Electron tomography and immunonanogold electron microscopy for investigating intracellular trafficking and secretion in human eosinophils

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Published Version</td>
<td>doi:10.1111/j.1582-4934.2008.00346.x</td>
</tr>
<tr>
<td>Citable link</td>
<td><a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:28993616">http://nrs.harvard.edu/urn-3:HUL.InstRepos:28993616</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a></td>
</tr>
</tbody>
</table>
The classical picture of the cell secretory pathway includes protein synthesis within the endoplasmic reticulum, transport of cargo inwards towards the Golgi apparatus and then through the Golgi and trans-Golgi network en route to the plasma membrane, all carried by the transport vesicles [1]. Human eosinophils, leukocytes of the innate immune system, with functions in allergic, inflammatory and immunoregulatory responses [2], additionally show a novel secretory pathway organization (reviewed in Reference [3]).

Recent studies based on fully automated electron tomography (ET) and refined immunonanogold electron microscopy (EM) revealed that, during eosinophil secretion, a distinct population of large, tubular transport vesicles, termed eosinophil sombrero vesicles (EoSVs) (Fig. 1), bud from the cytoplasmic secretory granules (also referred to as specific granules), and in conjunction with small, round vesicles, transport granule-stored products to the plasma membrane for extracellular release [4]. This vesicle-mediated process of cell secretion (piecemeal degranulation) [5], was until recently believed to be accomplished only by small, round vesicles. However, recent data have provided conclusive evidence for both the active formation of large vesiculotubular carriers (EoSVs) in response to cell activation (Figs. 1 and 2A) and their participation in granule-to-plasma membrane trafficking [4]. Combining pre-embedding immunonanogold EM for precise epitope preservation, highly specific monoclonal antibodies and subcellular localization associated with very small gold particles (1.4 nm) as a probe, EoSVs were positively immunolabelled for typical granule products such as major basic protein (MBP) (Fig. 2B and C) and interleukin 4 (IL-4) [4, 8]. MBP is one of the most abundant cationic proteins stored within and recognized as a marker of the eosinophil specific granules, while IL-4, also stored in the specific granules, is a hallmark eosinophil cytokine, typical of allergic and anti-helminthic parasite immune responses [9].

Studies performed with ET and computer-based modelling, powerful approaches for understanding cellular architecture [10], revealed the three-dimensional (3D) structure of EoSVs (Fig. 2D) [4]. They are folded, flattened tubular carriers, larger (150–300 nm in diameter) and more pleomorphic than the conventional small (~50 nm in diameter), spherical vesicles. EoSVs present substantial membrane surfaces (Fig. 2D) and represent a dynamic system with a remarkable ability to change their shape and to interact with the secretory granules (Figs. 1 and 2A) [3, 4]. The curved morphology of EoSVs provides a higher surface-to-volume ratio system,
likely important for the specific transport of the membrane-bound proteins. In fact, further studies by our group confirmed that these vesicles transport IL-4 through a membrane-bound receptor-mediated mechanism [11]. This, importantly, might underlie the remarkable ability of the eosinophils to participate in distinct immune responses [12].

Studies focussed on the mechanisms of eosinophil secretion are critical not only to understand the normal leucocyte function, but also to understand the pathological basis of allergic and inflammatory diseases. Moreover, the identification of EoSVs as important secretory vesicles within the eosinophils is defining a broader role for large vesiculotubular carriers in intracellular trafficking and secretion of proteins, as described in different cell secretory pathways [3, 13, 14].

Acknowledgements

The work of the authors is supported by the National Institutes of Health grants AI033372, AI020241, AI051645, AI022571, HL070270 (United States) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil). We thank Rita Monahan-Earley, Tracey Sciuto and Ellen Morgan (Electron Microscopy Unit, Department of Pathology, BIDMC) and Wim Voorhout of FEI Company (Eindhoven, The Netherlands) for previous electron microscopy assistance.
Fig. 2 Eosinophil sombrero vesicles (EoSVs) are open, tubular-shaped carriers actively involved in the eosinophil secretory pathway. (A) An image from conventional transmission electron microscopy (TEM) shows EoSVs (lumens highlighted in pink) around a secretory granule (Gr) with a disarranged core. In (B) and (C) EoSVs within activated eosinophils are immunolabelled for major basic protein (MBP). In (D), a three-dimensional (3D) model generated from 4-nm thick serial slices by electron tomography shows EoSVs as curved, tubular and open structures surrounding a cytoplasmic centre. The cells were stimulated with stem cell factor (A) or eotaxin (B-D) and processed for conventional TEM or immunonanogold EM as before [4]. Tilt series were acquired, fully automatically at 200 kV, on a FEI Tecnai Sphera microscope (FEI’s Nanoport-Eindhoven, The Netherlands). Tomograms were generated using Xplore 3D software (FEI) [4]. Modelling was carried out using IMOD software (The Boulder Laboratory for 3-D Electron Microscopy of Cells, University of Colorado) [15]. Bars: 250 nm (A), 190 nm (B and C), 150 nm (D).

References


3. Melo RCN, Spencer LA, Dvorak AM, Weller PF. Mechanisms of eosinophil secretion: large vesiculotubular carriers mediate transport and release of granule-derived...