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Eosinophil-derived cytokines in health and disease: unraveling novel mechanisms of selective secretion

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Abstract

Over the past two decades, our understanding of eosinophils has evolved from that of categorically destructive effector cells to include active participation in immune modulation, tissue repair processes, and normal organ development, both in health and disease. At the core of their newly appreciated functions is the capacity of eosinophils to synthesize, store within intracellular granules, and very rapidly secrete a highly diverse repertoire of cytokines. Mechanisms governing the selective secretion of preformed cytokines from eosinophils are attractive therapeutic targets, and may well be more broadly applicable to other immune cells. Here we discuss recent advances in deciphering pathways of cytokine secretion, both from intact eosinophils, and from tissue-deposited cell-free eosinophil granules, extruded from eosinophils undergoing a lytic cell death.

Biological significance of eosinophil-derived cytokines

Eosinophils are innate immune leukocytes recruited in large numbers to sites of allergic inflammation and parasitic infections. Within the context of allergic and parasitic diseases, eosinophils have historically been categorized as terminal effector cells, mediating cytotoxic effects on parasites or asthmatic airways through deposition of a group of granule-derived, cationic proteins. More recently appreciated are additional pleiotropic effects of recruited eosinophils impacting immunomodulation and tissue homeostasis and repair. Many of these emerging functions rely upon the eosinophils' capacity to respond to environmental cues with the very rapid secretion of an array of cytokines, stored preformed within intracellular granules. Immediate availability of ready-made cytokines distinguishes eosinophils from most other leukocytes, which rely upon de novo protein synthesis for cytokine secretion.

Eosinophil-derived cytokines may influence the local tissue immune profile. For example, in murine models, eosinophils are among the earliest IL-4-secreting cells recruited in response to *Nippostrongylus brasiliensis* (1), *Schistosoma mansoni* eggs (2), or intranasal administrations of ovalbumin (3), and contribute to the local cellular immune microenvironment in allergic lungs through secretion of chemokines implicated in the specific recruitment of Th2 effector memory subsets (4, 5) and CCR1-expressing dendritic cells (DCs) (6). Likewise, in humans, the majority of IL-4⁺ cells detected within nasal mucosa of allergic rhinitis patients 6 hours after allergen challenge are eosinophils (7). Of

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Conflict of interest

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note, eosinophils express cytokines with a broad range of effects, including those associated with regulatory responses, and Th1 and Th2 immunity (8–10).

At tissue injury sites eosinophils promote healing through secretion of cytokines (e.g. TGF- α (11)), and in chronic inflammation, eosinophil-derived factors, including TGF- β , IL-13, and VEGF contribute to tissue remodeling and angiogenesis (12–16). Eosinophil-derived cytokines have been implicated in fibrotic processes in allergic asthma (17, 18), idiopathic pulmonary fibrosis (19), eosinophilic esophagitis (20), scleroderma (21), liver fibrosis (22), and eosinophilic endomyocardial fibrosis (23).

Local innate immune cells, and cells of the nervous and muscular systems are also responsive to eosinophil-derived cytokines (e.g. NGF, SCF). Moreover, eosinophils are themselves responsive to many of the cytokines that they also secrete (e.g. IL-5, CCL11). Thus, activated eosinophils mediate their own recruitment, activation, and survival through autocrine stimulation.

In addition to the contributions of cytokine-secreting eosinophils recruited within the context of disease, several lines of data have emerged from murine studies to suggest that cytokine secretion by tissue resident eosinophils is vital to maintaining immunologically and metabolically relevant cells, including splenic IgM⁺ B cells (24, 25), bone marrow plasma cells (26), and adipose tissue alternatively activated macrophages (27). Moreover, functions for eosinophils in mammary gland development (28) and uterine maturation (29) depend upon cytokine secretion by eosinophils within tissues. Thus, at baseline and in disease, eosinophil-derived cytokines elicit broad biological effects. Figure 1 provides an overview of some of the effects of eosinophil-derived cytokines on homeostatic and immune processes, in health and disease. Mouse models have in many ways led the march toward recognitions of eosinophils, through cytokine-driven processes, as tissue and immune modulators. However, how closely mouse eosinophils recapitulate human eosinophil modes of secretion remains controversial (30–35). The remainder of this review will therefore focus exclusively on data derived with human eosinophils.

Stimulating eosinophil cytokine secretion

Eosinophil cytokine secretion is a finely regulated and stimulus-dependent process. Numerous molecules function as eosinophil secretagogues. Eosinophil chemoattractants (e.g. eotaxins) elicit cytokine secretion from eosinophils in parallel with their induced migration (36, 37). Various cytokine stimuli representative of immune polarized or inflammatory microenvironments (8), bound IgG or IgA (38), lipid mediators (e.g. platelet activating factor (PAF)), complement fragments, and interactions with pathogens or their secreted products (39) directly and indirectly evoke eosinophil cytokine secretion. Cytokine secretion is thereby induced downstream of a plethora of eosinophil-expressed receptor subtypes, including G-protein coupled receptors (GPCRs), types I or II cytokine receptors, TNF family receptors, Ig-like receptors, adhesion molecules, and toll-like receptors (TLRs). Priming by so-called eosinophilopoietins (i.e. IL-5, GM-CSF, IL-3), present in higher amounts within tissues and plasma in conjunction with eosinophil-associated disorders, induce eosinophil cytokine secretion and lower the threshold for subsequent stimulus-dependent secretion (40, 41). A recent study demonstrating PKC β II-mediated phosphorylation of the actin bundling protein L-plastin in response to GM-CSF stimulation may provide a mechanistic insight directly linking priming signals to eosinophil cytoskeletal machinery (42). Of note, the pro-inflammatory cytokine TNF- α appears to uniquely evoke a robust synergistic effect on the generation of eosinophil-derived cytokines, including IL-8 (43), Th1 (CXCL9, CXCL10) and Th2 (CCL17, CCL22) chemokines (44), and MMP-9 (45) when combined with other stimuli (i.e. GM-CSF, IL-3, IL-4, IL-5 or IFN- γ), through an

NF- κ B-mediated mechanism. The vast variability in receptor activation that may induce eosinophil cytokine secretion is depicted in Fig. 2.

Heterogeneity in receptors mediating cytokine secretory responses is mirrored in the variety of signaling pathways implicated in eosinophil cytokine secretion. Intracellular cascades, including MAPK (MEK/ERK, p38, and JNK), PI3K-Akt pathways, and NF- κ B activation, have all been implicated in eosinophil secretion. Some stimuli elicit intracrine mediators or requisite integrin engagement downstream of receptor activation to accomplish cytokine release. For example, IL-16- or eotaxin-1 (CCL11)-elicited secretion of IL-4 is dependent upon induction of intracellular lipid body-derived LTC₄ (Fig. 2A) (46), and other stimuli (e.g. IL-5 family ligands (GM-CSF, IL-5, IL-3) and IgG) enable integrin-dependent signals in series with receptor activation to elicit robust cytokine secretion (Fig. 2B,C) (47, 48). These observations suggest adhesive events within tissues spatially orient activated eosinophils and coordinate cytokine secretion *in vivo*.

Modes of eosinophil cytokine secretion

Eosinophil-secreted cytokines may be derived through *de novo* gene transcription, translation of nascent mRNA pools, or from preformed, granule-stored cytokine caches. Stimulus-driven *de novo* gene transcription and new protein translation occurs in eosinophils through the canonical pathway of synthesis within endoplasmic reticulum and secretion through the Golgi apparatus, as in most other nucleated cells (Fig. 2D and reviewed in (49)). In addition, eosinophils maintain nascent pools of mRNA, expressions of which can be modulated through cytosolic proteins affecting mRNA stabilization (Fig. 2E). For example, GM-CSF mRNA is constitutively expressed in human eosinophils, but undergoes rapid decay under resting conditions. Eosinophil activation promotes stabilization of cytoplasmic GM-CSF mRNA, likely through interactions of the peptidyl-prolyl isomerase Pin1 with AU-rich element (ARE) binding partners (50). Similarly, Pin1 regulates cytoplasmic mRNA levels of TGF- β within eosinophils (51). How secretion of cytokines synthesized in their pro forms and requiring subsequent processing (e.g. IL-1 β) is achieved and regulated in eosinophils remains poorly defined.

In addition to canonical methods of *de novo* secretion, the existence of intracellular granule cytokine reserves sets eosinophils apart from many other leukocytes and enables very rapid secretion from eosinophils by bypassing the potentially time consuming steps of gene transcription and protein translation (Fig. 2F). Attesting to the breadth of their preformed cytokine repertoire, human eosinophils isolated from the circulation of healthy and mildly atopic donors uniformly contained preformed stores of Th1, Th2, proinflammatory, and immunoregulatory cytokines, that were rapidly and differentially released upon exposure to distinct cytokine stimulatory environments (8).

Pathways of preformed eosinophil cytokine secretion

Secretion of preformed, granule-stored cytokines is accomplished through four distinct degranulation processes: 1) *Classic Exocytosis*, whereby intracellular granules fuse with plasma membrane, eliciting the wholesale release of single granule contents; 2) *Compound Exocytosis*, whereby two or more intracellular granules fuse with one another prior to fusion with the cell membrane, eliciting the more robust simultaneous release of multiple granule contents; 3) *Piecemeal Degranulation* (PMD), whereby specific cytokines are selectively depleted from granules and shuttled within secretory vesicles to the plasma membrane for extracellular release; and 4) *Cytolysis*, whereby intact granules are liberated into extracellular spaces through a ruptured or disintegrated plasma membrane. Physiological evidences for classical and compound exocytosis are limited to eosinophils adhered to helminth parasite surfaces *ex vivo* (52), or tissue eosinophils from patients with advanced

inflammatory bowel diseases and invasive parasitic infections; *in vivo* evidence of granule-membrane fusion events are absent or exceedingly rare in tissue sections from patients with allergic diseases or asthma. Rather, the most commonly observed mechanisms of eosinophil degranulation in human diseases *in vivo* are PMD and cytolysis. As such, the remainder of this review will focus on these two mechanisms.

Ultrastructural characteristics and intracellular mechanisms of selective PMD

PMD as a mechanism for the discharge of granule-stored cytokines was first described in basophils over three decades ago (53). It is now recognized as a secretory mechanism also employed by eosinophils, mast cells and endocrine cells. PMD is the mode of degranulation most often encountered by eosinophils *in vivo* within tissue sections from diseased patients, and is readily induced *in vitro* upon exposure of human eosinophils to physiological stimuli (54). In contrast to classic or compound exocytosis, whereby entire granule contents are extruded en bloc, PMD delivers discrete packets of granule-derived cytokines, without spending whole granules, thereby maintaining intracellular granules competent for subsequent rounds of degranulation. Ultrastructural evidence for PMD, as shown in Figs. 3 and 4, includes changes apparent within intact intracellular granules (disorganization of the crystalline core, loss of electron density, and appearance of an intricate web of intragranular membranes), and the appearance of numerous small round and long tubular vesicles (Fig. 4, termed eosinophil sombrero vesicles, or EoSVs, in recognition of their concentric sombrero hat-shaped appearance in cross-section) observed throughout the cytoplasm (55). Structural studies of EoSVs demonstrated that these vesicles are curved tubular structures with remarkable plasticity and ability to interact with secretory granules (56). EoSVs present substantial membrane surfaces and are larger and more pleiomorphic than the small, spherical vesicles classically involved in intracellular transport. EoSVs seem particularly relevant for rapid delivery of eosinophil preformed cytokines or other proteins from secretory granules (Fig. 4D) (56).

Origin of secretory vesicles

Granule-stored cytokines are loaded into secretory vesicles, including small vesicles and EoSVs, which traverse the cytosol, and fuse with plasma membrane, releasing vesicular contents extracellularly. The origin of secretory vesicles has been the subject of some debate. Two lines of evidence suggested secretory vesicles shuttling granule-derived proteins might be extragranular in origin. First, granules do not appear to shrink in size during degranulation events, and second, the vesicle associated membrane protein VAMP-2, implicated in IFN- γ -induced transport and secretion of RANTES from human eosinophils, was detected in permeabilized whole eosinophils, but not on isolated eosinophil granules, by flow cytometry (57), or western blotting (58, 59). These observations prompted the hypothesis that secretory vesicles and granules exist as separate entities, with vesicles acquiring granule-derived content through a “kiss and run” mechanism of transient fusion events with granules (60). In contrast, compelling ultrastructural evidences have emerged that argue in favor of granule-derived secretory vesicles. Intracellular granules of stimulated eosinophils undergoing PMD display an intricate network of membranous folds beneath the granule limiting membrane (Fig. 4 and (54)). Should this membranous pool supply the emerging vesicular pool, one might explain the overall lack of size differential between degranulating and resting granules. In support of this interpretation, inhibition of eosinophil PMD through treatment with brefeldin A (BFA) collapses the intragranular membranous network, evidenced by the appearance of BFA-inducible lipid deposits within granules (54). It is also feasible that VAMP-2, although not readily detectable on outer granule membranes, is expressed by the intragranular membranous networks. In the authors’

experiences, eosinophil granule contents are exceedingly difficult to access through traditional wet-prep permeabilization (i.e. exposure to saponin within an aqueous environment); in contrast, exposing cells on slides to air-drying prior to permeabilization improved granule content labeling (61). In favor of VAMP-2 expression within eosinophil intracellular granules, low level VAMP-2 immunoreactivity was detected in association with eosinophil granules by immunoelectron microscopy (62). Moreover, tracking vesicle formation in serial sections of degranulating eosinophils by automated three-dimensional (3D) electron tomography revealed both small, round vesicles, and larger EoSVs, with membranes contiguous with intragranular membranes, apparently emerging from granules, suggesting vesicles can be derived directly from granules (Fig. 4) (56).

Receptor-mediated trafficking of cognate cytokines

Human eosinophils store numerous cytokines, with dichotomous immune potentials, within intracellular granules. However, stimulus-dependent release of individual cytokines is differential, suggesting mechanisms exist to govern parsimonious secretion of granule-derived cytokines. Indeed, mechanisms directing selectivity in mediator secretion remain poorly defined for any cell type. We have previously identified a novel mechanism by which IL-4 is differentially sorted from eosinophil intracellular granules, through chaperoning by its cognate receptor ligand (63). This pathway is likely more generally applicable to the selective trafficking of other granule-derived cytokines, in eosinophils as well as other cell types.

The seminal observation prompting investigation of cognate receptor chains as potential convoys for selective cytokine secretion was the luminal side membrane association of IL-4 within secretory vesicles following stimulation with eotaxin, an agonist known to induce secretion of granule-stored IL-4. This staining pattern was in contrast to that observed for staining of granule-derived eosinophil major basic protein (MBP; (61) and Fig. 4D). This may suggest distinct mechanisms govern packaging and secretion of cationic proteins and cytokines. With no transmembrane domain of its own, the association of IL-4 with vesicular membranes suggested involvement of a docking molecule; a prime candidate being its cognate receptor, the IL-4 receptor alpha chain (IL-4R α). Utilizing a combination of complementary methods (flow cytometry, immunoelectron microscopy, subcellular fractionation and western blotting), IL-4-bound IL-4R α chains were revealed within secretory vesicles emerging from mobilized granules and traversing the cytosol (63). Importantly, no evidence of common gamma (γ_c) chain movement was observed, suggesting granule-mobilized IL-4R α chains were not trafficking as functional heterodimers. The implication being that IL-4-bound IL-4R α would not induce activating signaling cascades during transit, and without the stabilizing effect of a heterodimerized γ_c chain, IL-4 might be more readily released extracellularly.

Taken together, these data suggest a model by which exogenous cell stimulation elicits specific mobilization of cytokine receptor chains associated with intragranular membranes, as depicted in Fig. 5. Once mobilized, receptors sequester granule-derived cognate cytokine ligands, and are packaged into discrete vesicles (Fig. 5Ai). Cytokine cargo is chaperoned within cognate receptor-expressing vesicles through the cytoplasm, to the plasma membrane for extracellular release. Importantly, recognition of receptor-chaperoned transport of cytokines in eosinophils provides a functional basis for the large surface area to volume ratio of the tubular EoSVs, characteristic of activated eosinophils (Fig. 4).

Of note, eosinophils express receptors for most, if not all, of the cytokines they also secrete, fueling the hypothesis that cognate receptor chaperoning is a mechanism broadly utilized by eosinophils in the selective transport of granule-derived cytokines for secretion. Fully supportive, several additional receptors have been detected within eosinophil intracellular

pools (IL-13R α , IL-6R α and CCR3 (63) and unpublished observations). Receptor chaperoning of cytokines may be applicable to other cells as well. Expressions of IL-10R on neutrophil granule membranes (64), and CCR3 by mast cell granules (65) may suggest a similar mechanism functions in the secretion of IL-10 and CCR3 ligands (eotaxins and RANTES) by neutrophils and mast cells, respectively. Moreover, subsequent to our work, IL-15R α chain chaperoning of IL-15 through endoplasmic reticulum (ER) and golgi was reported in dendritic cells (66) (reviewed in (67)). In contrast to receptor-mediated cytokine secretion from human eosinophils, IL-15-IL-15R α complexes remained intact at the dendritic cell surface, and were detected as complexes within cell supernatants.

Accessory molecules involved in eosinophil vesicle trafficking

Vesicle-mediated secretion of granule-derived cytokines depends upon assembly of SNARE complexes at membrane junctures, i.e. vesicle to plasma membrane fusions (59, 68) (Fig. 5Aii). Human eosinophils express the SNARE vesicle-associated membrane protein (VAMP)-2, VAMP-7 and VAMP-8, expressed on vesicle (VAMP-2) and granule (VAMP-7, 8 and low levels of VAMP-2) membranes (58, 59, 62). At the plasma membrane, eosinophils express the cognate SNARE molecules SNAP-23 and syntaxin-4 (69). Assembly of SNARE complexes is in many cells regulated by protein kinase C (PKC) isoforms, activated by Ca²⁺ transients to act upon the Sec/Munc family of conserved syntaxin-binding proteins. Eosinophils express multiple isoforms of PKC, and eosinophil granules express Munc18c. SNARE-mediated mechanisms of eosinophil mediator secretion has been reviewed in greater detail by Logan et al (60).

Key questions remain unanswered

Several key issues remain to be resolved, including an understanding of how stimulus activation and downstream signal transduction mediates cytokine receptor mobilization within granules. The potential existence of additional signaling intermediaries (such as intracrine LTC₄, discussed above) and their granule-expressed receptors (described in the next section) is an intriguing possibility.

Also incompletely understood is the degree of homogeneity in cytokine potential of eosinophils, both circulating and within tissues. In a recent study, preformed expression of seven diverse cytokines within blood eosinophil lysates from healthy and mildly atopic donors was investigated (8). Although disparities in absolute quantities of individual cytokines were observed between blood eosinophils from individual donors, all seven cytokines were detectable in cells from every donor analyzed, and for most of the cytokines analyzed, their concentrations, relative to one another, were conserved between donors (8). Therefore, although quantities of granule-stored cytokines vary, it is probable that human circulating eosinophils uniformly maintain a diverse cytokine repertoire. In contrast, immunofluorescence analyses of gastrointestinal (70), ocular (71), and skin (72) tissue eosinophils have revealed variable patterns of cytokine expression in association with disease status. Taken together, these findings may suggest blood eosinophils exhibit a broader range of preformed cytokine potential, while tissue-homed eosinophils modulate preformed cytokine content in response to local environmental cues. However, confirmation of this hypothesis awaits further vetting of the different methods, due to challenges inherent in effectively accessing and distinguishing intracellular compartments that house cytokine proteins within eosinophils, i.e. granules and secretory vesicles. For example, we have previously found granule-stored proteins to be inaccessible to antibody detection, while vesicular pools are strongly apparent, in saponin-permeabilized eosinophils maintained in an aqueous environment (61). Being mindful of this distinction is critical to deciphering cytokine competency, as vesicle contents may signify those cytokines en route to secretion

in real time, while granule contents would presumably represent the total available preformed cytokine content of the cell.

Ultrastructural characteristics of cytolysis and extracellular secretory competence of cytolysis-liberated free granules

Cytolysis is characterized ultrastructurally by nuclear alterations, (i.e. chromatolysis and dissolution of the nuclear envelope) and rupturing of the plasma membrane, and is accompanied by deposits of clusters of free granules (cfegs) within surrounding tissue (Fig. 6) (73, 74). Therefore, unlike PMD, throughout which eosinophils remain viable and competent to undergo multiple rounds of degranulation, cytolysis results in eosinophil cell death. Of note, tissue-deposited cfegs appear as a heterogeneous pool of both fully membrane-bound, intact organelles, and those exhibiting varying degrees of dissolution (73).

Cytolytic eosinophils and clusters of liberated eosinophil granules are readily observed *in vivo*, for example within human allergic airways (74) and nasal polyps (75), yet cytolysis remains an enigmatic mode of eosinophil degranulation, and mechanisms triggering this secretion process are poorly understood. Experimentally, cytolysis may be induced *in vivo* following allergen challenge, or in response to mechanical epithelial denudation (reviewed in (77)). *In vitro*, exposures to the divalent cation ionophore A23187 or secretory IgA-opsonized beads (and to a lesser extent IgG-coated beads), produce cytolytic eosinophils ((76) and reviewed in (77)).

Depositions of cell-free eosinophil granules in disease

Although the biological relevance of tissue-deposited cfegs was not yet appreciated, the earliest recorded observation of free eosinophil granules, identified within sputum of asthmatics, dates back to the early 1880s (77). More recently, clusters of cell-free eosinophil granules, frequently retaining an intact trilaminar delimiting membrane, have been reported in tissues from patients with atopic dermatitis (78), upper airways mucosal disorders (including nasal allergy (79), and chronic rhinosinusitis (80, 81)), asthma (77), urticaria (82), eosinophilic esophagitis (20), and *Onchocerca volvolus* patients treated with amocartazine (83). In addition to allergic and parasitic diseases, cfegs have been detected in patients with advanced gastric carcinoma (84), and subcutaneous fat necrosis lesions in newborns (85). Cytolysis and PMD are not mutually exclusive, but rather are observed concurrently within tissue biopsies.

Surface phenotype and secretory competence of cell-free granules

Recent new findings have attached biological significance to those cell-free granules exhibiting intact granule membranes within tissues. Neves et al demonstrated granule membrane expression of GPCR CCR3, cytokine receptor IFN- γ R α chains, cysteinyl leukotriene receptors 1 and 2, and purinergic receptor P2Y₁₂ (86–88). Granule-expressed receptors are topologically oriented to engage their cognate ligands, and trigger phosphorylation of intragranular signaling kinases upon activation (88). Stimulation of cell-free granules with IFN- γ or the CCR3 agonist eotaxin elicited degranulation of cationic proteins and differential secretion of cytokines. IFN- γ -induced degranulation of isolated, cell-free granules was attenuated in the presence of inhibitors of tyrosine kinases, PKC, and p38 MAPK (but not PI3K inhibitors), while eotaxin-1-elicited secretion was abolished by inhibitors of PKC, p38 MAPK, and PI3K, and by pertussis toxin, an inhibitor of Gi-coupled GPCRs. Taken together, these findings suggest eosinophil intracellular granules, cytolytically released from eosinophils as membrane-intact free granules, may remain

secretory-competent organelles within tissues, capable of stimulus-dependent, parsimonious cytokine secretion akin to that elicited from intact eosinophils.

Key questions remain unanswered

In vivo significance of cytolysis-deposited cfegs in disease remains to be delineated. Additional questions include longevity of free granules within tissues, and mechanisms by which cytokine secretion is achieved. Are extracellular granule contents mobilized and released through a vesicular-based pathway akin to PMD? Are tissue cfegs competent for multiple rounds of degranulation? Moreover, the mechanisms by which human eosinophils can cytolytically release intact granules extracellularly, and the breadth of potential free granule secretagogues has yet to be fully appreciated.

Conclusion

It is now established that eosinophils synthesize, store, and secrete a vast armamentarium of cytokines with broad biological effects in health and disease. Unique approaches, including electron tomography, have enabled an unprecedented glimpse into the inner machinations of eosinophil secretion of granule-stored cytokines. Moreover, discoveries of granule membrane-expressed receptors linked to intragranular signal transduction cascades within extracellular, cell-free eosinophil granules ushers in a new frontier in the study of eosinophil secretory responses. Key questions remain to be resolved, including determinations of the cytokine potentials of intact blood and tissue eosinophils at baseline and in disease, and a full delineation of the breadth of secretagogues effective at eliciting cytokine secretion from tissue-deposited cell-free granules. Continued study in this burgeoning field will undoubtedly reveal novel therapeutic targets for treatment of asthma and other eosinophil-associated disorders, and will likely bring to light mechanisms more broadly applicable to cytokine secretion from other innate immune cells.

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Figure 1. Biological effects of eosinophil-derived cytokines in health and disease

Eosinophils secrete numerous cytokines with varied biological functions in health (left panels) and disease (right panels). Shown is an abridged list of eosinophil-derived cytokines compiled from (6, 89, 90). M2=alternatively activated macrophage; PC=plasma cell; B=B cell; BV=blood vessel; Eos=eosinophil; T=T cell; DC=dendritic cell.

Figure 2. Stimulus-induced activation of intracellular cytokine secretory pathways in eosinophils
Eosinophil cytokine secretion is initiated through interactions of a wide variety of eosinophil secretagogues, including soluble factors and tissue components, with their respective receptors. Intracellular signal transduction pathways (vertical gray arrows) are initiated downstream of single receptor activation (narrow gray arrows), or following the synergistic activation of multiple receptors (wide gray arrows). Stimuli may elicit intracellular signaling intermediaries (A), or promote expression and/or activation of eosinophil-expressed adhesion molecules (B, C), in order to accomplish cytokine secretion. Secreted cytokines are either synthesized *de novo* and shuttled through golgi apparatus for packaging and secretion (D), translated from nascent pools of cytoplasmic mRNA (E), or sorted and mobilized from preformed intracellular granule caches into secretory vesicles for delivery to cell surface (F).

Figure 3. Ultrastructural images of human eosinophils showing normal appearance (A) and piecemeal degranulation (PMD) characteristics (B)

In (A), the cytoplasm is packed with specific (secretory) granules (Gr) containing an internal often electron-dense crystalline core surrounded by an electron-lucent matrix. (B) After stimulation with physiological stimuli, granules (Gr) exhibit progressive emptying of their contents classically associated with PMD. Disassembled cores and reduced electron-density are frequently observed. Cells were stimulated with eotaxin as described (54) and prepared for conventional transmission electron microscopy. Nu, nucleus. Bar, 900 nm.

Figure 4. Morphology of human stimulated eosinophils undergoing piecemeal degranulation (PMD)

(A) Enlarged, emptying secretory granules (Gr) and large tubular carriers termed eosinophil sombrero vesicles (EoSVs, highlighted in pink) are observed in the cytoplasm. In (B), a highly mobilized granule (Gr) with intragranular membranes (arrowheads) is seen in high magnification. Note different profiles of EoSVs surrounding or in contact with the granule. In (C), a three-dimensional (3D) model based on electron tomographic slices show the structural organization of EoSVs (pink). The granule limiting membrane is highlighted in blue and intragranular membranes in green. (D) Immunogold electron microscopy (EM) for major basic protein (MBP) reveals labeling at EoSVs lumina (arrows). Cells were stimulated with eotaxin (A, C, D) or platelet-activating factor (PAF) (B) as before (54) and prepared for conventional (A, B) or immunogold (C) EM. The 3D model was constructed from serial virtual slices extracted from reconstructions of a human eosinophil analyzed by fully automated electron tomography at 200 kV. Figure 4C was reprinted from (91) with permission. Nu, nucleus; LB, lipid body. Bar, 400 nm (A), 200 nm (B, C), 500 nm (D).

Figure 5. Proposed model of receptor-mediated chaperoning of cognate cytokines from intragranular stores

Cytokine receptor chains, expressed within granules and mobilized downstream of cell stimulation, sequester and chaperone cognate cytokine ligands into emerging secretory vesicles (Ai). Unlike MBP, which exhibits a free luminal pattern of expression within secretory vesicles (red circles), granule-derived cytokines (blue circles) remain bound to receptor chaperones within secretory vesicles. Docking and fusion of secretory vesicles to plasma membrane is directed through complexes formed between vesicle-expressed VAMP-2 and plasma membrane-expressed Syntaxin-4 and SNAP-23 (Aii).

Figure 6. Ultrastructure of a tissue human eosinophil undergoing cytolysis
Note the disintegrating nucleus (Nu) and the entire membrane-bound secretory granules (arrows) in the surrounding tissue. Tissue eosinophils were present in a skin biopsy performed on a patient with breast cancer who underwent treatment using rhSCF (recombinant human Stem Cell Factor). CF, collagen fibrils. Bar, 800 nm.