparison with subjects having fewer α-tryptase copies [75]. It has also been demonstrated that individuals harbouring a triplication of the *TPSAB1* gene encoding α-tryptase present with a multisystem disorder [76]. However, the molecular basis for the impact of the α-tryptase allele on these conditions, especially considering that α-tryptase is enzymatically inactive, remains to be explored.

MC proteases in animal models for asthma

An important approach to explore the role of the MC proteases in asthma has been to evaluate corresponding knockout mice in models for allergic airway inflammation. By using an ovalbumin-based model, Waern *et al.* [77] showed that airway reactivity, somewhat unexpectedly (since MCs are thought to promote allergic airway inflammation), was enhanced in mice lacking the expression of the chymase Mcpt4. Moreover, it was demonstrated that airway eosinophilia was profoundly enhanced in *Mcpt4*$^{-/-}$ animals and that the absence of Mcpt4 led to an increase in the thickness of the SMC layer [77]. By using a model induced by repeated sensitisation with house dust mite extract, Waern *et al.* [78] confirmed a protective role of chymase (Mcpt4) in airway responses [78] and it was suggested that the protective role of chymase was due to its ability to degrade interleukin (IL)-33 [78]. Further support for a protective role of chymase comes from a study where protection against allergic airway hyperresponsiveness in $\alpha_v\beta_6$-integrin-deficient mice could be attributed to increased expression of Mcpt4, which inhibited IL-13-induced epithelial-dependent enhancement of contractility [79]. Importantly, since Mcpt4 represents the functional homologue to human chymase [23–25], it is likely that functions ascribed to Mcpt4 are shared by human chymase. In agreement with this notion, there is clinical evidence suggesting that human chymase can have a protective role in asthma [33, 35].

To study the role of tryptase in asthma, mice lacking Mcpt6 have been evaluated in an ovalbumin-based model of allergic airway inflammation. In this study it was shown that Mcpt6 contributed profoundly to the airway reactivity in methacholine-challenged animals [80]. In contrast, eosinophil infiltration and other inflammatory parameters were not affected by the absence of Mcpt6 [80]. This suggests that MC tryptase selectively affects airway narrowing, possibly by affecting SMC contraction, without contributing to tissue inflammation. However, the underlying mechanism behind this effect has not yet been revealed.

To date, animals lacking expression of CPA3 have not been evaluated in models of asthma.

In addition to studies based on knockout mice, a number of investigations have addressed the role of the MC proteases in asthma by administering recombinant/purified MC proteases in experimental systems. In an early study it was shown that administration of β-tryptase into the airways of allergic sheep caused bronchoconstriction, supporting a detrimental role for tryptase in asthma [81], and tryptase has also been demonstrated to cause constriction in isolated guinea pig and human bronchi [82, 83]. It has also been demonstrated that instillation of γ-tryptase into the trachea of mice causes airway hyperresponsiveness [84]. In agreement with a protective role for chymase in allergic lung inflammation, Sundaram *et al.* [85] showed that administration of recombinant chymase to human bronchial rings prevented cytokine-enhanced bronchoconstriction and it was demonstrated that such protective activity could be attributed to chymase-mediated degradation of fibronectin.

Substrates for the MC proteases

To understand the mechanism by which the MC proteases impact on asthma it is imperative to identify their proteolytic targets. Indeed, by adopting various approaches, a multitude of reports have identified substrates that could be potential targets for tryptase, chymase or CPA3 in asthma, either by experiments in purified systems, cell biological approaches or *in vivo* experimentation (table 2).

Tryptase

In the tryptase tetramer, all of the active sites are facing a central narrow pore, which causes restricted access for large protein substrates [86]. Accordingly, several of the identified substrates for tryptase are small peptides, including vasoactive intestinal peptide (VIP) [87, 88], calcitonin gene-related peptide (CGRP) [88] and peptide histidine-methionine [88]. However, somewhat unexpectedly, tryptase also has the ability to cleave a number larger proteins such as human/mouse fibrinogen [89, 90], gelatin (from porcine sources) [91, 92], rodent and human proteinase-activated receptor (PAR)-2 [93–95], human RANTES [96], human eotaxin-1/CCL11 [96, 97], human pro-matrix metalloproteinase (MMP) 1 [98], human/mouse pro-MMP3 [99, 100], human/mouse pro-MMP13 [100], human complement factors [101] and mouse/human histones [102]. Several of the identified tryptase substrates may be candidate proteolytic targets in the context of allergic airway inflammation. For example, VIP has recently been implicated as a potential asthma therapeutic due to its relaxing impact on SMCs [103, 104]. The degradation of VIP by tryptase could thus contribute to the detrimental impact of tryptase on asthma, a notion being supported

TABLE 2 Substrates for the mast cell (MC) proteases

Protease/substrate	Setting	Implication	Reference(s)
Tryptase			
VIP	Purified dog and human tryptase; purified system	Degradation; blockade of VIP-induced SMC relaxation	[87, 88]
CGRP	Purified dog and human tryptase; purified system	Degradation	[88]
PHM	Purified dog and human tryptase; purified system	Degradation	[88]
Gelatine	Recombinant human β-tryptase; purified system	ECM remodelling	[91, 92]
Pro-MMP1, -3, -13	Tryptase added to cell-conditioned medium; data from *Mcpt6*$^{-/-}$ MCs	Activation of pro-collagenase activity	[98–100]
PAR-2	Cell biological approaches	Receptor activation	[93–95]
RANTES/CCL5	Recombinant/purified human β-tryptase; chemotaxis assays	Degradation; blockade of eosinophil chemotaxis	[96]
Eotaxin	Recombinant/purified human β-tryptase; chemotaxis assays	Degradation; blockade of eosinophil chemotaxis	[96, 97]
Histones	Cell culture, *Mcpt6*$^{-/-}$ MCs	Degradation; removal of N-terminal ends	[102]
Fibrinogen	Purified β-tryptase; *in vivo* findings using *Mcpt6*$^{-/-}$ mice	Degradation; regulation of blood coagulation	[89, 90]
Complement factors (C3, C5)	Purified system	Generation of anaphylatoxins (C3a, C5a)	[101]
Chymase			
Fibronectin	Purified systems; cell biological approaches; *in vivo* data from *Mcpt4*$^{-/-}$ mice	ECM remodelling	[23, 85, 107, 108]
Pro-MMP2	*In vitro* data; *in vivo* data from *Mcpt4*$^{-/-}$ mice	Processing to active MMP2; ECM remodelling	[111, 113]
Pro-MMP9	*In vitro* data; *in vivo* data from *Mcpt4*$^{-/-}$ mice	Processing to active MMP2; ECM remodelling	[110–112]
Latent TGF-β	Purified/recombinant human chymase; chymase inhibitor; cell biological approaches	Activation; pro-fibrotic activity; ECM remodelling	[123–125]
Pro-collagenase	Purified human chymase; purified system	Activation of collagenolytic activity; ECM remodelling	[109]
Pro-IL-1β	Purified human chymase; purified system	Activation to IL-1β; pro-inflammatory activity	[116]
IL-6	Human purified chymase; human skin MCs	Degradation; blockade of pro-inflammatory activity	[114, 115]
IL-13	Human purified chymase; human skin MCs	Degradation; blockade of pro-inflammatory activity	[114]
Pro-IL-18	Recombinant human chymase; purified systems and cell biological approach	Activation; proposed pro-inflammatory activity in atopic disease	[115, 117]
IL-33	Human chymase, purified mMCP4; purified systems and *in vivo* data from *Mcpt4*$^{-/-}$ mice	Degradation; blockade of pro-inflammatory activity	[78, 115, 127]
TNF	*In vivo* data from *Mcpt4*$^{-/-}$ mice	Degradation; blockade of pro-inflammatory activity; increased survival in sepsis	[118]
CCL6, CCL9, CCL15, CCL23	Human recombinant chymase; purified system/cell biological approaches	Activation; promotes CCR1 stimulation	[119]
CTAP-III	Human skin MCs	Processing to active NAP-2/CXCL7	[131]
Chemerin	Purified human chymase; chemotaxis assays	Degradation; abolished chemotactic activity	[130]
Substance P	Purified dog chymase	Degradation	[87]
HMGB1	*In vivo* data from *Mcpt4*$^{-/-}$ mice	Degradation; blockade of pro-inflammatory activity	[127]
Thrombin	Purified mMCP4; *in vivo* findings from *Mcpt4*$^{-/-}$ mice	Degradation; regulation of blood coagulation	[23, 121, 122]
Tight junction proteins	Cell biological approaches; *in vivo* data from *Mcpt4*$^{-/-}$ mice	Degradation; Increased endothelial/epithelial permeability	[113, 128]
Big-endothelin-1	*In vitro*; *in vivo* data from *Mcpt4*$^{-/-}$ mice	Gained vasoconstrictor activity	[129]
Hemidesmosomes (BP180)	*In vivo* data from *Mcpt4*$^{-/-}$ mice	Skin blistering	[112]
Angiotensin I	Purified systems; cell biological approaches; *in vivo* data from *Mcpt4*$^{-/-}$ mice	Activation to angiotensin I; degradation of angiotensin I; effects on vasoconstriction	[120, 135, 136]
VIP	Purified dog chymase; *in vivo* data from *Mcpt4*$^{-/-}$ mice	Degradation; potential detrimental impact on asthma	[87, 126]
CPA3			
Endothelin	*In vivo* data from mice with mutated *Cpa3*	Degradation	[9, 137]
Neurotensin	Purified human CPA3; purified system	Degradation	[133, 134]
Kinetensin	Purified human CPA3; purified system	Degradation	[133, 134]
Neuromedin N	Purified human CPA3; purified system	Degradation	[134]
Angiotensin I	Purified human CPA3; purified system; cell biological approach	Degradation	[135, 136]

VIP: vasoactive intestinal peptide; SMC: smooth muscle cell; CGRP: calcitonin gene-related peptide; PHM: peptide histidine-methionine; ECM: extracellular matrix; MMP: matrix metalloproteinase; PAR-2: proteinase-activated receptor-2; TGF: transforming growth factor; IL: interleukin; TNF: tumour necrosis factor; NAP-2: neutrophil activating peptide-2; CTAP-III: connective tissue-activating peptide-III; HMGB1: high mobility group box 1; CPA3: carboxypeptidase A3.

by *ex vivo* findings in ferret tracheal rings [105]. Conversely, CGRP secreted from pulmonary neuroendocrine cells has recently been suggested to represent a pathogenic factor in asthmatic responses [106] and its degradation by tryptase could hence serve a protective function, in seeming contradiction to the proposed detrimental impact of tryptase in asthma. Furthermore, eotaxin and RANTES are strong eosinophil chemoattractants, and their degradation by tryptase could thus dampen eosinophil influx in the context of asthma, again in apparent discrepancy with a proposed harmful impact of tryptase in allergic lung inflammation.

Importantly, it should be noted that it has not been confirmed that any of the candidate tryptase substrates are indeed targets for tryptase *in vivo* under asthmatic conditions. Clearly, to understand the function of tryptase in asthma it will be important to identify its *in vivo* targets. This could be accomplished by, for example, using unbiased approaches such as comparing the lung tissue or BALF proteome of wild-type *versus* tryptase-null animals in models of asthma. Additional information could be obtained by comparing the transcriptome of lungs from tryptase-sufficient *versus* tryptase-deficient animals, or after tryptase inhibition, to potentially identify gene expression pathways that are targeted by tryptase.

Chymase

In contrast to tryptase, chymase is a monomeric protease and is thus not hampered by macromolecular constraints in its ability to cleave substrates. Accordingly, a large number of substrates for chymase have been identified, including fibronectin [23, 107, 108], pro-collagenase [109], pro-MMP9 [110–112], pro-MMP2 [111, 113], IL-6 [114, 115], IL-13 [114], IL-15 [115], IL-33 [78, 115], pro-IL-1β [116], pro-IL-18 [115, 117], tumour necrosis factor [118], chemokines CCL6/9/15/23 [119], angiotensin I [120], thrombin [23, 121, 122], latent transforming growth factor (TGF)-β [123–125], VIP [87, 126], substance P [87], high mobility group box 1 [127], tight junction proteins [113, 128], Big-endothelin-1 [129], chemerin [130] and connective tissue-activating peptide-III [131] (table 2). Notably, in many cases chymase causes degradation of the respective substrates, *i.e.* abrogating their biological activities (*e.g.* fibronectin, thrombin, IL-33, IL-13, IL-6, VIP and substance P), whereas in other cases chymase causes activation of the respective compound by exerting limited proteolysis (table 2). Examples of the latter include pro-MMP2/9, pro-IL-1β, pro-IL-18, latent TGF-β and Big-endothelin-1 (table 2).

It is clear that the effects of chymase on several of its identified substrates could have the ability to influence the pathology of asthma. For example, cleavage (degradation) of IL-13, IL-33 and IL-6 could potentially contribute to a protective role of chymase in asthma, by attenuating the pro-inflammatory responses to these compounds and/or by affecting SMC contraction. Alternatively, activation of other cytokines such as IL-1β and IL-18 could have an opposite, *i.e.* pro-inflammatory, impact on airway inflammation. Overall, it is noteworthy that many of the identified chymase substrates are implicated in extracellular matrix (ECM) remodelling and it is thus possible that chymase can regulate such processes occurring in the context of asthma. For example, it is well established from both *in vitro* and *in vivo* studies that chymase has the ability to cleave and thereby activate pro-MMP2 and pro-MMP9 [110–113], which could serve to prevent excessive ECM deposition in the allergic airways. Activation of pro-collagenase by chymase could also contribute to this. Furthermore, it is well established from multiple approaches, including *in vivo* studies of lung tissue, that fibronectin is a major substrate for chymase [23, 85, 107, 108] and it is thus plausible that chymase could have a role in preventing excessive deposition of fibronectin under asthmatic conditions. Altogether, the combined effects of chymase on multiple ECM components could thus have a protective effect by dampening airway remodelling. Conversely, chymase has also been shown to cleave and thereby activate latent TGF-β, with the potential to promote ECM deposition in the allergic airways [123–125]. However, it remains to be established whether chymase has this ability *in vivo*; in fact, there are studies challenging this notion by showing that the levels of TGF-β did not differ between wild-type and *Mcpt4*$^{-/-}$ animals in a model of lung fibrosis [132].

CPA3

In comparison with tryptase and chymase, there is to date very limited insight into the substrate cleavage profile of CPA3. Previous studies in purified systems have shown that CPA3 can cleave neurotensin [133, 134], kinetensin [133, 134], neuromedin N [134] and angiotensin I [135, 136]. Moreover, there is evidence from *in vivo* approaches that endothelin-1 is a major substrate for CPA3 [9, 137]. Intriguingly, endothelin-1 has strong vasoconstrictor and pro-fibrotic properties; hence, it is possible that CPA3 could serve to regulate such processes in the context of asthma. However, this notion needs to be verified by dedicated approaches. The substrate cleavage profile of CPA3 has also been mapped by using a mass spectrometry-based approach [138].

Effects of the MC proteases on airway cells

When MCs degranulate, large quantities of the MC proteases are released. To some extent, the released MC proteases may diffuse to sites distant from the degranulating MCs and even enter the circulation. However, the MC proteases are secreted in large aggregates, complexed with serglycin proteoglycans, and these complexes tend to accumulate locally [139, 140]. It is therefore likely that the impact of the MC proteases on asthmatic settings is largely due to the effects on cells residing in the local environment, *e.g.* SMCs, epithelial cells or fibroblasts. As elaborated in the following subsections, a number of studies have approached this topic (figure 2).

SMCs

The effect of chymase on airway SMCs has only been scarcely studied, but in one study it was shown that chymase degrades fibronectin and CD44 in the pericellular matrix of primary airway SMCs, and that chymase can block epidermal growth factor-induced SMC proliferation [141]. In line with these findings, chymase was shown to have potent pro-apoptotic effects on vascular and uterine SMCs [142–144]. This effect was dependent on fibronectin degradation, leading to decreased pro-survival Akt (protein kinase B) and NF-κB signalling [142, 143]. Based on these findings, one plausible scenario could be that the protective effect of chymase in asthma can be attributed, at least partly, to its inhibitory action on airway SMCs.

Tryptase has been shown to stimulate SMC proliferation [145, 146]. Mechanistically, there is indirect evidence that tryptase triggers SMC activation through PAR-2 activation [147, 148] and by triggering extracellular-regulated kinase (ERK) 1/2 signalling [146]. However, other studies have suggested that

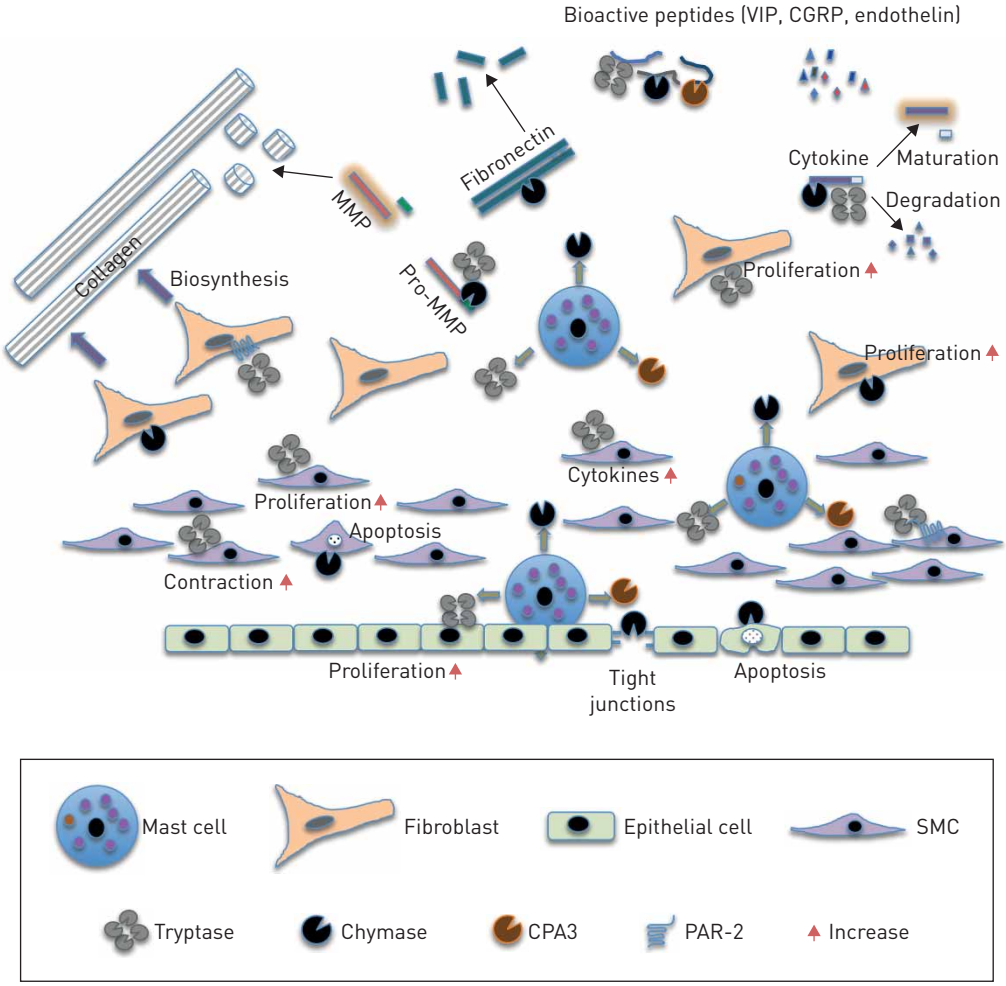

FIGURE 2 Effects of the mast cell proteases on lung cell populations. CPA3: carboxypeptidase A3; PAR-2: proteinase-activated receptor-2; SMC: smooth muscle cell; VIP: vasoactive intestinal peptide; CGRP: calcitonin gene-related peptide; MMP: matrix metalloproteinase.

activation of airway SMCs by tryptase is independent of PAR-2 [149, 150]. Finally, several studies have shown that tryptase can induce or potentiate cytokine/chemokine release from airway SMCs [150–152], and it has also been shown that tryptase can induce and proteolytically activate latent TGF-β [153, 154].

Fibroblasts

Chymase has been shown to promote proliferation and collagen production in various types of fibroblasts, and there is evidence suggesting that chymase acts on fibroblasts by inducing the TGF-β signalling pathway [155–157]. However, it was shown in one study that chymase decreases pro-collagen output from cardiac fibroblasts and, intriguingly, it was shown that chymase is taken up into fibroblasts by dynamin-dependent endocytosis [158].

Tryptase can also affect fibroblasts. In particular, numerous studies suggest that tryptase can stimulate proliferation and collagen synthesis in fibroblasts of various origin [159–168], and there is substantial evidence that tryptase acts on fibroblasts through PAR-2, ERK1/2 and peroxisome proliferator-activated receptor-γ [160, 165–168]. It has also been shown that tryptase can induce chemokine synthesis in fibroblasts and promote fibroblast chemotaxis [169, 170]. Clearly, these findings suggest that effects of tryptase on airway fibroblasts could contribute to the extensive connective tissue remodelling that occurs in asthma. However, the effect of tryptase on primary lung fibroblasts has only been scarcely studied [165]. Interestingly, tryptase can also promote myofibroblast differentiation [167, 171].

Epithelial cells

Chymase can affect epithelial cells, as exemplified by a recent study where it was shown that chymase causes dissociation of airway epithelial cells from the basement membrane of human bronchial rings [172]. Mechanistically, a number of studies have shown that chymase can cleave epithelial tight junction proteins such as claudin-3–5, occludin and zonula occludens-1 [113, 172–175], and chymase-mediated degradation of hemidesmosomes has also been reported [112]. Chymase can additionally stimulate mucin expression in epithelial cells [176], inhibit epithelial cell growth [177] and induce epithelial cell apoptosis [178].

The effect of tryptase on airway epithelial cells has been studied to some extent. In one study it was demonstrated that tryptase is a mitogen for an epithelial cell line of lung origin [179], and it has also been shown that tryptase stimulates prostaglandin E_2 release from primary small airway epithelial cells [180] and IL-8 production in retinal epithelial cells [181]. In other studies it was shown that tryptase, in contrast to chymase (see earlier), does not cause increased permeability of airway or retinal epithelial cells [181, 182]. There are, however, conflicting studies indicating that tryptase can in fact affect the epithelial barrier function of epithelial cells [183, 184].

Summary of effects of the MC proteases on airway cells

Altogether, these findings indicate that the MC proteases can have profound effects on cell populations residing in the vicinity of MCs in lungs of asthmatic subjects and it is thus plausible that the impact of MCs on asthma pathology could be due to such effects. However, it should be emphasised that many of the studies on this issue were performed on either transformed cells or on cells of non-lung origin and it will therefore be important to assess whether the MC proteases impose corresponding effects on primary airway cells. Finally, it remains to be investigated whether CPA3 has an impact on any of these airway cell populations.

MC proteases as drug targets

Based on the notion that the MC proteases can have a pathogenic role in asthma, their inhibition may have therapeutic potential. To address this, previous efforts have mainly focused on tryptase inhibitors (table 3). In early reports, a beneficial effect of the tryptase inhibitor APC-366 was seen in a sheep model of allergic asthma induced by *Ascaris suum* sensitisation and challenge [185]. APC-366 has since been evaluated in allergic pigs and was proven to have beneficial effects [186]. These findings formed the basis for evaluating APC-366 in a small-scale clinical trial involving mild atopic asthmatic subjects. In this trial, APC-366 had a significant positive effect on the late-phase airway response against allergen [187]. However, it was later revealed that some APC-366-treated patients developed bronchospasm and the use of APC-366 for clinical purposes was abandoned. It should be noted that APC-366 is a very poor tryptase inhibitor, being extremely slow acting and with very limited selectivity for tryptase over other trypsin-like proteases (table 3) [185, 188], and it is therefore difficult to ascertain that its effects *in vivo* are due to targeting tryptase as opposed to other trypsin-like proteases. To account for this issue, more selective and more efficient tryptase inhibitors have been developed. These new-generation tryptase inhibitors are typically dibasic, *i.e.* they have dual active site-interacting domains [189, 190]. One of them, AMG-126737, was assessed in allergen-induced airway responses in guinea pigs and sheep, and was proven to have efficacy [191]. Another potent, dibasic tryptase inhibitor is nafamostat [192]. It was shown to efficiently suppress both airway reactivity and inflammation in an ovalbumin-based mouse model of asthma [193,

TABLE 3 Tryptase inhibitors used for treatment of allergic airway responses

Inhibitor	K_i/IC_{50}	Selectivity over trypsin fold	Setting	Outcome	Reference(s)
APC-366	0.33–450 µM	0.5	Antigen-challenged allergic sheep (*A. suum*)	Inhibition of late-phase airway reactivity; reduced inflammatory response	[185]
			Antigen-challenged allergic pigs (*A. suum*)	Reduced airway responsiveness; reduced histamine levels in urine	[186]
			Clinical trial; mild atopic asthmatic subjects	Positive effect on the late-phase allergen response	[187]
BABIM	5 nM	18	Antigen-challenged allergic sheep (*A. suum*)	Inhibition of late-phase airway reactivity	[185]
Nafamostat	0.016 nM	160	Antigen-challenged allergic mice (ovalbumin); chronic model (Der p)	Reduced airway reactivity; reduced airway inflammation	[193–195]
Gabexate	3.4 nM	500	Antigen-challenged allergic mice (ovalbumin); chronic model (Der p)	Reduced airway reactivity; reduced airway inflammation	[193, 195]
AMG-126737	90 nM	28	Antigen-challenged allergic sheep (*A. suum*); antigen-challenged allergic guinea pigs (ovalbumin)	Reduced airway reactivity	[191]
RWJ-58643	10 nM	1	Antigen-challenged allergic sheep (*A. suum*)	Effects against early- and late-phase airway responses in allergic sheep	[198]
BMS-262084	4 nM	18	Antigen-challenged allergic guinea pigs (ovalbumin)	Reduced airway reactivity and inflammation	[196]
MOL 6131	45 nM	24	Antigen-challenged allergic mice (ovalbumin)	Reduced airway inflammation; no effect on inflammation	[197]

K_i: inhibition constant; IC_{50}: median inhibitory concentration; *A. suum*: *Ascaris suum*.

194], as well as in a chronic model of asthma in mice [195]. Other tryptase inhibitors include BMS-262084, MOL 6131, gabexate, ulinastatin and RWJ-58643, all of which show beneficial effects in models of asthma [193, 195–198]. However, none of these new-generation tryptase inhibitors has been evaluated in humans.

More limited knowledge is available concerning the effects of chymase inhibitors in asthma. In one study it was shown that RWJ-355871 suppressed early- and late-phase airway reactivity in a sheep asthma model [199], this being in seeming discrepancy with the reported beneficial effects of chymase on asthma [33, 35, 77, 78]. However, RWJ-355871 is a dual cathepsin G/chymase inhibitor and its inhibitory effect on allergic airway responses could thereby be attributed to its effect on cathepsin G (or other chymotrypsin-like proteases) rather than chymase. More selective chymase inhibitors are now available [200], but have not been evaluated in models of asthma.

Based on novel findings implicating CPA3 in the pathogenesis of asthma [34, 38, 65, 68, 69], it would be of great interest to evaluate CPA3 inhibitors in asthma models. However, this has not yet been done.

Conclusions and future directions

As discussed here, there is now strong support for a contribution of the MC proteases to the manifestations of asthma, both from clinical investigations and experimental approaches in animal models. Based on this notion, it may be foreseen that in the near future the MC proteases could be further exploited for therapeutic and/or diagnostic purposes in asthma. However, several important issues remain to be resolved to fully understand the role of the MC proteases in asthmatic disease. One of these is to identify the proteolytic targets for the MC proteases *in vivo*, in asthmatic settings. Another important task is to further evaluate the impact of highly selective and efficient MC protease inhibitors in asthma.

Overall, it is intriguing to note that the MC proteases can constitute a double-edged sword in their impact on asthma (figure 3). For example, whereas tryptase has generally been shown to have detrimental functions in asthma, several lines of evidence point to a protective role for chymase. It is even more intriguing to note that each of the MC proteases can have potentially both detrimental and beneficial impacts on asthma. This is exemplified by tryptase, which promotes bronchial hyperreactivity in a variety of settings, but also has the ability to degrade pathogenic cytokines and chemokines. Similarly, chymase has activities that can potentially promote airway responses, but also numerous activities that can serve to dampen manifestations of asthma. Clearly, it will be challenging to reconcile these observations and to

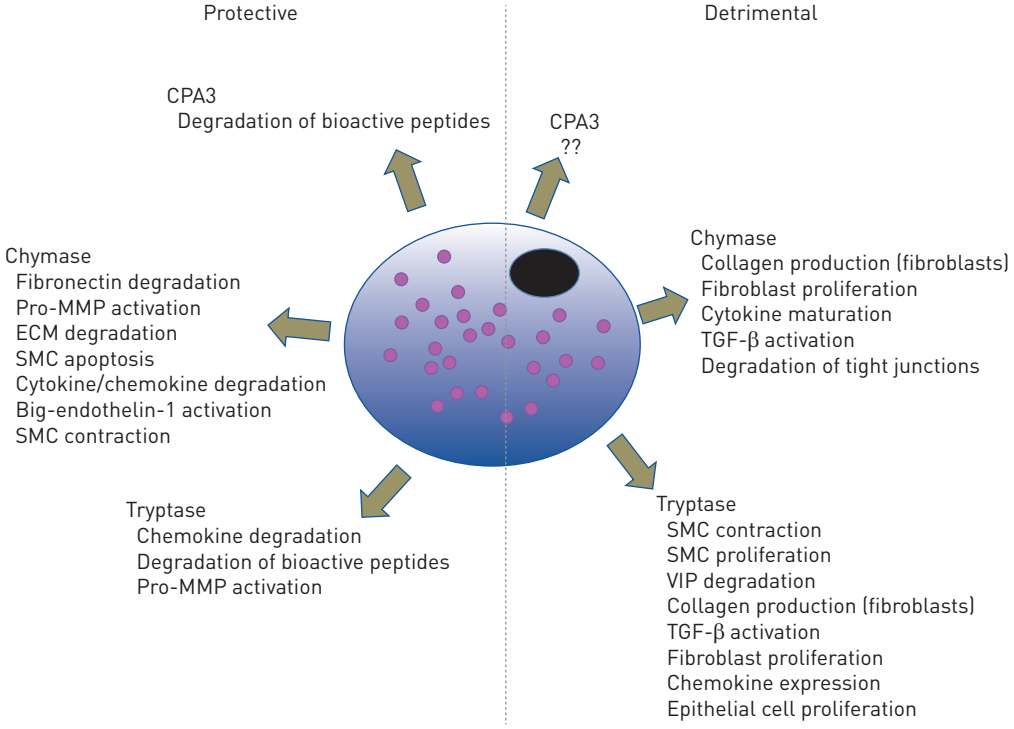

FIGURE 3 Pro- and anti-asthma effects of the mast cell proteases. CPA3: carboxypeptidase A3; MMP: matrix metalloproteinase; ECM: extracellular matrix; SMC: smooth muscle cell; TGF: transforming growth factor; VIP: vasoactive intestinal peptide.

outline at the molecular level how the MC proteases influence the pathology in various settings and phases of asthma.

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