



Mast cell tryptases in allergic inflammation and immediate hypersensitivity

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Dysregulated mast cell-mediated inflammation and/or activation have been linked to a number of human diseases, including asthma, anaphylaxis, chronic spontaneous urticaria, and mast cell activation syndromes. As a major mast cell granule protein, tryptase is a biomarker commonly used in clinical practice to diagnose mast cell-associated disorders and -mediated reactions, but its mechanistic roles in disease pathogenesis remains incompletely understood. Here, we summarize recent advances in the understanding of human tryptase genetics and the effects that different genetic composition may have on the quaternary structure of tetrameric mature tryptases. We also discuss how these differences may impact clinical phenotypes including allergic inflammation, immediate hypersensitivity, and others seen in patients with mast cell-associated disorders. With the increased application of next-generation sequencing, we foresee that human genetic approaches will be a major focus of understanding human tryptase functions in various human mast cell disorders and in new therapeutic development.

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Introduction

Mast cells are immune cells derived from hematopoietic progenitors in the bone marrow [1,2]. Developing mast cells subsequently migrate to the peripheral tissues and undergo terminal differentiation under the influence of local tissue growth factors, such as stem cell factor (SCF; also known as KIT ligand) and interleukin-3 (IL-3) [2].

Mature mast cells are mostly found in tissues where external pathogens enter the body, including the skin, gastrointestinal tract, and airway. Therefore, mast cells are ideally situated as first responders against external pathogens and other environmental stimuli and toxins [3,4].

Mast cells are morphologically distinguished by high content of electron-dense lysosome-like secretory granules [5]. Mast cell granules are prefilled with large quantities of immunomodulatory mediators, including histamine, cytokines (TNF, IL-4, IL-5), and mast cell-specific proteases such as tryptases and chymases [5–7]. Upon mast cell degranulation, mast cell secretory granules fuse among themselves and with the plasma membrane to rapidly release the granule mediators into the extracellular space and exert their functions contributing to immune regulation and host defense [5–7]. Distinct types of stimuli may induce differential release of mast cell mediators [8,9]. Among the pre-formed mediators, tryptases are the most abundant granule proteins (up to 25% of total cellular protein content) in human mast cells [10,11]. Tryptases have a broad range of biological activities and have been shown to regulate immune responses, contribute to host defense and toxin neutralization, and modulate the stromal environment through extracellular matrix remodeling and promotion of fibrosis in preclinical animal models [6,11]. While the functions of tryptases in human diseases are not yet fully understood, serum tryptases have been commonly used in clinical practice to diagnose mast cell-associated disorders including anaphylaxis, mastocytosis, and a number of other secondary or idiopathic mast cell activation syndromes [12,13]. This review focuses on recent advances in our understanding of tryptase genetics and their role in allergic inflammation and immediate hypersensitivity reactions in humans.

Tryptase genetics, structure, and function

Human tryptase isoforms and structure

Human tryptases are comprised principally of four isoforms, including secreted α - and β -tryptases (encoded at *TPSAB1* and/or *TPSB2* -see genetics section below for more detailed discussion), δ -tryptase (*TPSD1*), and membrane anchored γ -tryptase (*TPSG1*) (Table 1). Human δ -tryptase has a C-terminal truncation and lacks essential substrate binding residues [14]; thus human δ -tryptase is considered to be a protein with no biological activity [14]. α -tryptase and β -tryptase are the most studied secreted tryptases. Whereas essentially all trypsin-like serine proteases including β -tryptase have a glycine (G) residue at

Table 1

Biochemical features and enzyme formats of different human tryptase isoforms

Tryptase isoforms	Gene	Activity	Protein/Enzyme format	Variation
α -tryptase	<i>TPSAB1</i>	Inactive ^a	Inactive pro-monomer, Inactive homotetramer, Active α/β heterotetramer	Structural-variable or increased allelic copy number
β I-tryptase	<i>TPSAB1</i> or <i>TPSB2</i>	Tryptic	Inactive pro-monomer and monomer, Active tetramer	Structural-variable copy number ^b
β II-tryptase	<i>TPSAB1</i> or <i>TPSB2</i>	Tryptic	Inactive pro-monomer and monomer, Active tetramer	Structural-variable copy number ^b
β III-tryptase	<i>TPSB2</i>	Tryptic	Inactive pro-monomer and monomer, Active tetramer	β III ^{FS} -tryptase p.M123Dfs*14 (inactive); Structural -variable copy number ^b
γ -tryptase	<i>TPSG1</i>	Tryptic	^c Active; Membrane-anchored	None known
δ -tryptase	<i>TPSD1</i>	Inactive	Truncated; likely monomeric	None known

^a α -tryptase does not have a functional active site and α -tryptase does not have any intrinsic catalytic activity. However, α -tryptase pairs with β -tryptase to form active α/β -tryptase heterotetramers.

^b Increased and decreased allelic copies of β -tryptase have been reported, but the location(s) and isoform(s) of these structural variants are currently undefined.

^c Activity determined on mutant lacking the hydrophobic membrane-anchoring tail.

position 216 (chymotrypsinogen numbering), α -tryptase has essentially no catalytic activity due in part to an aspartic acid (D) substitution at this position. In α -tryptase, the p. G216D and p.D189K residues result in conformational changes in the 214–220 segment that partially fill the S1 pocket and block peptide substrates from binding in the active site [15]. β -Tryptase has three major isoforms, including β I-tryptases, β II-tryptases, and β III-tryptases. These three β -tryptase isoforms have over 95% homology and similar catalytic activity [16^{••}]. Among β -tryptases, about 23% of humans with European ancestry carry a nonsense variant in β III-tryptase resulting from a single base pair insertion that leads to a frameshift and premature stop codon (β III^{FS}) [17]. If expressed, the resulting protein would have a large C-terminal truncation and lack an active site. The ratio of active β -tryptase alleles versus inactive ones (including α -tryptases and β III^{FS}-tryptase) determines the amount of tryptic activity pre-stored in the mast cell granule, and mast cells with a higher number of active β -tryptase alleles have increased total tryptic activity within the mast cell granule [16^{••}]. The genetic basis of frequent and recurrent loss-of-function variants that have resulted in α -tryptase, truncated β III^{FS}-tryptase, and δ -tryptase during human evolution, and how active versus inactive tryptase alleles affect human diseases and host defense, are incompletely understood.

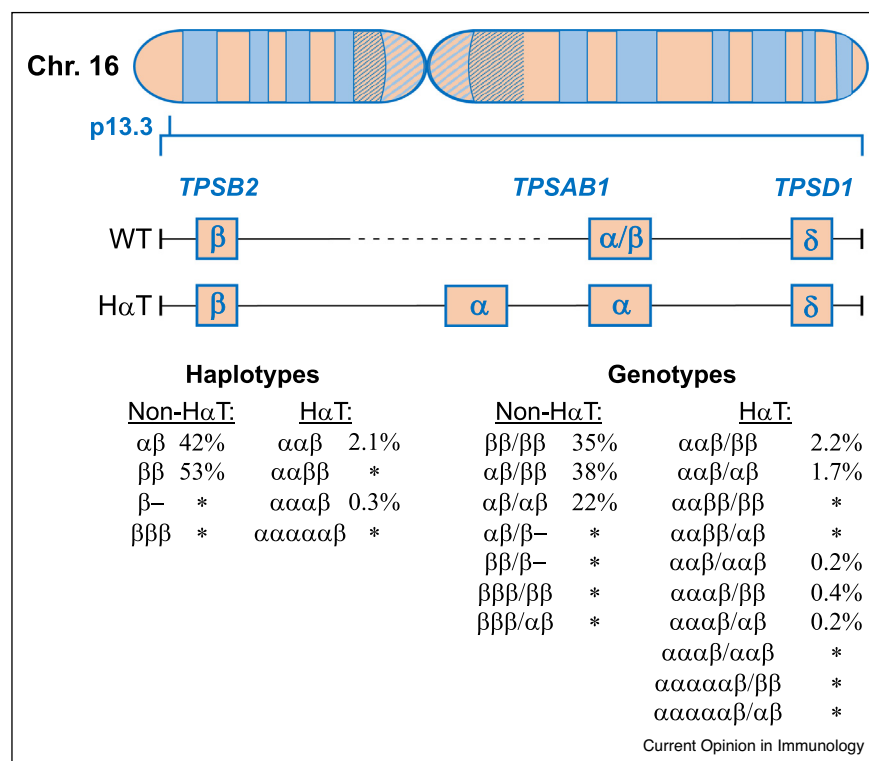
Tryptase is a tetrameric trypsin-like protease with a tightly controlled mechanism governing assembly. Full length tryptases contain a pro-peptide at their N-terminus (pro-tryptase zymogen); pro-peptide cleavage by cathepsins in the mast cell granule appears to be essential for tryptase activation [18]. Mature tryptase monomers (with pro-peptide sequences removed) form tetramers in mast cell secretory granules, which have an acidic pH environment and abundant heparin glycosaminoglycan [11,19]. Since tryptase monomers have negligible

catalytic activity under physiological conditions, tetrameric tryptases are the predominant enzymatically active protease in mast cell granules [19–21]. Tetramer formation is facilitated under these low pH conditions and through the binding to heparin glycosaminoglycan [11,19]. Structural analysis of mature tryptases show a toroidal, donut-like tetramer comprising protomers that contact their neighbor at both the large and small interfaces [22,23]. In this tetrameric form, each tryptase protomer serves as a co-factor to stabilize the neighboring tryptase in a catalytically active conformation [11,20,22,23]. Although α -tryptase does not have any intrinsic catalytic activity, the α -tryptase promoter can stabilize and activate neighboring β -tryptase in an α/β -tryptase heterotetramer format [24^{••}]. Intriguingly, α/β -tryptase heterotetramers have been reported to have altered catalytic activities against certain substrates (e.g. EMR2 and PAR2) when compared with β -tryptase homotetramers (see below in the section on tryptase substrates and biological activities), suggesting that α -tryptase may have a unique effects in interacting with neighboring β -tryptases to regulate overall β -tryptase catalytic conformations and/or substrate accessibility [24^{••}]. The interactions between promoters within tryptases are stabilized by heparin, which is negatively charged and binds to a large positively charged surface spanning both of the small interfaces [25,26]. Since there are no known endogenous inhibitors for tetrameric tryptase, the low extracellular heparin concentration in peripheral circulation may serve as a natural inactivating mechanism where this gradient favors gradual dissolution of tetrameric tryptases into inactive monomers to restrict active enzyme half-life [27].

Human tryptase genetics

The human tryptase locus exists within the subtelomeric region of the short arm of chromosome 16 at position

Figure 1



Identified tryptase haplotypes and genotypes encoded at *TPSAB1* and *TPSB2* and their estimated frequencies.

Haplotypes and resulting genotypes from Wild-type (WT) alleles and hereditary α-tryptasemia (HαT)-associated alleles. HαT results from increased *TPSAB1* copy number encoding α-tryptase on a single allele. *Insufficient data exist to estimate frequencies for these rare haplotypes and genotypes.

p13.3 [28] (Figure 1). All four known tryptase encoding genes — *TPSG1*, *TPSB2*, *TPSAB1*, and *TPSD1* — reside within this locus. As is typical of subtelomeric regions, the tryptase locus contains large segments of homologous repetitive sequences that facilitate gene conversion and replication [29,30]; it is believed that human tryptases evolved in this region via tandem duplication which may help to explain the high degree of homology between human tryptase isoforms [31]. The two best studied loci are *TPSB2* and *TPSAB1*, which are believed to encode the biologically important secreted tryptase isoforms comprised of α and β-tryptase. Whereas *TPSAB1* may encode either α or β-tryptases, *TPSB2* has only been reported to encode β-tryptases (βI, βII, or βIII-tryptase),

In addition to the principal α- and β-tryptase isoforms described, additional structural variations have been described at this locus, and indeed for 16p13.3 generally wherein large duplications (OMIM # 613458) and deletions (OMIM # 610543) have been described to result in syndromic presentations in humans [32,33]. In addition to these larger structural variants — that largely have not been reported to include the tryptase locus — gene replication and copy number loss have also been shown

to occur for human tryptase alleles [34–36]. This phenomenon was first discovered when it was recognized that elevated basal serum tryptase (BST) — composed of constitutively secreted α-pro-tryptase and β-pro-tryptase zymogens — was inherited in an autosomal dominant manner in humans [37]. This increase in BST results from *TPSAB1* replications that encode α-tryptase, a genetic trait known as hereditary alpha-tryptasemia (HαT). However, the increases in BST are far in excess of what has been reported for differences between tryptase haplotypes [38], or what would be expected based purely upon copy number excess. It has been speculated that the increased BST principally results from overexpression of tryptases on the replication-harboring allele, but this is unproven. Indeed, individuals carrying up to four additional *TPSAB1* copies have been reported to date, and BST levels have been shown to follow a gene-dosage effect with each additional *TPSAB1* replication increasing BST by approximately 9.5 ng/mL [34].

Copy number loss has also been anecdotally reported at the tryptase locus, but it is unclear whether these structural variants exist at the *TPSAB1* or *TPSB2* locus based upon current reports [36]. Similarly, increased β-tryptase

encoding copy number has been reported, but where present has not been shown to segregate with elevated BST and it is currently unknown whether these replications exist at *TPSAB1* or *TPSB2* [Wu *et al. Curr Allergy Asthma Rep* (in press)]. Ultimately the location of these replications or deletions may be arbitrary given that either locus may encode any of the known active versions of β -tryptases (β I-III).

Whereas β -tryptases have serine proteinase activity in their complete enzymatic (mature) form, α -tryptases contain two defining variants: one that renders them partially resistant from processing to mature tryptase from immature zymogen, and another at the catalytic site (p.G216D) effectively rendering them enzymatically inactive [15,39,40]. One exception to β -tryptase activity has been identified to occur at *TPSB2* where a nonsense variant, c.980_981insC, p.M123Dfs*14, has been reported in the coding sequence for the β III-tryptase isoform [17]. This single base-pair insertion results in a frame-shift and premature stop codon before the enzyme active site (β III^{FS}). Expression of this truncated protein has not been demonstrated, and the transcript may be unstable and subject to nonsense mediated decay. However, in the unlikely event that β III^{FS}-tryptase is expressed, it would lack intrinsic enzymatic activity. This variant has only been observed in cis with β -tryptase sequences at *TPSAB1*, and thus a tryptase null allele in humans has not been reported [17].

Because the human tryptase locus is small, spanning only approximately 40 kB, the genes within a given locus are in near complete linkage disequilibrium and inherited as a haplotype, one from each parent [28]. While studies are limited, the frequencies and various compositions of these haplotypes have been shown to be somewhat racially and/or ethnically restricted [17]. For example, H α T is principally observed among individuals of Caucasian ancestry [35], whereas the truncated β III-tryptase sequence appears to be lacking among individuals of Asian descent [17]. Moreover, α -tryptase containing alleles appear to be more common among Asians and β -tryptase containing alleles more common among individuals of African descent, with Caucasians residing somewhere in the middle. While the reasons for these differences may be multifactorial, the evolution of human tryptases is very recent, with orthologs even in non-human primates being poorly conserved. Thus, selective population pressures are likely to have been important in establishing these differences.

Tryptase substrates and biological function

Various tryptase substrates and biological targets have been identified through tryptase incubation with purified proteins, or the addition of purified tryptases to cell culture, *ex vivo* tissue, or animal models [11]. As trypsin-like serine proteases, tryptases are capable of

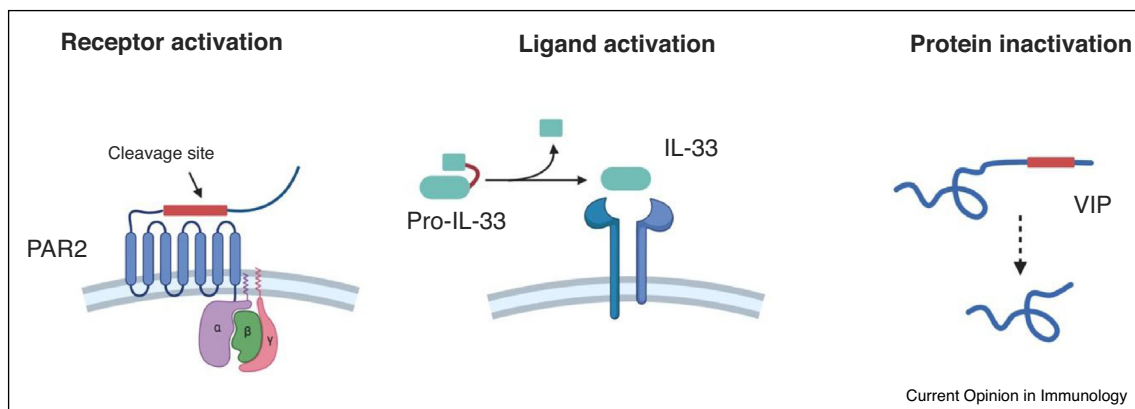
activating protein targets through the cleavage of cell surface receptors (e.g. PAR2, EMR2) or secreted proteins (e.g. IL-33) in order to trigger cell signaling [24^{••},41] (Figure 2 left and middle panels). Conversely, tryptases can also inactivate a protein or peptides thus altering biological activity (e.g. VIP, collagen, fibrinogen) (Figure 2, right panel). However, it remains to be determined whether any of these identified substrates are also biologically relevant targets *in vivo*, and whether one or more of these substrates has a clinically significant impact on health or disease in humans. The species differences among mouse, cynomolgus monkey, and human tryptase isoforms have posed further challenges in the use of mouse and/or non-human primate model systems to study human tryptase biology [31]. Here we summarize a few identified tryptase substrates and discuss their potential biological relevance in humans.

Potentiation of mast cell degranulation

Two mechanisms have recently been proposed whereby tryptases may augment mast cell degranulation in an autocrine manner [16^{••},24^{••}]. EGF-Like Module-Containing Mucin-Like Hormone Receptor-Like 2 (EMR2) is an adhesion G-protein coupled receptor (GPCR) that is highly expressed in human mast cells and other myeloid cells. EMR2 is catalytically cleaved into two noncovalently associated subunits, a proteoglycan binding EMR2 α domain and the GPCR-linked EMR2 β subunit [42–44]. EMR2 α statically inhibits EMR2 β signaling but mechanical force can transiently separate the two subunits to allow EMR2 β to signal and activate mast cells [43,44]. α / β -Tryptase heterotetramers, but not β -tryptase homotetramers, have been shown to be uniquely capable of cleaving EMR2 α and weakening the association of EMR2 α /EMR2 β association to potentiate vibration-dependent mast cell degranulation [24^{••}]. Consistent with this observation, individuals with H α T who have increased α -tryptase copy number have frequently been reported with cutaneous symptoms including flushing and erythema often elicited by vibratory stimuli [24^{••}].

Although the cleavage of EMR2 α on mast cells seems to be α / β -tryptase heterotetramer-specific, recombinant β -tryptase or purified lung tryptases can also induce mast cell degranulation in a catalytic dependent manner [16^{••},45]. Inhibition of tryptase by small molecule inhibitors and inhibitory anti-tryptase antibodies has a mast cell stabilizing effect and reduces IgE-mediated mast cell release of histamine and prostaglandin D2 (PGD₂) generation [16^{••},45]. Beyond EMR2 cleavage, which may not be a generalizable effect on all mast cells given their heterogeneity partly depending upon localization in tissue, the substrates and mechanism(s) underlying autocrine potentiation of mast cell activation by tryptases remain undefined. However, a G α (i)-protein coupled receptor mechanism seems likely to be involved as

Figure 2



Three mechanisms by which tryptase regulates biological activity.

Left panel, direct cleavage of cell surface receptors (e.g., PAR2, EMR2) by tryptase leads to receptor activation; middle panel, tryptase cleavage of a pro-peptide leading to protein activation and subsequent receptor signaling (e.g., IL-33); right panel, the cleavage of a protein (e.g. VIP, gelatin) by tryptase leading to its degradation and inactivation. PAR2, protease-activated receptor 2; EMR2, EGF-Like Module-Containing Mucin-Like Hormone Receptor-Like 2; IL-33, interleukin 33; VIP, vasoactive intestinal peptide.

β -tryptase-induced mast cell degranulation is pertussis toxin sensitive [45].

Promotion of vascular permeability

A link between tryptase enzymatic activity and alterations in vascular permeability were first demonstrated in a Dengue virus associated hemorrhagic fever (DHF) model [46]. In humans, severity of infection has been shown to be associated with elevated serum tryptase levels and a possible role in severity of disease has been suggested [47]. Prolonged incubation of endothelial cells with mature tryptases *in vitro* results in basement membrane (BM) degradation and contributes to vascular leak *in vivo* when injected intravascularly into mice [46]. Moreover, enzymatic inhibition of tryptases using the broad-spectrum serine protease inhibitor nafamostat mesylate can reduce vascular leak resulting from Dengue infection in mice.

In addition to BM degradation resulting from prolonged endothelial cell exposure to mature tryptases, an acute effect on human vascular umbilical vein endothelial cells (HUVECs) has been demonstrated [48^{••}]. Exposure of HUVEC monolayers to tryptases in a Transwell® system results in vascular leak within minutes [48^{••}]. While the effect on vascular BM has been shown to result from mature β -tryptase exposure, only α/β -tryptase heterotetramers were shown to be capable of inducing acute vascular leak via a PAR2-dependent pathway [48^{••}]. While both mechanisms are provocative, their roles in human disease remains speculative. However, a positive association between α -tryptase containing alleles and disease severity has been reported among individuals

with DHF and Dengue shock syndrome [49], suggesting that tryptase isoform composition may be important in disease pathogenesis and severity. Additional considerations regarding a potential role of tryptases on vascular leak in humans are discussed in the context of immediate hypersensitivity below.

Regulation of sensorineural input

Mast cells are often found to be closely associated with nerve fibers in peripheral tissues [50]. Bidirectional communication between mast cells and nerves has been shown to regulate itch, pain, and inflammatory responses in model systems [50,51]. Sensory neurons release neuropeptides, including substance P, which is a potent activator of Mas-related G-protein coupled receptor-X2 (MRGPRX2) present on certain subsets of mast cells [51,52]. Recently it was reported that neuropeptide substance P-mediated mast cell activation through MRGPRB2, a mouse homolog to human MRGPRX2, promoted neuroinflammation associated with pain and itch responses in a mouse preclinical system. Mouse tryptase B2 (*Tpsb2*) is suggested as a key mediator from mast cells for sensory neuron activation through protease-activated receptors, including PAR1, PAR2, and PAR4, which are highly expressed on neurons and have been shown to regulate itching and scratching behaviors [9[•],53–55]. The connection between tryptase B2 and neuroinflammation are largely derived from mouse models and it remains unclear whether human tryptases are capable of activating PAR2 on sensory nerves *in vivo*, and whether in this context α/β -tryptases and β -tryptases may significantly and/or differentially regulate neuroinflammation and sensory neuron activation.

In addition to ostensibly promoting pruritus, tryptase has also been shown to degrade neuropeptides, including vasoactive intestinal peptide, to regulate non-adrenergic neural control of airway contraction tone [16^{••},56,57]. Vasoactive intestinal peptide (VIP) is a neuropeptide that acts on airway nerve fibers to relax bronchial smooth muscle [58]. VIP expression has been reported to be absent in asthmatic lung tissues [59]. The ability of mast cells and mast cell tryptases to degrade VIP may counteract the smooth muscle relaxant effect by VIP in this context and promote airway hyperreactivity [56,57].

Tryptases in allergic inflammation and immediate hypersensitivity

Asthma

Mast cells have been proposed as key mediators in allergic inflammation and hyperreactivity in human airways [60,61]. Asthmatic patients have been reported to have increased numbers of tryptase-positive mast cells in the smooth muscle layers of airways, and mast cell density in the airway smooth muscle has been shown to directly correlate with the degree of airway hyperresponsiveness to methacholine [62,63]. Pharmacological inhibitors of KIT that decreased mast cell reactivity and survival — but also impact ILC2 cells and deplete other bone marrow-derived cells such as neutrophils and eosinophils — have been shown to improve airway hyperreactivity and asthma symptoms [64,65]. As a major mast cell granule protein, tryptase levels are elevated in bronchoalveolar lavage fluid and blood of severe asthma patients as compared with healthy controls [16^{••},66].

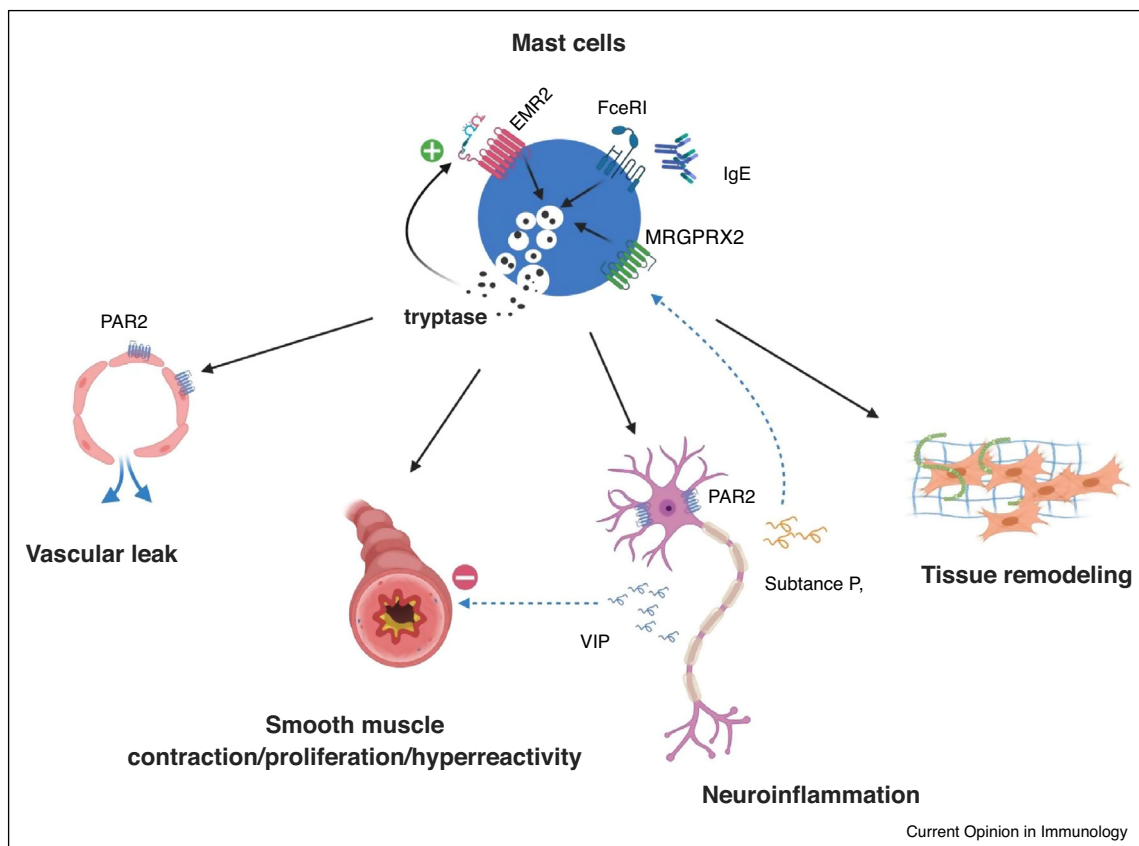
BST elevations in severe asthmatic patients have been shown to occur independently of established type 2 inflammatory biomarkers such as peripheral eosinophilia or periostin levels [16^{••}]. Moreover, a *post hoc* analysis of phase III studies involving asthmatic patients treated with the IgE-neutralizing monoclonal antibody omalizumab revealed that the efficacy was principally enriched in severe asthma subjects with fewer functional β -tryptase alleles [16^{••}]. Patients with fewer functional β -tryptase alleles exhibited improved lung function and asthma symptoms with omalizumab as compared to placebo. In contrast, patients with greater numbers of functional β -tryptase alleles had reduced placebo-adjusted lung function and asthma symptom improvement with omalizumab treatment [16^{••}]. These data suggest that increased β -tryptase activity may potentially contribute to type 2 (T_H2) inflammation-independent asthma pathology. Since catalytically active β -tryptase may enhance mast cell degranulation in an autocrine fashion, we postulate that higher numbers of functional β -tryptase alleles may potentiate mast cell degranulation in an IgE-independent manner. It is worthwhile to point out that while tryptase genetics contributed to clinical responses to omalizumab, β -tryptase allele distributions did not differ between controls and asthmatic patients [16^{••}].

Therefore, tryptases are more likely to modify asthmatic phenotypes rather than to represent a risk factor for asthma incidence.

Tryptases have been demonstrated to exert multiple biological activities *in vitro* and in model systems that are relevant to asthma pathogenesis (Figure 3). As a mast cell granule protein, tryptase has been shown to augment mast cell degranulation in an autocrine manner and inhibition of tryptase activity reduces the magnitude of mast cell degranulation [16^{••},45]. Tryptases have also been shown to increase the sensitivity and magnitude of the contractile response of bronchial smooth muscle [67,68]. β -Tryptases directly promote airway smooth muscle proliferation and contraction in a catalytic dependent manner [16^{••},69,70]. Moreover, tryptases may also regulate non-adrenergic neural control of airway contractile tone. Mast cell tryptases degrade VIP and counteract the smooth muscle relaxant effect of VIP [56]. Finally, tryptases can promote vascular permeability and affect extracellular matrix remodeling and fibrosis (Figure 3). β -Tryptases have been shown to degrade gelatin and aggrecan, and promote fibroblast proliferation and collagen secretion [71,72].

Tryptase inhibition improved airway inflammation and hyperreactivity in preclinical animal models and generated encouraging results in early clinical settings. Mast cell protease-6 (MCP6, the closest ortholog for human β -tryptase) deficient mice exhibited reduced airway hyper-responsiveness in an OVA-sensitized mouse asthma model [73]. Several small molecule protease inhibitors have also been shown to ameliorate asthmatic symptoms in preclinical models involving sheep, rats, and guinea pigs [74–77]. One small molecule peptidomimetic inhibitor (APC366) significantly ameliorated the late asthmatic response in a human Phase II allergen challenge study [78]. However, APC366 was not developed further due to clinical challenges relating to the inhaled formulation and tolerability, as well as moderate potency, poor selectivity, and a lack of oral bioavailability. The unique tetrameric structure of β -tryptases complicates the design and optimization of pharmacological small molecule inhibitors with desirable drug-like properties. Moreover, there are a large number of serine proteases with tryptic-like specificity involved in essential physiological processes including food digestion, blood coagulation, complement activation, and many others. Many of these trypsin-like proteases have similar active sites to β -tryptase which impose additional challenges on rational design methods for improving inhibitor selectivity towards tryptases. While development of small molecule inhibitors of mature tryptases would be clinically desirable, it remains challenging to generate molecules with suitable specificity, *in vivo* pharmacological properties, and oral bioavailability.

Figure 3



Pathophysiologic role for mast cell tryptases in asthma, allergic inflammation and reactions.

Mast cell tryptase exerts multiple biologic effects that may contribute to the pathogenesis of asthma and allergic inflammation, as well as to the severity of immediate hypersensitivity reactions. Tryptases promote mast cell degranulation in an autocrine manner through mechanosensing G-protein coupled receptor EMR2. Tryptases increase acute vascular permeability through PAR2 activation. Tryptases directly promote airway smooth muscle cell proliferation and contraction, increase sensitivity of the bronchial smooth muscle contractile responses, and are capable of cleaving the bronchodilator VIP. In addition, tryptases also affect extracellular matrix remodeling and fibrosis as seen in the context of chronic allergic inflammation. EMR2, EGF-Like Module-Containing Mucin-Like Hormone Receptor-Like 2; PAR2, protease-activated receptor 2; VIP, vasoactive intestinal peptide; MRGPRX2, Mas-related G-protein coupled receptor-X2; FcεRI, high affinity IgE receptor.

Recently, an allosteric monoclonal antibody (31A.v11) was developed to inhibit tetrameric tryptase activity [16^{••}]. This antibody potentially inhibits tetrameric β -tryptase in biochemical and cell-based assays and attenuates IgE-mediated systemic anaphylaxis in a humanized mouse model [16^{••}]. Similar tetramer dissociation phenomena have also been described for other anti-tryptase antibodies and for lactoferrin [79–81]. The crystal structure of the β I-tryptase-Fab complex and biochemical data revealed that 31A.v11 binds to an unexpected exosite, allosterically and non-competitively inhibiting enzymatic activity by destabilizing the tryptase tetramer interfaces, which leads to dissociation into catalytically inactive monomers [16^{••}]. 31A.v11 binds to α -tryptases, β I-tryptases, β II-tryptases, and β III-tryptases with similar affinities. In addition to inhibiting homotetrameric β -tryptases, it also inhibits α/β -tryptase heterotetrametric tryptase

activity through rapid dissociation of heterotetrametric tryptases (Maun HR, Jackman JJ, Lazarus RA., unpublished observations). 31A.v11 exhibited favorable pharmacokinetic properties and inhibited airway tryptase activity in non-human primates and healthy human subjects in a dose-dependent manner [16^{••},82]. Currently, this antibody (also known as MTPS9579A) is under investigation in a Phase II clinical trial for the treatment of patients with moderate-severe asthma (NCT04092582).

Hereditary alpha-tryptasemia (H α T)

Elevated basal serum tryptase (BST) has been shown to be variably present in 3–6% of populations in which this phenomenon has been studied — which to date have all been Western and predominantly Caucasian [36,48^{••},83,84,85^{••}]. The cause for elevated BST in the

substantial majority of these individuals is the autosomal dominant genetic trait hereditary alpha-tryptasemia (H α T). This trait results from increased *TPSAB1* copy number encoding α -tryptase. *TPSAB1* replications have thus far only been identified on alleles also containing α -tryptase sequence and thus frequently described as duplications where two are present, triplications where three are present, and so on. Alleles containing up to four α -tryptase encoding *TPSAB1* copies have been reported in the literature.

Because tryptase is a serum protein that is restricted to allergic effector cells [86,87], it has rarely been studied outside of allergic diseases and reactions. Thus, data related to phenotypes associated with H α T in unselected populations are limited. Moreover, because most studies involving individuals with H α T have been from institutions that focus on genetic and/or mast cell-related disorders, referral biases likely exist in currently reported phenotypes. One small cohort study has examined symptoms among unselected ostensibly healthy adults [34]. Among the small number identified with H α T approximately one-third were found to have minimal clinical complaints, one-third had moderate symptoms, and the remaining one-third reported pervasive multisystem complaints. However, systemic immediate hypersensitivity reactions to stinging insects were found to be nearly ten times more common among individuals with H α T in this study. Of these complaints, irritable bowel syndrome (IBS)-like symptoms and/or cutaneous flushing and/or pruritus were the most prevalent and are likewise the commonest complaints reported across symptomatic cohorts [35]. Moreover, the findings of an increased prevalence and severity of anaphylaxis among individuals with clonal mast cell disorders and stinging insect allergy, respectively, have now been demonstrated in large-scale cohort studies [48^{••},85^{••},88]. While additional clinical manifestations have been associated with H α T including but not limited to: connective tissue abnormalities such as joint hypermobility and retained primary dentition, symptoms of autonomic dysfunction, eosinophilic gastrointestinal disease, food intolerances, chronic pain and fatigue, studies are ongoing in order to test these prior associations. Intriguingly, several of these phenotypes have been associated with elevated BST in population or cohort studies involving ungenotyped participants suggesting that H α T may contribute to or modify the clinical presentations of several of these clinical phenotypes [83,89–92].

Similar to the gene-dosage effect that has been described for BST in individuals with H α T, a gene-dosage effect on symptoms was first reported among a large cohort of families, many of whom were found to have more than one extra *TPSAB1* copy, resulting either from higher order copy number on a single allele, or inheritance of multiple haplotypes containing duplications and/or

triplications [34]. While one small cohort study did not support this initial finding [36] — likely due to a small sample size — a recent larger study involving patients with systemic mastocytosis demonstrated a similar significant gene-dosage effect on mast cell mediator-associated symptoms as determined using a validated questionnaire [85^{••}].

How α -tryptase may contribute to, modify and/or provoke clinical phenotypes and symptoms associated with H α T remains unclear. However, α/β -tryptase heterotetramers have been posited as one possible explanation [24^{••},48^{••}]. Indeed, the demonstrable activity of heterotetramers on PAR2 and the mechanosensing GPCR EMR2, which at ostensibly physiological concentrations *in vitro*, appears to be selective, is provocative. However, mature tryptases are principally released *en masse* following mast cell activation [28], and are not observable in carefully handled and preserved tissue sections or baseline peripheral blood samples [93], making this explanation for many of the chronic symptoms somewhat unsatisfactory. Moreover, a number of studies have now demonstrated increased numbers of mast cells in bone marrow and in the GI epithelium when compared to healthy volunteers, disease controls, and to patients without H α T who have shared clinical symptoms [37,88,115,116]. These data would suggest that H α T may affect mast cell homeostasis in some manner.

A compelling case for this might also be made on the basis of large cohort as well as Mendelian genetic studies that have demonstrated H α T to be present in up to 17% of individuals identified with the clonal mast cell disease systemic mastocytosis (SM) [48^{••},85^{••},94]. In these studies, H α T was also shown to modify the severity of symptoms among SM patients — including doubling the prevalence of anaphylaxis — likely resulting in a detection bias, where individuals with concomitant H α T and SM are more likely to come to medical attention because they are more symptomatic. However, a similar bias would not exist or explain the increased mast cell number in bone marrow and gut mucosae of individuals with H α T in the absence of SM. The mechanisms underlying these findings, and whether heterotetramers, or some other unidentified role of α -tryptases, contribute (s) to these clinical associations, remains unproven.

Immediate hypersensitivity

Mature tryptases are stored in mast cell secretory granules where they are stabilized under low pH by heparin bound to serglycin [11,19,95]. Degranulation occurs *en masse* following mast cell activation that occurs canonically via antigen-mediated cross-linking of cognate IgE bound to the high affinity IgE receptor (Fc ϵ RI) present on the mast cell surface [28,60,86]. IgE-independent mechanisms of mast cell activation have also been described, most notably via activation of the activating Mas relating

GPR family member X2 (MRGPRX2) receptor by cationic or similarly charged small molecules [51]. In addition to tryptase, a large number of pre-formed mediators exist within mast cell secretory granules, including histamine and platelet activating factor, that have been shown to contribute to the symptoms of immediate hypersensitivity reactions [5,96]. These symptoms include redness, swelling, and pruritus that may be localized, or result in systemic anaphylaxis due to vascular leak resulting in rhinorrhea, diarrhea, and in severe cases hypotensive syncope and loss of control of bodily functions.

A number of tryptase activities, including anaphylatoxin generation and contact system activation, have been demonstrated *in vitro* that may be capable of contributing to symptoms of immediate hypersensitivity [97–100]. However, differences in human and murine tryptases and targets have historically limited more definitive studies [31]. Recently, a humanized model employing the NOD.scid IL2rg^{null}SCF/GM-CSF/IL-3 (NSG-SGM3) mouse has demonstrated a major contribution of mature tryptases in modeled IgE-dependent anaphylaxis [16^{••}]. In these mice neutralization of the enzymatic activity of mature human tryptases resulted in a significant reduction in anaphylaxis severity *in vivo*, as measured by core temperature drop following sensitization and allergen challenge of the animals. In addition to the acute effects on endothelial monolayer permeability resulting from PAR2 activation by α/β -tryptase heterotetramers described above [24^{••}], mature recombinant β -tryptase was shown to promote mast cell degranulation — increasing histamine and β -hexosaminidase release — and to augment PGD₂ generation.

Anaphylaxis is associated with an increase in total serum tryptase resulting principally from release of mature tryptases from mast cell secretory granules during degranulation [101,102]. Indeed, more severe anaphylaxis is generally associated with higher serum tryptase levels [103,104], most notably following parenteral antigen exposures (e.g., envenomation or intravenous medication). The specificity of this finding for mast cell degranulation has resulted in serum measurements of tryptase within 4 hours of symptom onset being the most reliable biomarker currently available for the confirmation of mast cell mediated reactions [105], though notable exceptions and limitations exist.

Despite the importance of serum tryptases in confirming the clinical diagnosis of anaphylaxis, the role of tryptases in immediate hypersensitivity reactions in humans remains undefined. While α -tryptase deficiency is fairly common [106], β -tryptase deficiency has not been reported and a null β -tryptase haplotype still has yet to be identified [17]. However, numerous population studies have identified elevated BST as associated with an increased prevalence and/or severity of anaphylaxis in

humans in a number of contexts, most notably in the context of Hymenoptera envenomation [83,89,90,92,107–114]. While older studies did not determine the cause for elevated BST, multiple recent studies have confirmed that H α T is the common cause for elevated BST [36,48^{••},85^{••}]. Moreover, H α T has now been shown to be associated with an increased prevalence of severe anaphylaxis that results from insect stings or that occurs spontaneously (i.e. idiopathic anaphylaxis), and from any cause among individuals with SM. In all disease-specific cohort analyses, H α T was associated with a two-fold to three-fold increase in severe anaphylaxis relative to controls and healthy volunteers. While rare inherited and acquired genetic variants have been described in association with increased mast cell reactivity and anaphylaxis — including the acquired *KIT* p.D816V pathogenic variant commonly associated with SM — H α T is the first common heritable risk factor identified for severe anaphylaxis.

Whether elevated BST composed principally of pro-tryptases [102] — and in the case of H α T likely α -pro-tryptases — may exert any physiological effect(s) that contribute to these clinical associations remains unknown. The unique properties and potentially expanded substrate specificity of α/β -tryptase heterotetramers [24^{••},48^{••}], resulting in increased mast cell reactivity to vibration and selectively promoting endothelial cell permeability at physiologic levels, may provide some of the bases for the clinical associations of H α T with anaphylaxis. However, whether heterotetramers may directly activate mast cells in a manner similar to recombinant mature β -tryptase [16^{••}] has not been demonstrated, and mechanism(s) underlying the association between mast cell number, clonal disease, and H α T remain elusive. Despite these limitations, the clinical and recent *in vitro* and *in vivo* experimental advances are provocative, suggesting an important contributory role of tryptases in severe immediate hypersensitivity reactions. Ultimately, pharmacologic inhibition of mature tryptase activity in clinical trials using monoclonal antibodies or small molecule inhibitors will provide the strongest evidence for a putative role of mature tryptases in severe anaphylaxis.

Concluding remarks and future perspectives

Historically, mast cell tryptases have been extensively studied in both *in vitro* and *in vivo* models of allergic pathogenesis, inflammation and remodeling. Increased serum tryptase levels have also been clinically associated with clonal mast cell disorders, and in immediate hypersensitivity reactions basal levels have been associated with increased prevalence and/or severity of reactions whereas acute elevations are consistent with mast cell activation. However, the complex structural variation of the human tryptase locus has now begun to be appreciated and the impact of increased α -tryptase encoding

TPSAB1 copy number on BST and the severity of immediate hypersensitivity reactions in certain contexts now understood. The association of hereditary alpha-tryptasemia and severe anaphylaxis suggests that α -tryptases have an important role in mast cell activation and/or effector cell responsiveness, such as vascular permeability. Active β -tryptase copy number differences and recently described altered substrate specificity of α/β -tryptase heterotetramers add further complexity to our understanding of how tryptase genetic variation may regulate mast cell function and disease phenotypes. With the increased application of next-generation sequencing and available genotyping methods to detect α -tryptase and β -tryptase copy number variation and active β -tryptase allele counts, we foresee that genetic approaches will be a major focus of in various human mast cell disorders and in therapies targeting this compartment or immediate hypersensitivity reactions.

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Conflict of interest statement

T.Y. is a current employee of Genentech, a member of the Roche group. J.J.L. has no competing interests to declare.

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