The Current State of RSV Vaccine Development

2014 AAAAI Annual Meeting
4304: How Close Are We to Preventing Asthma by Vaccination?
San Diego, CA
Barney S. Graham, MD, PhD
March 3, 2014

Outline

• RSV epidemiology and pathogenesis
• History and challenges for RSV vaccine development
• F glycoprotein function, structure, and antigenic sites
• Vaccine antigen design and immunogenicity
• Considerations for vaccine development

Respiratory Syncytial Virus
Global RSV Disease Burden

- RSV kills more children <1 year than any other single pathogen except malaria

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RSV Disease Burden and Market Potential

- Leading cause of hospitalization in children under 3 years of age
- Leading cause of medically attended respiratory infection in children
- Cause of excess mortality in elderly similar to seasonal influenza
Challenges for a RSV Vaccine

- Young age of serious disease
- Multiple mechanisms to interfere with induction and effector function of Type 1 interferons
- Failure of natural immunity to protect against reinfection
- Difficult to boost responses in adults
- Legacy of vaccine-enhanced disease
  - Properties to Avoid
    - Antibodies with poor NT activity → Immune complex deposition
    - CD4+ Th2-biased response → Allergic inflammation

FI-RSV Vaccine-Enhanced Disease

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>n</th>
<th>Infected (%)</th>
<th>Hospitalized (%)</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI-RSV</td>
<td>31</td>
<td>20 (65)</td>
<td>16 (80)</td>
<td>2</td>
</tr>
<tr>
<td>FI-PIV-1</td>
<td>40</td>
<td>21 (53)</td>
<td>1 (5)</td>
<td>0</td>
</tr>
</tbody>
</table>

Kim et al. Am J Epidemiol 1969;89:422

Overview of RSV Vaccine Clinical Development

<table>
<thead>
<tr>
<th>Efficacious</th>
<th>None</th>
<th>Low/No Efficacy – Inappropriate Immune Response</th>
<th>Low/No Efficacy – No Immediate Safety Concerns</th>
<th>Efficacy unknown – Currently in Clinical Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>Subunit vaccine G protein – streptococcal conjugate</td>
<td>Subunit vaccine F glycoprotein in alum in adults</td>
<td>Live attenuated RSV nasally in children</td>
<td></td>
</tr>
<tr>
<td>Formalin-inactivated</td>
<td>Live virus vaccine delivered IM in children</td>
<td>BPIV-RSV live chimeric virus nasally</td>
<td>Post-Aucion F Rosettes</td>
<td></td>
</tr>
</tbody>
</table>

FI-PIV-1
Options for Vaccine Development

<table>
<thead>
<tr>
<th>Platforms</th>
<th>Antigens</th>
<th>Delivery</th>
<th>Target Populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live-attenuated</td>
<td>F</td>
<td>Respiratory tract</td>
<td>Neonate (&lt;2 mo)</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>Parenteral</td>
<td>Infants and children (&gt;6 mo)</td>
</tr>
<tr>
<td>Gene-based vectors</td>
<td>SH</td>
<td>Other mucosal site</td>
<td>Siblings and parents of neonates</td>
</tr>
<tr>
<td>Subunit or particle-based</td>
<td>Multiple</td>
<td></td>
<td>Young adult women</td>
</tr>
<tr>
<td>Whole-inactivated RSV</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RSV Vaccine Pipeline

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**RSV Genome Organization and Protein Functions**

- NS1 and NS2: Nonstructural proteins
- M2-1 and M2-2: Membrane spikes
- G: Envelope spikes
- F: Fusion protein
- P: Polymerase
- L: Nucleocapsid

**Organization of RSV F Glycoprotein**

- **F1** has a cysteine-rich domain and a relatively large interspace between the two heptad repeats
- Short cytoplasmic tail
- Type I integral membrane protein
- pH-independent class I viral fusion protein
- Two furin cleavage sites
- Two heptad repeat regions that form an anti-parallel six-helix bundle in post-fusion state
- **F2** has cysteine-rich domain and a relatively large interspace between the two heptad repeats

**Comparison of other Class I Fusion Proteins in Native Prefusion Conformation**

Mechanism of F-mediated membrane fusion

1. Receptor triggering
2. Fusion pore established
3. Delivery of nucleocapsid and genome

Antigenic sites present on post-fusion F are not major targets for *in vivo* NT activity

Stabilization of Prefusion RSV F with Human mAbs (D25/AM22) Reveals New Antigenic Site

Vaccine Antigen Selection: Rationale for Choosing F

Reasons for choosing F:
- Target of Synagis
- Higher sequence conservation than G
- Unlike G, F is absolutely required for virus entry

RSV F Has One Extremely Mobile Domain and Another that is Relatively Fixed
Antigenic Site Ø

Properties of Antigenic Site Ø mAbs

Antigenic Sites on RSV F Associated with Neutralization
Implications of Using Prefusion or Postfusion F

Antigenic Sites on RSV F Under Immune Pressure

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S155C/S290C mutations (DS) can form disulfide bond only in prefusion state

DS version of stabilized prefusion RSV F can be purified to homogeneity

DS version of prefusion RSV F induces potent neutralizing activity
DS does not stabilize antigenic site Ø

Design Approach for Stabilizing Antigenic Site Ø on Trimeric F

Alternative Method of Stabilization: Cavity-Filling
Combining Mutations Alters Physical Stability

<table>
<thead>
<tr>
<th></th>
<th>Cubic pH 9.5</th>
<th>Cubic pH 5.5</th>
<th>Tetragonal pH 5.5</th>
<th>Cubic pH 9.5</th>
<th>Cubic pH 5.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield</td>
<td>1.9</td>
<td>2.2</td>
<td>1.9</td>
<td>1.3</td>
<td>1.9</td>
</tr>
<tr>
<td>DS binding</td>
<td>0.29</td>
<td>0.23</td>
<td>0.15</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>10°C</td>
<td>0.3</td>
<td>0.8</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>pH</td>
<td>3.5</td>
<td>0.1</td>
<td>0.7</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Osmolality</td>
<td>10</td>
<td>3.0</td>
<td>1</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Freeze-thaw</td>
<td>X10</td>
<td>0.3</td>
<td>0.6</td>
<td>0.7</td>
<td>0.3</td>
</tr>
</tbody>
</table>

DS-Cav1 is More Physically Stable Than DS

Immunogenicity Achieved by Stabilization is Additive

1/7/2014
DS-Cav1 induces NT activity in NHP against both subtype A and B RSV

Matching Vaccine Platform to Target Population

<table>
<thead>
<tr>
<th>Target Population</th>
<th>Challenges</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate (&lt;2 mo)</td>
<td>Vaccine-enhanced disease</td>
<td>Gene-based vector +/- Subunit protein +/- Nanoparticle boost + Protein alone</td>
</tr>
<tr>
<td>Infants and children (&gt;6 mo)</td>
<td>Poor immunogenicity, Maternal antibody</td>
<td></td>
</tr>
<tr>
<td>* Sero-negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Siblings and parents of neonates</td>
<td>Repeated infections despite prior infections, Difficulty boosting pre-existing antibody</td>
<td>Subunit protein Nanoparticle + Gene-based vector prime</td>
</tr>
<tr>
<td>Young adult women</td>
<td>Pregnancy-related concerns</td>
<td></td>
</tr>
<tr>
<td>* Pregnant women + Women of child-bearing age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elderly (&gt;65 yr)</td>
<td>Poor immunogenicity, Difficulty efficacy endpoint</td>
<td></td>
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Candidate RSV Vaccine Products Based on Stabilized Prefusion F Trimer

- Stabilized prefusion F trimer
  - Pregnant women
  - Elderly

- Gorilla-derived rAd vector
  - Infants < 6 months
  - Seronegative children >6 months

Considerations for Immunizing RSV-Naïve Infants

- Opportunity to prevent or delay first RSV infection
  - Reduced primary morbidity
  - Reduced childhood wheezing

- Opportunity to establish future immune response patterns (antibody specificity and T cell phenotype)
  - Improved immunity against reinfection

- Target age is critical
  - Peak age of hospitalization ~2.5 mo
  - ~50% of hospitalization occur >6 mo
  - If incidence is ~60% in first year, ~70% are RSV-naïve at 6 mo

Selecting Target Age for Initiating Vaccination

<table>
<thead>
<tr>
<th>Efficacy</th>
<th>&lt;6 mo</th>
<th>&gt;6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatic mutation</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Dendritic cell and APC maturation</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Clearance of maternally-derived antibody</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>No longer breast-feeding</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

| Safety                                        |       |       |
| Idiosyncratic apnea and other rare adverse events | +     | -     |
| Small airway size                             | ++    | -     |
| Relative Th2 bias                             | ++    | -     |
Convergence of Technologies Has Produced a New Vaccine Development Paradigm

New Technologies Have Made an RSV Vaccine Possible

NIAID Vaccine Research Center

Viral Vaccines

Major Conceptual and Technological Advances

NIAID Vaccine Research Center

Viral Pathogenesis Laboratory

Structural Biology Section