Resolution of Inflammation

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Boston, MA USA
Outline

Define inflammation resolution at the tissue, cellular and molecular levels

Chemical mediators of resolution for injury, noxious stimuli and infection - their biosynthesis and bioactions

Distinguish anti-inflammation from promoting resolution

Translation to human disease
Define inflammation resolution at the tissue, cellular and molecular levels

Chemical mediators of resolution for injury, noxious stimuli and infection - their biosynthesis and bioactions

Distinguish anti-inflammation from promoting resolution

Translation to human disease
Uncontrolled Inflammation Is a Pathological Feature of Common Diseases

Cardiovascular diseases
(Atherosclerosis)

Neurological disorders
(Alzheimer’s, Parkinson’s)

Inflammatory bowel diseases
(Colitis, Crohn’s)

Asthma

Cancer

Diabetes

Autoimmune diseases

How does the acute inflammation resolve?
What are the mechanisms/components underlying the resolution process?
Resolution Of Acute Inflammation
Key Features in Tissue Resolution

Blood vessel → Fluid and proteins → Lymphatic vessel

Monocyte → Drainage of edema fluid and proteins into lymphatics

Neutrophil → Necrotic tissue, microbes

Macrophage → Growth factors

Phagocytosis and clearance of necrotic tissue, microbes

New blood vessels → Fibroblasts

Repair

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Resolution Defined In Operative and Quantitative Terms

Resolution defined in operative and quantitative terms

PMN magnitude:
\[ Y_{\text{max}} = 14 \times 10^6 \]
\[ T_{\text{max}} = 12 \text{ h} \]

Resolution indices:

- \( \Psi_{\text{max}} \): maximal PMN numbers
- \( T_{\text{max}} \): time when PMN numbers reach maximum
- \( R_{50} \): 50% of \( \Psi_{\text{max}} \)
- \( T_{50} \): time when PMN numbers reduce to 50% of \( \Psi_{\text{max}} \)
- \( R_i \) (resolution interval): time interval from maximum PMN (\( \Psi_{\text{max}} \)) to 50% reduction point (\( R_{50} \)) (i.e., \( T_{50} - T_{\text{max}} \)).
- \( t_{\text{PMN=mono}} \) (point of intersection): time point when increase in mononuclear cells intersects the decrease in PMN (i.e., PMN numbers = mononuclear cell numbers).

New Concepts of Resolution of Inflammation

Previous concept (passive process):

- Passive termination of inflammation
- Disappearance of local chemotactic stimuli and pro-inflammatory mediators

New concept (active process):

- Rapidly turn on after acute inflammatory challenge
- Active cellular events and biochemical pathways
- Generation of anti-inflammatory and pro-resolution mediators

Courtesy of Nan Chiang
Resolution Circuits in Inflammation

Host Defense

PMN infiltration → Acute Inflammation

Chemical Mediators

Chemical Mediators
Amplification

Chronic Inflammation

Chemical Mediators
“New & Uncharted”

Resolution
Decision Paths in Acute Inflammation: 
Identification of Specialized Mediators During Resolution

Acute Inflammation → Resolution (Ideal outcome) → Return to homeostasis

Excessive “unresolved”

Prostaglandins 
Leukotrienes

Chronic Inflammation → Fibrosis

Lipid mediator class switching
PGE2, PGD2

Specialized pro-resolution mediators

- Lipoxins (LXs)
- Aspirin-triggered Lipoxins

Resolvin Es (RvEs)
Resolvin Ds (RvDs)
[Neuro]Protectins (PDs)

Omega-3 derived

Courtesy of Nan Chiang
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Translation to human disease
Resolution of Acute Inflammation Is An Active Process
Polyunsaturated Fatty Acid (PUFA)-derived Lipid Mediators

Injury / infection → Host Defense

Chronic Inflammation → Acute Inflammation

PMN

Acute Inflammation → Resolution

Switching phenotype

Pro-inflammatory mediators

Leukotrienes
Prostaglandins

Aspirin-triggered Lipoxins (ATL)

PMN

Lipoxins (LXs)

Resolvin Es (RvEs)

Resolvin Ds (RvDs)

[Neuro]Protectins (PDs)

Polyunsaturated Fatty Acid (PUFA)-derived Lipid Mediators

EPA

DHA

Aspirin

Cell-cell interaction

OH

COOCH₃

F

HO

OH

COOH

PMN

Courtesy of Nan Chiang
Eicosanoid Mediators in Inflammation

Phospholipids → cPLA$_2$ → Arachidonic Acid

COX 1 & 2 → Aspirin → Prostaglandins

5-Lipoxygenase → Anti-LTs → Leukotrienes

Cell-Cell Interactions → Transcellular Biosynthesis

Lipoxins

Initiation → Resolution
Transcellular Lipoxin & ATL Biosynthesis

- Arachidonic Acid
- COX-II
- Aspirin
- TNFα
- IL-1β
- 15-LO
- IL-13
- IL-4
- Platelets
- 12-LO
- 5-LO
- 15-LO
- PMN
- Leukocytes
- 15 epi-LXA₄
- 15 epi-LXB₄
- LXA₄
- LXB₄
- IL-1β
- COX-II
- Epithelial cells or Endothelial cells
- Apoptotic bodies
- 15S-LO
- 5-LO
- IL-13
- IL-4
Aspirin Initiates a **Switch** in Biosynthesis & Chirality

**Switch in chirality**
- *Enhances bioactivity*
- *Slow metabolic inactivation*

ASA-triggered 15 epi-Lipoxins
Cell Type Specific Responses:

Neutrophils - *Stops* Adhesion, Chemotaxis, Transmigration, Degranulation

Eosinophils - *Stops* Chemotaxis, Allergen-Induced Trafficking

T-Lymphocytes - *Stops* Cytokine release, NK cell cytotoxicity

Monocytes - *Stimulates* Adhesion, Chemotaxis, Phagocytosis of Apoptotic PMN

Epithelial Cells - *Stops* Cytokine Release and Promotes Bacterial Killing
Polyunsaturated Fatty Acids Are Essential to Health

**Omega-3 PUFA**

- **EPA**
- **DHA**

**Milligrams to grams daily**

**Eye**

- ↓ Dry eye
- ↓ Macular degeneration

**Cardiovascular**

- ↓ Thrombosis
- ↓ Hypertension
- ↓ Sudden death
  
  *GISSI study*

**Brain**

- ↑ Brain functions
- ↓ Alzheimer’s
- ↓ Stroke
- ↓ Depression

**Oral**

- ↓ Tooth loss
- ↓ Periodontal disease

**Aspirin**

**Skin disorders**

**Immune systems**

**Cancer**

**Diabetes**

**Arthritis and Gout**

**Lungs**

- ↓ Allergies
- ↓ Asthma

**GI tract**

- ↓ Inflammatory Bowel Diseases
  
  (Colitis, Crohn’s disease)

**Polyunsaturated Fatty Acids Are Essential to Health**

*Molecular mechanisms of action?*

*Can EPA and DHA be precursors to generate bioactive mediators?*
Resolvin Es Derived from EPA

Eicosapentanoic Acid (EPA)

Vascular Endothelial Cells

Aspirin: COX2

Leukocytes

5-LOX

P450

EPA

Aspirin-independent pathway

Healthy Individuals Taking EPA (1g) and Aspirin (160mg)

<table>
<thead>
<tr>
<th>ng/ml plasma</th>
<th>RvE1</th>
<th>18-HEPE</th>
<th>EPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA with ASA (n=6)</td>
<td>0.18 0.06</td>
<td>0.74 0.27</td>
<td>15.95 4.03</td>
</tr>
<tr>
<td>EPA w/o ASA (n=3)</td>
<td>0.11 0.02</td>
<td>0.36 0.15</td>
<td>14.20 5.20</td>
</tr>
</tbody>
</table>

© Serhan et al., 2000 J. Exp. Med. 192:1197-1204
© Arita et al., 2005 J Exp. Med. 201:713-722
Resolvin E1: Mechanisms of Action

Epithelium
↑ apical CD55 expression
↑ PMN clearance

ChemR23

PBMC
↑ MAPK activation

Resolvin E1

BLT1

Neutrophils
↓ migration
↓ superoxide anion

Dendritic cells
↓ Migration
↓ IL-12 production

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Disease model</th>
<th>Dose, duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>Dorsal air pouch</td>
<td>100 ng/mouse, 4 hours</td>
</tr>
<tr>
<td></td>
<td>Peritonitis</td>
<td>100 ng/mouse, 2-24 hours</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>Peritonitis</td>
<td>10-300 ng/mouse, 2 hours</td>
</tr>
<tr>
<td></td>
<td>Colitis</td>
<td>50 µg/kg, 4-12 days</td>
</tr>
<tr>
<td></td>
<td>Eye retinopathy</td>
<td>10 ng/day, 17 days</td>
</tr>
<tr>
<td>Topical</td>
<td>Periodontitis</td>
<td>4 µg/tooth, 6 weeks</td>
</tr>
</tbody>
</table>

Resolvin Ds Derived from DHA

Docosahexanoic Acid
DHA

Leukocytes

LOX

H(O)O₂

17S-H(p)DHA

LOX

7S-hydroperoxy,17S-HDHA

4S-hydroperoxy,17S-HDHA

7S(8)-epoxide intermediate

4S(5)-epoxide intermediate

Resolvin D1

Resolvin D2

Resolvin D3

Resolvin D4

Neutrophils
↓ Transendothelial migration

Microglia
↓ IL-1β expression

© Serhan et al., 2002 J. Exp. Med. 196:1025-1037
# Resolvin D1 *In Vivo* Actions

<table>
<thead>
<tr>
<th>Disease model</th>
<th>Action</th>
<th>Dosage (Route)</th>
<th>Duration of post-exposure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin (Dorsal Air pouch)</td>
<td>Reduces PMN infiltration</td>
<td>100 ng/mouse (intrapouch)</td>
<td>4 h</td>
<td>Serhan et al. (JEM 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hong et al. (JBC 2003)</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>Reduces PMN infiltration</td>
<td>100 ng/mouse (IV)</td>
<td>2 h</td>
<td>Hong et al. (JBC 2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sun et al. (JBC, 2007)</td>
</tr>
<tr>
<td>Kidney Ischemia-Reperfusion</td>
<td>Protects in renal perfusion injury by limiting PMN infiltration</td>
<td>5 µg/mouse (IV, SC)</td>
<td>24-48 h</td>
<td>Duffield et al. (Jl, 2006)</td>
</tr>
<tr>
<td>Eye (Retinopathy)</td>
<td>Reduces vaso-oblitration by neovascularization</td>
<td>10 ng/day (IP), from postnatal day 6 to day 17</td>
<td>17 days</td>
<td>Connor et al. (Nat. Med., 2007)</td>
</tr>
</tbody>
</table>
Protectins Derived from DHA

This research was originally published in the Journal of Biological Chemistry. Hong et al. “Novel Docosatrienes and 17S-Resolvins Generated from Docosahexaenoic Acid in Murine Brain, Human Blood, and Glial Cells.” J Biol. Chem. 2003, 278:14677-14687. © The American Society for Biochemistry and Molecular Biology

**Protectin D1: Potent Stereoselective Actions**

![Chemical Structure]

### Cell type | Bioactions
---|---
PMN | ↓ Transendothelial migration
Macrophage | ↑ Non-phlogistic phagocytosis of apoptotic PMN
T-cell | ↓ TNF-α and IFN-γ secretion promotes apoptosis
Microglia | ↓ IL-1β expression
Epithelia | ↓ Oxidative stress-induced apoptosis in retinal pigment epithelia

**Neuroprotectin D1**

**Protectin D1 (PD1)**

*Low nanomolar range*

---

<table>
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</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>Peritonitis</td>
<td>100 ng/mouse, 2 hours</td>
</tr>
<tr>
<td></td>
<td>Asthma</td>
<td>2-200 ng/mouse, 4 days</td>
</tr>
<tr>
<td></td>
<td>Kidney ischemia-reperfusion</td>
<td>5 µg/mouse, 1-2 days</td>
</tr>
<tr>
<td>Perfusion</td>
<td>Stroke</td>
<td>0.4 µg/mouse, 48 hours</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>Peritonitis</td>
<td>0.1-300 ng/mouse, 2-24 hours</td>
</tr>
<tr>
<td></td>
<td>Colitis</td>
<td>50 µg/kg, 4-12 days</td>
</tr>
<tr>
<td></td>
<td>Eye (retinopathy)</td>
<td>10 ng/day, 17 days</td>
</tr>
<tr>
<td>Topical</td>
<td>Eye (wound healing)</td>
<td>1 µg/mouse, 48 hours</td>
</tr>
<tr>
<td></td>
<td>Periodontitis</td>
<td>4 µg/tooth, 6 weeks</td>
</tr>
</tbody>
</table>

Responses To Tissue Injury
Restitution of Injured Bronchial Epithelia

500μl HCl 0.1N, pH = 1.5

Normalize pH to 7.0 with PBS

5min, 37°C
5% CO₂

Exposed to mediators
Transmission Electron Microscopy: Morphological features

NHBE cells without acid

5 min after acid injury

2h after acid injury

6h after acid injury

Acid induced ALX expression

LXA₄ Stimulated Basal NHBE Cell Proliferation

LXA₄ Inhibited Acid-Induced IL-6 Release

**LXA$_4$ Inhibited PMN Transmigration Across Differentiated NHBE**


with permission from the American Society for Investigative Pathology.
# Responses To Noxious Stimuli

## Animal Protocol for Allergic Asthma

<table>
<thead>
<tr>
<th>Allergen Sensitization</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day: 0 7</td>
<td>14 15 16 17 18</td>
</tr>
<tr>
<td>OVA: 10 µg 10 µg</td>
<td>6% _______</td>
</tr>
<tr>
<td>i.p. i.p. i.p. i.p. i.p.</td>
<td>Aerosol ___</td>
</tr>
</tbody>
</table>

+/- LX analog i.v. (30 min prior)

On Day 18, determine:

1. Airway Inflammation
   - Lipid Profile
   - Histology/BAL
2. Airway Reactivity
OVA-Induced Allergic Airway Inflammation

**Interleukin-13 in BAL**

<table>
<thead>
<tr>
<th></th>
<th>Veh.</th>
<th>ZK-994</th>
<th>ATLa</th>
<th>Monte.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-13 (pg/ml)</td>
<td>500</td>
<td>400</td>
<td>300</td>
<td>200</td>
</tr>
</tbody>
</table>

**Interleukin-4 in BAL**

<table>
<thead>
<tr>
<th></th>
<th>Veh.</th>
<th>ZK-994</th>
<th>ATLa</th>
<th>Monte.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4 (pg/ml)</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
</tr>
</tbody>
</table>

**Lymphocytes in BAL**

<table>
<thead>
<tr>
<th></th>
<th>Veh.</th>
<th>ZK-994</th>
<th>ATLa</th>
<th>Monte.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes x 10^4</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

* P < 0.05 vs vehicle

ZK-994 and ATLa are **orally active** in allergic airway inflammation, modulate Th2 cytokine production and lymphocyte infiltration.

3-oxa-ATL efficacy at least equivalent to oral montelukast, modulatory profile broader

*Levy et al. FASEB J. 21,3877-3884. 2007 © FASEB*
Impact Of LX Stable Analog and Montelukast On Allergic Airway Inflammation: OVA

Control  Montelukast  ATLα

40x magnification
Representative of n > 4

vascular
airways

Levy et al. FASEB J. 21,3877-3884. 2007 © FASEB
**Animal Protocol for Cockroach Allergen Sensitization and Airway Challenge**

<table>
<thead>
<tr>
<th>Allergen Sensitization</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day: 0</td>
<td>21</td>
</tr>
<tr>
<td>Day: 14</td>
<td>23</td>
</tr>
<tr>
<td>CRA: 10 μg i.p.</td>
<td>4 μg i.t.</td>
</tr>
<tr>
<td>CRA: 10 μg nasal</td>
<td>4 μg i.t.</td>
</tr>
</tbody>
</table>

+/- LX analog (2h prior)

On Day 24, determine:
1. Airway Inflammation
   - Lipid Profile
   - Histology/BAL
2. Airway Reactivity
CRA-induced Allergic Airway Inflammation

- Peribronchial eosinophilia markedly ↓ by ZK-994
- Oral ZK-994 modulates lung cytokine/chemokine networks significantly:
  - Cytokines: ↓ IL-5 (Th$_2$), ↑ IFN$_{\gamma}$ (Th$_1$)
  - Chemokines: ↓ C10, ↓ RANTES, ↓ eotaxin

N = 6
*p = <0.05

Levy et al. FASEB J. 21, 3877-3884. 2007 © FASEB
CRA-induced Allergic Airway Inflammation

Intraperitoneal

<table>
<thead>
<tr>
<th>Change in Resistance (cmH₂O/ml/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>3-oxa-LXA₄ analog (µg/kg)</td>
</tr>
</tbody>
</table>

Oral

<table>
<thead>
<tr>
<th>Change in Resistance (cmH₂O/ml/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>

10 µg/ml Blinded

Levy et al. FASEB J. 21,3877-3884. 2007 © FASEB
LXs and Resolution of Allergic Airway Inflammation

Time (d) OVA sensitization

OVA aerosol challenge

Resolution

LX analog

Vehicle
LX analog

ATLa

LXA₄

Total BALF cells (× 10⁶)

Day 18 21

Vehicle LX analog

n ≥ 3, *P < 0.05

Protocol Day 21

Expression of Human ALX in Transgenic Mice Decreases Pulmonary Inflammation

Protectin D1 Dampens Airway Inflammation

**Lung histopathology**

*Vehicle*  

*Vehicle*  

*Vehicle*  

**Allergen-driven leukocyte infiltration**

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Chemical mediators of resolution for injury, noxious stimuli and infection - their biosynthesis and bioactions

Distinguish anti-inflammation from promoting resolution

Translation to human disease
Distinguishing Anti-Inflammation From Pro-Resolution

Inhibition of Neutrophils  Stimulation of Monocytes/Macrophages
Anti-Inflammation is Distinct From Promoting Resolution

*Dual Actions of Lipoxins*

**Anti-inflammation – (Stop Signals)**
- Inhibition of PMN recruitment and activation
- Inhibition of dendritic cell motility/NK cell cytotoxicity
- Block cytokine release from activated T-cells

**Pro-resolving – (Go Signals)**
- Stimulate non-phlogistic recruitment of monocytes
- Stimulate uptake of apoptotic PMN by macrophages
- Reduce MMP release from fibroblasts
- Enhance host defense/bacterial killing
Non-Lethal Experimental Model of ALI

Timed intervals
(2, 12, 48, 72h)

Harvest BAL
(Time)

Acid
(pH 1.5, 0.1 N, 50μl)

Anterior

Posterior

The Kinetics of Inflammation and Resolution After Acid-initiated Acute Lung Injury

Leukocytes in BALF (10^3 cells)

Time (h) after acid instillation

Protein (μg/ml BAL)

PMN
Lymphs

Involvement of COX In Host Response To Acid Initiated Lung Injury

<table>
<thead>
<tr>
<th>Time (h) after acid instillation</th>
<th>Total leukocytes in BALF (10^4 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>48</td>
<td>48</td>
</tr>
</tbody>
</table>

*COX-2 inhibitor

*Vehicle

Effect of COX Inhibition on Airway Inflammation 48h After Acid Injury

Total leukocytes in BALF (x $10^4$ cells)

<table>
<thead>
<tr>
<th>ASA</th>
<th>COX-2 inhibitor</th>
<th>COX2 -/- mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Impact of COX-2 Inhibition on Airway LXA$_4$
After Acid Initiated Injury

LXA$_4$ (pg/ml BAL)

Vehicle

COX-2 inhibitor

Time after acid instillation (h)

0 2 12 48

(pg/ml) 0 10 20 30 40

* 

Eicosanoid 'CLASS SWITCH' to **Resolution**

When & Where-------treatment?
ASPIRIN
It’s a painkiller, a blood thinner and a heart saver as well. But taking aspirin in combination with ibuprofen (in the...
Responses to Bacterial Infection

Murine model of aspiration pneumonia

HCl (pH1, 0.1N, 25 μl)  E.coli (1-2×10⁵ CFU)

12, 24 or 48h  24h  Collect left lungs

→Calculate BGI

Bacterial growth index (BGI): \[ \frac{\text{lung CFU}}{\text{original inoculum instilled}} \]
Acid injury transiently impairs airway host defense

Bacterial growth index

Time interval from HCl or PBS to *E. coli* (h)

Mean ± SEM (n>4, each)

*, P<0.05 vs HCl (-)

Effect of RvE1 in a model of aspiration pneumonia

RvE1 (100 ng), EPA (100 ng) or saline i.v. 30min prior to HCl instillation or 2h after E. coli inoculation

* Collect left lungs
  - Calculate BGI
  - Measure MPO
  - Measure inflammatory mediators
  - Lung histopathology
RvE1 enhances bacterial clearance in a model of aspiration pneumonia

Mean ± SEM (n>12, each)
* $P<0.05$ vs saline / HCl (-) / E. coli (+)
† $P<0.05$ vs saline / HCl (+) / E. coli (+)

RvE1 blocks leukocyte accumulation after aspiration pneumonia

Mean ± SEM (n>12, each)
*, P<0.05 vs RvE1 (-) / HCl (-) / E. coli (-)
†, P<0.05 vs RvE1 (-) / HCl (+) / E. coli (+)

Outline

Define inflammation resolution at the tissue, cellular and molecular levels

Chemical mediators of resolution for injury, noxious stimuli and infection - their biosynthesis and bioactions

Distinguish anti-inflammation from promoting resolution

Translation to human disease
What window into human pathophysiology can murine models of asthma provide?
Neutrophils In Severe Asthma

Hypothesis: Is Severe Asthma a Lipoxin Deficient Condition?

Status Asthmaticus

Healthy

Histology courtesy of K. Haley

Anti-CD15, 1:250 dilution
Lipoxin A₄ Generation in Activated Whole Blood

*P<0.05 c/w health, **P<0.05 c/w moderate

Leukotriene Generation in Activated Whole Blood

*P<0.05 c/w health, **P<0.05 c/w moderate

Relationship Between Lipoxygenase-Derived Eicosanoids In Whole Blood and Airflow Obstruction

5-LO Products In Severe Asthma –
Increased LTs
Decreased LXs

$LXA_4 / CysLT$ Ratio in Non-Stimulated Whole Blood

$FEV_1$ (% Predicted)

$P=0.003$

LXA₄ and 15S-HETE Levels in BALF

- iLXA₄ (pg/ml BALF)
- LXA₄/CysLTs

* p < 0.05

Lipoxin Biosynthetic Gene Expression in Multiple Anatomic Compartments

Fold change = $2^{-\Delta\Delta CT}$

Blood

BAL cells

EBBs

LXA₄ Receptor Gene Expression in Peripheral Blood

Expression of ALX Receptors in Peripheral Blood Leukocyte Subsets

PMN  Eosinophils  Monocytes  Lymphocytes

Summary and Conclusion

Today, we have presented evidence that:

- LXA$_4$ levels are decreased in peripheral blood and BAL fluids in severe compared to not severe asthma subjects

- CysLTs and 15-HETE levels are increased in both severe and not severe asthma subjects

- 5-LO, 15-LOA, 15-LOB and COX-2 are under distinct regulatory control that varies by anatomic compartments and asthma severity

In conclusion, our findings indicate that severe asthma is characterized decreased lipoxin biosynthesis. In conjunction with the decreased LXA$_4$ receptor expression, these data suggest that more severe variants of asthma may result from a defect in counter-regulatory signaling.
Lipoxin Defects In Other Diseases of Chronic Inflammation

Asthma –
  Severe Asthma
  Exercise induced Asthma
  
Cystic Fibrosis –

Inflammatory Bowel Disease –
  Ulcerative Colitis

Vasculitis –
  Henoch–Schönlein purpura

Prost. & Other Lipid Med. 2006, 79:84
PLEFA. 2009, 80:177.
Non-Invasive Technique For Sampling Airway Biomarkers

*Exhaled Breath Condensates*
### Characteristics of Subjects

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Asthma Exacerbation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (n)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>28 +/- 1</td>
<td>41 +/- 6</td>
</tr>
<tr>
<td>M:F</td>
<td>1:2</td>
<td>2:2</td>
</tr>
<tr>
<td>Race</td>
<td>3 other</td>
<td>2 Caucasian, 1 African American, 1 other</td>
</tr>
<tr>
<td>Current cigarette smoker</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*EBC was collected during 10 min of tidal breathing from individuals in the BWH emergency department with acute asthma exacerbation and a control group of healthy subjects. Plus-minus values are means +/- SD.

Generation of Protectin D1 in asthma

Human EBC 17(S)-Hydroxy-DHA and PD1 Levels

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>17(S)-hydroxy-DHA</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>PD1</td>
<td>2.23 +/- 1.55 ng</td>
<td>Trace</td>
</tr>
<tr>
<td>Sample size (n)</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

*Plus-minus values are means +/- SEM.

d₄-PGE₂ i.s.

Calibration curve r² = 0.991

Chronic Inflammation in Disease (Asthma) - A Loss Of Counter-Regulatory Signaling?
Resolution of inflammation is an active process that is orchestrated by specific cells and signals, including PUFA-derived lipid mediators.

Key features in tissue resolution include blocking PMN influx and functions and enhanced Macrophage-mediated clearance of apoptotic PMN.

Early events in acute inflammation are crucial to timely resolution

pus bonum et laudible

Lipoxins and their bioactive stable analogs are anti-inflammatory and pro-resolving.
Outline

Define inflammation resolution at the tissue, cellular and molecular levels

Chemical mediators of resolution for injury, noxious stimuli and infection - their biosynthesis and bioactions

Distinguish anti-inflammation from promoting resolution

Translation to human disease
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