

Electron Tomography reveals 3D Structure of Secretory Organelles in Eosinophils

Rossana C. N. Melo,^{1,2,3} Ann M. Dvorak³ and Peter F. Weller²

1. Department of Biology, Federal University of Juiz de Fora, Brazil. 2. Department of Medicine and 3. Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA.

BIOGRAPHY

Rossana Melo earned her MSc and PhD degrees in Cell Biology from the Federal University of Minas Gerais, Brazil and received post-doctoral training from Harvard Medical School, USA. She is a researcher in cell ultrastructure and a professor of cell biology at Federal University of Juiz de Fora, Brazil. Her interests include advanced TEM techniques applied to the studies of cellular mechanisms involved in inflammation.



ABSTRACT

We describe recent applications of electron tomography (ET) for the study of cell secretion. ET revealed a new view of unique secretory organelles in human eosinophils. Eosinophil secretory granules are compartmentalized organelles with internal membranous domains imaged in 3D models as flattened tubular networks. ET also unveiled the 3D structure of distinct secretory vesicles (termed eosinophil sombrero vesicles – EoSVs) as large, folded, and elongated tubules with substantial membrane surfaces, important for membrane-bound intracellular transport. Electron tomography has provided novel insights into the structural mechanisms related to secretion of eosinophil proteins during inflammation.

KEYWORDS

transmission electron microscopy, electron tomography, three dimensional reconstruction, eosinophils, cell secretion, intracellular transport, membrane trafficking

ACKNOWLEDGEMENTS

I thank Wim F. Voorhout of FEI Company, Eindhoven, The Netherlands, for assistance. Supported by NIH grants AI33372, AI20241, AI22571, and Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil.

AUTHOR DETAILS

Rossana C. N. Melo, PhD, Laboratório de Biologia Celular, Dept de Biologia, ICB, Universidade Federal de Juiz de Fora, Campus, 36036-330, Juiz de Fora, MG, Brazil. Tel: +55 32 3229 3206 ext. 215 Email: rossana.melo@ufjf.edu.br

Microscopy and Analysis 21(1):15-16 (UK), 2007

INTRODUCTION

The characterization of the three-dimensional (3D) structure of organelles is essential to understand cellular processes, especially cell secretion. Eosinophils, leukocytes involved in the pathogenesis of asthma, allergies and other diseases, have a remarkable ability to secrete specific proteins in response to a particular stimulus [1].

Eosinophils contain morphologically unique secretory organelles – a dominant population of cytoplasmic granules termed specific or secretory granules (Figure 1a), which store numerous proteins, and an intriguing and distinct population of large secretory vesicles termed eosinophil sombrero vesicles (EoSVs) (Figure 3) [2-4]. The role of eosinophils in a range of allergic and inflammatory diseases is based on their secretory responses; granule-stored proteins are released into extracellular spaces mainly by vesicular trafficking [5-7].

To understand in more detail this kind of cell secretion, which is also common in other types of inflammatory cells, human eosinophils from peripheral blood were studied, for the first time, by electron tomography (ET) [2,3]. This technique allowed not only imaging of the 3D structure of secretory organelles but also revealed novel functional aspects underlying cell secretion.

MATERIALS AND METHODS

Eosinophils were isolated from the blood of healthy donors as described [3]. Aliquots (10^6 cells ml^{-1}) were stimulated with 100 ng ml^{-1} of recombinant human eotaxin (R&D Systems, Minneapolis, MN, USA), a potent eosinophil activator [1], or medium alone, for 1 h.

Intact or fractioned eosinophils were prepared for conventional transmission electron microscopy (TEM) or immuno-nanogold EM as before [2]. Epon sections 200 or 400 nm thick were collected from eotaxin-stimulated eosinophils for analysis by ET.

Tilt series were acquired fully automatically

at 200 kV on an FEI Tecnai Sphera microscope using FEI Xplore 3D software. Digital images of the structures of interest were recorded on a 1K Gatan 794 slow scan CCD camera as they were tilted from -65° to $+65^\circ$ at 1° intervals. The tomograms were generated using Xplore 3D software. All tilted images were aligned to a common tilt axis using cross-correlation and the volume was reconstructed by real-space back-weighted projection. Modeling was carried out using IMOD software [8].

RESULTS AND DISCUSSION

Electron tomography has been highlighted as a robust tool to understand the complex architecture of cell organelles [9]. Here, electron tomography provided novel insights into the structural mechanisms related to secretion of proteins by human eosinophils. These multifunctional leukocytes were used as a model for our electron tomographic studies because they are cells that are specialized in secretion and ready to secrete, i.e., contain preformed proteins with multiple biological activities within their cytoplasmic granules. In response to diverse stimuli, eosinophils are recruited from the circulation into inflammatory foci, where they modulate immune responses through the release of granule-derived products [10]. Eosinophil granules are notable not only for their ultrastructural morphology, that includes a crystalline core (Figure 1a, arrowhead; Figure 3 a,b), but also because they contain several dozen preformed cytokines, mediators that enhance the immune response, as well as four cationic proteins [2].

TEM and tomography showed that eosinophil specific granules are not merely storage stations, but elaborate and compartmentalized organelles with internal membranous vesiculotubular domains able to sequester and relocate granule products upon stimulation (Figure 1b, Figure 2, and also see Movies 1 and 2 available on line at: www.traffic.dk/videos/6_10.asp) [2]. This means that

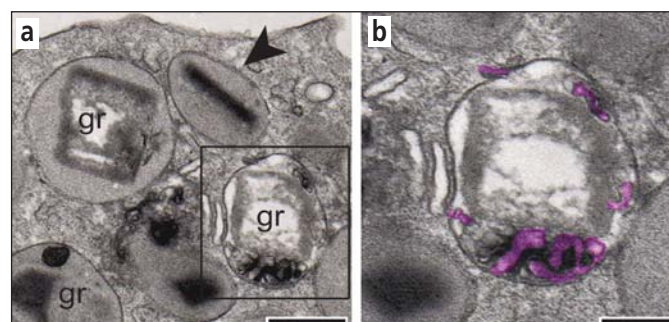


Figure 1: Morphology of secretory granules in a human eosinophil after physiological stimulation. (a) A mixed population of intact (arrow) and enlarged emptying granules (gr) with reduced electron-density is seen in the cytoplasm. (b) Emptying granule (boxed in (a)) with internal membranous tubules (pink). Scale bars: (a) 630 nm, (b) 300 nm.

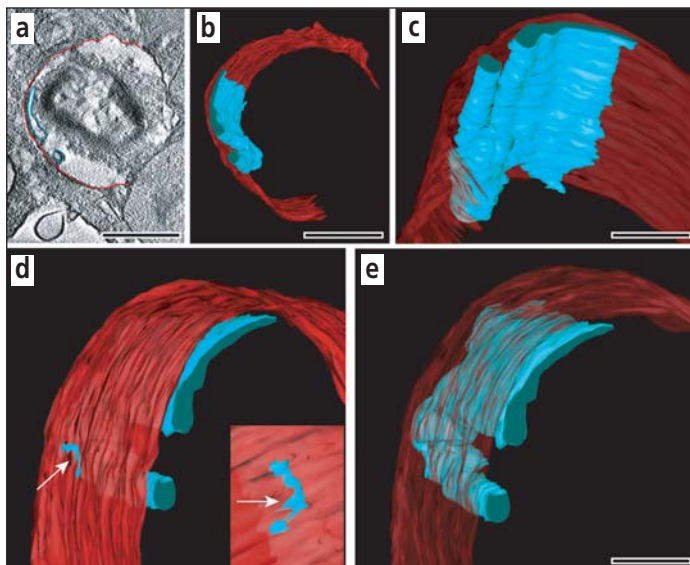


Figure 2: Tomographic slice and 3D models from a secretory granule. 75 serial single virtual slices were extracted from a tomogram. The outer granule membrane was partially traced in red and intragranular vesiculotubular structures were outlined in blue (a) to generate 3D models. (b-e) 3D models of the same granule show intragranular membrane domains (blue) organized as a flattened tubular network and tubules. (d) and (e) show rotated views. An area of continuity between the intragranular membranous network and the limiting granule membrane is indicated in (d) by arrows. The slices (~4 nm thick) were extracted from 3D reconstructions of a 400 nm eosinophil section analyzed by automated ET at 200 kV. Cells were stimulated as described [3]. Scale bars: (a) 450 nm, (b) 400 nm, (c) 180 nm, (d,e) 150 nm. Fig. 2 was published in *Traffic* [2] and is reproduced with permission of the copyright holder.

upon stimulation, eosinophil granule contents can be rearranged within intragranular compartments.

These findings have the important functional implication that specific proteins may be sorted within granules before being sent to the cell surface for release [2]. Interestingly, the most common process of cell secretion in eosinophils and other inflammatory cells is not based on granule-to-granule or granule-to-plasma membrane fusion. A prominent vesicular trafficking from the cytoplasmic granules to the cell surface enables the rapid transport of small packets of granule products, a secretion process termed piecemeal degranulation (PMD) [11]. As a result, secretory granules undergoing PMD appear at the ultrastructural level as emptying granules with progressive loss of their contents and reduced electron-density (Figure 1 a,b).

Within stimulated eosinophils, a distinct population of large tubular vesicles is increased and takes part in the transport of granule products [2-4]. These tubular carriers (~150-300 nm in diameter) are termed by us 'eosinophil sombrero vesicles' (EoSVs) because of their typical mexican hat (sombbrero) appearance in cross-sections (Figure 3a). EoSVs were frequently observed attached or surrounding specific granules (Figure 3b), can be isolated as a discrete population by subcellular fractionation [3] and were able to transport typical eosinophil proteins such as major basic protein (Figure 3c) and interleukin-4 [2,3]. To delineate the spatial organization of these intriguing EoSVs and to track vesicle formation from granules, we used high-resolution 3D analysis by electron tomography [3].

As visualized by electron tomographic reconstructions and 3D models, EoSVs are folded, flattened and elongated tubules with substantial membrane surfaces (Figure 4), important for membrane-bound intracellular transport [12]. Along the length of EoSVs,

there were both continuous fully connected cylindrical and circumferential domains and incompletely connected and only partially circumferential curved domains (Figure 4, and also see Movie 1 available on line at: www.traffic.dk/videos/6_12.asp). These two domains explain both the C-shaped morphology of these vesicles and the presence of elongated tubular profiles very close to typical EoSVs, as frequently seen in 2D cross-sectional images of eosinophils (Figure 3a). While tubular extensions from the surfaces of eosinophil granules were documented by conventional TEM [3], electron tomography revealed the contributions of these tubular extensions to forming EoSVs. This technique showed that EoSVs interact with and arise from granules through a tubulation process in response to stimulation (see Movie 2 available on line at: www.traffic.dk/videos/6_12.asp) [3].

CONCLUSIONS

This work has demonstrated the efficacy of electron tomographic analyses for the study of cell secretion. Electron tomography revealed the 3D structure of secretory organelles within human eosinophils – cytoplasmic granules with compartmentalized internal membranes and secretory large tubular vesicles with a distinct morphology that enables rapid cell secretion. In addition, electron tomography has provided evidence for the origin of these large vesicular compartments from granules, highlighting the importance of this technique in the study of the membrane-traffic field. Electron tomography revealed insights into the regulated secretion of preformed proteins by eosinophils and has proven its worth in the understanding of cell secretion mechanisms during inflammation.

REFERENCES

- Gleich G. J. *J. Allergy Clin. Immunol.* 105:651-663, 2000.
- Melo R. C. N. et al. *Traffic* 6:866-879, 2005.

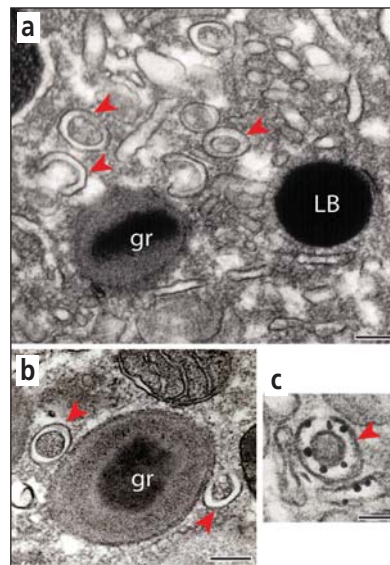


Figure 3: Ultrastructure of distinct secretory vesicles (eosinophil sombrero vesicles – EoSVs). (a) TEM shows EoSVs (arrowheads) distributed within a human eosinophil. Note the presence of typical eosinophil organelles in the cytoplasm – a specific granule with crystalloid (gr) and an electron-dense lipid body (LB). In (b) and (c) EoSVs (arrowheads) are associated with a granule and labeled for major basic protein, respectively. Scale bars: (a) 450 nm, (b) 400 nm, (c) 180 nm.

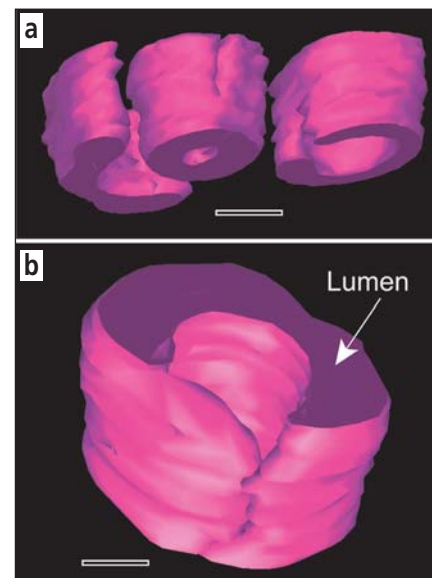


Figure 4: 3D models of distinct secretory vesicles (EoSVs). (a) EoSVs are imaged as curved tubular and open structures surrounding a cytoplasmic center. In (b) the arrow points to the tubular lumen. The models were generated from 4-nm thick serial slices obtained using automated ET. Scale bars: (a) 150 nm, (b) 100 nm. Figure 4 was published in *Traffic* [3] and is reproduced with the permission of the copyright holder.

- Melo R. C. N. et al. *Traffic* 6:1047-1057, 2005.
- Melo R. C. N. et al. *Int. Arch. Allergy Immunol.* 138:347-349, 2005.
- Dvorak A. M. et al. *Histol. Histopathol.* 9:339-374, 1994.
- Dvorak A. M. et al. *Hum. Pathol.* 11:606-619, 1980.
- Ahlstrom-Emanuelsson C. A. et al. *Eur. Respir. J.* 24:750-757, 2004.
- Kremer J. R. et al. *J. Struct. Biol.* 116:71-76, 1996.
- Koster A. J. et al. *J. Nat. Rev. Mol. Cell Biol. Suppl.* S56-10, 2003.
- Rothenberg M. E. et al. *Ann. Rev. Immunol.* 24:147-174, 2006.
- Dvorak A. M. et al. *Clin. Exp. Allergy* 24:10-18, 1994.
- Spencer L. A. et al. *PNAS. USA* 103:3333-3338, 2006.

©2007 John Wiley & Sons, Ltd