Adaptive Immune Responses in Rhinovirus Infection

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Learning Objectives
- To describe the utility of using autologous mDC to evaluate CD4 AND CD8 RV-specific immune responses
- To understand the phenotype of RV-specific CD4 and CD8 cells in:
  - immune surveillance, and
  - in response to RV infection

September (and May) Epidemics of Asthma Exacerbations in Children and Adolescents in North America

and this occurs despite compelling evidence that RV infections only occur in September (and May)

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

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:
      - sensitivity to ragweed, alternaria, dermatophagoides
    - Spring exacerbation requires:
      - 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)

Children with Positive Tests for Viral Infection (qPCR)

* p<0.03 for the percentage of RV positive tests in Feb and Oct
Probability of Asthma Exacerbation Based on Titters 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 (?)
    - Asthmagenic Rhinovirus (Strain C?)*
  - **The soil= the asthmatic**
    - Innate immune deficiency in asthmatics
      - Inability to control virus\(\rightarrow\) increased viral load + exacerbations
    - Robust immune response to viral infection
      - Inflamed lungs of asthmatics + cytokine barrage from RV \(\rightarrow\) exacerbations
    - And this immune response can be directed to either:
      - the virus itself
        - bystander allergens
    - Does the virus multiply better or worse in the asthmatic?

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

RV qPCR for RV in Nasal Washes from Children with Asthma Exacerbations, Acute Rhinitis, and Controls in the ER
Conclusions, part II

- RV-induced asthma exacerbations cannot be ascribed to differences in viral load
  - or, by extension, to differences in innate immune responses

If the viral load is the same, why do asthmatics exacerbate?

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)

Immune Response to RV

Tetramer-guided epitope mapping (TGEM) of RV39 capsid proteins

- PBMCs were stimulated with pooled HRV39 peptides (P11-15) for 14 days before staining with tetramers containing each of the peptides

- VP1 epitope: MFTYRFDSEIT (overlapping peptides 14 + 15) maps to a region that is highly conserved among RV groups A, B, and C;
  - implies memory CD4+ T cells induced by one strain can respond to a different strain;
  - thereby allowing a rapid T cell recall response
  - could facilitate recruitment of RV-specific CD4+ T cells to the respiratory tract soon after RV infection

Kwok, W and Woodfolk, J unpublished
Tetramer-guided epitope mapping (TGEM) of RV39 capsid proteins

- Identified DR4-restricted T cell epitopes within 2 RV capsid proteins: VP1 and VP2
  - Present at frequency of ~11.8 X 10^6 CD4+ T cells.

- VP2 (P60) epitope (P60) CCR7+ suggestive of a central memory phenotype (preferentially home to secondary lymphoid organs)

In order to test this we needed to develop DC model of RV antigen presentation

Adaptive Immune Response to RV

- Adaptive Immune Responses
  - CD4+ T cells engaging antigens presented by APCs on MHC class II
  - CD8+ cytotoxic T cells engaging MHC class I on infected epithelium

- RV-associated asthma exacerbations are linked to the presence of an increased type 2 cytokine signature (IL-4, IL-5, and IL-13)

- Hypothesis: RV-specific adaptive T cells in asthmatics who develop an asthma exacerbation demonstrate a Th2 cytokine bias
  - HOWEVER, we cannot rule out the possibility that RV infection merely acts to induce/exacerbate an adaptive Th2 effector immune response to bystander allergens

Autologous Dendritic Cells as RV Antigen-Presenting Cells

- PBMCs from volunteers are cultured to generate an expanded population of autologous DC
- CD14+ monocytes (>95%) enriched using magnetic affinity purification
- CD14+ cells cultured in medium with 10% autologous serum containing hGM-CSF (1000 U/ml) and hIL-4 (1000 U/ml) for 5 days with the addition of fresh media and cytokines at 3-day intervals

DC Phenotyping

- DC identified by flow cytometry morphologic criteria:

<table>
<thead>
<tr>
<th>DC markers</th>
<th>Activation markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC IIhi</td>
<td>CD80+</td>
</tr>
<tr>
<td>MHC IIhi</td>
<td>CD83+</td>
</tr>
<tr>
<td>CD11chi</td>
<td>CD86+</td>
</tr>
<tr>
<td>CD14low</td>
<td></td>
</tr>
<tr>
<td>CD1c+</td>
<td></td>
</tr>
</tbody>
</table>

DC Phenotyping

- CD80+ APC iso
- CD83+ HLA-DR
- CD86+ HLA-DR
Antigen Loading
• DCs cannot be infected by RV
• Used RV itself as the means of DC maturation
  – not requiring maturation cocktails that would influence
    T-cell immune deviation
• RV (50 µg/ml (10^{5.6} pfu/ml)) for 48 hrs at 37°C
• Measured RV-induced DC maturation by:
  – cell surface staining
  – mRNA expression of activation markers
  – ability to support RV-specific T cell proliferation

RV-Induced DC HLA-DR Expression

RV-Induced DC CD80 Expression
CD80 increased in 13/14 subjects

RV-Induced DC CD86 Expression
CD86 increased in 10/14 subjects

RV-DC CD83 Expression
CD83 decreased in 11/14 subjects

RV-induced DC maturation in control subjects: mRNA

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>ΔCT Medium</th>
<th>ΔCT RV</th>
<th>Fold change</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homeostatic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>c-kit</td>
<td>9.12±1.30</td>
<td>8.07±1.28</td>
<td>2.07</td>
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<tr>
<td>SCF</td>
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<td>8.91±0.81</td>
<td>1.77</td>
<td>0.07</td>
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<tr>
<td>Innate</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TNF-α</td>
<td>4.76±0.67</td>
<td>4.82±0.91</td>
<td>0.96</td>
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</tr>
<tr>
<td>IL-1β</td>
<td>7.67±0.51</td>
<td>7.23±0.58</td>
<td>1.36</td>
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</tr>
<tr>
<td>IL-15</td>
<td>8.08±1.15</td>
<td>8.60±0.70</td>
<td>1.12</td>
<td>0.08</td>
</tr>
<tr>
<td>Th1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-12p35</td>
<td>8.88±0.95</td>
<td>7.83±0.97</td>
<td>2.07</td>
<td>0.027</td>
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<tr>
<td>IL-12p40</td>
<td>6.63±0.72</td>
<td>6.86±0.70</td>
<td>0.85</td>
<td>N.S.</td>
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<tr>
<td>IL-18</td>
<td>4.43±0.67</td>
<td>4.60±0.74</td>
<td>0.98</td>
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<tr>
<td>p28</td>
<td>10.22±1.06</td>
<td>9.75±0.90</td>
<td>1.39</td>
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<tr>
<td>Th2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSLP</td>
<td>9.59±1.21</td>
<td>10.59±1.50</td>
<td>0.5</td>
<td>N.S.</td>
</tr>
<tr>
<td>Th17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>8.07±0.66</td>
<td>7.74±0.73</td>
<td>1.26</td>
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<tr>
<td>IL-23</td>
<td>5.64±1.00</td>
<td>6.16±0.98</td>
<td>0.70</td>
<td>N.S.</td>
</tr>
<tr>
<td>Treg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β</td>
<td>3.47±0.49</td>
<td>2.44±0.63</td>
<td>2.04</td>
<td>0.03</td>
</tr>
<tr>
<td>IL-10</td>
<td>7.04±0.64</td>
<td>6.56±0.50</td>
<td>1.39</td>
<td>0.03</td>
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<tr>
<td>EBI3</td>
<td>11.86±0.86</td>
<td>11.59±0.86</td>
<td>1.23</td>
<td>N.S.</td>
</tr>
<tr>
<td>N.S. = not significant</td>
<td></td>
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</tbody>
</table>
Conclusions, part III

- Even though RV does not productively infect DC
- RV does provide DC maturation signals
  - and via expression of RV-associated peptides these newly activated DC can be used to induce both
    - MHC class I activation of CD8s and
    - MHC class II activation of CD4s

RV-Specific Memory T cells in Healthy Subjects

- PBMCs isolated from healthy subjects to generate mature DCs and collect T cells
- T cells labeled with the dilution sensitive dye CFSE prior to co-culture RV-loaded or control DC
  - CFSE<sup>low</sup> cells represent the CD4<sup>+</sup> and CD8<sup>+</sup> T cells which have proliferated in response to the RV-matured DC
- DC presenting RV antigen co-cultured at a T cell:DC ratio of 10:1 using 2x10<sup>5</sup> cells per culture for 7 days

% CFSE<sup>low</sup> and intracellular cytokines in DC:T cell co-cultures: healthy subjects

<table>
<thead>
<tr>
<th></th>
<th>CD4</th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFSE&lt;sub&gt;low&lt;/sub&gt; (%)</td>
<td>8.34±0.4</td>
<td>3.46±0.74</td>
</tr>
<tr>
<td>IFN-γ (%) of proliferating cells</td>
<td>74 ± 9</td>
<td>43 ± 10</td>
</tr>
<tr>
<td>IL-4 (%) of proliferating cells</td>
<td>50 ± 8</td>
<td>8 ± 2</td>
</tr>
</tbody>
</table>
Secreted cytokines from DC:T cell co-cultures: healthy subjects

<table>
<thead>
<tr>
<th></th>
<th>IFN-γ</th>
<th>IL-4</th>
<th>IL-13</th>
<th>IL-10</th>
<th>IL-17A</th>
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</thead>
<tbody>
<tr>
<td>-RV</td>
<td>101±3</td>
<td>5.5±1.2</td>
<td>50.5±17.1</td>
<td>109±37</td>
<td>21.3±0.9</td>
</tr>
<tr>
<td>-RV</td>
<td>145±580</td>
<td>19.05±5.52**</td>
<td>145±53.2***</td>
<td>105±20</td>
<td>34.3±17.8</td>
</tr>
</tbody>
</table>

n=4, pg/ml, * p<0.05, ** p<0.01, *** p<0.001

Activation and Phenotyping of RV-Specific T-Lymphocytes: RV Challenge

- PBMCs isolated from 2 subjects undergoing RV challenge prior to infection to generate mature DCs
- At day 5 of infection T cells isolated from blood
- ICCS staining on CFSE^low_ population

% CFSE^low_ and intracellular cytokines in DC:T cell co-cultures: asthmatic subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>CD4</th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>24.4</td>
<td>17</td>
</tr>
<tr>
<td>#2</td>
<td>47.2</td>
<td>13.6</td>
</tr>
<tr>
<td>IFN-γ + (% of proliferating cells)</td>
<td>32.9</td>
<td>10.2</td>
</tr>
<tr>
<td>IL-4+ (% of proliferating cells)</td>
<td>2.9</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Conclusions, part IV

- Circulating RV-specific effector memory CD4s – and to a lesser extent CD8s – can be identified in the circulation of healthy subjects
  - where they are presumably involved in immune surveillance
- These cells recognize epitopes shared across RV-A, -B, and -C clades
  - and via heterologous immunity this will allow rapid (day 5-7) immune responses to RV strains _without need for previous exposure_
- Effector memory cells in healthy subjects primarily express Th1 (IFN-γ) signature
- Increased numbers of circulating RV-specific effector T cells are observed during infection
  - in healthy subjects these cells display a mixed Th1>Th2 cytokine signature
  - a Th2 bias could be present in asthmatics who exacerbate

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