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Review article

Vesicular trafficking of immune mediators in human eosinophils revealed by immunoelectron microscopy

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ABSTRACT

Electron microscopy (EM)-based techniques are mostly responsible for our current view of cell morphology at the subcellular level and continue to play an essential role in biological research. In cells from the immune system, such as eosinophils, EM has helped to understand how cells package and release mediators involved in immune responses. Ultrastructural investigations of human eosinophils enabled visualization of secretory processes in detail and identification of a robust, vesicular trafficking essential for the secretion of immune mediators via a non-classical secretory pathway associated with secretory (specific) granules. This vesicular system is mainly organized as large tubular-vesicular carriers (Eosinophil Sombrero Vesicles – EoSVs) actively formed in response to cell activation and provides a sophisticated structural mechanism for delivery of granule-stored mediators. In this review, we highlight the application of EM techniques to recognize pools of immune mediators at vesicular compartments and to understand the complex secretory pathway within human eosinophils involved in inflammatory and allergic responses.

Contents

1. Introduction

Cells from the immune system communicate by secreting mediators, which govern the course of immune responses. However, before being transferred from one group of cells to another, immune mediators need to be intracellularly mobilized, trafficked and secreted. Thus, the knowledge of secretory pathways and their related intracellular compartments are important to understand immune responses. In this sense, a central question is: how does a specific immune mediator traverse from intracellular sites to the cell surface in order to be released upon cell activation?

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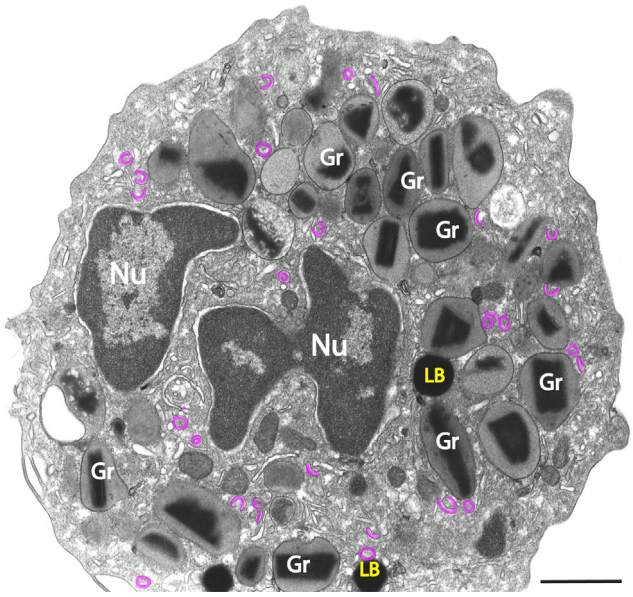

Fig. 1. Conventional transmission electron microscopy (TEM) of a human eosinophil. The eosinophil cytoplasm contains a major population of secretory (specific) granules (Gr), lipid bodies (LBs), vesiculotubular structures termed Eosinophil Sombrero Vesicles (EoSVs, highlighted in pink) and an usually bi-lobed nucleus (Nu) with condensed, marginated chromatin. Note the unique morphology of specific granules (Gr) with an internal often electron-dense crystalline core surrounded by an electron-lucent matrix. Scale bar, 800 nm.

In leukocytes such as eosinophils, immune mediators are mostly transported en route to the plasma membrane in a ER-Golgi-independent manner, i.e., these messengers are stored as preformed pools within secretory (specific) granules, a major population present in the eosinophil cytoplasm (Fig. 1), from where they are mobilized and released in response to cell activation. Granules can fuse with the plasma membrane in order to secrete their contents, but the most frequent mechanism for the delivery of eosinophil mediators involve vesicular carriers, which recruit cargos directly from secretory granules, a secretory process termed piecemeal degranulation (reviewed in [1–3]). Thus, a granule-derived, vesicle-mediated secretion takes place within human eosinophils, enabling rapid release of specific mediators [3].

Our group has been using conventional transmission electron microscopy (TEM), immunonanogold EM and electron tomography to understand the cellular mechanisms involved in the release of immune mediators from human eosinophils activated by inflammatory stimuli [4–11]. Because TEM provides a comprehensive view of the interior of a cell at nanometer scale, application of this technique has enabled the identification of a consistent, granule-associated vesicular system represented by large vesiculotubular structures formed in response to eosinophil activation [5].

Combined with molecular detection methods (immunonanogold), TEM provides sufficient resolution to localize proteins to intracellular compartments [12]. The use of an improved approach for ultrastructural detection of proteins in leukocytes (pre-embedding immunonanogold EM) [13] led to the first ultrastructural identification of a vesicle-based transport of interleukin-4 (IL-4) [5] and major basic protein (MBP-1) [7] from eosinophil secretory granules. More recently, this technique located SNARES at secretory granules and vesicles [11] and identified an active intracellular CD63 trafficking connected to eosinophil granule-derived secretory pathways [14]. Here, we review the use of TEM to visualize and understand intracellular trafficking and secretion of immune mediators in eosinophil leukocytes during immune responses.

2. Eosinophil activation triggers formation of large transport carriers

It is well documented that large vesicular-tubular structures are fundamental to transfer secretory cargos within different cell types and secretory pathways [15–18]. These structures can show different sizes and shapes and complex plasticity [17].

In human eosinophils, vesiculotubular structures, considered as microgranules in the past, constitute one important morphological feature of these cells and are clearly identified in the cytoplasm by TEM [19] (Figs. 1 and 2). These structures have a typical and unique morphology, which differ from tubular vesicles found in other cells. We coined the term Eosinophil Sombrero Vesicles (EoSVs) for these eosinophil vesiculotubular carriers in 2005 when we identified them as morphologically distinct vesicles resembling a “sombrero hat” and involved in the trafficking of eosinophil products [5].

As demonstrated by electron tomography, a technique that enables three-dimensional observation at high resolution [20,21], EoSVs represent a dynamic and pleiomorphic vesicular system within human eosinophils with substantial membrane surfaces and remarkable ability to change their shape and to interact with secretory granules [17] (Fig. 2A). Three-dimensional reconstructions and models generated from serial sections revealed that individual EoSVs are curved tubular structures with cross-sectional diameters of approximately 150–300 nm, surrounding a cytoplasmic center – an intriguing architecture that confers the “sombrero hat” morphology. In addition to this typical aspect, large tubular vesicles with a “C” shaped appearance and elongated tubular profiles are frequently observed close to standard EoSVs in eosinophil thin sections [5] (Fig. 2A-C). Electron tomography showed that along the length of EoSVs, there were both continuous fully connected cylindrical and circumferential domains and incompletely connected and only partially circumferential curved domains (Fig. 2C). Thus, these two domains explain the different views of EoSVs in cross-sectional images of eosinophils [5] (Fig. 2A).

EoSVs are transport vesicles consistently formed in response to cell activation [17]. Stimulation with inflammatory stimuli such as CCL11 [5] and tumor necrosis factor alpha (TNF-α) [14] induces increased formation of EoSVs. Moreover, eosinophils naturally activated as observed in hypereosinophilic disorders also exhibit amplified numbers of EoSVs [7]. Ultrastructural analyses of blood eosinophils from patients with hypereosinophilic syndrome [7] or tissue eosinophils found in esophageal biopsy specimens from patients with eosinophilic esophagitis [22] revealed marked cytoplasmic vesiculation of EoSVs.

3. EoSVs are formed from secretory granules

As noted, EoSVs are typically found in mature human eosinophils [19]. Maturation of these cells is accompanied by increased numbers of EoSVs in parallel with the formation of secretory granules [23]. Conventional TEM has frequently identified EoSVs around and/or attached to secretory granules in the cytoplasm of human activated eosinophils (Fig. 2A). Ultrastructural studies showed that the increased genesis of EoSVs within these cells is associated with secretion. In eosinophils stimulated with inflammatory stimuli, not only did the total number of EoSVs increase but also did the number of EoSVs in contact with secretory granules undergoing release of their contents [5,14]. Ultrastructural images obtained by conventional EM applied to human eosinophils have also indicated that EoSV were projecting from granule boundaries. Indeed, by tracking serial images with electron tomography and using ultrastructural immunodetection