The Surprising Role of IFN-γ in AERD

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Conflict of Interest

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Learning Objectives

• to understand inflammatory pathways of AERD, in particular roles of novel leukotriene signaling pathways
• to understand mechanisms of aspirin/NSAID triggering of non-IgE mediated anaphylaxis in AERD

AERD as a Disease of Excessive Cysteinyl Leukotriene Production and Responsiveness

• Constitutive over-production of CysLTs
• Over-expression of CysLT receptors
• “Surge” in CysLT production on exposure to aspirin and other non-selective (cox-1) inhibitors
• Protection from the anaphylactoid response via LT modifiers
• Therapeutic Implications

LTE₄ Increases After Aspirin Challenge in Aspirin Sensitive Subjects

Urinary LTE₄, following aspirin (closed symbols) and placebo (open symbols) in aspirin-intolerant (circles) and aspirin tolerant (triangles) subjects
Responsiveness of subjects with AERD to LTE_4 pre and post desensitization


Hyperresponsiveness was not observed for LTC_4
Christie PE Eur Respir J 1993;6:1466-73

AERD – Therapeutic Implications

- Aspirin Desensitization
- Leukotriene Modifiers
  - Leukotriene receptor antagonists (e.g., montelukast, zafirlukast) improve lung function, decrease rescue bronchodilator use, reduce symptoms, and improve qol
  - Zileuton – may be uniquely effective including in reducing upper airway symptoms

Aspirin Desensitization Treatment for AERD:
Patients treated 1-3 yrs (n=29)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>ASA Rx</th>
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</thead>
<tbody>
<tr>
<td>Sinusitis (yr)</td>
<td>0-1.12</td>
<td>0-0.12</td>
</tr>
<tr>
<td>Sinus surgery/yr</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Hospitalizations/yr</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>ED visits/yr</td>
<td>0.2-2</td>
<td>0.1-1.5</td>
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<tr>
<td>Olfaction score</td>
<td>0.1-5</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Asthma Response

| Prednisone (mg/d)   | 7.9 ±0.9 | 1.8 ±0.7        |
| Nasal steroid (mg/day) | 137 ±25.6 | 90 ±22.3         |
| Inhaled steroid (µg/d) | 640 ±146 | 885 ±226        |

Decrease in Leukotriene-Receptor Expression on Nasal Mucosal Inflammatory Cells after Aspirin Desensitization

Nasal biopsy specimens immunostained for CysLT1 before and after desensitization with topical lysine aspirin

Decrease in Leukotriene-Receptor Expression on Nasal Mucosal Inflammatory Cells after Aspirin Desensitization


Why So Much CysLTs?

- also, why so many CysLT receptors?
I: Too many eosinophils:


Eosinophils


AERD Associated With Eosinophilic Inflammation:


And, II: Too many eosinophils expressing LTC₄S


Relative expression of CysLTs and LTC₄S mRNA

Why the Upregulation of Cysteinyl Leukotrienes in AERD: Role of Cytokines

Th1/Th2 Cytokine Signature in Chronic Sinusitis (qPCR)

Image Stream

Intracellular Cytokine Staining of CCR3 +ve Eosinophils in CHES

Intracellular Cytokine Staining of CCR3 +ve Eosinophils in AERD

Eosinophil Intracellular Cytokine Expression

<table>
<thead>
<tr>
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<th>CHES</th>
<th>AERD</th>
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<tbody>
<tr>
<td>IL-4</td>
<td>33.2±8.8</td>
<td>24.6±10.1</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>15.6±3.4</td>
<td>28.2±10.7</td>
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</table>
**IFN-γ Immunofluorescence: CHES**


**IFN-γ Immunofluorescence: AERD**


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**IL-4 Regulates IgE-dependent CysLT production by Mast Cells:**

Profound Induction of LTC₄ Synthase expression by IL-4:

![Graph showing LTC₄ Synthase expression by IL-4](image)


**Effect of IL-4 priming of human MCs on IgE mediated CysLT release**

**Effect of IL-4 priming on S-LO/LTC₄ pathway protein expression by hMCs**

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**Cytokines NOT Associated With Upregulation of LTC₄S mRNA in Eosinophils:**

- IL-4
- IL-5
- IL-13
- GM-CSF

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**IFN-γ Potentiation of Eosinophilopoiesis**

IL-5/IL-3 x 3 weeks 400x
CD34+ hematopoietic stem cells cultured for 3 days with SCF, TPO, Flt3L, IL-3, and IL-5 then 3 weeks with only IL-3/IL-5 + IFN-γ

+ IFN-γ


**Enhanced eosinophilopoiesis in additional presence of IFN-γ:**

-Siglec 8
-CCL3

+IFN-γ

**Enhanced Eosinophilopoiesis by IFN-γ**

![Graph showing enhanced eosinophilopoiesis by IFN-γ](image)


**IFN-γ Activation of Eosinophilopoiesis**

<table>
<thead>
<tr>
<th>Siglec8</th>
<th>ECP</th>
<th>CysLT1</th>
<th>CysLT2</th>
<th>PTX12</th>
<th>PTX</th>
<th>LTC4</th>
<th>Cox2</th>
<th>F0D2</th>
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<tr>
<td>-IFN-γ</td>
<td>0.051</td>
<td>0.045</td>
<td>0.051</td>
<td>0.051</td>
<td>0.051</td>
<td>0.051</td>
<td>0.051</td>
<td>0.051</td>
</tr>
<tr>
<td>+IFN-γ</td>
<td>0.018</td>
<td>0.018</td>
<td>0.018</td>
<td>0.018</td>
<td>0.018</td>
<td>0.018</td>
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Fold increase: 2.88* 1.46* 2.11* 0.66 4.23* 3.75*

qPCR data reflect relative expression in comparison to housekeeping gene (EF1α) (2−ΔΔCT) (n=12)

*Fold increase induced by IFN-γ in comparison to IL-5 and IL-3 alone
*p<0.05 compared to without IFN-γ


**What About the CysLT Receptor Over-Expression?**

![Graph showing EDN production](image)


**CysLT Production**

![Graph showing CysLT production](image)


**CysLT1 Receptor Expression on Nasal Mucosal Inflammatory Cells in AERD**

![Graph showing CysLT1 receptor expression](image)


*Immunoreactive cells were counted in specimens of nasal submucosa*
Why don’t aspirin tolerant or even non-asthmatics respond to a reduction in PgE$_2$ (what is the mechanism of cellular activation)?

- More eosinophils in AERD
- Eosinophil activation may occur in aspirin tolerant asthma, but PgE$_2$ acting ELSEWHERE is protective
- More LTC$_4$S in tissue eosinophils in AERD
- PgE$_2$ primarily acts to block CysLT production (5-LO activity)
- It is OK to activate eosinophils and release CysLTs as long as LTE$_4$R is not simultaneously over-expressed

OR...

Asparin/Salicylate Activation of Eosinophils

*but again, no obvious difference between aspirin tolerant and AERD

Hawley SA et al. Science 2012;336:918-22
Din FVN et al. Gastroenterology 2012;142:1504-15
Conclusions

- AERD is a disease of over-expression of LTC₄ synthase, increased production of CysLTs, and increased expression of CysLT receptors
- AERD reflects over-expression of Th2 (IL-4/IL-13) and Th1 (IFN-γ)-associated cytokines
- IFN-γ increases expression of LTC₄S in newly developing eosinophils
- IFN-γ increases expression of CysLT receptors
- Aspirin can directly activate eosinophils (and mast cells)
  - And this includes CysLTs if those eosinophils were allowed to mature in a "high" IFN-γ milieu
  - IFN-γ will contribute to a milieu in which allergic sensitization (atopy) might not develop