Rhinovirus-Induced Asthma Exacerbations: How that Happens and Implications for Treatment

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• Consultant; uncompensated
  – Allakos
• Compensated DSMB
  – PluriStem

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LEARNING OBJECTIVES

• Examine the role of bystander allergic reactions in the pathogenesis of RV-induced asthma exacerbations.
• Describe the role of aberrant innate immune responses in RV-associated asthma exacerbations.
• Clarify therapeutic implications for treating and preventing asthma exacerbations.
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September (and May) Epidemics of Asthma Exacerbations in Children and Adolescents in North America

Number of hospitalizations of children age 5 to 15 yo by week of the year in Ontario

Children (age ≥3) and Adolescents Hospitalized for Asthma in Charlottesville, VA:

Heymann, P et al JACI 2004;114:239-47
and this occurs despite there being no compelling evidence that RV infections only occur in September (and May)

- RV infections occur year round
- i.e., this cannot just be explained by kids returning to school
Seasonal incidence of respiratory viral infections in hospitalized patients

Evidence of an "allergic" reaction:

- Eosinophilia
- High ECP
- High leukotrienes

High total IgE

- Allergic sensitization (atopy)


<table>
<thead>
<tr>
<th>TOTAL SERUM IGE [IU/mL]</th>
<th>WHEEZE n=79</th>
<th>CONTROL n=77</th>
<th>ASTHMA n=33</th>
<th>CONTROL n=34</th>
<th>ASTHMA n=20</th>
<th>CONTROL n=21</th>
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<td>&lt; 3 YEARS OLD</td>
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Asthma exacerbations link to a highly allergic phenotype
Asthma exacerbations link to a highly allergic phenotype

• Evidence of an “allergic” reaction:
  – eosinophilia, high ECP, high leukotrienes

• High total IgE
  – allergic sensitization (atopy)
  – Hypothesis:
  – Autumn exacerbation requires RV AND:
    • sensitivity to ragweed, alternaria, dermatophagoides
  – Spring exacerbation requires RV AND:
    • sensitivity to grass

So, if this were true what would we observe in an environment where allergen exposure is perennial?

**Hypothesis:**
If the allergen exposure is perennial, and RV infections occur perennially then exacerbations will occur perennially without a seasonal “spike”
Costa Rica ER Study
Hospital Nacional de Niños, San José
(Children Ages 7 to 12 years)

- **February** enrollment when children start school
- **October** enrollment
- Enrollment included:
  96 children treated for acute asthma exacerbations, and
  126 non-asthmatic controls
  65 children who required treatment for asthma
  within the previous 12 months (i.e., “stable asthma” at the time of enrollment)

Soto-Quiros, M et al. *J. Allergy Clin Immuno* 2012;129:1499-1505
Children with Positive Tests for Viral Infection (qPCR)

- Children with Positive Tests for Viral Infection (qPCR)
  - N = 44 51 35 29 57 69
  - % Children with Positive PCR for RV, HEV, or RSV
  - *p < 0.001

- February
- October

- Rhinovirus
- RSV
- Enterovirus

- Exacerbating asthmatics
- Stable Asthma
- Control

- *p < 0.03 for the percentage of RV positive tests in Feb and Oct

Total IgE levels in Costa Rica

- Asthma Exacerbation (n=95): 482
- Stable Asthma (n=64): 332
- Controls (n=123): 86

Costa Rica: Titers of allergen-specific IgE antibody in children and adolescents having an asthma exacerbation:


GM values (in blue) include only children whose titers were ≥ 0.35 IU/ml

% Positive: 93 90 53 38 42 4 12 25 16 11
Probability of Asthma Exacerbation Based on Titers of IgE ab to *D. pteronyssinus* and Tests for Rhinovirus (Costa Rica)

Conclusions, part I

• Most asthma exacerbations in children and adolescents occur in association with RV infection

• This reflects concomitant presence of allergic sensitization to aerollergens expressed at the time of the infection
Two Schools of Thought

The seed = the virus

- Three virus clades
  - A (ICAM/LDL), B (ICAM/LDL) and C (Cadherin-related family member 3)
- Asthmagenic* Rhinovirus (Strain C?)
Order Picornavirinae
Family Picornaviridae
Genus Enterovirus

Clades/ Species
HRV-A (75 serotypes)
HRV-B (25 serotypes)
HRV-C (60 genotypes)

RV Serotypes and Frequency of Inducing of Inducing Asthma Exacerbations

*this could reflect *either* increased inherent virulence *or* altered immune response

MSI – moderate to severe illness

## Viral Analysis

<table>
<thead>
<tr>
<th></th>
<th>Asthma (n=68)</th>
<th>Acute Rhinitis (n=32)</th>
<th>Control (n=14)</th>
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<tbody>
<tr>
<td>qPCR for RV, % positive</td>
<td>57.1*</td>
<td>56.2*</td>
<td>7.1</td>
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<tr>
<td>RV-A, % positive</td>
<td>50</td>
<td>59</td>
<td>0</td>
</tr>
<tr>
<td>RV-C, % positive</td>
<td>50</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>RV-B, % positive</td>
<td>0</td>
<td>6</td>
<td></td>
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<tr>
<td>Other viruses, % positive</td>
<td>21</td>
<td>6</td>
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*p<0.01 compared to control

RV-C

- Unique binding receptor is cadherin-related family member 3 (CDHR3) – Previously linked to increased susceptibility to severe asthma exacerbations in GWAS study, (in contrast to RV-A/-B which bind ICAM-1 or LDLR)

- Identified and grown using sinus epithelial explants
  - Could sinus infection and “sinobronchial” reflexes trigger the exacerbation?

Bochkov YA et al. Proc Nat Acad Sci (USA) 2015
Computed Tomographic Study of the Common Cold

Day 4 of illness

Day 13

Two Schools of Thought

The seed = the virus

– Three virus clades
  • A (ICAM/LDL), B (ICAM/LDL) and C (Cadherin-related family member 3)
  – Asthmagenic* Rhinovirus (Strain C?)

• The soil = the asthmatic
  – Innate immune deficiency in asthmatics
    • Inability to control virus → increased viral load + exacerbations

*this could reflect either increased inherent virulence or altered immune response
Decreased IFN-β mRNA expression from bronchial epithelial cells in response to RV infection

- with similar results shown for type III IFNs and IL-15
- and linked to higher viral loads

RV may replicate better in the lungs of asthmatics

- Infection (worsened infection) of the lower airway
  - in association with reduced innate immune response (type I IFN, type III IFN, and IL-15)

Mosser et al, J Infect Dis. 185:734-743, 2002
But does RV even need to infect the lower airway to drive an asthma exacerbation?
Humoral Mechanisms of RV-induced upper respiratory rhinitis driving lower respiratory asthma exacerbations

another consideration might be that RV-C uniquely infects the sinuses and acute sinusitis drives the asthma exacerbation
• More recent studies have not confirmed significant differences in innate immune responsiveness in the lungs of asthmatics

• and pre-treatment with IFN-β was associated with lots of SEs without robust evidence for much benefit
Does RV replicate better in the nose of asthmatics?
however, this isn’t necessarily comparing viral load on the same day post-infection therefore, need to do viral challenge approach

**RV qPCR for RV in Nasal Washes from Children with Asthma**

Asthma Exacerbation: n=28
Acute Rhinitis: n=32
Control: n=14

Viral loads in Controls and “Low” and “High” IgE Asthmatics:

Kennedy J et al. *Am J Respir Crit Care Med* 2014; 189:532-9
Despite similar viral loads, symptoms are worse in allergic asthmatics (and last longer):
Alternative (complementary) hypothesis: Could diminished innate immune response alter the *pathological* response to RV?

- RV infection – in healthy subjects – *produces no discernible pathology* in nasal biopsies:
  - No pathology
  - No identifiable infected/necrotic cells
Absence of Cytopathology in RV Rhinitis: Airway Epithelial Cells Efficiently Engulf Apoptotic Epithelial Cells

Development of a flow cytometry based apoptotic cell phagocytosis assay:

and this engulfment is associated with generation of anti-inflammatory mediators TGF-β and PGE$_2$* (and requires engagement of PS receptors)

*and IL-10

Could diminished innate immune response alter the pathological response to RV?

- *normal* innate immune response – *apoptosis* of RV infected cells – *enhanced* IL-10/PgE2/TGF-β – *no inflammation*

- *diminished* innate immune response – *necrosis* of RV infected cells – *reduced* IL-10/PgE2/TGF-β – *inflammation*
  – and, possibly, with IL-33 secretion
Conclusions, part II

• RV-induced asthma exacerbations do not appear to reflect differences in viral load
  – or, by extension, do not reflect differences in innate anti-viral immune responses
  – (but this could be associated with conversion of apoptosis to necrosis)
  – (or, alternatively, perhaps this is limited to the lower airway)
But how would any of this explain the enhanced “allergy” requirement?

i.e., it’s the soil

(there may be “asthmagenic” viruses but it is not their inherent virulence -- but the nature of the immune response they generate that is the problem)

and if asthmatics have such an awful innate capacity to respond to viruses, why aren’t they have horrific responses to influenza*, adenovirus, etc., etc., etc.?
Two Schools of Thought

The seed = the virus

- Three virus clades
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  - Asthmagenic Rhinovirus (Strain C?)

- The soil = the asthmatic
  - Innate immune deficiency in asthmatics
    - Inability to control virus → increased viral load + exacerbations
  - Robust immune response with viral infection
    - And this immune response is being directed to:
      - bystander allergens
So how do allergic immune responses get generated?

- In genetically predisposed individuals there develops a key central role for epithelial-derived:
  - IL-25
  - IL-33
  - TSLP

Allergen → Epithelial damage → IL-25, IL-33, TSLP → DC → Th0 → Th2

Microbes → TSLP → DC → Th0 → Th2

IL-4, IL-5, IL-9, IL-13
Except that epithelium doesn’t actually need Thelper2 lymphocytes to generate the Th2 cytokine milieu.
And once epithelial cells are epigenetically differentiated to produce these cytokines, they – and their daughter cells – constitutively and permanently retain that tendency.
And this epigenetic “differentiation” of the epithelium includes programming to secrete cytokines/chemokines that drive eosinophilia (which also make Th2 cytokines)

- **IL-33**
- **TSLP**
- **IL-25**
- **GM-CSF, CCL5, CCL11, CCL24, CCL26**
- **IL-25**
- **IL-33**
- **TSLP**
- **Mast Cell**
- **Eosinophil**

- **ILC2**
  - IL-5
  - IL-13

- **IL-4, IL-5, IL-9, IL-13**
- **IL-4, IL-13**
Methods

• Primary epithelial cell cultures
  – Infected with RV39 (3 TCID$_{50}$/mL) at 33°C
    • Placed in 37°C incubator and harvested cells at 2, 4, 24, and 48 hours.
  – Evaluate mRNA and protein expression of different cytokines and chemokines
Epigenetically-programmed rapid upregulation of IL-25/IL-33/TSLP mRNA from RV-Infected Respiratory Epithelium

n=6 (Asthmatics)
n=9 (Controls)
And in our viral infection models there is a “suggestion” of a systemic Th2 signature immune response: Serum total IgE (by day of study)

*increased IgE in 9/10 subjects
Local IgE Production in the Nose of Allergic and Non-Allergic Rhinitis Patients

- Quantification of allergen-specific and total IgE in the circulation and in both nasal secretions / interstitial nasal fluid
- The concept being that "diffusion" of IgE would be allergen non-specific, and as such a ratio of specific and total IgE in the nares compared to the circulation >1 would signify local production

<table>
<thead>
<tr>
<th></th>
<th>Local DP IgE</th>
<th>Local BT IgE</th>
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<tbody>
<tr>
<td>Allergic</td>
<td>34%</td>
<td>0%</td>
</tr>
<tr>
<td>Non-Allergic</td>
<td>0%</td>
<td>0%</td>
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Local IgE Production in the Nose of Allergic Rhinitis Patients With and Without RV Infection

- no virus
- RV/HEV+

![Bar chart showing percentage of local IgE production in the nose of allergic rhinitis patients with and without RV infection for allergens DP and BT.](chart.png)
Despite similar viral loads, symptoms are worse in allergic asthmatics:

Reflecting an exacerbated and prolonged allergic state?
so, is IgE important to RV-induced asthma exacerbations?

and will targeting IgE-mediated bystander allergic responses protect against RV-induced exacerbations?

Antihistamines / Intranasal CCS – never studied
Allergy Immunotherapy – never studied
Omalizumab?
Omalizumab in Allergen-Exacerbated Asthma: Reduction in Exacerbations

Including exacerbations during the rhinovirus-associated May – September asthma epidemics

Seasonal Variation in Frequency of Exacerbations
(width of the bands represents the 95% CI)

Conclusions, part III

• RV-mediated asthma exacerbations in children and adolescents are dependent upon an enhanced IgE-driven immune response to bystander allergens
  – this may be attenuated by treating the allergies:
    • Intranasal CCS / Anithistamines – unstudied
    • IT – perhaps
    • Omalizumab

• Proof of this concept will require a randomized controlled trial of omalizumab in patients undergoing experimental RV challenge:
  – in progress