

Update review

Tryptase genetics and anaphylaxis

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Tryptases secreted by tissue mast cells and basophils can enter the bloodstream. In human subjects tryptases are encoded by several genes and alleles, including α , β , γ , and δ . Common variations include complete absence of α genes. Until recently, α tryptase was considered to be the major tryptase secreted at baseline and in mastocytosis. However, lack of α tryptase genes has little effect on circulating tryptase levels, which are now thought mainly to consist of inactive pro- β tryptase secreted constitutively rather than stored in granules with mature tryptases. Pro- β tryptase levels thus might reflect total body mast cell content. In contrast, mature β tryptase can increase transiently in severe systemic anaphylaxis and confirm the diagnosis. However, it might fail to increase in food anaphylaxis or might increase nonspecifically in samples acquired after death. Thus pro- and mature β tryptase measurements are useful but associated with false-negative and false-positive results, which need to be considered in drawing clinical conclusions in cases of suspected anaphylaxis. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Anaphylaxis, tryptase, mastocytosis, mast cell, basophil

Tryptases are a subgroup of trypsin-family serine peptidases with shared enzymatic, structural, and phylogenetic features. Their connection to anaphylaxis arises from the fact that they are among the most specific products of mast cells and basophils.¹ Although mast cell subsets exhibit major variations in phenotype, almost all human mast cells, regardless of tissue location, make and store tryptases, which are the major proteins in their secretory granules. Some tryptases (notably β tryptase, see below) are released in bursts from mast cells stimulated by the combination of allergen and allergen-specific IgE. This set of properties makes them useful as markers of mast cell activation in anaphylaxis and anaphylactoid reactions.² Aside from serving as markers, it seems likely that exocytosed tryptases also help to produce clinical hallmarks of anaphylaxis, such as urticaria, angioedema,

and bronchospasm, as further considered below. Basophils, on the other hand, express tryptases in much smaller amounts and might play less significant roles than mast cells in acute anaphylaxis. Although several important features of mast cell tryptase were described 10 to 25 years ago, some were unexplained or misunderstood until recent years. In particular, there is recent appreciation that human mast cell tryptase encompasses products of as many as 4 gene loci and achieves additional diversity through allelic variation. This gives rise to major enzymatic and behavioral differences among expressed products of these genes.³ This newer knowledge allows reassessment of specific tryptases as markers and mediators of anaphylaxis.

TRYPTASE GENES: MULTIPLICITY AND VARIATION

All known human mast cell tryptase genes lie in a compact cluster near the end of the short arm of chromosome 16^{3,4} containing *TPSAB1* (α and $\beta 1$ alleles), *TPSB2* ($\beta 2$ and $\beta 3$ alleles), *TPSD1* (δ alleles), and *TPSG1* (γ alleles). The *TPSAB1* and *TPSB2* sites harbor alleles of classical soluble α and β tryptases. The surprise finding from dissection of this part of the locus is that the α allele, which was once presumed to occupy its own locus, occupies a site shared with $\beta 1$ alleles. A key prediction of this interpretation of the genetic data is that individuals inheriting 2 $\beta 1$ alleles at this site will be α null and that considering potential α and β alleles at the *TPSAB1* (α or β) and *TPSB2* (β only) sites, there are 3 fundamental genotypes: $\alpha\alpha:\beta\beta$, $\alpha\beta:\beta\beta$, and $\beta\beta:\beta\beta$. This prediction has been validated by identification of substantial subpopulations that are genetically deficient in α tryptase or that carry just one α gene.⁵ Furthermore, the commonly used human mast cell line HMC-1 does not contain an α gene,⁵ does not express α tryptase, and presumably derives from an α -null donor. Identification of α -null individuals further allowed testing of the conventional wisdom that α tryptases make the main contribution to baseline levels of circulating tryptase, which turns out not to be the case.⁶ This prompted searches for alternative explanations of the immunoassay results on which original interpretations were based, as discussed below.

The *TPSD1* site contains tryptase alleles that are transcribed at a low level, but the protein product is C-terminally truncated.⁴ At present, there is no evidence that δ tryptase appears in serum or that it plays a biologic role. The latter seems unlikely given that it has very

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Supported in part by National Institutes of Health grant HL024136.

Disclosure of potential conflict of interest: G. Caughey has declared that he has no conflict of interest.

Received for publication February 6, 2006; accepted for publication February 13, 2006.

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0091-6749

doi:10.1016/j.jaci.2006.02.026

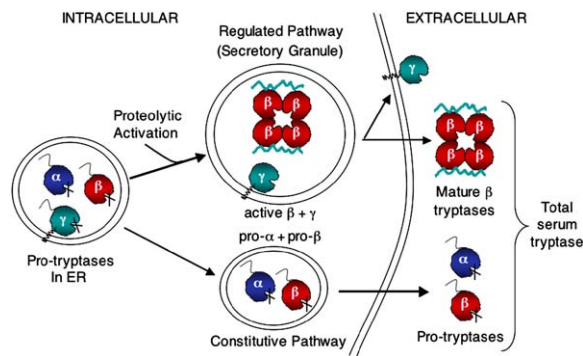


FIG 1. Activation and secretion of α , β , and γ tryptases. Catalytically inactive protryptases in the endoplasmic reticulum (ER) follow regulated or constitutive secretory pathways. β Tryptases processed by removal of the propeptide are assembled into catalytically active, heparin-stabilized tetramers and stored within the mast cell secretory granule, along with other preformed mediators. γ Tryptases activated by propeptide removal remain membrane associated. α Tryptases, which have a mutation preventing removal of the propeptide, are directly secreted, along with residual pro- β tryptase. On secretion, some mature β tryptases make their way to the bloodstream, where they are detected by tryptase immunoassays, along with secreted pro- α and pro- β tryptase. On release from the secretory granule, γ tryptase remains attached to the external surface of the mast cell plasma membrane.

little proteolytic activity,⁷ which is consistent with the phylogenetic evidence that δ is a chimera created by a gene conversion event involving an α/β -like tryptase gene and a gene similar to mouse tryptase mast cell protease 7.⁴ The *TPSG1* site encodes γ tryptase, which is expressed by some mast cells but is not as closely related to $\alpha/\beta/\delta$ tryptases as those tryptases are to each other.⁸ Its most distinct feature is a C-terminal peptide membrane anchor, which tethers γ tryptase to the mast cell surface after exocytosis. There is no evidence that γ tryptase is released or shed in a soluble form. Thus it might be incapable of joining the pool of circulating tryptases after anaphylaxis. Its role is uncertain, but studies of engineered, soluble versions of human γ tryptase suggest that it is an active peptidase that stimulates IL-13 production and promotes bronchial hyperresponsiveness when added to mouse airway.⁹ Another nearby gene is a pseudogene corresponding to tryptase-like mastin,³ which is expressed in mast cells of dogs, pigs, and mice but not in mast cells of human subjects.

Considered as a whole, the multigene human tryptase locus encodes a variety of tryptase-related genes, some classical and some crippled or converted to pseudogenes. Only α and β tryptases, which are the focus of the remainder of this update, are likely to contribute to circulating tryptase levels and to systemic features of anaphylaxis.

A WIDE FUNCTIONAL DIVIDE BETWEEN α AND β TRYPTASES

Relatively minor differences in amino acid sequence between α tryptase and various β tryptases¹⁰ cause

major differences in activation, secretion, and activity (Fig 1). α Tryptase appears to have a propeptide mutation that hinders removal and activation.¹¹ This has several consequences, including failure to adopt an active conformation and to accumulate in secretory granules. Instead, inactive monomeric proenzyme diverts to a constitutive secretion pathway.⁶ This is also the fate of unprocessed pro- β tryptase, which can be a large fraction of total tryptase output.⁶ Thus baseline immunoreactive tryptase in serum is now thought to consist mainly of pro- α and pro- β tryptases, with the latter making the major contribution, given that levels of immunoreactive tryptase change little in individuals without α genes.⁶ The situation can be expected to be similar in anaphylaxis. Because α tryptase is not stored, it will not contribute to an increase in tryptase levels with acute mast cell degranulation. α Tryptase has an additional defect in its catalytic domain,¹² which severely limits activity in preparations of enzyme in which the propeptide was artificially removed. In summary, α tryptase is not stored and seems to be doubly disadvantaged by defects in activation and catalytic domains. Therefore α tryptase is not a useful marker of mast cell burden or acute activation and is less likely than β tryptases to play a causative role in the pathophysiology of anaphylaxis.

CHANGES IN SERUM LEVELS OF TRYPTASES IN SYSTEMIC ANAPHYLAXIS

The concentration of total serum tryptase increases after severe anaphylaxis and anaphylactoid reactions.² The increase in total tryptase levels often, but not always, correlates with an increase in histamine levels. Part of the explanation of the imperfect correlation appears to be a difference in time course, with the increase in plasma histamine level being earlier and more transient.¹³ Tryptase levels are more likely to increase in severe than in mild reactions. For example, in insect sting challenges an increase in serum tryptase levels correlates with hypotension.¹⁴ Therefore a normal tryptase level does not rule out anaphylaxis, but it does make severe anaphylaxis less likely if the sample is drawn within a few hours of the onset of symptoms. Greater specificity in the diagnosis of anaphylaxis might be gained from selective measurement of mature β tryptase in addition to total tryptase (pro- β and pro- α tryptase plus mature β tryptase). Discrimination between mature β tryptase and total serum tryptase is particularly likely to be helpful when anaphylaxis occurs in the setting of systemic mastocytosis,¹⁵ in which baseline levels of protryptase tend to increase. Increases of serum tryptase levels in postmortem and forensic cases can support anaphylaxis as a cause of sudden infant death syndrome and other cases of unexplained death. However, serum levels of tryptase can increase nonspecifically shortly before death in trauma and other cases of severe illness, thereby increasing tryptase concentrations in samples obtained after death.¹⁶

Thus a high serum tryptase level in a postmortem specimen by itself might be inadequate to establish a diagnosis of anaphylaxis without other supporting clinical evidence.

FAILURE OF TRYPTASE LEVELS TO INCREASE IN FOOD-INDUCED ANAPHYLAXIS

Tryptase levels might be normal in food-induced anaphylaxis. In part, this could be a timing issue. Compared with bee venom-induced anaphylaxis, food-induced anaphylaxis can be slower to develop and more sustained and is more likely to be biphasic, resulting in less of a spike in tryptase level.¹⁷ To the extent that mast cell degranulation in food-induced anaphylaxis is limited to the gut, as suggested by a mouse model,¹⁸ there might be less tryptase release overall compared with truly systemic release. In this regard it is worth noting that mucosal mast cells tend to have less tryptase per cell than skin mast cells,¹ despite similar levels of histamine, which is perhaps another reason that serum tryptase levels increase less dramatically in gut anaphylaxis. Furthermore, some tryptase might end up in the gut lumen rather than the bloodstream (as is true of mast cell protease in a model of intestinal anaphylaxis in parasitized rats challenged with worm antigen¹⁹). Activation of gut basophils, rather than mast cells, is another potential reason for an increase in histamine level without a concomitant increase in tryptase level. Finally, some food-induced "anaphylaxis," especially after ingestion of bacterially contaminated fish, is really histamine poisoning, which is easily mistaken for allergic anaphylaxis and will not be associated with an increase in serum levels of β tryptases.

INVOLVEMENT OF TRYPTASES IN THE PATHOGENESIS OF ANAPHYLAXIS

Although several of lines of evidence in animals and human subjects suggest a role for tryptases in the pathogenesis of asthma and allergic inflammation,^{20,21} there is no direct evidence of a role for tryptases in shock and other clinical manifestations of anaphylaxis. Speculatively, tryptases contribute to the pathology of anaphylaxis by spreading the degranulation signal from mast cell to mast cell.²² In theory blood-borne tryptase could spread the signal throughout the body, but there is no evidence that tryptase in plasma is catalytically active. Tryptases can increase the egress of plasma from blood vessels by inactivating procoagulant proteins²³ and promoting fibrin clot lysis²⁴ and can promote bronchoconstriction,²⁵ which could give them a role in rash, tissue swelling, and bronchospasm in anaphylaxis. With specific regard to anaphylactic shock, the time course is such that the increase in tryptase level in blood occurs later than the onset of shock and rash and well after the serum histamine peak.¹³ Therefore the appearance of tryptases in blood most likely

reflects mast cell activation in a variety of tissue locations but is not itself a cause of anaphylactic shock.

REFERENCES

- Schwartz LB, Irani A-MA, Roller K, Castells MC, Schechter NM. Quantitation of histamine, tryptase, and chymase in dispersed human T and TC mast cells. *J Immunol* 1987;138:2611-5.
- Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. *N Engl J Med* 1987;316:1622-6.
- Caughey GH. New developments in the genetics and activation of mast cell proteases. *Mol Immunol* 2002;38:1353-7.
- Pallaoro M, Fejzo MS, Shayesteh L, Blount JL, Caughey GH. Characterization of genes encoding known and novel human mast cell tryptases on chromosome 16p13.3. *J Biol Chem* 1999;274:3355-62.
- Soto D, Malmsten C, Blount JL, Muilenburg DJ, Caughey GH. Genetic deficiency of human mast cell α -tryptase. *Clin Exp Allergy* 2002;32:1000-6.
- Schwartz LB, Min HK, Ren S, Xia HZ, Hu J, Zhao W, et al. Tryptase precursors are preferentially and spontaneously released, whereas mature tryptase is retained by HMC-1 Cells, Mono-Mac-6 Cells, and human skin-derived mast cells. *J Immunol* 2003;170:5667-73.
- Wang HW, McNeil HP, Husain A, Liu K, Tedla N, Thomas PS, et al. Delta tryptase is expressed in multiple human tissues, and a recombinant form has proteolytic activity. *J Immunol* 2002;169:5145-52.
- Caughey GH, Raymond WW, Blount JL, Hau LW-T, Pallaoro M, Wolters PJ, et al. Characterization of human g-tryptases, novel members of the chromosome 16p mast cell tryptase and prostatic gene families. *J Immunol* 2000;164:6566-75.
- Wong GW, Foster PS, Yasuda S, Qi JC, Mahalingam S, Mellor EA, et al. Biochemical and functional characterization of human transmembrane tryptase (TMT)/tryptase γ . TMT is an exocytosed mast cell protease that induces airway hyperresponsiveness in vivo via an interleukin-13/interleukin-4 receptor α /signal transducer and activator of transcription (STAT) 6-dependent pathway. *J Biol Chem* 2002;277:41906-15.
- Vanderslice P, Ballinger SM, Tam EK, Goldstein SM, Craik CS, Caughey GH. Human mast cell tryptase: multiple cDNAs and genes reveal a multigene serine protease family. *Proc Natl Acad Sci U S A* 1990;87:3811-5.
- Sakai K, Ren S, Schwartz LB. A novel heparin-dependent processing pathway for human tryptase: autocatalysis followed by activation with dipeptidyl peptidase I. *J Clin Invest* 1996;97:988-95.
- Huang C, Li L, Krilis SA, Chanasyk K, Tang Y, Li Z, et al. Human tryptases alpha and beta/II are functionally distinct due, in part, to a single amino acid difference in one of the surface loops that forms the substrate-binding cleft. *J Biol Chem* 1999;274:19670-6.
- Schwartz LB, Yunginger JW, Miller J, Bokhari R, Dull D. Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. *J Clin Invest* 1989;83:1551-5.
- van der Linden PW, Hack CE, Poortman J, Vivie-Kipp YC, Struyvenberg A, et al. Insect-sting challenge in 138 patients: relation between clinical severity of anaphylaxis and mast cell activation. *J Allergy Clin Immunol* 1992;90:110-8.
- Florian S, Krauth MT, Simonitsch-Klupp I, Sperr WR, Fritsche-Polanz R, Sonneck K, et al. Indolent systemic mastocytosis with elevated serum tryptase, absence of skin lesions, and recurrent severe anaphylactoid episodes. *Int Arch Allergy Immunol* 2005;136:273-80.
- Edston E, van Hage-Hamsten M. Mast cell tryptase and hemolysis after trauma. *Forensic Sci Int* 2003;131:8-13.
- Golden DB. Patterns of anaphylaxis: acute and late phase features of allergic reactions. *Novartis Found Symp* 2004;257:101-10.
- Brandt EB, Strait RT, Hershko D, Wang Q, Muntel EE, Scribner TA, et al. Mast cells are required for experimental oral allergen-induced diarrhea. *J Clin Invest* 2003;112:1666-77.
- Scudamore CL, Thornton EM, McMillan L, Newlands GF, Miller HR. Release of the mucosal mast cell granule chymase, rat mast cell protease-II, during anaphylaxis is associated with the rapid development of paracellular permeability to macromolecules in rat jejunum. *J Exp Med* 1995;182:1871-81.

20. Oh S-W, Pae CI, Lee D-K, Jones F, Chiang GKS, Kim H-O, et al. Tryptase inhibition blocks airway inflammation in a mouse asthma model. *J Immunol* 2002;168:1992-2000.
21. Krishna MT, Chauhan A, Little L, Sampson K, Hawksworth R, Mant T, et al. Inhibition of mast cell tryptase by inhaled APC 366 attenuates allergen-induced late-phase airway obstruction in asthma. *J Allergy Clin Immunol* 2001;107:1039-45.
22. Molinari JF, Scuri M, Moore WR, Clark J, Tanaka R, Abraham WM. Inhaled tryptase causes bronchoconstriction in sheep via histamine release. *Am J Respir Crit Care Med* 1996;154:649-53.
23. Thomas VA, Wheelless CJ, Stack MS, Johnson DA. Human mast cell tryptase fibrinogenolysis: kinetics, anticoagulation mechanism, and cell adhesion disruption. *Biochemistry* 1998;37:2291-8.
24. Stack MS, Johnson DA. Human mast cell tryptase activates single-chain urinary-type plasminogen activator (pro-urokinase). *J Biol Chem* 1994; 269:9416-9.
25. Johnson PRA, Ammit AJ, Carlin SM, Armour CL, Caughey GH, Black JL. Mast cell tryptase potentiates histamine-induced contraction in human sensitized bronchus. *Eur Respir J* 1997;10: 38-43.