Mechanisms of Eosinophil Degranulation in Allergic Disease

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Disclosures

I have no financial interests or relationships to disclose.
Eosinophil Functions in Allergic Diseases

**Tissue Damage, Repair and Remodeling**
- Epithelial cell damage (ROS, cytotoxic granule prtns)
- Fibroblast activation
- ECM production
- Fibrosis
- Sm. muscle activation/contraction
- Angiogenesis

**Host Defense**
- ROS
- Cytotoxic granule proteins
- Extracellular DNA Traps

**Cell Activation**
- “Itch” response
- Eosinophil:Mast Cell Allergic Effector Unit (AEU)

**Immunomodulation**
- DC activation
- T cell polarization
- T cell recruitment

**Humoral Immunity**
- Plasma cell survival
- IgA class switching

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### Eosinophil-Derived Mediators

<table>
<thead>
<tr>
<th>Cationic Proteins</th>
<th>Cytokines</th>
<th>Chemokines</th>
<th>Growth Factors</th>
<th>Lipid Mediators</th>
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<tbody>
<tr>
<td>MBP</td>
<td>IL-1β</td>
<td>Eotaxin-1 (CCL11)</td>
<td>NGF</td>
<td>LTE4</td>
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<tr>
<td>EPX</td>
<td>IL-2</td>
<td>GM-CSF</td>
<td>MCP-1 (CCL2)</td>
<td>PDGF</td>
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<tr>
<td>ECP</td>
<td>IL-3</td>
<td>SCF</td>
<td>MIP-1α (CCL3)</td>
<td>SCF</td>
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<tr>
<td>EDN</td>
<td>IL-4</td>
<td>TGF-α</td>
<td>RANTES (CCL5)</td>
<td>VEGF</td>
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<tr>
<td>IL-5</td>
<td>TGF-β</td>
<td>MCP-2 (CCL8)</td>
<td>HB-EGF-LBP</td>
<td>PGE2</td>
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<tr>
<td>IL-6</td>
<td>TNF-α</td>
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<tr>
<td>IL-8</td>
<td>TNF-β</td>
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<td>MDC (CCL22)</td>
<td>Thromboxane A2</td>
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<td>IL-11</td>
<td>MIP-1α (CCL9)</td>
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<td>IL-12</td>
<td>MIP-3 (CCL23)</td>
<td>protectins</td>
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<td>IL-13</td>
<td>Gro-α (CCL1)</td>
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<td>SDF-1 (CCL12)</td>
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<td>IL-18</td>
<td>IP-10 (CCL10)</td>
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<td>IL-25</td>
<td>TARC (CCL17)</td>
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<td>IL-23</td>
<td>Mig (CCL9)</td>
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Human Eosinophils Maintain Preformed Stores of Diverse Cytokines Within Intracellular Granules

Adapted from LA Spencer Eosinophils in Health and Disease, eds. Lee and Rosenberg, Elsevier, 2014

Mechanisms of eosinophil degranulation
**Piecemeal Degranulation (PMD)**

1. Cell stimulus

2. Selective loading of granule-stored cytokines into vesicles

3. Cargo-loaded vesicles bud from intracellular granules

4. Granule-derived cargo is transported within secretory vesicles

5. Secretory vesicles fuse with plasma membrane and release granule-derived cargo.

**Piecemeal Degranulation of Preformed, Granule-Store Cytokines**

Formation of eosinophil sombrero vesicles (EoSVs)

Discrete packets of cytokine released extracellularly.

Cell membrane

Deconvolution and 3D rendering
Granule-Stored Cytokines Are Differentially Released from Human Blood Eosinophils Through Piecemeal Degranulation (PMD)

Eosinophil cytokine secretion within 30 minutes of stimulation

- **Pro-inflammatory stimulation**
  - Donor #1
  - Donor #2
  - Donor #3

- **Th1 stimulation**
  - Donor #1
  - Donor #2
  - Donor #3

Cytolysis with release of free granules

- Cluster of cell-free granules
- DNA strands
- Nuclear and plasma membrane dissolution
Cytolysis is cell death distinct from apoptosis or necrosis

<table>
<thead>
<tr>
<th>Anti-Fas</th>
<th>Heat</th>
<th>A23187</th>
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<tbody>
<tr>
<td>Apoptosis</td>
<td>Necrosis</td>
<td>Cytolysis</td>
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</tbody>
</table>


Eosinophils Undergoing Cytolytic Cell Death
Expulse Cell-Free Granules

Ca^{2+} ionophore-elicited cytolysis of human blood eosinophils ex vivo

Arrows indicate cell-free granules

Eosinophils undergo Extracellular DNA Trap Cell Death (ETosis)

Free granules liberated from cytolytic eosinophils exhibit an intact delimiting membrane.
Cell-Free eosinophil granules express ligand-binding domains for IFN-γRα and CCR3

Might cell-free eosinophil granules respond to stimuli?

Cell-Free Eosinophil Granules Respond to Stimuli

Transient pH changes measured in cell-free granules stimulated with eotaxin

Ueki et al. Blood 2013;121:2074-2083
Stimulus-Induced Secretion of Cationic Proteins and Cytokines from Cell-Free Eosinophil Granules

- Secreted ECP
- IFN-γ
  - 100 ng/ml
  - 200 ng/ml
  - 400 ng/ml
- Eotaxin

Stimulus: Granule stimulus

Cytolysis with release of free granules

Differential Secretion
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Functions of Eosinophils in Allergic Diseases
Functions of Eosinophil Cell-Free Granules in Allergic Diseases

CLCs are associated with EETosis in human tissues

Frontal sinus, allergic

Colon, ulcerative colitis


Nasal polyps, ECRS
ETosis-mediated Charcot-Leyden crystal (CLC) formation

CLCs induce the secretion of IL-1β from primary human macrophages
Summary

- Eosinophil secretion is central to diverse effector functions of eosinophils in allergic diseases
- Eosinophils undergo *de novo* cytokine synthesis and secretion and also rapidly and differentially secrete cytokines from preformed granule stores
- Two main physiological mechanisms of secretion of preformed granule‐stored cytokines: 1) Piecemeal degranulation (PMD); and 2) Cytolysis with deposition of clusters of cell-free granules
- Cell-free granules deposited from cytolytic eosinophils maintain stimulus-dependent secretory competence; therefore *eosinophil secretory functions may continue to contribute to disease pathogenesis in the absence of intact eosinophils*
- Charcot-Leyden crystal (CLC) formation is associated with cytolytic eosinophil cell death and contribute to macrophage IL-1β secretion