

Intelligent granules: are eosinophil crystalloid granules inimitable?

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The controversy surrounding the effector function of eosinophils in health and disease continues at both the clinical and basic discovery levels. Two recent studies reported that an anti-human IL-5 mAb (mepolizumab) specifically targeting eosinophils significantly reduced asthma exacerbations, providing interesting insights into the potential role of this cell in asthma.^{1,2} Similarly, there is increasing evidence that eosinophils play a major role in tissue remodeling within asthmatic airways.³ In addition, eosinophils appear to play major roles in orchestrating the T_H2 bias associated with atopic conditions through production of specific chemokines, cytokines, and metabolic enzymes.

Previous studies from our laboratory provided ample evidence that eosinophils synthesize, store, and release a wide range of proinflammatory mediators, including cationic proteins, cytokines, chemokines, and growth factors.⁴ The vast majority of eosinophil-derived mediators are prestored in unique granular structures known as crystalloid or secondary granules. After their release to the extracellular environment, crystalloid granule mediators are known to be widely associated with allergic inflammatory events.⁵

Exocytosis describes intracellular fusion of granules or secretory vesicles with the plasma membrane after cell activation, leading to the release of granule contents to the extracellular space. Despite the unifying importance of the process of exocytosis to all immune and inflammatory cell types, the precise molecular and intracellular mechanisms that regulate mobilization of secretory granules/vesicles are only recently becoming clear. It is generally believed that eosinophil mediators are synthesized in the Golgi compartment, processed, prepackaged, and stored in crystalloid granules.⁶ Three different mechanisms are known to facilitate mediator release, namely classical exocytosis (whereby a granule at a time fuses with the plasma membrane en route to the exterior; this is rarely observed in eosinophils),

compound exocytosis, and piecemeal degranulation. A fourth pattern of degranulation is associated with cytolysis, which is not a true exocytotic process (Fig 1).^{7,8} In compound exocytosis a number of granules fuse intracellularly to form a large degranulation chamber or cavity, which in turn fuses with the cell membrane before discharging its contents to the extracellular space.⁹ In piecemeal degranulation mediators prestored in crystalloid granules are selectively released from an intragranular pool, leaving portions or the entire granules empty in the intact cell.^{10,11} A large body of evidence, including our own work, has documented the molecular processes involved in selective mediator mobilization and release from eosinophil crystalloid granules.¹² In the fourth pattern of eosinophil mediator release (ie, cytolysis), the cell membrane loses its integrity, which allows the deposition of a large body of extracellular crystalloid granules within the affected tissue.¹³ Indeed, free and intact granules have been localized to various tissues in association with a number of disease states, including asthma, allergic rhinitis, helminth infections, dermatitis, and urticaria.¹⁴ The role of these free-floating granules in inflammatory events remains ill understood. To our knowledge, there are no studies that have documented the numbers of these cell-free granules or have correlated the quantity of free eosinophil granules with clinical parameters.

Over the last several years, many observations directly or indirectly started to question the definitions of crystalloid granules as simple storage depots. Indeed, the capacity of crystalloid granules to select specific mediators for release to the extracellular space was indicative of the presence of a rather intelligent molecular mechanism within the intragranular space capable of recognizing and selectively packaging mediators for release to extracellular space.

Although questions regarding the intragranular machinery of piecemeal degranulation remain widely unanswered, a series of questions were also raised by cytolysis in relation to the fate of intact crystalloid granules in the extracellular space.

In 2000, while still at the University of Alberta in Edmonton, Alberta, Canada, our laboratory made a serendipitous, intriguing, and controversial observation. We observed that cell-free crystalloid granules from eosinophils expressed functional cytokine receptors. Curious to understand the biological significance of this observation, we carried out a series of *in vitro* experiments to determine whether eosinophil crystalloid granules could respond to agonist stimulation in a cell-free environment. Applying cell fractionation techniques routinely used in our laboratory, we purified these granules to 100% homogeneity and examined their capacity to respond to agonist stimulation. We observed that such stimulation induced a dose-dependent mediator release from crystalloid granules in a cell-free system. We showed that inhibitors of tyrosine kinase and phosphoinositide 3-kinase, as well as other signal transduction pathways, blocked mediator release

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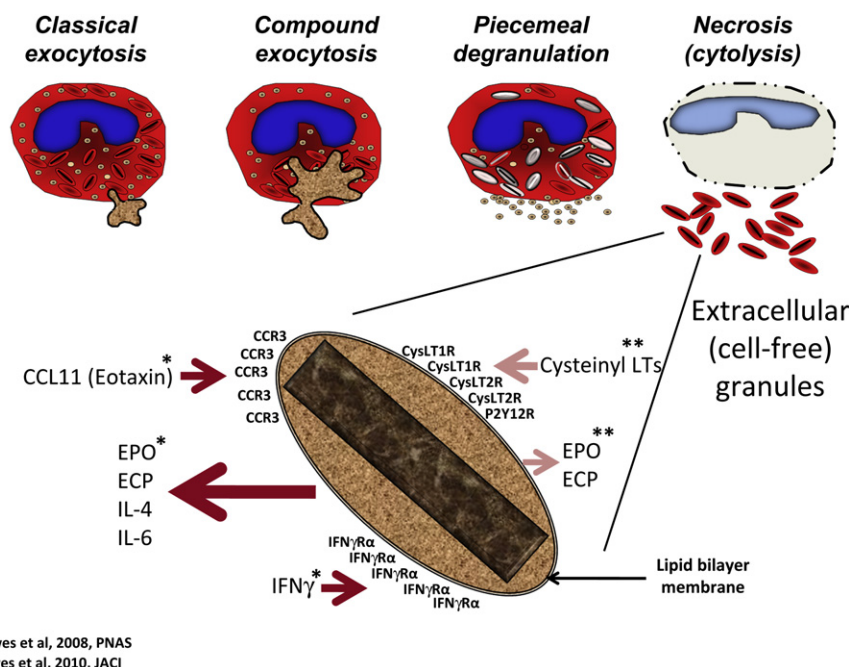


FIG 1. Upper panel, The 4 patterns of eosinophil granule mediator deposition after activation: classical exocytosis involving membrane fusion of individual granules with the plasma membrane, leading to mediator secretion; compound exocytosis, whereby granules fuse together before exteriorization of the fused mediators from a single fusion site; piecemeal degranulation involving rapidly mobilizable small secretory vesicles shuttling granule contents to and from the plasma membrane; and cytolysis, in which the cell loses its membrane integrity, releasing intact membrane-bound granules to the extracellular space. Lower panel, Magnified view of an extracellular intact eosinophil granule expressing biologically active receptors for CCL11 (eotaxin and CCR3) and IFN- γ (IFN- γ R α). Stimulation with relevant ligands induces the release of stored granule cationic proteins (eosinophil cationic protein [ECP] and eosinophil peroxidase [EPO]), as well as cytokines (IL-4 and IL-6).⁷ A recent study showed that the cysteinyl leukotrienes LTC₄, LTD₄, and LTE₄ stimulated cell-free eosinophil granules to secrete ECP and EPO, but not cytokines, through CysLT1R, CysLT2R, and P2Y12R.⁸

from appropriately stimulated cell-free granules. In 2002, we were fortunate to collaborate with the groups of Peter Weller and Anne Dvorak at the Harvard Medical School, who agreed to help complete and confirm our observation. The result was an exciting study published in the *Proceedings of the National Academy of Sciences of the United States of America*.⁷

The Harvard group provided ultrastructural evidence to support the presence of membranotubular structures in the granules as physical pathways for secretion (inhibitable by brefeldin A) and to visually demonstrate mediator release from cell-free intact granules by using acridine orange dye methodology. In addition, the study provided evidence of localization and functional presence of signal transduction molecules in eosinophil crystalloid granules.

This fascinating new observation has been further strengthened by a recent *Journal of Allergy and Clinical Immunology* study by the Weller laboratory showing that, in addition to functional cytokine and chemokine receptors on eosinophil granule membranes, bioactive leukotriene receptors (cysteinyl leukotriene receptors [CysLTRs]: CysLT1R, CysLT2R, and the purinergic P2Y12 receptor) were expressed.⁸ Stimulating the cell-free granules with the agonists of CysLT1R and CysLT2R (ie, leukotriene [LT] C₄, LTD₄, and LTE₄) at a nanomolar range of concentrations resulted in the release of eosinophil cationic protein (stored in eosinophil crystalloid granules) but not eosinophil-derived cytokine or chemokine secretion. Montelukast, an inhibitor of CysLT1R, as well

as the P2Y12 receptor antagonist MRS 2395, inhibited eosinophil cationic protein release after LTC₄/LTD₄/LTE₄ stimulation of eosinophil cell-free granules. This elegant study has further unveiled the intricacy and mediator selectivity of the cell-free granules and their receptor machinery that leads to secretion from these organelles once they are deposited outside the cell.

These studies suggest that cell-free granules might act as an extracellular source of cationic proteins, as well as immune-regulating cytokines and chemokines. This constellation of granules found within the airway and other inflammatory sites might, like “cluster bombs,” contribute to the perpetuation of the inflammatory process in an affected organ. Indeed, we have recently reported the presence of intact eosinophils and cell-free granules in infant thymic tissue. This observation suggests a potential role for the eosinophil and possibly its cell-free granular components in this primary lymphoid tissue in the absence of any insult or danger signal.¹⁵

Many questions regarding the nature and function of eosinophil crystalloid granules (and probably other granular cell phenotype) inside or outside the cell still remain unanswered. Are eosinophils unique in having secondary granules that can function as independent cell-free organelles capable of agonist-induced mediator secretion? Does this observation therefore have implications for other granule-containing cell phenotypes? We would venture to suggest that intracellular granule populations that exhibit bioactive membranes (ie, expressing appropriate receptors) are likely

to have the potential to exert extracellular effects when deposited in extracellular spaces within the context of immune, inflammatory, or both milieus. Thus it now appears appropriate to think that receptors on granule membranes have the ability to respond to their relevant ligands extracellularly, an opinion hitherto regarded as anathema within the realm of cell biology! In addition, the presence inside the granules of an intricate network of membranotubular mechanisms with all the necessary signal transduction components to effect secretion renders these cell-free granules capable of secreting (even differentially, as per intracellular piecemeal degranulation patterns) appropriate mediators that can influence the local inflammatory milieu. It is tempting to speculate that these data, including the new observation on CysLTR expression,⁸ expand the current appreciation of the “intelligence, capacity, and sophistication” of these granules both as intracellular organelles and extracellular compartments. They appear to be capable of sensing and responding to various triggers and ligands within the affected tissue site.

The novel observations referred to above (summarized in the lower half of Fig 1) have important implications for antieosinophil therapies that target the cell but ignore the potential ensuing tissue damage exerted by cell-free granules acting as “cluster bombs” in the absence of intact cells. New therapeutic strategies might be needed to target cell-free eosinophil granules and block their ability to secrete their mediator content by engaging agonist ligands to biologically functional receptors.

REFERENCES

1. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009;360:973-84.
2. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009;360:985-93.
3. Flood-Page P, Menzies-Gow A, Phipps S, Ying S, Wangoo A, Ludwig MS, et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003;112:1029-36.
4. Moqbel R, Lacy P. New concepts in effector functions of eosinophil cytokines. *Clin Exp Allergy* 2000;30:1667-71.
5. Adamko D, Lacy P, Moqbel R. Eosinophil function in allergic inflammation: from bone marrow to tissue response. *Curr Allergy Asthma Rep* 2004;4:149-58.
6. Mahmudi-Azer S, Velazquez JR, Lacy P, Denburg JA, Moqbel R. Immunofluorescence analysis of cytokine and granule protein expression during eosinophil maturation from cord blood-derived CD34 progenitors. *J Allergy Clin Immunol* 2000;105:1178-84.
7. Neves JS, Perez SA, Spencer LA, Melo RC, Reynolds L, Ghiran I, et al. Eosinophil granules function extracellularly as receptor-mediated secretory organelles. *Proc Natl Acad Sci U S A* 2008;105:18478-83.
8. Neves JS, Radke AL, Weller PF. Cysteinyl leukotrienes acting via granule membrane-expressed receptors elicit secretion from within cell-free human eosinophil granules. *J Allergy Clin Immunol* 2010;125:477-82.
9. Sceppek S, Moqbel R, Lindau M. Compound exocytosis and cumulative degranulation by eosinophils and their role in parasite killing. *Parasitol Today* 1994;10:276-8.
10. Lacy P, Mahmudi-Azer S, Bablitz B, Hagen SC, Velazquez JR, Man SF, et al. Rapid mobilization of intracellularly stored RANTES in response to interferon-gamma in human eosinophils. *Blood* 1999;94:23-32.
11. Melo RC, Perez SA, Spencer LA, Dvorak AM, Weller PF. Intragranular vesiculotubular compartments are involved in piecemeal degranulation by activated human eosinophils. *Traffic* 2005;6:866-79.
12. Moqbel R, Coughlin JJ. Differential secretion of cytokines. *Sci STKE* 2006;2006:pe26.
13. Erjefalt JS, Andersson M, Greiff L, Korsgren M, Gizycki M, Jeffery PK, et al. Cytolysis and piecemeal degranulation as distinct modes of activation of airway mucosal eosinophils. *J Allergy Clin Immunol* 1998;102:286-94.
14. Erjefalt JS, Greiff L, Andersson M, Matsson E, Petersen H, Linden M, et al. Allergen-induced eosinophil cytolysis is a primary mechanism for granule protein release in human upper airways. *Am J Respir Crit Care Med* 1999;160:304-12.
15. Tulic MK, Sly PD, Andrews D, Crook M, Davoine F, Odemuyiwa SO, et al. Thymic indoleamine 2,3-dioxygenase-positive eosinophils in young children: potential role in maturation of the naive immune system. *Am J Pathol* 2009;175:2043-52.