The Cotton Rat Model of Respiratory Viral Diseases

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Sigmovir Biosystems Inc.
Rockville, MD
**Sigmodon hispidus**

- Member of the family *cricetidae*
- *New world rodent*
- In many regions of southern United States most abundant wild rodent
- Natural host of several viruses (*i.e.*, Hantavirus, VEE virus, Arenaviruses)
- Inbred for >70 generations at SBI
THE EXPERIMENTAL TRANSMISSION OF POLIOMYELITIS TO THE EASTERN COTTON RAT, SIGMODOON HISPIDUS

By Charles Armstrong, Senior Surgeon, Division of Infectious Diseases, National Institute of Health, United States Public Health Service

Through the courtesy of Dr. Max Peet, of the Department of Surgery, University of Michigan, we received on August 28, 1937, a sample of brain and cord from an 18-year old boy, one of several bulbar cases of poliomyelitis which occurred at Lansing, Mich., during that summer. A strain of virus was recovered from the material which has now been through 15 monkey passages and which clinically, and pathologically as reported by Surgeon R. D. Lillie, is apparently a strain of poliomyelitis. Neutralization tests with this virus have not been done.

On November 8, 1937, several species of rodents, including a cotton rat received through the courtesy of Dr. A. Pachchanian, of the National Institute of Health, were inoculated with a fourth monkey passage of the virus. The cotton rat remained apparently well until the twenty-fifth day, when it appeared nervous and tremulous. On the following day it was paralyzed in both hind legs and was sacrificed.
• Polio epidemics in the U.S. in 1930’s
• Modeling paralytic disease in monkeys was impractical
• Search for a smaller animal model
• Dr. Charles Armstrong (NIH) dispatches several workers throughout the Southern United States to live-trap and bring back any small mammal adaptable to use in a laboratory setting
• Many different species were obtained and all animals were inoculated with polio virus from a human fatal case
• Of all the animals, only cotton rat *Sigmodon hispidus* developed paralysis
Important Infectious Diseases in the Cotton Rat

1937: Endemic typhus
1939: Polio (1, 2 & 3)
1940: *M. bovis*
1940: *C. diphtheriae*
1942: Epidemic typhus
1944: Filariasis
1967: *R. rickettsii*
1970: VEE
1971: RSV
1981: Parainfluenza (1, 2 & 3)
1984: Adenoviruses (2, 4, 5, 7, 8)
1985: HSV-1
1987: Lyme disease
1987: Influenza (A & B)
1992: Measles
1993: Venezuelan hemorrhagic fever
1995: Hantavirus
2002: Monkeypox
2004: hMPV
2006: HSV-2
Respiratory Syncytial Virus

• Leading cause of pneumonia and bronchiolitis in children <2 years and death in elderly and immunosuppressed groups.

• RSV vaccine is not available.

• FI-RSV failed vaccine trials in the 60’s (Lot 100 vaccine) impacted negatively in subsequent RSV vaccine development projects

• Immune-prophylaxis is available for selected population of infants.
RSV Infection in Cotton Rats

- **Viral replication**: peaks on d4 in the lung, clearance by d7. Slightly longer in the nose (peaks on d5, clearance by d9).
- **Permissiveness**: 100-fold more permissive and 10-fold more immunologically responsive than the mouse model.
- **Life-long susceptibility to RSV infection**.
- **Disease**: Primarily inflammatory, peaks at day 6 p.i. in the lung.
- **Viral mediation of host**: Rapid clearance, but only short-term immunity.
- **Antibody response**: RSV neutralizing antibody titers peak on day 9 post RSV infection.
- **Vaccine-enhanced disease** is induced by FI-RSV vaccination.
Primary RSV infection in Cotton Rats, Lung Pathology

Uninfected

Day 6 post-infection
RSV Infection, Reinfection, and FI-RSV vaccination in cotton rats

Pulmonary Viral Titer

Days Post-Infection

Log_{10} pfu/gram

Primary Infection
Secondary Infection
FI-RSV

Pulmonary Pathology

<table>
<thead>
<tr>
<th></th>
<th>Peribronchiolitis</th>
<th>Bronchitis</th>
<th>Alveolitis</th>
</tr>
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<tbody>
<tr>
<td>Primary</td>
<td>+++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Secondary</td>
<td>+++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>FI-RSV</td>
<td>++++</td>
<td>+++</td>
<td>++</td>
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</table>
Parallels to FI-RSV “Vaccine-Enhanced” Disease in the Cotton Rat (Lot 100)

• Alveolitis (primarily neutrophilic, eosinophilic and lymphocytic infiltrates) as the characteristic histopathological parameter seen in the fatalities of FI-RSV

• Inverse relationship between neutralizing antivirus titre and pathology.

• Exacerbated immuno response characterized by a cytokine/chemokine storm
FI-RSV Vaccination Results in Enhanced RSV-induced Histopathology
Alveolitis - a primary histopathology marker of FI-RSV vaccine-enhanced disease in Cotton Rat

Regular RSV Disease
Day 5 post-infection

FI-RSV Vaccine-enhanced Disease
Day 5 post-infection

Adapted from Boukhvalova et al., Vaccine 2006 24(23):5027-35
FI-RSV Augments the Cytokine/Chemokine Response to RSV Infection
Low Neutralizing Antibody Response during FI-RSV-enhanced Disease in Cotton Rats

Surrogates of RSV Vaccine Efficacy and Safety in Cotton Rats

- Viral titer in the lung and nose (plaque assay)
- Lung histopathology (direct comparison with Lot 100)
- Total anti-RSV IgG (ELISA)
- Neutralizing RSV antibodies (plaque reduction assay)
- Measurement of mucosal IgA (RSV Specific ELISA)
- Quantification of Antibody expressing cells (APCs) by ELISPOT
- Quantification of CD4 and CD8 primed cells (Spleen cell isolation and re-stimulation).
Monophosphoryl lipid A (MPL) Reverses Cytokine Storm Accompanying FI-RSV Vaccine-enhanced disease

SIGMOVIR BIOSYSTEMS, Inc.
CpG ODN/F-protein Vaccine disease enhancement

F protein

F protein + CpG ODN

Adapted from Prince et al., J Virol. 2003 December; 77(24): 13156–13160
Replication of RSV in the Presence of Neutralizing Antibodies

![Graph showing viral titer (PFU/g) vs. neutralizing antibody titer for lungs and nose. The graph includes data points for Ab-treated and control groups.]
Clinical Relevance of the Cotton Rat Model of RSV

- Correctly predicted **efficacy and dose** of polyclonal IgG in preventing RSV disease (RespiGam®).

- Correctly predicted **efficacy and dose** of monoclonal IgG in preventing RSV disease (Synagis®).

- Correctly predicted **lack of efficacy** of polyclonal or monoclonal IgG in *treating* RSV disease.

- Correctly predicted **blocking of parenteral live RSV** immunization by maternally-derived passive IgG.
## Therapy of RSV Disease

<table>
<thead>
<tr>
<th>Sponsor</th>
<th>Formulation</th>
<th>Route</th>
<th>Results</th>
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<tbody>
<tr>
<td>Viratek</td>
<td>Ribavirin®</td>
<td>aerosol</td>
<td>safe, little or no benefit</td>
</tr>
<tr>
<td>Sandoz</td>
<td>Generic IgG</td>
<td>i.v.</td>
<td>safe, no benefit</td>
</tr>
<tr>
<td>Sandoz</td>
<td>Generic IgG</td>
<td>aerosol</td>
<td>Phase I: safe, no benefit Sandoz</td>
</tr>
<tr>
<td>Sandoz</td>
<td>Generic IgG</td>
<td>aerosol</td>
<td>Phase II: safe, no benefit</td>
</tr>
<tr>
<td>MedImmune</td>
<td>RespiGam®</td>
<td>i.v.</td>
<td>High risk: safe, no benefit</td>
</tr>
<tr>
<td>MedImmune</td>
<td>RespiGam®</td>
<td>i.v.</td>
<td>Normal risk: safe, no benefit</td>
</tr>
<tr>
<td>SmithKline</td>
<td>MAb</td>
<td>i.m.</td>
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</tr>
<tr>
<td>MedImmune</td>
<td>Synagis®</td>
<td>i.m.</td>
<td>safe, reduced virus, no benefit</td>
</tr>
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</table>
Comparison of Anti-RSV Treatments in the Cotton Rat

**Viral Replication**

- IgG
- Triam
- Triam + IgG
- Untr

- Viral Titer (log_{10}, pfu/g)

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<th>Type of Treatment</th>
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<th>n=15</th>
<th>n=15</th>
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<tbody>
<tr>
<td>IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triam</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triam + IgG</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Untr</td>
<td></td>
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</table>

**Pathology**

- % of Total Affected

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<th>n=12</th>
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<tr>
<td>IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triamcinolone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triamcinolone + IgG</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Type of Treatment**

- Alveoli
- Bronchioles

SIGMOVIR BIOSYSTEMS, Inc.
Cotton Rat as the Model of RSV Disease in Highly Susceptible Cohorts:

Elderly
Immunosuppressed
Infants
Cotton Rat as the Model of RSV Disease in the Elderly

Aged cotton rats are incapable of efficient RSV clearance

Cotton Rat as the Model of RSV Disease in the Immunosuppressed

RSV persistence in immunosuppressed cotton rats

Adapted from Ottolini et al., Bone Marrow Transplant, 24: 41, 1999.
Cotton Rat as the Model of RSV Disease in the **Immunosuppressed**

RSV persistence in immunosuppressed cotton rats

Model of Effectiveness of **Immunotherapy**

Adapted from Ottolini et al., *Bone Marrow Transplant*, 24: 41, 1999.
Cotton Rat as the Model of RSV Disease in the Immunosuppressed

RSV persistence in immunosuppressed cotton rats
Model of Effectiveness of Immunotherapy

Adapted from Ottolini et al., Bone Marrow Transplant, 24: 41, 1999.
Cotton Rat as the Model of RSV Disease in Infants

Enhanced RSV replication in the upper respiratory tract

Cotton Rat as the Model of Antibody-mediated Immunosuppression in Infants

Presence of maternally derived antibodies at the time of RSV infection decreases the antibody response of human infants to RSV

(Murphy et al., 1986; Kimman et al., 1987)
Cotton Rat as the Model of Antibody-mediated Immunosuppression in Infants

RSV

-28

d0

birth

d0

d20

Immunize

Immunize

Viral Titer (log_{10}, pfu/g)

Naïve Mom

Immune Mom

C

RSVi.m.

d37

d20

Immunization (day postpartum)

SIGMOVIR BIOSYSTEMS, Inc.
Immunosuppressive Effect of Ab can be Modeled by Immune Serum Infusion Prior to Vaccination

- Immunize (live RSV i.m.)
- Anti-RSV serum
- RSV
- Immunized: -  +

Viral Titer (log_{10} pfu/g)

- control serum
- αRSV serum
Cotton Rat model of RSV Disease and Vaccine-enhancement

- Accurately recapitulates human RSV disease and vaccine-enhanced disease
- Accurately predicts efficacy and dose of RSV Ab prophylaxis
- Accurately predicts lack of efficacy of RSV Ab therapeutics
- Life-long susceptibility allows for long-term studies
- Special-need cohorts have been modeled (e.g., elderly, immunosuppressed, infants)
- Large number of reagents and assays available

RSV Drug / Vaccine Candidate

Effective and Safe

Not Effective OR Effective but Unsafe
The Cotton Rat Model of Influenza infection and disease
### The Cotton Rat Model of Influenza Infection

<table>
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<th>Type (Subtype)</th>
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<th>Strain</th>
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<td>B/HK/73</td>
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<tr>
<td></td>
<td>No</td>
<td>B/Sichuan/379/99</td>
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<td>B/HK/330/01</td>
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<tr>
<td>A (H1N1)</td>
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<td>A/New Caledonia/20/99</td>
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<td>No</td>
<td>A/California/04/09</td>
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<td></td>
<td>No</td>
<td>A/Netherland/09</td>
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<td>A/Brisbane</td>
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<td>A/Bayern/95</td>
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<td>No</td>
<td>A/Malaya/302/54</td>
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<td>A/PR/8/34 (not mouse adapted)</td>
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<tr>
<td>A (H3N2)</td>
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<td>X-31</td>
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<td>A/Wuhan/359/95</td>
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<td>A/Duck/HK/375</td>
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<td>A (H5N1)</td>
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<td>A/Vietnam/1203/04</td>
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<tr>
<td>A(H9N2)</td>
<td>No</td>
<td>A/Guinea Fowl/HK/WF10/99</td>
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<td>A(H9N2)</td>
<td>No</td>
<td>A/Guinea Fowl/HK/WF10/99</td>
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</table>
Sialic Acid Receptors in the Respiratory Tract of Cotton Rats

Trachea

- **SA_{α2,3}**
- **SA_{α2,6}**
- Tubuline

Lung

- **SA_{α2,3}**
- **SA_{α2,6}**
Replication and Clinical Signs of Influenza Infection in Cotton Rats

Dynamics of Influenza Replication in Cotton Rats is Similar to Influenza Replication in Humans

**Cotton Rats**

**Humans**


Cytokine Response to Influenza in the Cotton Rat Model

Cotton Rat Model of Influenza Vaccine: FluLaval (2006-2007)

**FluLaval:**
- d0  FluLaval i.m.
- d28 FluLaval i.m.
- d50  live H1N1 A/New Caledonia/20/99 10^6 TCID\(_{50}\) i.n.

**Live H1N1:**
- d0  live H1N1 A/New Caledonia/20/99 10^6 TCID\(_{50}\) i.n.
- d50  live H1N1 A/New Caledonia/20/99 10^6 TCID\(_{50}\) i.n.
Cotton Rat Model of Influenza Antivirals: Zanamivir

Zanamivir: twice daily, starting d-1

<table>
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<tr>
<th></th>
<th>Lung</th>
<th>Nose</th>
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<tr>
<td></td>
<td>Viral Titer (TCID_{50}/g)</td>
<td>Viral Titer (TCID_{50}/g)</td>
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<tr>
<td>day 1</td>
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<td></td>
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<tr>
<td></td>
<td>5.0</td>
<td>5.0</td>
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<tr>
<td>day 4</td>
<td></td>
<td>5.0</td>
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- **Lung**: Mock (black) and Zanamivir (grey)
- **Nose**: Mock (black) and Zanamivir (grey)
Cotton rats can be infected with unadapted human influenza isolates.

Dynamics of viral replication is rapid and resembles that seen in humans.

Heterosubtypic immunity has been modeled.

Has been successfully used to test Influenza antivirals against human influenza strain.

Has been successfully used to test Influenza vaccines against human influenza strain.

Large number of reagents and assays available.
Development of Cotton Rat Reagents

- cDNA for >270 cotton rat genes
- Sequences are immediately deposited in GenBank
- R&D Systems, Inc. expresses gene product, produces antibody
- Over 71 cotton rat reagents in the current R&D Systems online catalog
# Cotton Rat Genes and Reagents

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<tr>
<th>Cytokines:</th>
<th>Chemokines:</th>
<th>Cell surface molecules:</th>
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<tbody>
<tr>
<td>IFN-γ. (A, B, C, D)</td>
<td>MIP-1α. (A, B, C)</td>
<td>CCR5</td>
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<td>IFN-α. (A,B)</td>
<td>MIP-1β. (A, B, C)</td>
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<td>RANTES. (A, B)</td>
<td>CD4 (C)</td>
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<tr>
<td>IL-1 β. (A, B)</td>
<td>IP-10. (A, B)</td>
<td>CD8a (C)</td>
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<td>GRO/IL-8. (A, C)</td>
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<td>MCP-1/JE. (A,C)</td>
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<td>IL-10. (A, B, C)</td>
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<td>CD62L (L-selectin)</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>Mx1/Mx2</td>
<td>β-2 microglobulin</td>
</tr>
</tbody>
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A: recombinant protein; B: Polyclonal antibody; C: Monoclonal antibody; D: ELISA