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Diagnostic Value of Tryptase in Anaphylaxis and Mastocytosis

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Tryptase background

The principal protein component of human mast cell secretory granules was first detected as trypsin-like activity by histoenzymatic stains [1–3] and then as releasable trypsin-like activity [4]. The enzyme accounting for greater than 90% of this activity was named tryptase [5,6], which was shown in vitro to be a marker of mast cell degranulation that was released in parallel with histamine and β-hexosaminidase.

Molecular biology

Two genes encode the major human mast cell tryptases, α-tryptase and β-tryptase [7–9]. These genes are clustered on human chromosome 16p13.3 [8,10]. The haploid genotype for tryptase is βα or ββ (ie, there are two tandemly arranged tryptase genes on human chromosome 16p13.3, a monomorphic copy of β-tryptase, and an allelic copy of α- or β-tryptase). Consequently, diploid individuals may have a βα/βα, ββ/βα, or ββ/ββ genotype. The βα and ββ haplotypes are common. In fact, almost 25% of subjects are α-tryptase deficient (ββ/ββ) [11–13]. The α/β-tryptases encode a 30–amino acid leader and a 245–amino acid catalytic sequence. The α-tryptases show an approximately 90% sequence identity to β-tryptases. Defining amino acid differences between α/β-tryptases seems to include Q/R^{-3} and D/G^{215}. The αI- and αII-tryptases and βI-, βII-, and βIII-tryptases show at least 98% identity within types. Each of these tryptase genes is organized into six exons and five introns, and alternative splicing has not been demonstrated.

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Also on chromosome 16p13.3 is δ-tryptase(s), initially named murine mast cell protease (mMCP)-7-like tryptase(s) [10]. The product of this gene shows close homology to α/β-tryptases over exons 1 through 4, having Q^{-3} like α-tryptase, but exon 5 is more closely related to mMCP-7. Although small amounts may be expressed by mast cells and other cell types, a seemingly premature stop codon terminates translation 40 amino acids earlier than α- and β-tryptases [14,15]. Western blot analysis of mast cell extracts using monoclonal antibodies (mAbs) prepared against purified β-tryptase but that recognize α- and β-tryptases has not revealed a protein band under 30,000 d (where δ-tryptase should migrate) (unpublished data), suggesting that these mAbs fail to recognize this protein or that little, if any, is stored in mast cells.

β-Protryptase is processed in two proteolytic steps. First, autocatalytic intermolecular cleavage at R^{-3} occurs, optimally at acidic pH and in the presence of heparin (or dextran sulfate). Second, the remaining pro′ dipeptide is removed, ostensibly by dipeptidyl peptidase I. The mature protein spontaneously forms enzymatically active tetramers at acidic pH in the presence of a polyanion like heparin [16], which also stabilizes the tetramer by binding to a cationic groove that spans each of two dimers in the tetramer [17,18]. The novel processing and stabilization mechanisms provide a teleologic explanation for why tryptase and heparin are coexpressed in human mast cells as well as in mast cells of many other species.

With respect to human αI-protryptase (and perhaps mMCP-7-like tryptase), the presence of Q^{-3} precludes optimal autocatalytic processing. Without a mechanism for processing α-protryptase, this protein remains enzymatically inactive. Further, even if the mature α-tryptase forms in vivo, based on in vitro data with recombinant mature α-tryptase, there would be negligible enzymatic activity against small synthetic substrates and no proteolytic activity [13,19,20].

Mature β-tryptase resides in secretory granules as an enzymatically active tetramer in a complex with proteoglycan, presumably heparin [5,6,17,18,21–23]. All the active sites face into the small central pore of the planar tetramer, thereby restricting inhibitor (and substrate) access and explaining previous observations of resistance to biologic inhibitors [24]. Skin mast cells were used to show that protryptase(s) are spontaneously secreted by mast cells at rest, whereas mature tryptase(s) are stored in secretory granules until their release by activated cells [13]. Indeed, when skin mast cells are cultured for 6 days, most of the tryptase is mature and retained by these cells, whereas spontaneously secreted tryptase includes α and β protryptases. Thus, α/β-protryptases are spontaneously secreted by resting mast cells, whereas mature β-tryptase is retained by mast cells until these cells are activated to degranulate.

Tryptase regulation

The quantity of catalytically active tryptase per mast cell (10–35 pg) [25] is dramatically higher than the levels of proteases found in other

granulocytes. What regulates tryptase activity after its release in vivo is uncertain, because the tetrameric enzyme resists inhibition by biologic inhibitors of serine proteases [24]. Regulation might occur, in part, when basic proteins, such as antithrombin III, dissociate the enzyme from heparin [24], but this is slow and incomplete, providing an unsatisfactory mechanism for tightly regulating catalytic activity.

Another possibility for regulation arises from observations that β-tryptase degrades fibrinogen approximately 50-fold faster at pH 6 than at pH 7.4 [26]. A similar acidic pH optimum had been noted for autoprocessing of β-protryptase [16] and for cleavage of low-molecular-weight kininogen [27] by lung-derived tryptase. In contrast, cleavage of small synthetic peptide or ester substrates occurs more readily at neutral than acidic pH, like classic serine proteases. Thus, release of β-tryptase at sites of acidic pH (asthmatic airway surface, foci of inflammation, and areas of poor vascularity [eg, solid tumor margins, wound healing sites]), would be optimal for proteolysis, whereas diffusion to sites of neutral pH would result in reduced proteolytic activity, limiting optimal activity to the local tissue site of release.

Catalytically active, tetrameric β-tryptase, in the absence of a stabilizing polyanion like heparin, converts to inactive monomers at neutral pH and physiologic ionic strength. Placing these inactive tryptase monomers into an acidic environment (at a concentration ≥ 1 μg/mL) leads to the complete reassociation of these monomers into a catalytically active tetramer [28]. The mechanism for reconstitution of active tetramer involves conversion of inactive monomers first to active monomers and then to tetramers [29]. At lower concentrations in an acidic pH environment with heparin, active monomers form without progressing to tetramers. Finally, the B12 antitryptase mAb inhibits β-tryptase at neutral pH and activates β-tryptase at acidic pH by converting heparin-stabilized tetramers to monomers [30].

Biologic activities of tryptase

The biologic activity(ies) of enzymatically active tryptase is not obvious from the involvement of mast cells in diseases like mastocytosis, anaphylaxis, urticaria, and asthma. The most relevant biologic substrate(s) of tryptase remain uncertain, although many potential ones have been evaluated, primarily in vitro. Predicted biologic outcomes might include anticoagulation, fibrosis and fibrolysis, kinin generation and destruction, cell surface protease-activated receptor (PAR)-2 activation, enhancement of vasopermeability, angiogenesis, inflammation, and airway smooth-muscle hyperreactivity. Showing the importance of these potential activities in vivo remains a challenge.

Tryptase as a clinical marker of anaphylaxis and mastocytosis

Because tryptase is selectively and abundantly produced by mast cells, tryptase levels in biologic fluids should provide a more precise measure of

local or systemic involvement of these cells than is possible to ascertain by clinical presentation or documentation of antigen-specific IgE. Basophils, the only other cell type that normally expresses tryptase, contain approximately 1/500th the amount [31]. Accordingly, mouse mAbs were prepared against human tryptase to develop specific tryptase immunoassays.

All mAbs prepared against tryptase in the author's laboratory against human β-tryptase recognize mature and precursor forms of α- and β-tryptases except one, which is called G5. The G5 mAb recognizes a linear epitope only on the mature forms of natural and recombinant β-tryptase and of recombinant α-tryptase [13]. This is clinically relevant, because α/β-tryptase precursors seem to be continuously secreted by human mast cells [13], with their levels in blood typically reflecting the burden or number of mast cells [32]. In contrast, mature tryptase, presumably β-tryptase, is stored in secretory granules and is released only during granule exocytosis, with levels thereby reflecting mast cell activation. Two immunoassays were developed, one that measures mature α/β tryptases and another that recognizes mature and precursor forms of α/β tryptases. Both use B12 mAb (conformational epitope) for capture, and then for detection the G5 mAb to measure mature α/β tryptases or the G4 mAb (linear epitope) to measure total tryptase (pro, pro′, and mature forms of α/β tryptases). Because only β-protryptase is thought to convert to mature β-tryptase in vivo, the mature tryptase immunoassay probably detects only β-tryptase in patient samples.

The total tryptase fluoroimmunoenzymatic assay (B12 capture, G4 detection) is available commercially (Phadia AB, Uppsala, Sweden), whereas the mature tryptase assay (B12 capture, G5 detection) is only available as an ELISA in the author's laboratory. In healthy subjects, mature tryptase levels in serum and plasma are undetectable (<1 ng/mL), whereas total tryptase levels range from 1 to 15 ng/mL and average approximately 5 ng/mL (Table 1).

The tryptase haplotype and gender have a modest effect on the total tryptase level of healthy individuals [33]. Tryptase haplotypes exhibit an approximately 1:2:1 (βα/βα:ββ/βα:ββ/ββ) distribution. The βα haplotype increases the total tryptase level by 0.5 ng/mL over the mean, whereas female gender

Table 1
Mature and total tryptase levels

Clinical condition	Tryptase levels (ng/mL)		Tryptase ratio (total/mature)
	Total	Mature	
Normal	1–15	<1	Not applicable
Systemic anaphylaxis (acute)	>Baseline	>1[a]	<10
Systemic mastocytosis (nonacute)	>20[b]	<1 to small elevations	>20

[a] Level related to clinical severity (hypotension), timing of sample collection in relation to onset of signs and symptoms, and nature of the anaphylactic stimulus.

[b] Speculated to reflect primarily the total body burden of mast cells.

also increases the level by 0.2 ng/mL over the mean. A similar study using subjects with systemic mastocytosis indicates no statistically significant effect of the α/β tryptase genotype on serum levels of total tryptase (data not shown). The small effects observed in normal subjects would not have been discernible in the mastocytosis patients, however, because of smaller patient numbers and because the distribution of total tryptase levels in such patients is much wider than in healthy subjects.

The impact of the α/β-tryptase genotype on basophil tryptase levels and the type of tryptase stored in these cells also were determined [33]. Tryptase in 19 of 20 basophil preparations was mature and enzymatically active. Tryptase quantities in basophils were less than 1% of those in tissue mast cells. Tryptase protein and mRNA levels per basophil were not affected by the tryptase genotype. Peripheral blood basophils obtained from active asthmatics and healthy subjects contained comparable amounts of tryptase [34]. How much α/β-protryptase is spontaneously released by these cells is not known.

Systemic anaphylaxis

β-Tryptase levels in serum or plasma (detected by the mature tryptase immunoassay) are elevated in most subjects with systemic anaphylaxis of sufficient severity to result in hypotension [35]. Although β-tryptase is released from mast cells in parallel with histamine, the protein diffuses through tissues more slowly than histamine, presumably because of its association with the macromolecular protease/proteoglycan complex. As illustrated in Fig. 1, during insect sting–induced anaphylactic hypotension, β-tryptase levels in the circulation are maximal 15 to 120 minutes after the sting,

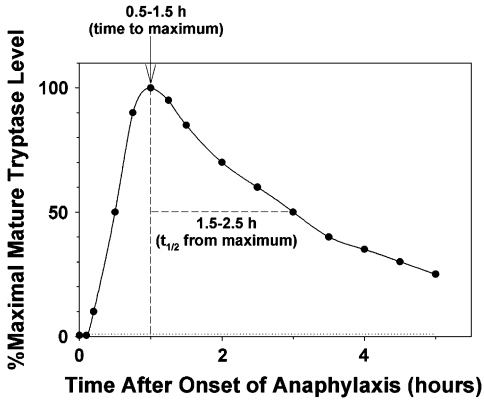

Fig. 1. Hypothetic time course for the appearance of mature tryptase in serum or plasma during systemic anaphylaxis. The maximal level is set at 100% in the figure; however, in reality, it varies depending at least in part on the clinical severity and nature of the anaphylactic stimulus, which, in turn, affects how long mature tryptase is in a detectable range. $t_{1/2}$, half-life.

whereas histamine levels peak at approximately 5 minutes and decline to baseline by 15 to 30 minutes [36,37]. Peak β-tryptase levels decline with a half-life of 1.5 to 2.5 hours. The practical consequence of these different time courses is that plasma samples for histamine levels must be obtained within 15 minutes of the onset of such reactions, whereas those for β-tryptase levels can be obtained up to several hours after the reaction begins, depending on its severity. In systemic anaphylaxis induced experimentally in 17 subjects by insect stings, peak levels of β-tryptase correlated closely to the drop in mean arterial pressure, indicating that the magnitude of mast cell activation and mediator release is a primary determinant of the clinical severity of systemic anaphylaxis [36]. Further, the ratios of total tryptase to β-tryptase were less than 6 in 16 of 17 subjects, and the ratio was 23 in the single outlier [38]. Thus, when β-tryptase is detectable in serum, a total-to–β-tryptase ratio of 10 or less suggests systemic anaphylaxis.

In addition to insect sting–induced anaphylaxis, mature tryptase levels have been used to investigate hypotensive episodes occurring during surgery [39–42], injection of fluorescein [43] or methylprednisolone [44], ingestion of nonsteroidal anti-inflammatory drugs [45], and exposure to latex [46,47] and other pharmaceutic and environmental stimuli. Elevated serum levels of mature tryptase in postmortem serum also serve as an indicator of premortem anaphylaxis [48]. In one study, β-tryptase levels in serum were determined in possible cases of fatal systemic anaphylaxis within 24 hours of death in 19 victims [48]. Elevated levels (>10 ng/mL) appeared in 9 of 9 subjects after Hymenoptera stings, in 6 of 8 after food ingestion, and in 2 of 2 in reaction to parenteral diagnostic or therapeutic agents. Levels were less than 5 ng/mL in 57 sequential sera collected postmortem from 6 control subjects. In general, β-tryptase levels were dramatically higher after parenteral rather than oral introduction of the allergen, in spite of a fatal outcome in each case.

Not all hypotensive reactions that clinically seem to be anaphylactic are associated with elevated levels of mature tryptase, however. For example, victims of fatal and near-fatal food-induced anaphylaxis often show no mature tryptase elevation [48,49], raising the possibility that some of these events may not be dependent on mast cell activation. Basophils have been suggested as an alternative effector cell, but direct evidence for this has not yet emerged. Other considerations might include overproduction through non-mast cell pathways of vasoactive mediators, such as complement anaphylatoxins, kinins, or lipids.

Against the diagnostic specificity of postmortem β-tryptase levels is a study that found elevated levels of β-tryptase (>10 ng/mL) in 5 of 49 cases thought to be nonanaphylactic deaths. One case was a salicylate overdose (23 ng/mL). Because mast cell activation occurs with anaphylactic and airway hypersensitivity reactions to cyclooxygenase inhibitors, this individual could have been aspirin sensitive. One subject had a diagnosis of atherosclerotic coronary vascular disease (β-tryptase of 33 ng/mL), as did 10 other subjects with levels less than 5 ng/mL. Details regarding drugs received

near the time of death, particularly those that might activate mast cells, such as morphine, were not available. Three subjects died of multiple trauma (β-tryptase values of 20, 24, and 106 ng /mL). Thus, careful consideration of the events near the time of death is needed to interpret postmortem levels of β-tryptase fully.

Systemic mastocytosis

Systemic mastocytosis is associated with mast cell hyperplasia in skin lesions (urticaria pigmentosa), the liver, the spleen, lymph nodes, and bone marrow [50,51] and is subdivided into mastocytosis that is indolent (indolent systemic mastocytosis [ISM]), smoldering (smoldering systemic mastocytosis [SSM]), systemic mastocytosis associated with a hematologic clonal non-mast cell disorder (SM-AHNMD), or aggressive (ASM). Mast cell leukemia (MCL) and sarcoma, each of which is quite rare, are malignant forms of mastocytosis. Activating mutations in the tyrosine kinase portion of Kit are associated with systemic or persistent disease [52]. In general, total tryptase levels are greater than 20 ng/mL in patients with systemic mastocytosis. Ratios of total to mature serum tryptase, when the latter is detectable, are greater than 20. Total tryptase levels in plasma correlate with the density of mast cells in urticaria pigmentosa lesions in adults with systemic mastocytosis [53]. For those with only cutaneous mastocytosis, normal levels of total tryptase are typically observed [32,38,54,55]. Total tryptase levels are recommended by the World Health Organization as a minor criterion for use in the diagnostic evaluation of systemic mastocytosis [50,51,56]. The total tryptase level in serum or plasma seems to be a more discriminating biomarker than urinary methylhistamine for the diagnosis of systemic mastocytosis [57]. Whether always to proceed with a bone marrow biopsy, given an elevated total tryptase level, should be based on additional clinical or laboratory features. For example, in adult and pediatric patients with an elevated total tryptase level and a clear clinical diagnosis of ISM, a bone marrow biopsy is considered optional by some experts. A marked increase in a patient's baseline tryptase level or a significant clinical change suggests that further evaluation is indicated, however, including a bone marrow biopsy, to evaluate the stage of disease.

Total tryptase levels measured early in the course of emerging systemic disease, when the mast cell burden is low, may be normal but become higher several months to a year later when the steady-state burden of mast cells has established itself. Typically, total tryptase levels do not change dramatically once systemic mastocytosis is established but can be followed as a measure of disease burden. Although total tryptase levels tend to be higher in more aggressive disease, the overlap between disease categories is substantial.

Anaphylactic or anaphylactoid reactions may be a presenting manifestation of systemic mastocytosis, particularly in response to insect stings [58,59] but also to radiocontrast media and narcotics. Ratios of total tryptase to

β-tryptase are variable when a subject with systemic mastocytosis experiences anaphylaxis. If mastocytosis is being considered because of an anaphylactic reaction to an insect sting or to another inciting event, one should wait at least 24 hours after clinical signs and symptoms have completely subsided before a baseline total tryptase level is obtained. Even in those individuals without mastocytosis, an increased mast cell burden (as reflected by total tryptase levels of 10–20 ng/mL) places them at increased risk for more severe anaphylactic reactions [38,60]. Patients with systemic mastocytosis and those at increased risk for systemic anaphylaxis should be premedicated with H1 and H2 antihistamines and glucocorticosteroids before administration of radiocontrast media or anesthetic agents capable of activating these cells through non-IgE-dependent pathways. Thus, total tryptase levels are a relatively noninvasive surrogate marker of the overall mast cell burden and, possibly, for the severity of future systemic anaphylactic reactions.

Total tryptase levels also can be used to monitor the response to mast cell reductive therapy. For example, three mastocytosis patients treated with cyclosporin A failed to respond clinically, and their total tryptase levels failed to decline [61]. In contrast, a case report of a patient with systemic mastocytosis (D816V) and urticaria pigmentosa showed a response to high-dose interferon-α (10 million U three times per week) manifested by a decrease in mast cell percentages in bone marrow aspirates from 50 to 5 or less, a 75% decrease in urinary prostaglandin F2α, a 75% decrease in urinary methyl histamine, a 98% decrease in serum total tryptase, a decrease in serum calcitonin, and resolution of urticaria pigmentosa [62]. Treatment of mastocytosis with cladribine also was associated with clinical improvement and a decline in total tryptase levels, but adverse drug-related side effects were a concern [63,64]. Elevated total tryptase levels also declined into the normal range in a patient with MCL treated with myeloablative stem cell transplantation [65].

Non-mast cell disorders associated with elevated total tryptase levels

Activating Kit mutations can be detected in mast cells and other myeloid cells from patients with systemic mastocytosis associated with various hematologic disorders [66,67] or in patients with myeloproliferative diseases associated with independent mutations [68]. For example, approximately 40% of patients with acute myeloblastic leukemia have elevated total tryptase levels, with the source of tryptase being blasts rather than hyperplastic mast cells [69]. These blasts display lineage markers for mast cells and other myeloid cells [70]. Elevated total tryptase levels also may be associated with various myelodysplastic disorders and can be produced by abnormal mast cells [71] and basophils [72] associated with such disorders. In the hypereosinophilic syndrome associated with the FIP1L1/PDGFRA mutation, total tryptase levels are elevated [73], perhaps related to an associated mast cell hyperplasia. Of further interest is that total tryptase levels decline along with the

eosinophil numbers in response to imatinib [74]. A similar response to imatinib has been reported in a patient with systemic mastocytosis and hypereosinophilic syndrome associated with the FIP1L1/PDGFRA mutation [75].

Elevated total tryptase levels have been reported in patients with end-stage kidney disease and occur with treatment of onchocerciasis, suggesting transient mast cell hyperplasia [76]. Idiopathic episodes of transient mastocytosis in adults may occur based on transiently elevations in total tryptase levels along with signs and symptoms associated with the release of mast cell mediators in a small number of patients [77].

Summary

Serum (or plasma) levels of total and mature tryptase measurements are recommended in the diagnostic evaluation of systemic anaphylaxis and systemic mastocytosis, but their interpretation must be considered in the context of a complete workup of each patient. Total tryptase levels generally reflect the increased burden of mast cells in patients with all forms of systemic mastocytosis (indolent systemic mastocytosis, smoldering systemic mastocytosis, systemic mastocytosis associated with a hematologic clonal non-mast cell disorder, aggressive systemic mastocytosis, and mast cell leukemia) and the decreased burden of mast cells associated with cytoreductive therapies in these disorders. Causes of an elevated total tryptase level other than systemic mastocytosis must be considered, however, and include systemic anaphylaxis, acute myelocytic leukemia, various myelodysplastic syndromes, hypereosinophilic syndrome associated with the FLP1L1-PDGFRA mutation, end-stage renal failure, and treatment of onchocerciasis. Mature (β) tryptase levels generally reflect the magnitude of mast cell activation and are elevated during most cases of systemic anaphylaxis, particularly with parenteral exposure to the inciting agent.

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