Outline

• Review of RSV-induced pathology
• History of FI-RSV vaccine-enhanced disease
• What can animal models tell us?
• What can assays tell us?
• Reinterpretation of FI-RSV antigenicity
RSV Bronchiolitis

Ciliated bronchiolar epithelium

Type 1 alveolar pneumocyte

RSV causes airway obstruction

RSV in Immunosuppressed Child

Courtesy of John DeVincenzo
Neutrophils are the dominant cell in BAL, while mononuclear cells are most common in tissue.
History of FI-RSV Vaccine Enhanced Disease in Clinical Trials

<1966 – Live and inactivated RSV given parenterally without benefit

1966-7 – 4 independent studies using Pfizer lot 100 formalin-inactivated RSV did not protect and caused enhanced disease

>1967 - Live RSV IM, live-attenuated RSV IN given without harm

Subunit F (F+G+M, FG, F+G) and G (BBG2Na) given to adults and children with pre-existing immunity (2-3 fold rise in NT; >10-20 fold rise in ELISA titers)
Pfizer Lot 100 FI-RSV

- Bernett strain of RSV isolated in primary human embryonic kidney from throat swab at NIH, May 1961 and passaged X3
- Passaged X10 and produced in vervet monkey kidney cells
- 3 early (day 4) and 11 late (day 7) sublot harvests combined
- Clarified by Millipore SM filtration
- **72 hours at 36°C with formalin 1/4000 wt/vol**
- 50,000 rpm pellet and 25-fold concentration in EMEM in Earle’s salt solution
- Alum precipitation (4 mg/ml) (?aluminum potassium sulfate)
- Storage at 4°C with polymyxin, neomycin, streptomycin, and benzethonium preservatives
- IM delivery on a 0,1 or 0,1,2 or 0,1,4 month schedule
- Control was Parainfluenza I then trivalent Para 1,2,3
Chemistry of aldehyde fixation

Formaldehyde - Cross-links free amino groups with –CH2- or causes an amino alkylation of a carbonyl group resulting in a β-amino-carbonyl compound.

Glutaraldehyde – can react amine, thiol, phenol, and imidazole functional groups on amino acids, but preferentially reacts with ε-amino groups to cross-link lysine residues.

Paraformaldehyde – fixation similar to formaldehyde.
Papers reporting FI-RSV vaccine-enhanced disease (Am J Epidemiol 1969)

- Chin et al. (0,1 schedule; 4 mo. – 10 year; 43 <1 yr.)
  - Eosinophilia
  - CF response <6 mo (36%), 6-18 mo (55%), 2-9 yrs. (84%)
  - Higher rate and severity of illness in vaccinees (?higher virus load)

- Fulginiti et al. (0,1,2 schedule; 6 mo.-7 yrs.)
  - 7/51 (13.7%) vaccinees vs. 1/65 (1.5%) TPV recipients hospitalized
  - Of 19 with >4X CF responses only 9 had NT responses

- Kapikian et al. (0,1,2 schedule; 5-65 mo. of age; only 2 <1yr.)
  - 9/13 vaccinees with pneumonia vs. 4/47 nonvaccinated (5 & 0 hosp.)
  - Relatively high CF antibody and NT response, and no eosinophilia

- Kim et al. (0,1,4 schedule; 2-7 mo. of age)
  - Peribronchial monocytic infiltrate with excess of eosinophils
  - Relatively high CF antibody response in vaccinees
  - High lymphoproliferation index in vaccinees
# 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>

* 2 received 1 injection, 8 received 2 injections
Noted 21/54 infected in 1962/3 trial and 10/21 required hospitalization

** 14 and 16 mo. of age, 3 injections starting at 2 and 5 mo. of age, one had no CF or NT response, the other had CF=128 and NT=48. both had bacterial pneumonia complicating RSV

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>% 4-fold rise</th>
<th>Mean fold rise</th>
<th>Post RSV mean fold rise</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT with C’</td>
<td>23</td>
<td>43</td>
<td>2.6</td>
<td>21 (12 of 16)</td>
</tr>
<tr>
<td>CF</td>
<td>23</td>
<td>91</td>
<td>30</td>
<td>165 (15 of 16)*</td>
</tr>
</tbody>
</table>

* Paraflu recipients after natural infection mean fold rise = 71 (13 of 14)
Histopathology in FI-RSV Vaccine-Enhanced Disease
FI-RSV Immunization Results in a Discordance Between Functional and Binding Antibody

Murphy et al JCM 1986; 24:197 & 1988; 26:1595
Animal Models of RSV

- Rodents
  - Mice
    - immunocompromised
    - Pneumovirus of mice
  - Cotton rats
  - Guinea pigs

- Large animal
  - Bovine
  - Ovine

- Nonhuman primates
  - Chimpanzees
  - African Green monkeys
  - Baboons

- Other
  - Ferret
  - Chinchilla
Determinants of FI-RSV Vaccine Enhanced Illness in BALB/c Mice

Gob-5

Muc5ac

Mucus by PAS

Alveolitis


Johnson et al.
J Virol 2004; 78:8753
Potential Advantages of Cotton Rats Over Mice

• 3d old cotton rats >1 log$_{10}$ pfu more replication in nose than adults with delayed viral clearance
• More prominent neutrophil infiltrate
• Slightly more permissive than mice, particularly neonates
RSV Replication in Cotton Rat Lung and Nose

FI-RSV Enhanced Disease Endpoint
in Cotton Rats

Problems with Rodent Models

**Mice**
- Semi-permissive
- Relatively high body temperature
- Type I IFN not inhibited by NS1/2
- Large inoculum directly to lung
- BALB/c model characteristics based largely on two unusual T cells – a Vβ13.2 M2_{82-90} CD8 and a Vβ14 G_{184-198} CD4

**Cotton Rats**
- Semi-permissive
- Large inoculum directly to lung
- Does not exhibit illness
- Limited immunological assessment based on inbred strains and reagents
- Difficult to distinguish neutrophils from eosinophils

**Caveats** – 1) cannot assess histopathology in absence of viral replication, 2) cannot reinfect after primary infection
Bovine model of bRSV

Lung consolidation

Infection of bronchiolar epithelium

Bronchiolitis

Alveolitis

Caveats – 1) bRSV is not hRSV, 2) generally small group sizes in each experiment, 3) limited immunological reagents

Geraldine Taylor. Current Topics in Microbiology and Immunology 2013
Immune Response Patterns Associated with FI-RSV Vaccine-Enhanced Illness

Antibodies with poor NT activity
↓
Immune complex deposition

CD4+ Th2-biased response
↓
Allergic inflammation

[Image of histological slides labeled A and B with C4d and preimmune sera]


[Image of blot analysis for α-tubulin, IL-4, and IFN-γ]

Graham et al. JI 1993;151:2032
Class I Fusion Glycoproteins

- RSV F
- PIV F
- Influenza HA
- HIV-1 gp160
- Ebola GP
- CoV S

Regions of high mobility:
- 27
- 70
- 116
- 126
- 212
- 313
- 322
- 333
- 343
- 358
- 367
- 382
- 393
- 416
- 422
- 439
- 550
- 574

Antigenic sites:
- V
- II
- III
- I
- V
- IV
RSV F mediates viral entry
Location and Conformations of F

RSV budding from a cell

F on the viral envelope

Prefusion native form of F

Post-fusion inactive form of F

Liljeroos L et al. PNAS 2013;110:11133-11138
RSV buds as a filamentous particle from cholesterol-rich lipid raft microdomains
RSV F conformation changes as virus morphology evolves

Liljeroos L et al. PNAS 2013;110:11133-11138
RSV morphology and protein content
Prefusion F has unique sites of vulnerability to neutralizing antibodies.
Conclusions

• FI-RSV caused enhanced illness in children <24 mo. old
• Antibody response showed a much greater fold increase in complement-fixing than neutralizing or fusion-inhibiting activity (i.e. high ratio of binding to functional antibody)
• Animal models and original studies suggest a Th2-biased immune response was associated with enhanced disease
• Most NT-sensitive epitopes are on pre-F conformation
• Heating virus at 37°C for 72 hours causes F to flip into post-F conformation
NIAID Vaccine Research Center

Viral Pathogenesis Laboratory

Sung-Han Kim, Syed Moin, Barney Graham, Kaitlyn Morabito, Azad Kumar, Kevin Graepel, Kayvon Modjarrad, Man Chen, Tracy Ruckwardt, Allison Malloy, Jason McLellan, Jie Liu, Erez Bar-Haim, Joan Ngwuta, April Killikelly

Special thanks to Peter Collins

Structural Biology Section

Peter Kwong, Jason McLellan, Gordon Joyce, Guillaume Stewart-Jones, et al.