Viruses and bacteria in asthma/allergy

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Introduction

• The microbiology of asthma is complex

• Role of viruses in asthma onset early in life

• Role of viruses in asthma exacerbations (AE)
  - Epidemiological studies
  - In vivo human experimental infection studies

• Recent mechanistic insights into how viruses cause AE
  - Virus and Th2 cytokine synergy
  - Impaired anti-viral responses in asthma

• Bacteria in asthma
  - the microbiome
  - bacteria as protective
  - bacteria causing asthma
The 7 stages of asthma

Asthma difficult to diagnose in children <5 yrs, present with "wheeze"
Bacteria and other microbes cause or effect of asthma?

Edwards et al Nat Micro 2010
The common mucosal immune system: Lung-Gut crosstalk?
Importance of asthma and AE

• Asthma affects 300 million people worldwide

• Majority of morbidity and mortality associated with asthma is via virus induced AE
  - Viruses account for 80% of AE
  - In the UK yearly mortality due to AE is approx 1200
  - Moderate asthmatics have approx 1-1.5 AE per year

• Current treatments are ineffective at controlling AE
  - Glucocorticoids and $\beta_2$ agonists
  - Better therapies are desperately required for asthma onset prevention and asthma exacerbations (AE)
What properties make a virus asthmagenic?

- **Physiochemical properties**
  - Stable in environment easily transmissible
  - Readily infect children
  - Diverse, high mutation rate

- **Interactions with the host response**
  - Not be overly lytic, not induce excessive cell death, Th1 inflammation
  - Interact with Th2 pathways in an additive or synergistic manner
  - Induce poor neutralising antibody
  - Promote encourage secondary bacterial infections
Viruses associated with wheeze and asthma onset

- **Respiratory Syncytial Virus (RSV)**
  - *Paramyxoviridae*
  - Infects all children <2 years of age
  - Epidemiological evidence (Sigurs et al AJRCCM 2000 & Sigurs et al Thorax 2010)
  - Mouse model studies with RSV and the mouse equivalent, mouse pneumovirus (Culley et al JEM 2004, Kaiko et al JACI 2013)

- **Human rhinovirus**
  - *Picornaviridae*
  - Growing epidemiological evidence (Jackson et al AJRCCM 2012 & Jackson et al AJRCCM 2008)
  - Variants at the 17q21 locus (ORMDL3 and GSDMB) are associated with asthma onset in RV-induced wheezers (Caliskan et al NEJM 2013)
RSV early in life predicts allergic sensitisation later in life

Asthma/wheeze

p < 0.001

B

rhinoconjunctivitis

p < 0.001

C

Total SPT +ve

p = 0.003

D

Perennial allergen SPT +ve

p < 0.001

Sigurs et al Thorax 2010
Palivizumab prevents wheeze during RSV infection early in life

429 Preterm Infants during the First Year of Life.

Blanken et al NEJM 2013
Viruses are commonly associated with AE

- RV 62%
- RSV 11%
- Coronavirus 9%
- Influenza 5%
- Adenovirus 2%
- Other 11%

Human rhinovirus (RV)

- Associated with asthma onset in children
- Accounts for 2/3 of virus induced AE
  - Picornavirus (ss +ve RNA) genome=7 kB
  - Enters via endosome, requires H⁺ environment of the endosome
- Replication is in the cytoplasm
- Can be divided into subgroups:
  - Receptor specificity major group (ICAM-1) and minor group (LDL receptor)
  - RNA sequence, RV-A, RV-B, RV-C
- Diverse; approx 150 serotypes and counting
1. Virus infections and asthma exacerbations in 9-11 yr old children

2. 

3. 

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SL Johnston et al  BMJ 1995
The September “asthma epidemic” is caused by RV infection

No. of emergency room admissions associated with asthma

Timeline

Johnston et al JACI 2005
The September Epidemic of RV Infection in Children

Corresponds to Labour Day, children return to school

April-March Mean = 373

752, 862, 646

No. of emergency room admissions associated with asthma

April, 2001  September 2001  March, 2002

NW Johnston et al  JACI 2005
Experimental models of AE: Increased symptom scores

- 10 atopic asthmatics
- 15 non-atopic non-asthmatics

S Message et al PNAS 2009
Experimental models of AE: Increased airway inflammation
During experimental infection, AA have higher virus loads.

Differences between asthmatic and normal are all non-significant.

S Message et al PNAS 2009
Evidence that virus load is related to clinical outcomes

A

Total chest symptom score

Asthmatics alone

\[ r = -0.795, P = 0.01 \]

B

Change in \( \text{PC}_{20} \) at d6 (doubling dilutions)

Asthmatics alone

\[ r = -0.77, P = 0.016 \]

Nasal lavage virus load (log₁₀ copies/mL)
RV infection synergises with allergen exposure

- Children: OR for asthma admission
  - Sensitised: +, -, -, +, -
  - Exposed: -
  - Virus: -
  - OR: ***p<0.001

- Adults: OR for asthma admission
  - Sensitised: +, -, -, +, -, +
  - Exposed: -
  - Virus: -
  - OR: **p<0.01

Interactions with Th2 immunity

Virus infections may produce:
IL-25, TSLP, IL-33, MDC and TARC

Interactions between Th2 immunity and various immune cells:
- **Th2 cell** secretes IL-4, IL-13, TGFβ.
- **IgE** binds to **mast cell**.
- **Allergen** triggers IgE.
- **DC** produces IL-25, TSLP, IL-33, MDC, TARC.
- **EC** secretes CCL17(TARC), CCL22 (MDC).
- **ASM** releases PGD2, LTC4/D, E4, histamine.
- **MBP**, ECP, TGFβ.
- **Eosinophil** interactions with Th2 cell.
anti-IgE therapy can reduce % of AE

Figure 1.
Impact of omalizumab therapy on virus-induced asthma exacerbations in 6-12 yr old children

Busse et al. NEJM2011
Interferons (IFNs) : Anti-viral Cytokines

- **Type I IFN-α** (14 subtypes) & IFN-β (1)
  - Chromosome 9
  - IFNAR1&2 complex
  - IFN-α mostly leukocytes, IFN-β all nucleated cells

- **Type III IFN-λ** (3 subtypes, IL-29, IL-28A, IL-28B)
  - Chromosome 19
  - IL-10R2 and IFN-λR1
  - Most cell types
  - Mice have IFN-λ (IL-28)

- **Type II IFN-γ**
  - leukocytes
  - a pleiotropic cytokine
# Impaired innate IFN in asthma

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cell type</th>
<th>IFN studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelhlar, <em>Clin Exp All</em> 2006</td>
<td>PBMCs†</td>
<td>IFN-αs</td>
</tr>
<tr>
<td>Wark <em>JEM</em> 2005</td>
<td>HBECs</td>
<td>IFN-β</td>
</tr>
<tr>
<td>Contoli <em>Nat Med</em> 2006</td>
<td>HBECs, BAL macs</td>
<td>IFN-λs</td>
</tr>
<tr>
<td>Uller <em>Thorax</em> 2010</td>
<td>HBECs</td>
<td>IFN-λs</td>
</tr>
<tr>
<td>Gill <em>J Imm</em> 2011</td>
<td>DCs‡</td>
<td>IFN-αs, IFN-λs</td>
</tr>
<tr>
<td>Sykes <em>JACI</em> 2012</td>
<td>BAL macs</td>
<td>IFN-αs, IFN-β, IFN-λs</td>
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<tr>
<td>Durrani <em>JACI</em> 2012</td>
<td>DCs</td>
<td>IFN-αs, IFN-λs</td>
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<tr>
<td>Baraldo <em>JACI</em> 2012</td>
<td>HBECs</td>
<td>IFN-β, IFN-λs</td>
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<tr>
<td>Edwards <em>Muc Imm</em> 2012</td>
<td>HBECs</td>
<td>IFN-β, IFN-λ</td>
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<tr>
<td>Collison <em>Nat Med</em> 2013</td>
<td>HBECs</td>
<td>IFN-λs</td>
</tr>
</tbody>
</table>

†Used Newcastle disease virus and RSV ‡used influenza virus NOT rhinovirus
Type I & type III IFN have anti-viral activity in models of RV infection

Data from normal HBEC cells n=4 experiments, RV1B MOI of 1, **p<0.01, *p<0.05 versus medium; Edwards unpublished observations

n=4-6 animals per group, n=2 independent experiment, p<0.01 between groups Bartlett, Slater & Edwards, EMBO Mol Med 2012
RV induced IFNs are impaired in STRA at 24hrs

- IL-8/CXCL8 & ENA-78/CXCL5 were not impaired in STRA
- IFN-β & IFN-λ protein was impaired in STRA

Edwards et al *Muc Immunol* 2012
RV load is elevated in STRA

Edwards et al Muc Immunol 2012
**Th2 antagonistic properties of IFNs**

IL-28 = IFN-λ2

Koltsida et al EMBO Mol Med 2011
Low asthma incidence on farms is associated with increased diversity.
Relationship between environmental microbial exposure and Asthma

Farm-derived Gram-positive bacterium *Staphylococcus sciuri* W620 prevents asthma in mice.
Lung microbial communities differ in asthma

Distribution of common phyla and genera in diseased and normal bronchi.

<table>
<thead>
<tr>
<th>PHYL A</th>
<th>Control</th>
<th>Asthma</th>
<th>P* Asthma vs. Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria</td>
<td>83</td>
<td>331</td>
<td>6.45E−14</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>282</td>
<td>239</td>
<td>1.13E−17</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>218</td>
<td>454</td>
<td></td>
</tr>
<tr>
<td>Fusobacteria</td>
<td>69</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>18</td>
<td>27</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>GENERA</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroidetes/Prevotella</td>
<td>247</td>
<td>137</td>
<td>3.23E−31</td>
</tr>
<tr>
<td>Other Bacteroidetes</td>
<td>35</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Firmicutes/Staphylococcus</td>
<td>13</td>
<td>74</td>
<td>7.16E−03</td>
</tr>
<tr>
<td>Firmicutes/Streptococcus</td>
<td>98</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>Firmicutes/Veillonella</td>
<td>69</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Other Firmicutes</td>
<td>38</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Proteobacteria/Haemophilus</td>
<td>17</td>
<td>154</td>
<td>1.44E−13</td>
</tr>
<tr>
<td>Proteobacteria/Neisseria</td>
<td>53</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Other Proteobacteria</td>
<td>13</td>
<td>100</td>
<td>1.01E−06</td>
</tr>
<tr>
<td>Fusobacteria</td>
<td>69</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>

The numbers of sequences are shown for each split level.
*Only significant P values are shown. The significance levels have been Bonferroni corrected for multiple comparisons.

doi:10.1371/journal.pone.0008578.t005

Certain bacteria are associated with wheeze & asthma onset

Table 3 | Association between bacteria and viruses and wheezy episodes in children up to age 3 years.

<table>
<thead>
<tr>
<th>Wheezy episodes</th>
<th>Bacteria</th>
<th>P value</th>
<th>Virus</th>
<th>P value</th>
<th>Bacteria adjusted for viruses</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>2.9</td>
<td>&lt;0.001</td>
<td>2.8</td>
<td>&lt;0.001</td>
<td>2.5</td>
<td>0.049</td>
</tr>
<tr>
<td>≤4 episodes</td>
<td>3.5</td>
<td>&lt;0.001</td>
<td>3.5</td>
<td>&lt;0.001</td>
<td>4.0</td>
<td>0.012</td>
</tr>
</tbody>
</table>

- Wheezy episodes were associated with higher numbers of *Strep pneumonia*, *Haemophilius influenzae*, *Morexella Catarrhalis*, *Staph aureus* and *Strep pyogenes* versus controls.

- Further evidence that *Proteobacteria* are pathogens

Can diet control asthma? Evidence for a gut-lung axis

Richness = no. of species; Diversity = no. of equally abundant species

Trompete et al Nat Med 2013
A high fibre diet, rich in SCFAs controls HDM-related asthma in mice

- A high fibre diet contain short chain fatty acids (SCFAs).
- SCFAs include acetate, butyrate and propionate.

Trompette et al Nat Med 2013
Summary

• The microbiology of asthma is complex with regard to type of asthma, stage (age) of asthma, environment, and impact of other organs (gut).

• Evidence suggests respiratory viruses cause wheeze and trigger AE

• Viruses may interact with Th2 pathways in a synergistic or additive manner triggering AE

• Anti-viral immunity may be lacking in asthma and contribute to AE pathogenesis

• Some bacteria may be protective, others harmful.

• More mechanistic studies are required to be understand this relationship.