The cotton rat as a model to study Respiratory Syncytial Virus (RSV) 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

Disadvantage:

- Lack of reagents
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
Important Infectious Diseases in the Cotton Rat

- 1937: Endemic typhus
- 1939: Polio (1, 2 & 3)
- 1940: *M. bovis*
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- 1944: Filariasis
- 1967: *R. rickettsii*
- 1970: VEE
- 1971: RSV
- 1981: Parainfluenza (1, 2 & 3)
- 1984: Adenoviruses (2, 4, 5, 7, 8)
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Common Characteristics of the Cotton Rat as a Model of Respiratory Viral Diseases:

- Semi-permissive (histopathology and output virus proportional to input virus)
- Tissue tropism (lungs and nose)
- No species adaptation necessary
Levels of Study in Cotton Rats

- Quantitative virology
- Serology
- Histopathology
- (Molecular)
Development of Cotton Rat Commercial Reagents

- cDNA for >270 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
Cytokines:
IFN-γ (A, B, C, D)
IFN-α (A, B)
IFN-β
IL-1α (A, B, C)
IL-1β (A, B)
IL-2 (A, B, C, D)
IL-4 (A, B, C, D)
IL-5
IL-6 (A, B, C)
IL-9
IL-10 (A, B, C)
IL-12 p40
IL-12 p35
IL-13
IL-18
TNF-α (A, B, C, D)
TNF-β
TGFβ1
GM-CSF

Chemokines:
MCP-5 analog
MIP-1α (A, B, C)
MIP-1β (A, B, C)
RANTES (A, B)
IP-10 (A, B)
GRO/IL-8 (A, C)
MIP-2/IL-8 (A, C).
MCP-1/JE (A, C)

Housekeeping genes:
β-actin
GAPDH
18S rRNA

Cell surface molecules:
CCR5
CD3
CD4 (B)
CD8 alpha
CD11b
CD14
CD16
CD18
CD25
CD45/B220
CD62L (L-selectin)
CD74 (MHC II)
CD83 (HB15)
CD86 (B7-2)
Ly-6
MHC I
MHC II A
MHC II E
β-2 microglobulin

Other genes:
IRF-2
IRF-8 (ICSBP)
Cox-2
Hsp70
Mx1 and Mx2
## Addtional Cotton Rat Genes
### Fully or Partially cloned

**Ribosomal proteins:**
- Elongation factor 1-a
- Ribosomal proteins L13, L30, L31, S16

**Structural proteins:**
- Alpha I type III collagen
- Alpha globin chain
- Anexin A2
- Beta gamma crystalline-like protein
- Calreticulin
- Cofilin 1
- Ficolin A
- Granulin (Grn)
- Gravin (PRKA anchor protein)
- Pro-alpha-1 (V) collagen
- Vimentin

**Ribonuclear proteins:**
- Heterogeneous nuclear ribonucleoprotein A/B (Hnrpab)
- Small nuclear RNA auxiliary factor (RNU2)

**Transcription factors:**
- Activating transcription factor-1 (ATF-1)
- IRF-1
- Max transcription factor
- p53 associated protein (mdm2)
- PHD finger protein 3 (PHF3)

**Cytokines/chemokines:**
- IL-15
- IL-23
- Eotaxin (SCYA-11)
- MCP-5
- MDC (SCYA-22)
- TARC (SCYA-17)
- G-CSF
- M-CSF
- TGFβ2
- VEGF

**Membrane assoc. proteins:**
- Atopy-related autoantigen
- CCR3
- CXCR4
- CX3CR
- CD11α and β
- CD28
- CD36 Scavenger receptor
- CD45/B220
- CD80
- CD132
- CD161
- CD206 Mannose Receptor
- IL-23 receptor
- aquaporin 9 (Aqp9)
- Prostaglandin E receptor
- TLR1, 2, 3, 4, 5, 7 and 10
- TREM-1

**Enzymes:**
- 4-amino butyrate aminotransferase
- Cyclooxygenase 1 (COX-1)
- Cytochrome C oxidase subunit III
- Farnesyl transferase
- Glutamine synthetase
- 3-hydroxyisobutyrate dehydrogenase
- i-nitric Oxide synthetase (i-NOS)
- Macrophage metalloelastase
- NADH dehydrogenase subunits 2 and 3
- Sphingolipid hydrolase glycoprotein
- Superoxide dismutase, mitochondrial
- Ubiquitin-conjugating enzyme 7 (Ubce7)
- Arginase I

**Other proteins:**
- Adrenomedullin
- Cell cycle checkpoint protein (CHFR)
- Complement protein C4
- Dystroglycan
- IgE heavy chain
- Protein kinase C
- Protein tyrosin phosphatase
- RAB7 (Ras oncogene family member)
- Ras suppressor protein 1
- Serine protease inhibitor 3
- Surfactant protein C
- Thrombospodin
- Thymopoietin
Cotton Rat: the “gold standard” model of human RSV infection
RSV in humans:

Most common cause of severe lower respiratory tract infections in infants and young children

A cause of severe lower respiratory tract infections in the elderly and immunocompromised individuals

Does not induce long-term immunity

History of formalin-inactivated RSV vaccine-enhancement

The only effective intervention: prophylaxis with anti-RSV antibodies RespiGam® and Synagis®
RSV in cotton rats:

Infects both upper and lower respiratory tract

Peak pulmonary replication: day 4, clearance by day 7

Disease is primarily inflammatory

Only short-term immunity

Most important parallels to human disease:
  - Antibody efficacy
  - Vaccine-induced immunopathology
Cotton Rat as a Model of RSV Passive Prophylaxis
## Prophylactic Effect of Anti-RSV Antibody in Cotton Rats

<table>
<thead>
<tr>
<th>Neutralizing Antibody Titer</th>
<th>Viral Titer (PFU/Gram)</th>
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<tbody>
<tr>
<td>Ab-treated</td>
<td>Lungs</td>
</tr>
<tr>
<td></td>
<td>Nose</td>
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<tr>
<td>10</td>
<td>20</td>
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<tr>
<td>100</td>
<td>1000</td>
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<td>1000</td>
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</tbody>
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**Legend:**
- **Ab-treated**
- **Control**
The Results

RespiGam®
Synagis®
The Cotton Rat Model of RSV:

- Correctly predicted **efficacy and dose** of RespiGam® and Synagis® in preventing RSV disease.
- Correctly predicted **lack of efficacy** of polyclonal or monoclonal IgG in *treating* RSV disease.
Cotton Rat as a Model of Formalin-inactivated RSV Vaccine-enhanced Disease
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 (78% vs. 5%)
- Two RSV-infected Lot 100 vaccinees died
FI-RSV Vaccine-Enhanced Disease

In mice (BALB/c):
- Lung Pathology: Characterized by pulmonary eosinophilia
- Augmentation of the Th2-type response

In humans:
- Lung Pathology: Characterized by alveolitis, or cellular infiltrates in alveolar spaces
- Infiltrating cells: neutrophils and lymphocytes
Alveolitis is the Primary Marker of FI-RSV Vaccine-enhanced Disease in Cotton Rats
Alveolitis is the Primary Marker of FI-RSV Vaccine-enhanced Disease in Cotton Rats

Prince et al., Lab Investig 1999 79(11):1385-92
Monophosphoryl lipid A (MPL) reverses FI-RSV vaccine-enhanced disease histologic marker

Cotton Rat Model of Vaccine-Enhanced Disease: Effect of Adjuvants (i.e., monophosphoryl lipid A, MPL)

Collect lung samples for mRNA analysis:

- 0, 6h, 12h, d1, d2, d4, d5, d7, 7 wk

FI-RSV or FI-RSV + MPL

Boukhvalova et al., Vaccine 2006 24(23):5027-35
Cotton Rat Model of Vaccine-Enhanced Disease
Effect of Adjuvants (i.e., monophosphoryl lipid A, MPL)

Collect lung samples for mRNA analysis:

0  6h  12h  d1  d2  d4  d5  d7  7 wk

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d0  d21

↑  ↑

FI-RSV

or

FI-RSV + MPL

Controls:

Mock  RSV

Boukhvalova et al., Vaccine 2006 24(23):5027-35
Conclusions:

Vaccine-enhanced disease is associated with an increase in not only Th2-type cytokines, but also Th1-type cytokines and chemokines.

Inclusion of a surrogate TLR4 agonist, MPL, with FI-RSV mitigates vaccine-enhanced disease by blunting both Th1- and Th2-type cytokine and chemokine responses.

Pro-inflammatory Th1-type cytokines and chemokines elaborated during early vaccine-enhanced disease may contribute to the development of pulmonary pathology.

Boukhvalova et al., Vaccine 2006 24(23):5027-35
Advantages of the Cotton Rat Model

- Correctly predicted **efficacy and dose** of RespiGam® and Synagis® in preventing RSV disease.

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

- Reflects histopathology of human FI-RSV vaccine-enhanced disease and currently **predicts efficacy and safety** of potential candidate vaccines.

- Allows analysis into the **mechanisms** of vaccine-enhancement and **adjuvant modification** of vaccine-enhanced disease.
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