Have We Missed A Role For Neutrophils In Asthma?

In Steroid-Refractory Asthma?

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Disclosures

- Dr. Gelfand discloses that he is on the advisory board for Boehringer Ingelheim
Neutrophils May Play a Role in Asthma

A Steroid-Insensitive Cell Type
Development of Airway Allergic Responses

Hawrylowicz and O’Garra 2005
Reasons for Failure to Achieve Control

- Compliance
- Asthma heterogeneity
- Wrong diagnosis
- Wrong target
- Failure to deliver drug to the target site

- **Insensitivity of the pathway or target cell to corticosteroids**
Membrane Phospholipids

- cPLA₂
- 5-Lipoxygenase
- 5-HPETE → 5-HETE → 5-oxo-ETE
- 5-Lipoxygenase
- Leukotriene A⁴
- LTA⁴ Hydrolase
- Leukotriene B⁴
- LTA⁴ Hydrolase
- Leukotriene C⁴
- BLT₁ receptor
- Leukotriene D⁴
- CysLT₁ receptor
- Leukotriene E⁴

- Leukotriene B⁴: Eosinophil chemoattractant, Neutrophil monocyte migration, Neutrophil activation
- Leukotriene C⁴: Bronchoconstriction, Mucus secretion
- Leukotriene D⁴: Vascular permeability, Airway smooth muscle contraction
- Leukotriene E⁴: Eosinophil infiltration

- Arachidonic Acid
- 5-Lipoxygenase
- FLAP

- Bronchoconstriction
- Mucus secretion
- Vascular permeability
- Airway smooth muscle contraction
- Eosinophil infiltration

Neutrophil chemoatraction
Monocyte migration
Trafficking of T-cells
Asthma

Neutrophils

LTB4

BLT1

Many Factors

Asthma

Mast Cells

LTB4

BLT1

Many Factors
Airway Neutrophilia

**Increased recruitment**

- **Receptors**
  - BLT1
    - LTB4
  - CXCR2

- **Chemokines**
  - Epithelial cell-derived potent chemoattractants
    - IL-8
    - MIP-2
    - KC
Airway Neutrophilia

Increased survival

• Inhibit neutrophil apoptosis
  – IL-13
  – TNF
  – GM-CSF
  – G-CSF
  – IFN$\gamma$
Corticosteroids

- Increased release of neutrophils from bone marrow
- Enhance neutrophil survival
EARLY RESPONSE

Transmigration of neutrophils

- Bronchoconstriction
- Neutrophil adhesion

Adhesion molecules

- Cytotoxicity
- IL-8
- Mucus hypersecretion

ROS

- Cytotoxicity
- Vascular permeability
- Mucus hypersecretion
- Bronchial hyperreactivity
- Release of ECP

MPO

- Cytotoxicity
- Mast cells → Histamine
- Platelets → Serotonin

TXA₂

Elastase

Neutrophil-Derived Serine Proteinases/Defensins

- Affect epithelial integrity

- Mucin secretagogues
  - Goblet cells
  - Submucosal gland cells
  - Vascular permeability
  - Airway hyperresponsiveness
Inflammatory Cell Composition in Asthmatic Airways

- Eosinophils
- Neutrophils
- Mixed
- Pauci-cellular
Are there distinct asthma phenotypes?
Asthma can be divided into at least two distinct molecular phenotypes defined by the degree of Th2 inflammation!

Woodruff PG et al., AJRCCM 180:388-395, 2009
Eosinophilic and Neutrophilic Inflammation in Asthma

Eosinophilic Asthma
- Thickening of basement membrane zone
- Corticosteroid responsive

Neutrophilic Asthma
- More severe airflow (low post BD FEV1 obstruction)
- Severe disease
- Relatively corticosteroid unresponsive

Analysis of Sputum Eosinophils in Mild-To-Moderate Asthma

McGrath et al., AJRCCM 185:612, 2012
Conclusions

“A sizeable subgroup of mild-to-moderate asthma has a disease phenotype that is not the usual eosinophilic, steroid-responsive subtype, but a different subtype whose mechanisms are poorly understood and for which new controller treatments are needed”

McGrath et al., AJRCCM 185:612, 2012
Conclusions: In this large heterogeneous population of adults with asthma, we have shown that prebronchodilator FEV\textsubscript{1} is associated with neutrophilic and eosinophilic airway inflammation, whereas sputum total neutrophil counts alone are associated with postbronchodilator FEV\textsubscript{1}. This supports the hypothesis that neutrophilic airway inflammation has a role in the progression of persistent airflow limitation in asthma and raises the possibility that this progression and the development of COPD share a common mechanism.

Steroid Insensitivity

- Neutrophils
- CD8 T cells
- Th17 cells
- Mast cell / basophil degranulation
- Antibody production
Steroid Insensitivity

Neutrophils

Steroid Insensitive Pathways Initiated by Certain Triggers
Mediators in Wheezing Children (WC) Compared to Normal Controls (NC)

Krawiec ME; AJRCCM 163:1338, 2001
Conclusion: Urine LTE4 increases with smoking

## Asthma Pathogenesis

<table>
<thead>
<tr>
<th>Trigger</th>
<th>Allergen Exposure</th>
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<th>Diesel Exhaust/Ozone</th>
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<td>Th1, LT</td>
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<td>CD4 T cells</td>
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My Patients Asthma Does Not Appear to Respond to Corticosteroids: Why?

It’s all about the the Trigger!

Triggers activate pathways and pathways define corticosteroid sensitivity!
Steroid Insensitivity

- Neutrophils
- CD8 T cells
- Th17 cells
- Mast cell / basophil degranulation
- Antibody production

**Activity on:**
- Macrophages
- NK cells
- B cells
- Eosinophils
- Mast cells
- Basophils
- B cells
- Neutrophils
- Macrophages
- B cells

**Protection from:**
- Intra cellular bacteria, fungi, protozoa
- Extracellular parasites
- Extracellular bacteria, fungi

**Involved in:**
- Chronic inflammatory disorders
- Autoimmune disorders
- Asthma
- Atopic disorders
- Chronic inflammatory disorders
- Autoimmune disorders
- Asthma
## Asthma Pathogenesis

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<td>Th17</td>
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Asthma

Adaptive Immunity (Allergic)  Innate Immunity (Non-Allergic)
Asthma

Adaptive Immunity (Allergic)

T cells (Th2, Tc2) → Eosinophils

Innate Immunity (Non-Allergic)

ILC1

ILC2

ILC3

IL-17 → Neutrophils
Pathways Leading to Airway Pathology

**ENVIRONMENTAL TRIGGERS**

**GENES**

- IL-25
- IL-33
- TSLP

**INFLAMMATION**

- IL-5
- IL-13

**Airway epithelium**

**DC**

**Th2**

- IL-5
- IL-13

**ILC2**

- IL-8

**Neutrophils**

**ILC3**

- IL-17

**AHR**

**AIRWAY INFLAMMATION**

- Pollutants, microbes, glycolipids
<table>
<thead>
<tr>
<th>Study</th>
<th>Results</th>
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<tr>
<td>Al-Ramli et al., 2009 (14)</td>
<td>Higher number of IL-17A positive cells in patients with severe asthma compared with controls groups</td>
</tr>
<tr>
<td>Bullens et al., 2006 (15)</td>
<td>Increased expression of IL-17 in severe asthma Correlation between IL-17 and neutrophilia</td>
</tr>
<tr>
<td>Kim et al., 2014 (24)</td>
<td>Increased numbers of IL-17 secreting ILC3 in BAL of severe asthmatic patients</td>
</tr>
<tr>
<td>Molet et al., 2001 (32)</td>
<td>Higher number of cells expressing IL-17 in sputum and BAL of asthmatic subjects</td>
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<tr>
<td>Cosmi et al., 2010 (39)</td>
<td>Identification of a double IL-4/IL-17 producing T cells subset that is increased in severe patients</td>
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<tr>
<td>Irvin et al., 2014 (42)</td>
<td>Increased frequency of dual-positive T&lt;sub&gt;H&lt;/sub&gt;2/T&lt;sub&gt;H&lt;/sub&gt;17 cells in BAL of severe asthmatic patients</td>
</tr>
<tr>
<td>Brandt et al., 2013 (43)</td>
<td>Significant higher levels of IL-17 in asthmatic children exposed to diesel exhaust particles</td>
</tr>
<tr>
<td>Nanzer et al., 2013 (65)</td>
<td>Significant higher levels of IL-17 in asthmatic patients and healthy volunteers</td>
</tr>
</tbody>
</table>

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<tr>
<th>Response (Change from Baseline)</th>
<th>Placebo (N = 76)</th>
<th>140 mg (N = 74)</th>
<th>210 mg (N = 76)</th>
<th>780 mg (N = 76)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACQ</td>
<td>n – 76</td>
<td>n – 73</td>
<td>n – 75</td>
<td>n – 74</td>
<td></td>
</tr>
<tr>
<td>LS mean</td>
<td>0.431</td>
<td>0.498</td>
<td>0.506</td>
<td>0.544</td>
<td>0.3731</td>
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<tr>
<td>FEV₁, prebronchodilator</td>
<td>n = 76</td>
<td>n = 73</td>
<td>n = 75</td>
<td>n = 74</td>
<td></td>
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<tr>
<td>LS mean</td>
<td>0.056</td>
<td>0.009</td>
<td>0.034</td>
<td>0.037</td>
<td></td>
</tr>
<tr>
<td>AM PEF</td>
<td>n = 76</td>
<td>n = 74</td>
<td>n = 76</td>
<td>n = 75</td>
<td></td>
</tr>
<tr>
<td>LS mean</td>
<td>1.490</td>
<td>−15.357</td>
<td>−5.234</td>
<td>−3.999</td>
<td>0.7834</td>
</tr>
<tr>
<td>SABA use</td>
<td>n = 76</td>
<td>n = 74</td>
<td>n = 76</td>
<td>n = 75</td>
<td></td>
</tr>
<tr>
<td>LS mean</td>
<td>−0.561</td>
<td>−0.230</td>
<td>−0.795</td>
<td>−0.759</td>
<td>0.469</td>
</tr>
<tr>
<td>Daily symptom score, % change</td>
<td>n = 76</td>
<td>n = 74</td>
<td>n = 76</td>
<td>n = 75</td>
<td></td>
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<tr>
<td>LS mean</td>
<td>−10.59</td>
<td>−29.37</td>
<td>−24.47</td>
<td>−23.33</td>
<td>0.5603</td>
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<tr>
<td>Nighttime symptom score, % change</td>
<td>n = 76</td>
<td>n = 74</td>
<td>n = 76</td>
<td>n = 75</td>
<td></td>
</tr>
<tr>
<td>LS mean</td>
<td>39.01</td>
<td>16.83</td>
<td>26.67</td>
<td>24.52</td>
<td>0.4528</td>
</tr>
<tr>
<td>Symptom-free days</td>
<td>n = 76</td>
<td>n = 74</td>
<td>n = 76</td>
<td>n = 76</td>
<td></td>
</tr>
<tr>
<td>LS mean</td>
<td>0.243</td>
<td>0.181</td>
<td>0.226</td>
<td>0.201</td>
<td>0.5147</td>
</tr>
</tbody>
</table>

Airway Neutrophilia

Friend or Foe?
Neutrophils Release

Foe!

- Reactive oxygen species
- Cytokines
- Lipid mediators
- Enzymes
  - Elastic
  - Cathepsin G
  - Myeloperoxidase
  - Non-enzymatic defensins
Neutrophils - Friend

• Key immune defense cell

• BUT, even in death have important immunomodulatory activities
Resolution of Inflammation in Asthma

Inflammation resolution involves subsets of inflammatory cells, such as alternatively activated or M2 macrophages, that possess specific functional characteristics related to suppressing inflammation and cleaning up cellular debris.
Resolution of Inflammation in Asthma

Processes are mediated by:
• Anti-inflammatory cytokines
• Lipoxygenase-derived bioactive lipids
• Transcription factors
Resolution of Inflammation in Asthma

Successful resolution of inflammatory disease processes, often referred to as “catabasis”, requires a distinct series of processes:

• Inhibition of inflammatory cell recruitment
• Promotion of inflammatory cell egress
• Clearance of apoptotic cells (efferocytosis)
Anti-Inflammatory Potential of Apoptotic Neutrophils

• Apoptotic neutrophils are quiescent
  – Secretory processes shut down
• Provides important anti-inflammatory stimulus to other cells
• Resolution of inflammation
  – “Eat me” signals other phagocytic cells
    • M2 macrophages
      – IL-10
FOE

Neutrophils

Toxic Mediators

Enhance Asthma Pathobiology

Neutrophil Necrosis

Neutrophil Apoptosis

FRIEND
FOE\n
---

Neutrophils

Toxic Mediators

Phagocytosis of Pathogens

Protect Host

---

Enhance Asthma Pathobiology

Neutrophil Necrosis

Neutrophil Apoptosis

Resolution of Inflammation

FRIEND
Driving Neutrophil Apoptosis to Resolve Inflammation

- CDK inhibitors
- ERK1/2 inhibitors
- Bax inhibition
- PI3K inhibition
Do Neutrophils Play a Role in Severe Asthma in Severe Asthma?

- A large percentage of patients with severe asthma demonstrate airway neutrophilia.
- Neutrophils are not only steroid-insensitive but corticosteroids may prolong their survival and increase bone marrow release.
- Their role in contributing to a severe asthma endotype is unclear.
- Their role in defending against pathogens is clear.
- Neutrophils and neutrophil apoptosis may be an essential component for resolution of inflammation.