Nonhuman primate models of aerosol exposure to alphaviruses

Douglas S. Reed
Center for Aerobiological Sciences, USAMRIID

Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

The research described herein was sponsored by the U.S. Army Medical Research and Materiel Command Project No. 02-4-HH-078.
Nonhuman primate models Nonhuman primate models of aerosol exposure to alphaviruses

See also ADM001849, 2004 Scientific Conference on Chemical and Biological Defense Research. Held in Hunt Valley, Maryland on 15-17 November 2004., The original document contains color images.
Alphaviruses

- Small (11.4 kb), positive-stranded RNA viruses
- Venezuelan (VEEV), western (WEEV), and eastern (EEEV) equine encephalitis viruses are naturally transmitted by mosquitoes, but are also highly infectious by aerosol.
- By mosquito, disease caused by VEEV and WEEV is incapacitating but rarely lethal.
- EEEV outbreaks are rare but have a high mortality (>30%) and a large percentage of survivors have long-term neurological complications.
- There are no licensed vaccines for humans. Current IND vaccines have problems with reactogenicity and poor immunological response.
Aerosol exposure to alphaviruses

- Pathogenesis studies in animal models have shown that after natural infection, viremia leads to infection of the olfactory region and up through the olfactory nerves to the brain, causing encephalitis.

- Removing olfactory bulbs prevents or delays onset of encephalitis after subcutaneous inoculation of alphaviruses.

- Aerosol exposure may bypass the need for viremia because of direct infection of the olfactory region.
Rodent models

- **Mice (VEEV, WEEV, EEEV)**
  By far the most used. Lethal infection; cause of death is viral encephalitis.

- **Guinea pigs (EEEV)**
  Fatal viral encephalitis after aerosol exposure (Roy et al, submitted)

- **Hamsters (VEEV, EEEV)**
  For VEEV infection, cause of death is endotoxic shock caused by sloughing of the intestinal wall. EEEV model was recently reported, cause of death was fatal encephalitis with vascular complications.
Nonhuman primate models

- Rhesus & cynomolgus macaques have been used for studies with VEEV. Rhesus were used in the 1930s for studies with WEEV and EEEV (no publications since 1939).

- In 1998, Pratt et al (Vaccine 16:1056-64) at USAMRIID used radiotelemetry devices to monitor fever after aerosol exposure of cynomolgus macaques to a virulent epizootic strain of VEEV.

- In 2004, Reed et al (JID 189:1013-7) at USAMRIID reported that enzootic strains could cause illness equivalent to epizootic strains in cynomolgus macaques exposed by the aerosol route.
Aerosol Exposure of Nonhuman Primates (NHP)

1 µm particle – deep lung

Whole-body plethysmograph done prior to exposure

Dose confirmed by plaque assays from starting concentrations and aerosol sample collection
Instead of data collected once a day on an anesthetized monkey, physiological changes can be monitored continuously or intermittently at set intervals.

Center for Aerobiological Sciences
Fever after exposure to epizootic VEEV-IA/B

- Tmax = 3.3°C
- Duration = 6 days
- Fever-hrs = 217.8

Naive macaque aerosol exposed to 10^8 pfu of epizootic VEEV-IA/B

Center for Aerobiological Sciences
Similar fever responses to VEEV-IE

Naive macaque aerosol exposed to $10^8$ pfu enzootic VEEV-IE

Days Postchallenge

Tmax = 4.4°C
Duration = 5 days
Fever-hours = 238.4
Delayed fever with VEEV-III A

Naive macaque aerosol exposed to $10^7$ pfu

enzootic VEEV-III A

Center for Aerobiological Sciences

Days Postchallenge

Tmax = 4.0°C
Duration = 6 days
Fever-hours = 253.4
Fever response to WEEV

Naive macaque aerosol exposed to $10^7$ pfu WEE

Tmax = 3.9°C
Duration = 5 days
Fever-hours = 187.7
Changes in peripheral blood leukocytes

- Increase in neutrophils & monocytes
- Lymphopenia
Signs of encephalitis after aerosol exposure to WEEV

Clinical Score is the sum of:

1) Neurological signs
2) Activity
3) Behavior
4) Response to stimuli

Each scored as 5 = normal

Animals that develop signs of encephalitis can survive infection
Predicting outcome after exposure to WEEV

- Fever: $r = -0.87$
- Glucose: $r = -0.84$
- Granulocytes: $r = -0.66$

$n = 12$
Pathology in the CNS from aerosol exposure to WEEV

Viral antigen in Purkinje cells, cerebellum, rhesus macaque

Photo courtesy of Dr. Catherine Wilhelmsen

CNS, gray matter. Note the marked immunopositive tissue (brown staining) in this focal area of inflammation, increased cellularity of the neuropil, and apoptotic bodies. Immunoperoxidase stain, 10X & 4X.

Photo courtesy of Dr. Tom Larsen

To date, virus has only been found in the brain & CNS of euthanized macaques

Center for Aerobiological Sciences
CNS, gray matter. Comparative slide demonstrating normal on the left and WEE-infected tissue on the right. Note the increased cellularity on the infected animal, the marked perivascular cellular infiltrate, and vacuolization of the neuropil. Cynomolgus macaque, H&E, 10X.
Pathology from aerosol exposure to EEEV

**Corpus striatum, cerebrum, 40x.**
Note the prominent viral antigen staining in the gray matter of the brain.

**Hippocampus, 10x.**
Widespread antigen staining demonstrating viral antigen in neurons, their processes and astrocytes.

*Photos courtesy of Dr. Jo Lynne Raymond*
## Summary of NHP Response to Alphaviruses

<table>
<thead>
<tr>
<th></th>
<th>VEEV</th>
<th></th>
<th></th>
<th>WEEV</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IA/B</td>
<td>IE</td>
<td>IIIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Viremia</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>IP</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>IP</td>
</tr>
<tr>
<td>Leukocytosis</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+++</td>
<td>IP</td>
</tr>
<tr>
<td>Serum glucose</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>++</td>
<td>IP</td>
</tr>
<tr>
<td>Encephalitis*</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

* -determined by sum of changes in neurological signs, activity, behavior and stimuli response

ND = not determined; IP = in progress
Summary/Conclusions

- Cynomolgus macaques are a useful model of the human disease caused by aerosol exposure to alphaviruses.
- Aerosol exposure to enzootic VEEV caused disease in NHP.
- The duration & severity of the febrile response are comparable to epizootic strains of VEEV.
- Aerosol exposure to WEEV results in a more severe disease (in terms of fever, encephalitis and lethality) than VEEV in NHP.
- Outcome from exposure to WEEV correlates with increases in serum glucose levels, neutrophilia, and fever severity.
- EEEV is lethal by aerosol; pathology indicates a far more severe infection of the brain than is seen with WEEV.
Acknowledgements

Aerobiology
Matt Lackemeyer
Chad Roy
Louise Pitt

Vet Med
Pedro Rico
Keith Esham
Heather Esham
Scott Gamble

Virology
Bill Pratt
Mike Parker
Cathy Lind
Mary Kate Hart

Pathology
Dr. Tom Larsen
Dr. Jo Lynne Raymond

DSD
Larry Sullivan

Safety
Dr. Catherine Wilhelmsen