A new paradigm for eosinophil granule-dependent secretion

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Eosinophil granules are notable for their content of preformed proteins whose secretion underlies many of the contributions eosinophils make in allergic and anthelminthic responses. We have recently shown that cell-free eosinophil granules respond to eotaxin and IFNγ via cognate signaling-coupled granule membrane-expressed receptors, triggering phosphorylation of intragranular signaling molecules and eliciting specific secretion of granule-derived proteins. Thus, fully intact eosinophil granules deposited extracellularly within diseased tissues maintain a capacity to function as secretory competent organelles, demanding a fresh look at our understanding of the secretion of eosinophil granule-derived proteins, both intracellularly and extracellularly. Here, we review a series of our recent work revealing the unique ultrastructure of human eosinophil granules and mechanisms of intragranule sorting and mobilization of specific cytokine products from intact human eosinophils. Implications of these findings to granule secretory responses, both intracellularly and extracellularly, and a series of outstanding questions are discussed.

Eosinophils, prominent cells in allergic inflammation and anthelminthic host defense, are characterized by their abundance of cytoplasmic specific granules notable for their content of preformed proteins. Eosinophil specific granules contain multiple preformed proteins such as eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil-derived neurotoxin (EDN), enzymes and over three dozen cytokines and chemokines.1,2 Robust intragranular stores of preformed proteins uniquely enable eosinophils to very rapidly release, upon appropriate stimulation, a variety of cytokines with immunomodulatory and chemo-attractant capacities, distinguishing eosinophils from most other leukocytes that require new protein synthesis prior to cytokine secretion.

In contrast to the classic view of compound exocytosis taught in basic cell biology courses, the most physiological form of degranulation undertaken by eosinophils within tissues is “piecemeal degranulation” (PMD), a process whereby vesicles shuttle specific proteins from granules to the plasma membrane for release.3-6 Alternatively, a somewhat enigmatic mode of eosinophil degranulation results from deposition of intact membrane-bound eosinophil granules extracellularly.7 Fully intact free granules have been extensively observed in a diversity of disorders, including allergic asthma, dermatitis, eosinophilic esophagitis, urticaria and helminth infections,7,12 although the consequences of free granules within diseased tissues have been undefined. Recently, we demonstrated the ability of eosinophil granules to function extracellularly as independent secretory organelles capable of responding to IFNγ and eotaxin stimuli via cognate granule membrane-expressed receptors, topologically oriented with amino-terminal ligand-binding domains displayed externally on granule membranes.13,14 Granules not only express functional receptors on their surface membranes, but couple these receptors to intragranular signaling cascades and intragranular membranotubular network-based secretion responses.13,14 These findings reveal...
a physiological function for free granules found within diseased tissues, and provide a model for studying mechanisms of piecemeal secretion of granule-derived proteins. Here we discuss a series of studies from our laboratory delineating secretory processes of granule-derived proteins from intact human eosinophils and from free, extracellular granules, revealing a novel paradigm of secretion.

**Ultrastructure of Eosinophil Granules**

Despite the recognition for nearly 30 years of PMD as a principal mechanism of degranulation of innate immune cells, including eosinophils and basophils, structural mechanisms of PMD remained ill-defined. Through a series of electron microscopy and automated electron tomography studies using human eosinophils, we revealed a complex membranous network within intracellular eosinophil granules. Following physiological stimulation of intact eosinophils, membranous structures within granules formed tubular and spherical compartments within which specific protein cargoes were organized and sequestered. Subsequently, formed spherical and tubular vesicular carriers emerged from cytoplasmic granules and transported granule-derived proteins to the cell surface for extracellular release in a brefeldin A-inhibitable process.

**Receptor-Mediated Mechanism of Specific Cytokine Mobilization**

Studies of eosinophil secretion by our lab and others have revealed distinct patterns of specific, stimulus-induced secretion of preformed, granule-derived cytokines. Observations of sorting and sequestering of preformed granule-stored proteins and differential, stimulus-specific patterns of cytokine release from eosinophils suggested some mechanism of specific cytokine mobilization must occur within eosinophil intracellular granules to control differential cytokine mobilization.

A key finding leading to the elucidation of such a mechanism came with the observation that granule-derived IL-4 appeared to be membrane-associated within secretory vesicles. In a little-appreciated correlation, human eosinophils express receptors for many, if not all, of the over three dozen cytokines and chemokines stored preformed within intracellular granules. In a study combining immunoelectron microscopy with biochemical and immunological approaches, we localized substantial cytokine and chemokine receptor expression at intracranial granules of blood-derived human eosinophils. Upon specific stimulation of intact eosinophils, the ligand-binding chain of a functional type I IL-4 receptor heterodimer (IL-4Rα chain), but not the common γ accessory chain, was mobilized from within eosinophil intracellular granules in association with emerging vesicles, carrying IL-4 within its binding domain. IL-4-bound, IL-4Rα vesicles trafficked to the cell surface for secretion. Preliminary studies reveal similar mobilization of IL-6Rα chains and the G-protein-coupled receptor CCR3, suggesting that specific mobilization of intragranular cytokines and chemokines through binding to cognate receptors is likely a mechanism used by eosinophils to accomplish selective secretion from preformed intracellular granule stores.

**Extracellular Granules as Secretory-Competent Organelles**

In a recent publication we demonstrated that granules derived from human blood eosinophils express, on the outer granule membrane, functional receptors for IFNγ (IFNγRα) and eotaxin (CCR3), topologically oriented with ligand-binding domains facing outward. Extracellular stimulation of free eosinophil granules with IFNγ or eotaxin resulted in selective, stimulus-dependent release of granule contents, including a cationic protein, ECP and cytokines (IL-6 and IL-4). Stimulation of free, extracellular granules activated intragranular signal transduction pathways, and pre-exposure to brefeldin A inhibited secretion from extracellular granules, similar to results observed with intact cells.

Our findings that cell-free granules are organelles fully capable of ligand-elicited active secretory responses are notable because they expand the capacities of eosinophils to contribute to modulating host and immune responses after cell cytolysis and for the first time recognize common intracellular localizations for a functional GPCR, CCR3 and IFNγRα, that uniquely enable granules to function extracellularly and potentially, intracellularly, as regulators of granule secretion. These findings suggest a novel paradigm of innate cell secretion.

**Outstanding Questions**

Outstanding questions remain, including delineation of trafficking mechanisms governing specific movement of vesicles from granules to the cell surface, and processes undertaken by secretory vesicles at the cell surface. For example, do vesicles permanently fuse with the plasma membrane, leading to wholesale release of vesicular content, or might vesicles engage in a “kiss and run” scenario, as described in neurosecretory granules? What is the fate of vesicle-associated receptors following extracellular cargo release? How are proteins recycled, and membranes replenished within eosinophil granules? Elegant work by Moqbel and colleagues revealing an intricate interplay of vesicle, granule and membrane fusion and docking SNARE and Rab molecules, likely involved in the orchestration of vesicle trafficking, has begun to unravel these unknowns.

Also remaining to be delineated are intracellular signaling mechanisms enabling exogenous signals received by intact eosinophils to be transduced to the intracellular granule to mediate intragranular receptor mobilization. Previous work from our laboratory may shed light on this question, with the recognition of a function for intracellular LTC4 in mediating secretion of IL-4. Importantly, our demonstration of secretion-competent extracellular granules provides a unique methodology by which these questions might, for the first time, be effectively addressed.
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