


## INSIGHTS

### When alpha meets beta, mast cells get hyper

Michelle Shuling Ong<sup>1</sup> and Vinay Tergaonkar<sup>1,2,3</sup> 

**The evolutionary conservation of the catalytically inactive  $\alpha$ -tryptase gene has remained a mystery. In this issue of *JEM*, Le et al. (2019). *J. Exp. Med.* <https://doi.org/10.1084/jem.20190701> unveil the existence of a novel but natural tryptase, heteromeric  $\alpha/\beta$ -tryptase, a critical mediator of  $\alpha$ -tryptase-associated diseases.**

Tryptase is one of the key secretions of activated mast cells as well as basophils upon antigen challenge. It is a serine protease that is synthesized within secretory granules of these immune cells and is released during degranulation. The ubiquitous presence of tryptase in all mast cells makes it an excellent biomarker for the assessment of allergic responses in patients. Tryptase release promotes the inflammatory response and can lead to tumor angiogenesis (de Souza Junior et al., 2015), mastocytosis, and atopic, fibrotic, and autoimmune disorders (Jogie-Brahim et al., 2004; Cairns, 2005). X-ray crystallography revealed that tryptase exists as a homotetrameric ring-like complex with a central inward-facing catalytic site (Pereira et al., 1998). The buried active site of tryptase limits the accessibility of substrates, thereby conferring protection against the action of biological inhibitors. The tryptase genes are grouped into those producing membrane-bound ( $\gamma$ -tryptase produced from *TPSG1*) or soluble proteins ( $\alpha$ - and  $\beta$ I-tryptases from *TPSAB1*,  $\beta$ II- and  $\beta$ III-tryptases from *TPSB2*, and  $\delta$ -tryptase from *TPSD1*; Trivedi et al., 2007).

Despite high sequence homology between  $\alpha$ - and  $\beta$ -tryptase, only the latter is catalytically active and capable of promoting inflammation as a result of the potentiation of mast cell degranulation (Cairns, 2005). The lack of catalytic activity of  $\alpha$ -tryptase is due to an inherent mutation in the propeptide sequence, rendering it incapable of mediating cleavage of substrates otherwise targeted by its catalytically active counterpart  $\beta$ -tryptase (Cairns, 2005). Given the apparent lack of biological utility of  $\alpha$ -tryptase, why is it conserved throughout evolution?

The correlation between  $\alpha$ -tryptase overexpression and hereditary  $\alpha$ -tryptasemia spurred Le et al. to hypothesize the existence of a heteromeric form of tryptase in humans. In this issue of *JEM*, Le et al. not only verified their conjecture of a biologically functional  $\alpha/\beta$ -tryptase, but also successfully distinguished it from  $\alpha$ - and  $\beta$ -tryptases. In addition, they also elucidate the stoichiometric composition of the  $\alpha/\beta$ -tryptase heterotetramer as comprising two of each type of  $\alpha$ - and  $\beta$ -monomers. This naturally occurring  $\alpha/\beta$ -tryptase was not on the list of previously reported gene products of human tryptase genes, but this unique protein type is present in macaques (Trivedi et al., 2007).

Le et al. (2019) investigated the clinical role and implications of  $\alpha$ -tryptase in the context of individuals carrying an extra  $\alpha$ -tryptase-encoding gene. Individuals with this extra  $\alpha$ -tryptase-encoding gene are characterized by elevated  $\alpha$ -tryptase mRNA and protryptase levels, which lead to  $\alpha$ -tryptase-associated disorders such as hereditary  $\alpha$ -tryptasemia. Thus, clarification of the link between genotype, gene dosage, and phenotype by the Le et al. study in the current *JEM* issue represents a critical turning point in the field, as the findings of this study not only reveal a novel and functionally distinct type of tryptase, but also unseat the prior established belief of the preferential importance of  $\beta$ -tryptase over  $\alpha$ -tryptase. Moreover, the study explains the evolutionary conservation of the  $\alpha$ -tryptase-encoding gene.

Although  $\alpha$ -tryptase is constitutively expressed by mast cells, its seemingly redundant function is in contrast to the well-



Insights from Michelle Shuling Ong and Vinay Tergaonkar.

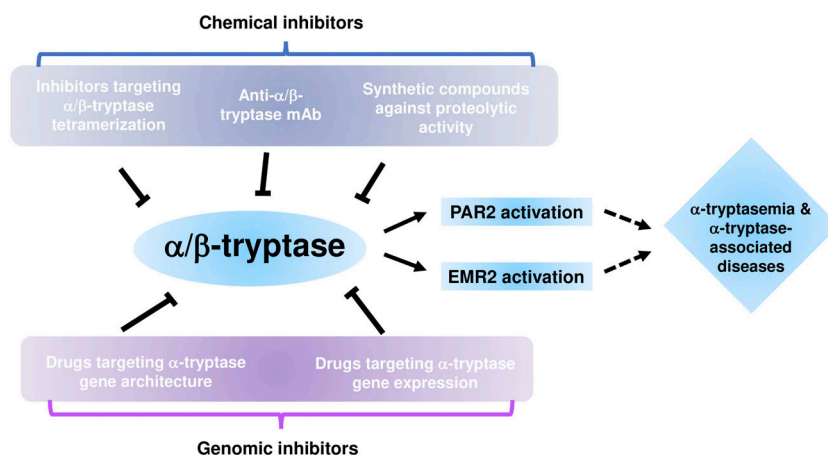
characterized role of  $\beta$ -tryptase in regulation of both innate and adaptive immunity. Despite the difficulty in pinpointing the function of  $\alpha$ -tryptase, the positive correlation of  $\alpha$ -tryptase genotypic dosage with total blood tryptase level (Min et al., 2004) and the association with  $\alpha$ -tryptasemia suggest that it may have biological functions. Indeed, Lyons et al. (2016) reported the contribution of  $\alpha$ -tryptase gene copy number to serum  $\alpha$ -tryptase levels and the severity of disease phenotype. These earlier findings from Lyons et al. (2016) are consistent with the observations from the Le et al. (2019) study, which showed that higher  $\alpha/\beta$ -tryptase gene dosage correlates with higher tryptase activity. Moreover, the higher relative stability of  $\alpha/\beta$ -tryptase compared with  $\beta$ -tryptase may account for the enhanced degranulation response observed in the Le et al. study.

Besides the role in inducing the release of cytokines and chemokines and in epigenetic modulation of gene expression (Cildir et al., 2017), tryptase has been reported to also recognize and cleave target substrates that constitute part of the signaling response pathway (Payne and Kam, 2004). In the Le et al. (2019) study, they confirmed that

<sup>1</sup>Institute of Molecular and Cell Biology, A\*STAR (Agency for Science, Technology, and Research), Singapore; <sup>2</sup>Department of Pathology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; <sup>3</sup>Faculty of Health Sciences, University of Macau, Macau, China.

Vinay Tergaonkar: [vinayt@imcb.a-star.edu.sg](mailto:vinayt@imcb.a-star.edu.sg).

© 2019 Ong and Tergaonkar. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>).



Schematic illustration of future potential drug development strategies such as chemical and genomic inhibitors, alone or in combination, against  $\alpha/\beta$ -tryptase, which targets EMR2 and PAR2 substrates for proteolytic cleavage. EMR2 and PAR2 activation can lead to  $\alpha$ -tryptasemia and  $\alpha$ -tryptase-associated diseases.

EMR2 (EGF-like module-containing mucin-like hormone receptor-like 2) and the known tryptase agonist PAR2 (proteinase-activated receptor 2; Payne and Kam, 2004; Cairns, 2005) are target substrates of  $\alpha/\beta$ -tryptase and are involved in potentiating mast cell degranulation. Vibration was used to trigger mast cell degranulation, as vibratory urticaria was reported to be a common symptom in patients with hereditary  $\alpha$ -tryptasemia. Le et al. (2019) also revealed the elements of time dependency and dose dependency in the mechanism of action of  $\alpha/\beta$ -tryptase in modulating downstream inflammatory responses. Hence, their findings suggest that patients with relatively higher  $\alpha$ -tryptase gene dosage could exhibit heightened mast cell responses and severity of resultant inflammatory/anaphylactic pathogenesis, particularly in tissues with a high abundance of resident mast cells as well as tryptase-resident cell types.

Given the role of tryptase in promoting inflammatory and angiogenic responses, inhibition of tryptase is a logical solution to treat or alleviate resulting allergic reactions, infections, and cancers. The catalytic prowess of mature  $\beta$ -tryptase led

scientists to presume its dominant role in modulating inflammatory/anaphylactic responses. Thus, compound development has been focused on the  $\beta$ -form. Development of mast cell stabilizers targeting  $\beta$ -tryptase were plagued by failures due to toxicity issues in preclinical and clinical trials. The promising inhibitor APC 366 that was developed against asthma is a noteworthy example that was discontinued due to bronchospasm in a phase 2a trial (Cairns, 2005). This compound was repurposed against rheumatoid arthritis but was deemed to qualify for use in palliative care only (Denadai-Souza et al., 2017). The failure of these early inhibitors led to the change in direction of lead development efforts against atopic and allergic diseases toward low molecular weight oral dosing compounds (Cairns, 2005; Ni et al., 2017). Tryptases are also implicated in tumorigenesis, tumor invasion, and metastasis (Ammendola et al., 2014). Hence, the discovery of the catalytically active  $\alpha/\beta$ -tryptase by Le et al. (2019) may also serve as a potential target in cancer drug development.

A primary factor impeding progress in the drug development front is the inherent tetrameric structure of tryptase with an

inward-facing active site at the heart of the complex, which poses a steric hindrance toward targeted binding by small molecule inhibitors (Pereira et al., 1998); thus, the leads generated tend to have poor target specificity and unintended dose-dependent toxic side effects. Moreover, the lack of tryptase orthologues in other species makes in vivo study with animal models difficult (Trivedi et al., 2007) and further hampers drug development efforts. The discovery by Le et al. (2019) of  $\alpha/\beta$ -tryptase as a novel clinical target for drug development studies throws in another layer of complexity and heralds an imminent shakeup in the future drug design and discovery landscape. Furthermore, the role of tryptase in mediating secretion of small molecule inflammatory mediators including chemokines further increases the difficulty in tryptase-targeted drug design and development. Hence, multi-target combination therapy may represent a more effective approach against mast cell-associated diseases. According to Le et al., genotypic dosage influences disease severity. Thus, this suggests the importance of factoring in a patient's genotype when designing a customized efficacious therapeutic regimen.

- Ammendola, M., et al. 2014. *BioMed Res. Int.* <https://doi.org/10.1155/2014/154702>
- Cairns, J.A. 2005. *Pulm. Pharmacol. Ther.* <https://doi.org/10.1016/j.pupt.2004.09.032>
- Cildir, G., et al. 2017. *J. Exp. Med.* <https://doi.org/10.1084/jem.20170910>
- Denadai-Souza, A., et al. 2017. *Arthritis Res. Ther.* <https://doi.org/10.1186/s13075-017-1326-9>
- de Souza Junior, D.A., et al. 2015. *BioMed Res. Int.* <https://doi.org/10.1155/2015/142359>
- Jogie-Brahim, S., et al. 2004. *J. Allergy Clin. Immunol.* <https://doi.org/10.1016/j.jaci.2004.02.032>
- Le, Q.T., et al. 2019. *J. Exp. Med.* <https://doi.org/10.1084/jem.20190701>
- Lyons, J.J., et al. 2016. *Nat. Genet.* <https://doi.org/10.1038/ng.3696>
- Min, H.K., et al. 2004. *J. Allergy Clin. Immunol.* <https://doi.org/10.1016/j.jaci.2004.04.008>
- Ni, W.W., et al. 2017. *Expert Opin. Ther. Pat.* <https://doi.org/10.1080/13543776.2017.1322064>
- Payne, V., and P.C. Kam. 2004. *Anaesthesia.* <https://doi.org/10.1111/j.1365-2044.2004.03757.x>
- Pereira, P.J., et al. 1998. *Nature.* <https://doi.org/10.1038/32703>
- Trivedi, N.N., et al. 2007. *J. Immunol.* <https://doi.org/10.4049/jimmunol.179.9.6072>