Respiratory Viruses II: Paramyxoviridae and Respiratory Infections

Susan Baker, Ph.D.

- Respiratory Infections – RSV, metapneumovirus and parainfluenza viruses
- Molecular Biology of Negative Strand Viruses

Common Causes of Respiratory Infection

82% viral!!

- Rhinovirus
- Parainfluenza virus
- Group A ßnemolytic streptococci
- Influenza viruses
- Respiratory syncytial virus
- Mycoplasma & other
- Adenoviruses
- Enteroviruses

- RSV isolates
- Influenza
- Parainfluenza

Classification of Viral Pathogens of the Paramyxoviridae Family That Infect Humans

- **Family**: Paramyxoviridae
  - **Subfamily**:
    - Paramyxovirinae
    - Pneumovirinae
  - **Genus**:
    - Respirovirus
    - Rubulavirus
    - Morbillivirus
    - Pneumovirus
    - Metapneumovirus
  - **Species**:
    - Parainfluenza types 1 and 3
    - Parainfluenza types 2 and 4
    - Human metapneumovirus types A and B

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**Respiratory Syncytial Virus**

- **Disease**
- **Symptoms**:
  - Lower respiratory tract infection in infants and young children
  - Milder URTI in adults
  - Pneumonia in elderly
  - Infects 1.9 million infants/children per year
  - Peak season October-April
  - Serious complications in premature or immunocompromised infants, or infants with congestive heart disease
  - Nosocomial infections are a common problem!
Percentage of specimens testing positive by antigen detection for respiratory syncytial virus, by region and week of report – United States, July 2003-November 2004

Epidemiology of RSV Infection in Infants

- Prevalence of antibodies by age 3 yrs: 100%
- Risk of infection in first year of life: 50%
- Risk of hospitalization: 1%
- Risk of mortality in high-risk hospitalized infants: 33%

Proportion of volunteers infected with RSV according to method of exposure to an infected infant

<table>
<thead>
<tr>
<th>Volunteers</th>
<th>Cuddlers</th>
<th>Touchers</th>
<th>Sitters</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. exposed</td>
<td>7</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>No. infected</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Afebrile URI**</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Febrile URI</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>0</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Incubation</td>
<td>4 days</td>
<td>5.5 days</td>
<td></td>
</tr>
</tbody>
</table>
RSV: Significant Mortality in Compromised Infants

- <1% mortality in previously healthy infants
- 30% mortality in infants with CHD (congenital heart disease)
- 70% mortality in infants with CHD and pulmonary hypertension

RSV: Pathology of Disease

- Necrosis of bronchiolar epithelium in 18-24 hours
- Submucosal edema
- Bronchioles occluded with mucus and cellular debris
- Hyperinflation and air trapping
Work Up of Respiratory Specimens (new way)

**Patient Nasopharyngeal Aspirate**

- 20 pathogens
- RSV, Influenza A, B
- Paraflu I, II, III
- Adenovirus

- **Film array RT-PCR** (1-3 hrs)
- **Viral Cultures**
  - 24 hrs - IF with McAbs
  - 48 hrs - IF with McAbs
  - 7 days - watch for CPE
### FilmArray Respiratory Panel

**Viral**
- Adenovirus
- Bocavirus
- Coronavirus 229E
- Coronavirus HKU1
- Coronavirus OC43
- Coronavirus NL63
- Influenza A
- Influenza A H1
- Influenza A H1 2009
- Influenza A H3

**Bacterial**
- Influenza B
- Parainfluenza 1
- Parainfluenza 2
- Parainfluenza 3
- Parainfluenza 4
- Respiratory Syncytial Virus
- Rhinovirus/Enterovirus

**Bacterial**
- Bordetella pertussis
- Chlamydophila pneumoniae
- Mycoplasma pneumoniae

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**Viruses are listed in descending order of frequency as a cause of bronchiolitis.**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Type</th>
<th>Approximate Frequency</th>
<th>Seasonality in North America</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory syncytial virus</td>
<td>A and B</td>
<td>50-80%</td>
<td>November through April</td>
</tr>
<tr>
<td>Human rhinovirus</td>
<td>1, 2, 4, and 6, 100 others</td>
<td>5-10%</td>
<td>Peak early in spring and summer</td>
</tr>
</tbody>
</table>
| Parainfluenza virus      | Types 3, 7, and 19, related to types 1, 2, and 4 | 5-25%                | Type 3 in mid winter during spring, summer, and fall in cold
| Human metapneumovirus    | Salmonella A and B         | 5-10%                 | Late winter and early spring, season outbreaks peak 1-2 mo before RSV peak |
| Adenovirus               | 0, 4, 7, 22, and 37       | 5-10%                 | Year round, although season for certain subtypes may be more pronounced |
| Influenza virus          | A and B                   | 1-3%                  | November through April       |
| Enteroviruses            | Echo virus and coxsackievirus | 5-6%                   | Generally June through October |

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**Preventive Treatment**

- **Humanized Immuloglobulins (palivizumab)**
  - expensive, effective for high-risk infants

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*Viruses are listed in descending order of frequency as a cause of bronchiolitis.*
Palivizumab

- Mouse monoclonal antibody that binds RSV F protein
- Replace CDRs of human IgG with the CDRs from the gene encoding the mouse McAb to RSV

Analysis of RSV Hospitalizations

<table>
<thead>
<tr>
<th>Incidence of RSV</th>
<th>Placebo</th>
<th>Palivizumab</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalization</td>
<td>53/500 (10.6%)</td>
<td>48/1002 (4.8%)</td>
<td>55%</td>
</tr>
<tr>
<td>Premature Infants</td>
<td>19/234 (8.1%)</td>
<td>9/506 (1.8%)</td>
<td>78%</td>
</tr>
<tr>
<td>BPD infants</td>
<td>34/266 (12.8%)</td>
<td>39/496 (7.9%)</td>
<td>39%</td>
</tr>
</tbody>
</table>

Vaccines for RSV

- A killed virus vaccine developed in the 1960s. Vaccinate children had more severe disease after RSV infection!!!
- An epitope-focused vaccine design offers new hope!!
Palivizumab can neutralize virus and protect from disease

How can we exploit that knowledge to develop a vaccine?

Take home messages from this diagram:
1. RSV fusion protein comes in 2 forms: prefusion and postfusion
2. We can identify the sites where antibodies bind to these distinct structures
3. Antibodies made against the prefusion F protein are the best for neutralizing the virus (red and orange sites)
4. We need to immunize with a stable form of the prefusion RSV F to generate neutralizing antibodies
5. Nanoparticles with stabilized RSV F protein are currently in stage 3 clinical trials
Conclusions: RSV F protein nanoparticle vaccine induced increases in measures of functional immunity to RSV in older adults and demonstrated an acceptable safety profile. Adjuvanted formulations provided additional immunogenicity benefit as compared to increasing antigen dose alone.

Take home message for RSV

- RSV is a significant cause of bronchiolitis and pneumonia for infants, elderly and immunocompromised patients
- Currently no treatment or vaccine
- PRETREATMENT of infants with palivizumab reduces the severity of disease
- NEW vaccine (stabilized fusion protein in nanoparticles) is in phase III clinical trials
Physician: I have several patients that present with what looks like RSV, but the Clinical Micro Lab reports that the cultures are negative? What is going on????

Research response: Identification of a new virus – human metapneumovirus (hMPV) in 2001. This virus grows poorly in tissue culture (takes 10-14 days to show CPE), but can be detected using RT-PCR. This virus is another important respiratory pathogen.

Classification of Viral Pathogens of the Paramyxoviridae Family That Infect Humans

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  - Respirovirinae

- **Genus**
  - Morbillivirus
  - Rubulavirus
  - Parainfluenza types 1 and 3
  - Human metapneumovirus types A and B
  - Parainfluenza types 2 and 4
  - Respiratory syncytial virus types A and B
  - Mumps
  - Rhinovirus
  - Human metapneumovirus types A and B

- **Species**
  - Human metapneumovirus

- **Chest Radiograph Obtained in a Six-Month-Old Infant with Human Metapneumovirus Bronchiolitis.**
  - Hyperinflation and diffuse perihilar infiltrates are evident.
Take home message:

• Different viruses can cause similar clinical presentations, such as Respiratory Syncytial Virus and Metapneumovirus

• PCR based testing can help you and your patients understand if they are suffering from the same agent or a new one!

Patient: A grumpy 2-year-old toddler with little appetite has a sore throat, fever, hoarse voice, and coughs with the sound of a barking seal. A high-pitched noise (stridor) is heard on inhalation. Flaring of the nostrils indicates difficulty breathing.

Physician: This is consistent with Croup, caused by parainfluenza viruses (and coronavirus NL63).

Epidemiology of Parainfluenza Virus Infections

• Disease/Viral Factors
  • Large relatively unstable enveloped virion, easily inactivated
  • Contagion period precedes symptoms and may occur in the absence of symptoms
  • Host range is limited to humans
  • Reinfection later in life can occur

• Transmission
  • Inhalation of large droplet aerosols
Epidemiology of Parainfluenza Virus Infections (cont.)

- Who is at risk?
  - Children: mild disease, croup
  - Adults: reinfection with milder symptoms
- Geography/Season
  - Ubiquitous and worldwide
  - Seasonal
- Modes of Control
  - None

Disease Mechanisms of Parainfluenza Viruses

- Four types of viruses
- Infection is limited to respiratory tract; upper respiratory tract disease is most common, but significant disease can occur upon lower respiratory tract infection

Disease Mechanisms of Parainfluenza Viruses (cont.)

- Parainfluenza viruses are not systemic and do not cause viremia
- Diseases include coldlike symptoms, bronchitis (inflammation of bronchiol tubes), bronchiolitis (inflammation of bronchioles), croup (laryngotracheobronchitis)
- Infection induces protective immunity of short duration
Another Virus Implicated in Croup!

- Human coronavirus NL-63 also causes croup (virus identified in 2004, associated with croup in 2005).
- Diagnosis by RT-PCR (one of 16 pathogens on the film array)
- Currently, no FDA approved antiviral or vaccine.

### Table 1: Viruses Demoted to Nonpathogenic Status from Hospitalized Children with Bronchiolitis

<table>
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<th>Type</th>
<th>Approximate Frequency</th>
<th>Seasonality in North America</th>
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</thead>
<tbody>
<tr>
<td>Respiratory syncytial virus</td>
<td>A and B</td>
<td>20-80</td>
<td>November through April</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>Subgroups A and B</td>
<td>5-10</td>
<td>Late winter and early spring; seasonally peaks 1-2 mo after RSV peak</td>
</tr>
<tr>
<td>Parainfluenza virus</td>
<td>Type 1: most common, followed by types 2, 3, and 4</td>
<td>3-25</td>
<td>Type 3 is most prominent during spring, summer, and fall in addicted patients</td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>24 species</td>
<td>5-10</td>
<td>Year-round, although season for certain serotypes may be more restricted</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>A and B</td>
<td>1-5</td>
<td>November through April</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>B and C</td>
<td>5-10</td>
<td>Generally June through October</td>
</tr>
</tbody>
</table>

### Paramyxoviridae and Respiratory Infections

- Respiratory Infections - RSV and Paraflu
- Molecular Biology of Negative Strand Viruses
**Paramyxoviridae**

*The Important Players*

**Human Pathogen**

- Respiratory Syncytial Virus (RSV)
- Human metapneumovirus
- Parainfluenzaviruses 1-4
- Mumps
- Measles

*Paramyxoviridae: Negative strand RNA viruses that induce cell fusion*
Structural and nonstructural proteins

**Negative Stranded Viruses**

- Structural and nonstructural proteins

**Positive Stranded Viruses**

+ Structural and nonstructural proteins

The appearance of RSV in tissue culture demonstrates the characteristic pattern of syncytia. The syncytia result from the amalgamation of cells with the loss of cell borders, multinucleated cells and intracytoplasmic inclusions.

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**Distinguishing Properties of Paramyxoviruses**

<table>
<thead>
<tr>
<th></th>
<th>Paramyxovirus</th>
<th>Mumps</th>
<th>Measles</th>
<th>RSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusion protein</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hemagglutinin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Antigenic types</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Immunity</td>
<td>short-lived</td>
<td>life-long</td>
<td>life-long*</td>
<td>short-lived**</td>
</tr>
<tr>
<td>Genus</td>
<td>Parainfluenza</td>
<td>Parainfluenza</td>
<td>Measles</td>
<td>RSV</td>
</tr>
<tr>
<td>Vaccine</td>
<td>none</td>
<td>live-attenuated</td>
<td>live-attenuated</td>
<td>in development</td>
</tr>
</tbody>
</table>

*vaccine-induced immunity 
**disease is of lesser severity