VIRUSES AND ASTHMA

“They asked if the sneezle came after the wheezle, or if the first sneezle came first”
First documented viral association of asthma attacks occurred during influenza epidemics at 4th National Boy Scouts Jamboree, Pennsylvania, 1957 followed a month later by the Centenary World Guide Camp, Ontario
REVIEW

Viruses as precipitants of asthma symptoms. 
I. Epidemiology

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Christopher Robin had wheezles and sneezles, they bundled him into his bed. They gave him what goes with a cold in the nose, and some more for a cold in the head. They wondered if wheezles could turn into measles, if measles would turn into mumps; They examined his chest for a rash, and the rest of his body for swellings and lumps. They sent for some doctors in sneezles and wheezles, to tell them what ought to be done. All sorts and conditions of famous physicians came hurrying round at a run. They all made a note of the state of his throat, they asked if he suffered from thirst; They asked if the sneezles came after the wheezles, or if the first sneezle came first. They said, ‘If you teaze a sneeze or wheeze, a measles may easily grow. But humour or pleazure the wheeze or sneeze, the measles will certainly go.’ They expounded the reazles for sneezles and wheezles, the manner of measles when new. They said ‘If he freezles in draughts and in breezles, then PHTHEEZLES may even ensue.’

Christopher Robin got up in the morning, the sneezles had vanished away. And the look in his eye seemed to say to the sky, ‘Now, how to amuse them today?’

A. A. Milne, *Now We Are Six*, 1927.
Viruses as precipitants of asthma symptoms: 38 studies

1. Resp viruses identified 5-17x as often in exacerbations as during well intervals; Bacteria similar at both times.

2. Viral Identification rates depended on

   1. Virology quality. rhinovirus expertise, and sensitive cell lines (Purpose-selected > hospital available (then))

   2. Intensity of follow-up
      Proactive monitoring for infections
      Interval between symptom onset and viral samples (prospective > p.r.n. community > p.r.n. hospital studies)

3. In wheeze episodes
   Highest rhinovirus ID (by culture) was 31.9%
   Most intensive study without HRV ID was 48% ~80%
Viruses as precipitants of asthma symptoms (2)

4. Every known respiratory virus & *M. pneumoniae* was found in at least one study, but no study found them all (or had adequate techniques to do so).

5. Double & triple infections were found

6. In prospective studies of children with asthma, wheezing occurred fairly predictably in about 50% of identified infections

7. Virus seroconversion occurred in some wheezing attacks without any other RTI symptoms in Davos
   
   (daily exam and spirometry, monthly + acute serology for one year)
   
   ? asthma exposing an infection that would otherwise be asymptomatic

4. Adult studies were few, poor in virology, numbers and follow-up and showed lower rates of viruses
Duration of lung dysfunction after influenza in children with asthma

Figure 3. Course of FEV₁ in three patients with an SRI caused by influenza A.

Roldaan & Masural. Eur J Respir Dis 1982;63: 140-50
The use of nucleic acid hybridization to detect human coronaviruses

Brief Report

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² MRC Common Cold Unit, Salisbury, England

Accepted January 6, 1989

Summary. We have applied an RNA:RNA hybridization test for the detection of human coronavirus 229E. This test is undergoing further development but already allows a diagnosis of HCV 229E infection within 48 hours.

Human coronaviruses are positive-strand RNA viruses that are thought to cause about 20% of all common colds. They have also been associated with other diseases [1] but only the association with respiratory tract infection is substantiated [2]. Coronaviruses have an unusual replication strategy which involves a 3' coterminal set of subgenomic mRNAs. Only the 5' proximal region of each mRNA generally codes for a protein. In the case of human coronaviruses the smallest of these subgenomic mRNAs codes for the nucleocapsid protein.

We have used an oligo-dT primer and a method based on that of Gubler and Hofmann [3] to create a library of human coronavirus (HCV) 229E cDNA clones. From this library, cDNA representing a complete copy of the nucleocapsid gene has been identified and subcloned into the 'riboprobe' vector pGEM-1. This vector has a promoter site for the attachment of T7 RNA polymerase, using which 32P-labelled single stranded RNA transcripts can be generated. These transcripts have been shown to be more sensitive than nick-translated double-stranded DNA probes in the detection of HCV 229E RNA [Myint et al., in prep.]. Their specificity has been examined by hybridization to a series of filters onto which had been bound RNA from 42 common cold pathogens (HCV 229E, HCV OC43, HCV 229E/Killick, 4 para influenza, 4 influenza and 31 rhinoviruses), only the HCV 229E group were detected (unpublished data).

This "riboprobe" has now been successfully applied to the detection of HCV 229E in nasal washings collected from volunteers infected with the virus.

fig. 1. Detection of RNA from 57 rhinovirus serotypes using synthetic oligonucleotide probes against conserved sequences in the 5' non-coding region. 250 µl tissue culture supernatant was taken for each virus indicated, treated as described in the Methods and applied to the filter. The film was exposed to the filter for 48 h.
Viruses identified in acute wheeze

- **No virus**: 18%
- **Rhinovirus**: 53%
- **Coronavirus**: 8%
- **RSV**: 5%
- **Para influenza**: 7%
- **Influenza**: 9%

Multiple viruses
In 15 episodes (5%)

Rhinovirus
12% when asymptomatic

Examples of charts of peak flow recordings and respiratory symptom scores for three children taking part in the study.

NEW VIRUSES
Bocavirus
Metapneumovirus
Coronavirus
NL63
HKU1
[SARS-CoV]
[MERS-CoV]
Parechovirus
Seasonal incidence of viruses in cohort, and child asthma admissions in Wessex

Correlation = 0.67, p<0.001

Publications: Virus AND {asthma OR wheeze}

1506 citations, currently ~60 citations/year
Viruses by RT-PCR in wheezy episodes

any virus

% of wheezy episodes positive for virus

Infants

Children

Adults

infancy
0-1 yr
0-1 yr
<2 yrs
<3 yrs
1-2 yrs
2-5 yrs
>3 yrs
3-15 yrs
>5 yrs
5-15 yrs
2-7 yrs
3 yrs
5 yrs
6 yrs
8 yrs
10 yrs
20 yrs
30 yrs
40 yrs
50 yrs
60 yrs
70 yrs
80 yrs
90 yrs
100%
Viruses by RT-PCR in wheezy episodes

[Bar chart showing the percentage of wheezy episodes positive for any virus and rhinovirus across different age groups: infancy, 0-1 yr, <2 yrs, 2-3 yrs, >3 yrs, 0-1 yrs, <2 yrs, 2-3 yrs, >3 yrs, 2-5 yrs, 2-6 yrs, 2-7 yrs, 3-15 yrs, 3-16 yrs, 3-17 yrs, 0-6 yrs, 2-7 yrs, adults, adults.]

- Any virus vs. rhinovirus across different age groups and time periods: infancy, 0-1 yr, <2 yrs, 2-3 yrs, >3 yrs, 0-1 yrs, <2 yrs, 2-3 yrs, >3 yrs, 2-5 yrs, 2-6 yrs, 2-7 yrs, 3-15 yrs, 3-16 yrs, 3-17 yrs, 0-6 yrs, 2-7 yrs, adults, adults.

- The chart highlights the prevalence of viruses in wheezy episodes, with a particular focus on rhinovirus.
Viruses by RT-PCR in wheezy episodes

- **Infants**
  - Any virus: 100%
  - Rhinovirus: 70%
  - RSV: 0%

- **Children**
  - Any virus: 90%
  - Rhinovirus: 50%
  - RSV: 20%

- **Adults**
  - Any virus: 80%
  - Rhinovirus: 30%
  - RSV: 10%
Genomic structure (ssRNA+) of Human Rhinoviruses

Low temperature preference? 
Detected on 40% cases' hands; 6% of home objects Survives up to 2h on human skin 
Nasopharynx first but can infect all resp cells including alveolar and interstitial Minimal epithelial destruction; disrupts tight junctions Minimal humoral cross-reactivity, but T-cell cross-reactivity potentiates higher cytokine response IL-8 activation is associated with worse symptoms, naproxen with less Asymptomatic in 12-32%

Classification of Human Rhinoviruses

- Family Picornaviridae
- Genus *Enterovirus*

- Genotypic Species:
  - HRV A (74 serotypes) 12 bind LDL-receptor, 62 bind ICAM-1 receptor
  - HRV B (25 serotypes) all bind ICAM-1 receptor
  - HRV C (50 serotypes) receptor unknown
rhinovirus 3
PDB: 1rhi
Epithelial cytokine responses to HRV

A potential role in airway remodelling

Saraya et al. *Frontiers in Microbiology*, 2014;5: 226
HRV promotion of bacterial infection

Rhinovirus C15 model
Basta, Sgro & Palmenberg
Virology 448 (2014) 176–184
HRV-C and ED acute asthma in Perth

**FIGURE 1.** Frequency of human rhinovirus (HRV) and other common respiratory viruses identified in 128 children with an asthma exacerbation.

**FIGURE 2.** Relationship between human rhinovirus (HRV)C infection and severity of asthma exacerbation in 128 children.

DO VIRUSES CAUSE ASTHMA?
The Tucson Study: Martinez et al 1980

• Birth cohort = 1246 472 children tested for viruses
• 207 children with RSV+ve LRTI in first three years of life,
Outcome: At 13 years, adjusted odds ratios for:
  • Infrequent wheeze(≤3/yr) Frequent (>3/yr) wheeze

The Swedish Study: Sigurs et al. 1989

**Design:**
- Index group: 47 children aged <1 year
  - hospitalised with RSV LRTI Dec 1989 - Apr 1990
- Controls: 93 age- and gender-matched children at same centres
- “Diagnosis of bronchiolitis was originally based on criteria published by Court*, but was also consistent with other later published criteria”

*Court, 1973: Acute bronchiolitis:
- “Illness mainly affecting infants, especially in the first 6 months of life. Rapid respiration, dyspnoea, wheezing, chest recession, cough, rhonchi and rales are very frequent. Visible distension of the chest and increased pulmonary translucency on the chest radiograph are frequent and of high diagnostic significance. Upper respiratory features, especially nasal discharge and a red pharynx are frequent. Fever is very frequent, but high fever uncommon.”
RSV Bronchiolitis and later asthma


Proportion With Asthma At 18 yrs

Any parent with asthma
RSV bronchiolitis

No No
No Yes
Yes Yes

5/66 3/26 8/28 10/18

p<0.001
**Table 2** Demographic data and family history of the respiratory syncytial virus (RSV) and control groups at age 18

<table>
<thead>
<tr>
<th>Variable</th>
<th>RSV (n = 46)</th>
<th>Controls (n = 92)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>65 (12)</td>
<td>70 (13)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174 (10)</td>
<td>174 (10)</td>
</tr>
<tr>
<td>Parental atopy</td>
<td>30/46 (65%)</td>
<td>52/92 (56%)</td>
</tr>
<tr>
<td>Parental asthma</td>
<td>18/46 (39%)</td>
<td>25/92 (27%)</td>
</tr>
<tr>
<td>Tobacco smoke exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>10/45 (22%)</td>
<td>14/92 (15%)</td>
</tr>
<tr>
<td>Passive (family member)</td>
<td>19/45 (42%)</td>
<td>40/92 (43%)</td>
</tr>
<tr>
<td>Indoor furred pets</td>
<td>24/46 (52%)</td>
<td>56/92 (61%)</td>
</tr>
</tbody>
</table>

Mean (SD) or proportions (percentages) of subjects are given. No significant differences were seen between the groups.
The Finnish Study: Hyvärinen et al. 1992

- Children hospitalised for wheeze in first 2 years of life
- Asthma at 11 yrs defined by continuous asthma meds, or recurrent wheeze/cough + positive exercise challenge

<table>
<thead>
<tr>
<th>Virus identified during early hospitalisation</th>
<th>Adjusted OR for asthma vs. no asthma at 11 yr</th>
<th>Fold risk over background asthma prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>0.41 (0.11-0.45)</td>
<td>5-fold</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>1.41 (0.40-4.94)</td>
<td>10-fold</td>
</tr>
</tbody>
</table>

The Perth study: Kusel et al. 1996

Odds Ratio for classification

Classification at 5 years old

LRI in 1st year
- HRV Wheezy LRI
- RSV Wheezy LRI
- RSV nonwheezy LRI
- HRV nonwheezy LRI

Perth study: the effect of early sensitisation

Predictors of current wheeze at 5 years in relation to time of atopic sensitisation

<table>
<thead>
<tr>
<th>Type of LRI</th>
<th>Never atopic OR</th>
<th>Atopic by 2 yrs OR</th>
<th>Atopic after 2 yrs OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole pop regardless of ARI history</td>
<td>0.4 (0.2-0.8)</td>
<td>3.1 (1.5-6.4)</td>
<td>2.9 (1.4-5.8)</td>
</tr>
<tr>
<td>Any wheezy LRI in 1st yr (wLRI)</td>
<td>1.4 (0.4-5.1)</td>
<td>3.4 (1.2-9.7)</td>
<td>0.5 (0.1-3.5)</td>
</tr>
<tr>
<td>≥2 wheezy LRI</td>
<td>1.0 (0.1-9.1)</td>
<td>7.1 (1.3-38.4)</td>
<td>NA</td>
</tr>
<tr>
<td>wLRI c RSV</td>
<td>1.6 (0.3-8.7)</td>
<td>3.6 (1.0-13.3)</td>
<td>Insufficient no.</td>
</tr>
<tr>
<td>wLRI v HRV</td>
<td>1.6 (0.38.7)</td>
<td>3.2 (1.1-9.5)</td>
<td>2.1 (0.3-18.5)</td>
</tr>
<tr>
<td>wLRI c HRV or RSV</td>
<td>0.8 (0.2-4.0)</td>
<td>4.1 (1.3-12.6)</td>
<td>0.9 (0.1-6.4)</td>
</tr>
</tbody>
</table>

The Wisconsin (COAST) Study: Lemanske et al. 1998

Percentage Of Children Wheezing in 3rd Year

The Wisconsin (COAST) Study: Lemanske et al. 1998

The Wisconsin (COAST) Study: Lemanske et al. 1998

![Bar chart showing asthma prevalence at 6 years by wheezing illness in 1st year and aeroallergen sensitisation in 1st year.](chart.png)

Asthma Prevalence at 6 yr

- Wheezing illness in 1st year:
  - No HRV wheezing
  - HRV wheezing

Aeroallergen sensitisation in 1st year

- No
- Yes

IFN-γ responses in infant wheeze

Infant wheeze risk doubles if no IFN-γ response detectable at birth

IFN-γ responses in adult asthma

IFN-γ response of bronchial cells to experimental HRV infection in normal and asthmatic subjects

bronchial cell IFN-γ response to RV16 and FEV1 fall in same subjects challenged with RV16

Asthma prevalence at 6y and IL-13 genotype in Dutch children hospitalised with RSV LRTI in infancy

![Graph showing the relationship between IL-13 polymorphism and asthma prevalence.](image)

**FIG 2.** Relationship between IL-13 Arg130Gln genotypes and proportion of children with wheeze at age 6 years. Wheeze at age 6 years was defined as 5 or more wheezing days during the winter season.
The Revenge of the Microbes

Nasopharyngeal colonisation with *Strep pneumoniae*, *H. influenzae* and/or *M. catarrhalis* at 1 month old and later wheeze

Strep pneumoniae* & RV found in weekly winter samples and risk of exacerbations


Kloepfer KM et al. JACI. 2014;133(5):1301-7
Model proposed by Fernando Martinez

Is this the basic instrument on which other asthma risk/protection factors play?
Treatments & Prevention for Rhinovirus

- **Treatments**
  - Capsid-binding
    - Pleconaril – phase 2 study (common cold and asthma) awaited
    - Vapendavir – phase 2 study (common cold and asthma) awaited
  - Nasal α-2 interferon – limited effects, side effects
  - Echinacea – insufficient data/evidence
  - Zinc supplementation – modest reduction in symptoms

- **Prevention**
  - Social distancing and masks  +
  - Hand hygiene ++
  - Interferons, echinacea, vitamin C  --
  - Probiotics +
    - Hao et al. Cochrane database of systematic reviews (Online). 2010 Dec 31;(9)
Viruses have been identified in up to 95% of wheezing episodes / asthma exacerbations, but at low rates at other times. Although all respiratory viruses have been identified, Human Rhinovirus (HRV) predominates in most studies in childhood and adults, and some studies in infants. HRV-C was the most common genotype associated with asthma attacks in Perth and was associated with more severe asthma than other HRVs. Severe HRV+/−RSV infections with wheeze in infancy are associated with an increased risk of wheezing and asthma in childhood. The risk is especially in children with early atopic sensitisation, suggesting that these children are already set up for more severe infections with wheezing in infancy. Markers of innate immunity are characteristically low in infancy in wheezy infants with HRV or RSV. Innate immunity, severe wheezy infections, and atopy genes seem to determine later wheezing phenotype. Other factors, such as breast feeding, may act via these pathways. There is no clear treatment for rhinovirus at this point but common sense preventive measures are appropriate.
Palivizumab in premature neonates and risk of recurrent wheeze to 2-5 yrs of age

<table>
<thead>
<tr>
<th>No family history of atopy or food allergies</th>
<th>Palivizumab treated</th>
<th>Palivizumab untreated</th>
<th>$P$ value</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined untreated groups</td>
<td>5/101 (5%)</td>
<td>16/100 (16%)</td>
<td>.012</td>
<td>0.31 (0.12-0.81)</td>
</tr>
<tr>
<td>Nonhospitalized untreated group</td>
<td>5/101 (5%)</td>
<td>13/75 (17%)</td>
<td>.011</td>
<td>0.29 (0.11-0.77)</td>
</tr>
<tr>
<td>Family history of atopy or food allergies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined untreated groups</td>
<td>10/90 (11%)</td>
<td>21/130 (16%)</td>
<td>.329</td>
<td>0.69 (0.34-1.39)</td>
</tr>
<tr>
<td>Nonhospitalized untreated group</td>
<td>10/90 (13%)</td>
<td>11/79 (14%)</td>
<td>.644</td>
<td>0.80 (0.36-1.78)</td>
</tr>
</tbody>
</table>

**FIG 1.** Effect of palivizumab on physician-diagnosed recurrent wheezing in infants with or without a family history of asthma or atopy or food allergies. Results are expressed as frequencies of outcome in univariate analyses. The results are graphically represented as point estimates of the RRs and 95% CIs.

IL-13 & IFN-γ responses by wheeze

Figure 6: Response to human rhinovirus infection

IL-13 responses by wheeze & virus

IFN-γ response by FEV1 after HRV infection