Melo locates subcellular Qa-SNAREs in Human Eosinophils

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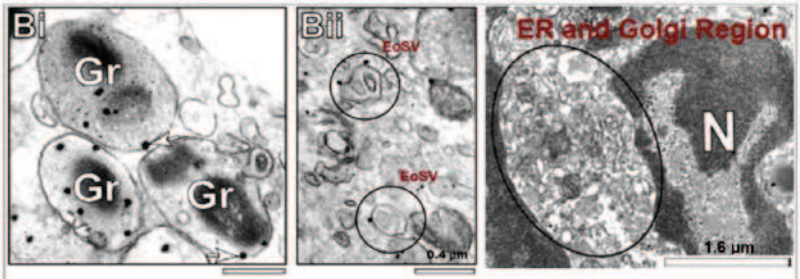
Secretion of intracellular contents is an essential component of cell function in health and disease. Cells of the immune system in particular, rely on secretion to perform their effector functions. Material transport between cell compartments and to and from the cell surface is mediated by vesicles. Membrane anchored soluble *N*-ethylmaleimide-sensitive-factor attachment protein receptors (SNAREs) work to mediate vesicular docking and fusion to target membranes leading to secretion of contents. This function is especially important to cells that contain pre-made mediators in storage for immediate release upon demand, such eosinophils. SNAREs are categorized by the conserved arginine (R-SNARE) or glutamine (Q-SNARE) residue in the central motif, and Q-SNAREs are further classified into subtypes (Qa, Qb, Qc, and Qb,c). Although R-SNAREs have been identified within eosinophils, Q-SNAREs (Qa-syntaxin 4 and Qb,c-SNAP-23) have only been identified on plasma membranes. Using flow cytometry and pre-embedded immunonanogold electron microscopy, Carmo *et al.* now report the identification of Qa-SNARE, syntaxin17 (STX17), on the membranes of secretory granules and eosinophil Sombrero vesicles (EoSV) of human eosinophils.



Images adapted from Carmo *et al.*, 2015 Figs 1 and 5. Human peripheral blood eosinophils sectioned and imaged after pre-embedding immunonanogold electron microscopy. (Bi) STX17 labeling within and on secretory granule (Gr) membranes, (Bii) STX17 labeling on eosinophil Sombrero vesicles (EoSV). Absence of STX17 labeling in ER and Golgi regions adjacent nucleus (N).

In steady-state, STX17 is localized to the membranes of secretory granules and EoSVs. Granule matrices also contain STX17. While known R-SNAREs are localized to either granules or vesicles, Qa-SNARE STX17 is localized to both compartments suggesting a function in membrane trafficking from secretory granules to the plasma membrane. Through its occurrence on smooth ER and rough ER-Golgi intermediate vesicles of secretory cells, STX17 has previously been deemed necessary for constitutive secretion. Its absence in eosinophil ER and Golgi suggests a more complicated role in these granulocytes. Stimulation with secretagogue CCL11, but not TNFα, resulted in an increase in STX17 expressing secretory granules. Since CCL11 induces piecemeal degranulation in eosinophils, the authors suggest a function for STX17 in this process.

Carmo LAS, Dias FF, Malta KK, Amaral KB, Shamri R, Weller PF, Melo RCN. Expression and subcellular localization of the Qa-SNARE syntaxin 17 in human eosinophils. Experimental Cell Research (2015).

We contacted senior author, Dr. Rossana Melo for a commentary on this article.

1. How long have you been interested in eosinophils?

   I have been interested in eosinophils for over a dozen years. My interest started mainly during my post-doctoral at Dr. Peter Weller´s lab (Boston, MA) when I had the opportunity to develop different projects focused on eosinophil biology.

2. What events led you to your current research showcased in this paper?

   My research interests include mechanisms of intracellular trafficking and secretion in eosinophils. The study of SNAREs is important to understand the complexity of vesicular traffic in these cells. Particularly, the Qa-SNARE syntaxin (SXT17) drew our attention because it is involved in constitutive secretion in other cells. We wondered if human eosinophils expressed STX17 and if this SNARE was associated with eosinophil secretory processes.

3. What are the implications of your findings to eosinophil-related diseases?

   A key function of eosinophils is to secrete a diversity of cytokines and other mediators during the course of an allergic, inflammatory and immunoregulatory response. It is thus increasingly important to understand how these mediators are trafficked and secreted. Our findings indicate that STX17 mediates membrane trafficking from secretory granules and that may be involved in mechanisms of piecemeal degranulation (PMD), a frequent secretory process during pathological conditions.

4. Do you think that these pathways apply to other cell types?

   Considering that SNAREs mediate membrane fusion during intracellular trafficking underlying immune responses, I think that STX17 may be involved in cargo delivery, specifically release of immune mediators from other cells of the immune system.

5. What are some caveats of your research (if any)?

   Our study is the first to identify STX17 in eosinophils, precisely at secretory granules and transport vesicles. However, the role of STX17 in specific eosinophil secretory pathways remains to be established.

6. What does the future hold for Rossana Melo?

   In addition to mechanistic studies of eosinophil secretion, future challenges include an Atlas on eosinophil ultrastructure and continued formation of young investigators in structural biology and immunobiology of eosinophils at my lab in Brazil.

7. What advice do you have for eosinophil biologists who may be interested in EM-based research?

   I strongly encourage eosinophil biologists to use EM as a tool to study eosinophils. EM has provided valuable insights into the functional capabilities of these cells. For example, EM-based research from our group identified, for the first time, a vesicular trafficking of cytokines and major basic protein in human eosinophils with the participation of distinct transport carriers (Eosinophil Sombrero Vesicles- EoSVs). Our advanced EM studies have also revealed a new view of eosinophil secretory granules as compartmentalized organelles with internal membranous domains. These works demonstrate the awesome power of EM and remind us all that a good picture is irreplaceable. The contributions of EM to understand secretion of immune mediators by human eosinophils were highlighted in a review (Melo et al., 2010. Microscopy & Microanalysis 16: 653).

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