Do mechanisms of eosinophil degranulation explain the impact on allergic diseases?

Lisa A. Spencer, PhD
Associate Professor
Gastrointestinal Eosinophilic Diseases Program (GEDP)
Mucosal Immunology Program (MIP)
GI and Liver Innate Immune Program (GALIIP)
University of Colorado School of Medicine

Disclosures

Funding:
• NIH (NIAID/NHLB)
• Holoclara, Inc.
• Past funding from AAAAI, APFED
Objectives

1. To highlight both helpful and harmful effector functions elicited through eosinophil degranulation events relevant to allergic diseases.

2. To characterize distinct mechanisms of eosinophil degranulation (i.e. PMD and cytolysis) and discuss their respective contributions to eosinophil effector functions in allergic diseases.

3. To discuss the effects of anti-IL-5 therapies on eosinophil tissue cytolysis.

4. Current implications and key unanswered questions

Eosinophil Functions in Allergic Diseases

Tissue Damage, Repair and Remodeling
- Epithelial cell damage (ROS, cytotoxic granule prtns)
- Eosinophil:epithelial cell
- 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
### Eosinophil-Derived Mediators

#### Cationic Proteins / Metalloproteinases
- MBP
- EPX
- ECP
- EDN
- MMP-9
- MMP-2
- TIMP1
- IL-10
- IL-11
- IL-12
- IL-13
- IL-16
- IL-17
- IL-18
- IL-25 (IL-17E)
- IL-23

#### Cytokines
- IL-3
- IFN-γ
- GM-CSF
- SCF
- TGF-α
- TGF-β
- TNF-α
- APRIL
- BAFF
- Gro-α
- SDF-1
- C10/CCL6
- IP-10 (CXCL10)
- TARC (CCL17)
- Mig (CXCL9)

#### Chemokines
- Eotaxin-1 (CCL11)
- MCP-1 (CCL2)
- MIP-1α (CCL3)
- RANTES (CCL5)
- MCP-2 (CCL8)
- MCP-3 (CCL7)
- MIP-1γ (CCL9)
- MIP-3α (CCL23)
- GRO-α (CXCL1)
- MIP-1α (CCL3)
- MIP-3α (CCL23)
- Gro-α (CXCL1)
- IP-10 (CXCL10)

#### Growth Factors
- NGF
- SCF
- VEGF
- MDC (CCL22)
- MDC-1 (CCL24)
- MDC-2 (CCL27)
- HB-EGF-LBP
- 15-HETE
- PAF
- PGE₂

#### Lipid Mediators
- LTC₄
- LTD₄
- PGE₁
- PGE₂
- Thromboxane A₂
- Resolvins
- Protectins

---

### Eosinophil Secretion

- **Preformed Granule Stores**
  - Cationic Proteins

- **De novo Synthesis**
  - Cytokines, Chemokines and Growth Factors

- **Arachidonic Acid Metabolism**
  - Lipid Mediators
How diverse is the repertoire of preformed cytokines in human eosinophils?

Human Blood Eosinophils Maintain Preformed Stores of Diverse Cytokines Within Intracellular Granules

Relative concentrations of cytokines within intracellular granules of blood eosinophils

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

1. To highlight both helpful and harmful effector functions elicited through eosinophil degranulation events relevant to allergic diseases.

2. To characterize distinct mechanisms of eosinophil degranulation (i.e. PMD and cytolysis) and discuss their respective contributions to eosinophil effector functions in allergic diseases.

3. To discuss the effects of anti-IL-5 therapies on eosinophil tissue cytolysis.

4. Current implications and key unanswered questions
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

<table>
<thead>
<tr>
<th>Secreted Cytokine</th>
<th>Subject #1</th>
<th>Subject #2</th>
<th>Subject #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-13</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-13</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>IL-15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Th1 stimulation

<table>
<thead>
<tr>
<th>Secreted Cytokine</th>
<th>Subject #1</th>
<th>Subject #2</th>
<th>Subject #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-13</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-13</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>IL-15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Piecemeal Degranulation (PMD)

#1 Cell stimulus

#2 Selective loading of granule-stored cytokines into vesicles

#3 Cargo-loaded vesicles bud from intracellular granules

Weller and Spencer Nature Reviews Immunology 2017; 17:746
Small vesicles and Eosinophil Sombrero Vesicles (EoSVs) Arise from Intracellular Granules

Formation of eosinophil sombrero vesicles (EoSVs) from intracellular granules

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.

Weller and Spencer Nature Reviews Immunology 2017; 17:746
PMD results in the release of discrete packets of cytokines

- Very rapid
- Stimulus-dependent, differential secretion
- Localized delivery of relatively small packets of cytokines into the microenvironment.

Biological significance of PMD:

Patient with undiagnosed diarrhea

Patient with active Crohn's; TI biopsy from non-involved site

Eosinophil:T cell interactions

CD3 = Nova Red
EPX (Eos) = DAB nickel
Cytolysis with release of free granules

Eosinophil cytolysis results in cell-free granules deposited within tissues

Diseases associated with cytolytic eosinophils include:
- Asthma
- Atopic dermatitis
- Allergic rhinitis
- Urticaria
- Nasal polyps
- Crohn’s disease
- Eosinophilic pneumonia
- Eosinophilic esophagitis
Cytolytic eosinophils expel granules and DNA traps

Ueki et al. Blood 2013

Microorganisms captured in cytolytic eosinophil DNA traps

Ueki et al. JACI 2016
Staining for EPX reveals eosinophil cell-free granules in intestinal biopsies

History of IBD/ Routine screen
Path report: TI – normal

Staining for EPX reveals extensive eosinophil cell-free granules in intestinal biopsies

Active CD
Path report: TI – active ileitis with evidence of focal erosion
Is there a biological significance to tissue-deposited cell-free eosinophil granules?

Free granules liberated from cytolytic eosinophils exhibit an intact delimiting membrane

Flow cytometry of isolated granules

Might cell-free eosinophil granules respond to stimuli?
Cell-free eosinophil granules respond to external stimuli

**Acidification of eosinophil cell-free granules in response to eotaxin stimulation**

![Image of Acridine orange-loaded cell-free granules](image)

**Stimulus-Induced Secretion of Cationic Proteins and Cytokines from Cell-Free Eosinophil Granules**

![Graph showing secretion of ECP and cytokines](image)
Cell-free granules released through eosinophil cytolysis are secretion competent

**Granule stimulus**

D Cytolysis with release of cell-free granules

- Nuclear and plasma membrane dissolution
- DNA traps – anti-microbial functions
- +/- free cationic granule protein deposition
- Secretion-competent clusters of cell free granules left behind in the tissue

**Biological significance of eosinophil cytolysis:**

- DNA traps – anti-microbial functions
- +/- free cationic granule protein deposition
- Secretion-competent clusters of cell free granules left behind in the tissue

**SEM of eosinophil DNA traps**

S. aureus  E. coli

Ueki et al, JACI 2016
Objectives

1. To highlight helpful and harmful effector functions elicited through eosinophil degranulation events relevant to allergic diseases.

2. To characterize distinct mechanisms of eosinophil degranulation (i.e. PMD and cytolysis) and discuss their respective contributions to eosinophil effector functions in allergic diseases.

3. To discuss the effects of anti-IL-5 therapies on eosinophil tissue cytolysis.

4. Current implications and key unanswered questions
Effects of anti-IL-5 therapy on eosinophil tissue cytolysis

Mepolizumab Attenuates Airway Eosinophil Numbers, but Not Their Functional Phenotype, in Asthma

Elizabeth A. Kelly¹, Stephanie Esmaut¹, Lin Ying Liu¹, Michael D. Evans², Mats W. Johansson³, Sameer Mathur¹, Deane F. Mosher¹, Loren C. Donlinber⁴, and Nizar N. Jarjour⁴

¹Allergy, Pulmonary and Critical Care Medicine Division, Department of Medicine, ²Department of Biostatistics and Medical Informatics, and ³Department of Biomolecular Chemistry, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin

Kelly et al. Am J Respir Crit Care Med 2017

Study Design

Participants with mild allergic asthma underwent segmental bronchoprovocation with antigen (SBP-Ag) before and after a single dose of mepolizumab. Blood, BAL, and tissue eosinophils were assessed at each time point.

Kelly et al. Am J Respir Crit Care Med 2017
Mepolizumab depletes allergen-induced circulating eosinophils

Mepolizumab decreases, but does not eliminate, allergen-induced BAL eosinophils

Kelly et al. Am J Respir Crit Care Med 2017
Mepolizumab decreases, but does not eliminate, eosinophil cytolysis in allergen-challenged bronchial mucosa

**Summary**

- Eosinophil secretion is central to diverse effector functions of eosinophils that promote both health and allergic diseases.
- In addition to cationic granule proteins, eosinophils rapidly and differentially secrete a highly diverse array of cytokines from preformed granule stores.
- Two main physiological mechanisms of secretion of preformed granule-stored cytokines in allergic diseases:
  1. **Piecemeal degranulation (PMD)** – differential, stimulus dependent secretion accomplishing localized delivery of relatively small packets of cytokines into cellular synapses and/or the immediate microenvironment.
  2. **Cytolysis with deposition of clusters of cell-free granules** – DNA traps, secretion-competent clusters of cell-free granules left behind in tissues.
- Anti-IL-5 therapies may not fully prevent cytolysis of residual tissue eosinophils.

**EPX staining in bronchial mucosa**

![EPX staining images](image)

**Summary table**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Ag-naive segment</th>
<th>20% AgPD_{20} segment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-mepolizumab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-mepolizumab</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(p=0.06)

Kelly et al. Am J Respir Crit Care Med 2017
Current Implications

- Successful depletion of circulating eosinophils may not protect against cytolysis of tissue eosinophils.
- New therapeutics should be evaluated for their impact on cytolysis of tissue eosinophils.
- Different mechanisms of eosinophil degranulation may have profoundly different effects on clinical outcomes.
- Defining triggers and mechanisms distinctive to eosinophil PMD and cytolysis may lead to novel therapeutic approaches.

Key Unanswered Questions

- Do subsets of tissue eosinophils differ from blood eosinophils in their preformed cytokine content?
- What are the mechanisms that link external stimuli to specific receptor mobilization within granules, and are these mechanisms similarly involved in selective secretion from cell-free eosinophil granules?
- How is the secretion of cationic granule proteins regulated?
- What are the functional and physical longevities of cell-free granules within tissues?

Acknowledgements

Beth Israel Deaconess Medical Center

HARVARD MEDICAL SCHOOL

Spencer Lab:
- Courtney Olbrich, BS
- Stephen Schworer, MD, PhD
- Kenya Koyama, MD, PhD

University of Colorado Anschutz Medical Campus

• Glenn Furuta, MD
• Dan Atkins, MD
• GEDP research and clinical teams

• Peter Weller, MD
• Rossana Melo, PhD
• Adam Cheifetz, MD