The Cotton Rat Model of Respiratory Viral Infections Pathogenesis and Immunity

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**Sigmodon hispidus**

- Member of the family *cricidae*
- In many regions of southern United States most abundant wild rodent
- Natural host of several viruses (*i.e.*, Hantavirus, arenaviruses)

**Advantages as an animal model:**

- Much more permissive for most viruses than mice (for RSV more than 100 fold)
- Inbred
- Reagents are available
Development of Cotton Rat Commercial Reagents

- cDNA for >290 cotton rat genes
- Sequences are immediately deposited in GenBank, none are being patented
- R&D Systems, Inc. expresses gene product, produces antibody
- 71 cotton rat reagents in the current R&D Systems online catalog
# Cotton Rat Genes and Reagents
(Selected from a total of 290 cDNAs)

<table>
<thead>
<tr>
<th>Cytokines:</th>
<th>Chemokines:</th>
<th>Cell surface molecules:</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ (A, B, C, D)</td>
<td>MCP-5 analog</td>
<td>CCR5</td>
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<tr>
<td>IFN-α (A, B)</td>
<td>MIP-1α. (A, B, C, D)</td>
<td>CD3</td>
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<tr>
<td>IFN-β.</td>
<td>MIP-1β. (A, B, C)</td>
<td>CD4 (C)</td>
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<td>IL-1α. (A, B, C)</td>
<td>RANTES. (A, B)</td>
<td>CD8alpha (C)</td>
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<td>IL-1β. (A, B)</td>
<td>IP-10. (A, B)</td>
<td>CD11b</td>
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<td>IL-5</td>
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<td>CD62L (L-selectin)</td>
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<td>IL-12p40.</td>
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<td>CD74(MHC II)</td>
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<td>CD86(B7-2)</td>
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<td>IL-18</td>
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<td>Ly-6</td>
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<td>TNFα. (A, B, C, D)</td>
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<td>MHC I</td>
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<td>TNFβ.</td>
<td>IRF-2</td>
<td>MHC II A</td>
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<td>TGFβ1.</td>
<td>IRF-8 (ICSBP)</td>
<td>MHC II E</td>
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<td>GM-CSF</td>
<td>Cox-2</td>
<td>TLR-2</td>
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<td>Hsp70</td>
<td>β-2 microglobulin</td>
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<td>Mx1 and Mx2</td>
<td></td>
</tr>
</tbody>
</table>

* A: recombinant protein; B: Polyclonal antibody; C: Monoclonal antibody; D: ELISA

2/08
<table>
<thead>
<tr>
<th>Year</th>
<th>Disease</th>
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<tbody>
<tr>
<td>1937</td>
<td>Endemic typhus</td>
</tr>
<tr>
<td>1939</td>
<td>Polio (1, 2 &amp; 3)</td>
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<tr>
<td>1940</td>
<td>M. bovis</td>
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<tr>
<td>1940</td>
<td>C. diphtheriae</td>
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<tr>
<td>1942</td>
<td>Epidemic typhus</td>
</tr>
<tr>
<td>1944</td>
<td>Filariasis</td>
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<tr>
<td>1967</td>
<td>R. rickettsii</td>
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<tr>
<td>1970</td>
<td>VEE</td>
</tr>
<tr>
<td>1971</td>
<td>RSV</td>
</tr>
<tr>
<td>1981</td>
<td>Parainfluenza (1, 2 &amp; 3)</td>
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<tr>
<td>1984</td>
<td>Adenoviruses (2, 4, 5, 7, 8)</td>
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<td>1985</td>
<td>HSV-1</td>
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<tr>
<td>1987</td>
<td>Lyme disease</td>
</tr>
<tr>
<td>1987</td>
<td>Influenza (A &amp; B)</td>
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<tr>
<td>1992</td>
<td>Measles</td>
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<tr>
<td>1993</td>
<td>Venezuelan hemorrhagic fever</td>
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<tr>
<td>1995</td>
<td>Hantavirus</td>
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<tr>
<td>2002</td>
<td>Monkeypox</td>
</tr>
<tr>
<td>2004</td>
<td>hMPV</td>
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<tr>
<td>2006</td>
<td>HSV-2</td>
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</table>
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
Cotton Rat Model of Respiratory Viral Diseases

Semi-permissive

(histopathology and output virus proportional to input virus)

Tissue tropism (lungs and nose)

No species adaptation is required

Functional Mx system

- **antiviral** type-I-interferon-inducible proteins
- important component of **innate** antiviral defense in humans
- Mx genes are **defective** in common laboratory strains of **mice**
  - lack of appropriate innate response
- Mx proteins are rapidly induced in infected cotton rats.
RSV
Respiratory Syncytial Virus
RSV in cotton rats:

Peak pulmonary replication: day 4, clearance by day 7

Disease is primarily inflammatory

Only short-term immunity

Most important parallels to human disease:

1. Antibody efficacy: Predicted efficacy and dose of RespiGam® and Synagis® in preventing RSV disease

2. Vaccine-induced immunopathology
LOT 100 TRIAL (1965)

Formulation:
- Formalin-Inactivated (FI-RSV)
- Alum-Precipitated, 100X Concentrated

Results:
- 16-fold higher hospitalization rate in RSV-infected Lot 100 vaccinees than in RSV-infected controls
- Two RSV-infected Lot 100 vaccinees died
- Lung Pathology: Characterized by alveolitis, or cellular infiltrates in alveolar spaces
Alveolitis is the Primary Marker of FI-RSV Vaccine-enhanced Disease in Cotton Rats
Monophosphoryl lipid A (MPL) reverses FI-RSV vaccine-enhanced disease histologic marker

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Boukhvalova et al., Vaccine 2006 24(23):5027-35
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Monophosphoryl lipid A (MPL) reverses FI-RSV vaccine-enhanced disease histologic marker

Boukhvalova et al., Vaccine 2006 24(23):5027-35
Real-time RT-PCR analysis of RSV replication in vivo
Abortive replication in the re-infection model

Infectious Virus Release
Viral Genome Replication

Effect of Aging on Cotton Rat Responses to RSV

Delayed viral clearance from the lungs of aged cotton rats:

Effect of Aging on Cytokine Responses

Boukhvalova et al., J Infect Dis 2007 195(4):511-8
Effect of Aging on Cytokine Responses

Boukhvalova et al., J Infect Dis 2007 195(4):511-8
Effect of Aging on Cytokine Responses

Boukhvalova et al., J Infect Dis 2007 195(4):511-8
Effect of Aging on Cytokine Responses

hMPV
Human Metapneumovirus
**hMPV:**

Newly-discovered paramyxovirus (2001)

More severe disease in infants, immunocompromized and elderly

Only short-term immunity

**hMPV in cotton rats:**

Peak pulmonary replication: day 5, clearance by day 12

Disease is primarily inflammatory

Vaccine-enhanced disease
Vaccine-enhanced disease in FI-hMPV-immunized cotton rats

Day 4  Day 7  Day 10

Primary Infection

Re-infection

FI-hMPV

mock

Yim et al., Vaccine 2007 25(27):5034-40
**FI-hMPV vaccine-enhancement is associated with Th2/Th1 imbalance**

Yim et al., *Vaccine* 2007 25(27):5034-40
Influenza
Influenza in cotton rats:

Infection is possible using **unadapted** influenza viruses.

Replication is rapid, inflammatory response lingers after viral clearance from the lungs.

Rapid activation of antiviral Mx response.

Abundant pulmonary cytokine response.

Heterosubtypic immunity.
# Replication of influenza viruses in cotton rats

<table>
<thead>
<tr>
<th>Type (Subtype)</th>
<th>Adaptation</th>
<th>Strain</th>
<th>Lung Titer&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Nose Titer</th>
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</thead>
<tbody>
<tr>
<td>B</td>
<td></td>
<td>B/HK/73</td>
<td>3.6</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B/Sichuan/379/99</td>
<td>5.2</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B/HK/330/01</td>
<td>5.5</td>
<td>5.2</td>
</tr>
<tr>
<td>A (H1N1)</td>
<td>Mouse Tissue culture</td>
<td>A/PR/8/34</td>
<td>N/D</td>
<td>4.7</td>
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<tr>
<td></td>
<td></td>
<td>A/PR/8/34</td>
<td>6.3</td>
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<td>A/Malaya/302/54</td>
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<td>6.3</td>
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<tr>
<td>A (H3N2)</td>
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<td>X-31</td>
<td>4.6</td>
<td>6.2</td>
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<tr>
<td></td>
<td></td>
<td>A/Wuhan/359/95</td>
<td>3.3</td>
<td>6.4</td>
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<sup>a</sup> Log<sub>10</sub> TCID<sub>50</sub>/g

# Replication of influenza viruses in cotton rats

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<tr>
<td>A (H1N1)</td>
<td>Mouse</td>
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<td>Tissue</td>
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\(^a\) Log\(_{10}\) TCID\(_{50}\)/g

Viral replication and clinical signs of influenza A infection in cotton rats

Induction of Mx response during influenza A infection in cotton rats

Pathology of influenza A infection in the cotton rat model

Heterosubtypic immunity to influenza in the cotton rat model

Viral Titer, Log₁₀ TCID₅₀/g

**Nose**
- **Wuhan/Wuhan**
- **None/Wuhan**
- **PR8/Wuhan**

**Lung**
- **Wuhan/Wuhan**
- **None/Wuhan**
- **PR8/Wuhan**

Time post-infection:
- day 1
- day 2
- day 4
- day 7

Heterosubtypic immunity to influenza in the cotton rat model

Advantages of the Cotton Rat Model

- Highly permissive to infection with unadapted human respiratory viruses, including unadapted influenza strains
- Carries functional Mx system
- Displays strong effect of aging on antiviral responses
- Reflects human FI-RSV vaccine-enhanced disease and predicts efficacy and safety of candidate vaccines
- Allows analysis into the mechanisms of vaccine-enhancement and adjuvant action.
- Predicts vaccine-enhanced disease for FI-hMPV
- Displays heterosubtypic immunity to influenza
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Dolores Harrigan
Tim Straight
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Gregory Prince
Jorge Blanco

David Porter