Tick-Virus-Host Interface: Nidus of Powassan virus Transmission

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Presentation Outline

• Introduction

• Powassan virus (POWV)

• Part 1: Tick-virus-host interface

• Part 2: Impact of tick saliva on POWV transmission and disease outcome

• Other Tick-borne Diseases research in my lab
To develop novel strategies to control tick-borne virus infections
Powassan virus, Tick-borne encephalitis virus, Heartland virus, Bourbon virus
• Development of novel methods to control Powassan virus infections.
  • salivary immunogens and small RNA molecules.

• Co-infections: Effect of co-infection on the clinical outcome of Lyme disease and Powassan encephalitis.

• Powassan virus Vs Deer tick virus: Comparative Neuropathogenesis.

• Heartland virus: Development of tick transmission model.

• Bourbon virus: Small animal model development.

• Ticks and tick-borne pathogen surveillance in Texas.
## Tick borne diseases in the US

<table>
<thead>
<tr>
<th></th>
<th>Disease</th>
<th>Causative agent(s)</th>
<th>Primary vector(s)</th>
<th>Primary geographical distribution of ticks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anaplasmosis</td>
<td><em>Anaplasma phagocytophilum</em></td>
<td><em>Ixodes scapularis, Ixodes pacificus</em></td>
<td>Northeastern and upper Midwestern U.S., Pacific coast</td>
</tr>
<tr>
<td>2</td>
<td>Babesiosis</td>
<td><em>Babesia microti</em></td>
<td><em>Ixodes scapularis</em></td>
<td>North-east and upper Midwest</td>
</tr>
<tr>
<td>3</td>
<td><em>Borrelia miyamotoi infection</em></td>
<td><em>Borrelia miyamotoi</em></td>
<td><em>Ixodes scapularis</em></td>
<td>Northeastern and upper Midwestern U.S.</td>
</tr>
<tr>
<td>4</td>
<td><em>Bourbon virus infection</em></td>
<td><em>Bourbon virus</em></td>
<td>Not yet identified</td>
<td>Kansas</td>
</tr>
<tr>
<td>5</td>
<td>Colorado tick fever</td>
<td><em>Cottivirus</em></td>
<td><em>Dermacentor andersoni</em></td>
<td>Western North America</td>
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<tr>
<td>6</td>
<td>Ehrlichiosis</td>
<td><em>EMLA</em></td>
<td><em>Ixodes scapularis</em></td>
<td>South-central and Eastern U.S.</td>
</tr>
<tr>
<td>7</td>
<td>Heartland virus infection</td>
<td><em>Heartland virus</em></td>
<td><em>Amblyomma americanum</em></td>
<td>South-central and Eastern U.S.</td>
</tr>
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<td>8</td>
<td>Lyme disease</td>
<td><em>Borrelia burgdorferi</em></td>
<td><em>Ixodes scapularis, Ixodes pacificus</em></td>
<td>Northeastern U.S. and upper Midwestern U.S., Pacific coast</td>
</tr>
<tr>
<td>9</td>
<td>Powassan encephalitis</td>
<td><em>Powassan virus</em></td>
<td><em>Ixodes scapularis, Ixodes cookei</em></td>
<td>Northeastern states and the Great Lakes region</td>
</tr>
<tr>
<td>10</td>
<td>Rocky Mountain spotted fever (RMSF)</td>
<td><em>Rickettsia sp.</em></td>
<td><em>Dermacentor variabilis, Rhipicephalus sanguineus</em></td>
<td>Throughout the U.S.</td>
</tr>
<tr>
<td>11</td>
<td>STARI (Southern tick-associated rash illness)</td>
<td><em>Tick bites</em></td>
<td><em>Amblyomma americanum</em></td>
<td>Southeastern and eastern U.S.</td>
</tr>
<tr>
<td>12</td>
<td>Tickborne relapsing fever</td>
<td><em>Borrelia</em></td>
<td>Ornithodoros spp.</td>
<td>Throughout the U.S.</td>
</tr>
<tr>
<td>13</td>
<td>Human monocytic ehrlichiosis</td>
<td><em>Ehrlichia chaffeensis</em></td>
<td><em>Amblyomma americanum</em></td>
<td>Southeastern and eastern U.S.</td>
</tr>
<tr>
<td>14</td>
<td>Tularemia</td>
<td><em>Francisella tularensis</em></td>
<td><em>Dermacentor variabilis, Dermacentor andersoni, Amblyomma americanum</em></td>
<td>Throughout the U.S.</td>
</tr>
</tbody>
</table>
How ticks are successful in transmitting variety of pathogens?

- Ticks inhabit almost every continent, with the number of species worldwide topping 850.

- Ticks have proven resilient and persistent in the environment, and the fossil records suggest that they originated 65–146 million years ago.

- Disruption of ecosystems by humans brings closer interaction with animals (at many interfaces) creating opportunities for zoonotic diseases transmission.

- Multiple life stages that require blood feeding. Constant contact with animals (multiple species).

- Egg laying potential (several thousands)

- Tick saliva
Powassan virus (POWV)

- First isolated in 1958 from the brain of a young boy who died of encephalitis in Powassan, Ontario.

- Tick borne flavivirus, closely related to West Nile Virus.

- One of the two North-American members of the *Tick-borne encephalitis virus* (TBEV) serological complex of flaviviruses.

- Two strains:
  - Lineage I, Prototype virus (POWV)
  - Lineage II, Deer tick virus
Clinical manifestations

• POWV can infect the CNS and cause encephalitis, meningoencephalitis, and aseptic meningitis

• Symptoms can include fever, headache, vomiting, weakness, confusion, loss of coordination, speech difficulties, and seizures

• POWV can cause fatal neuroinvasive disease in 10-15% of cases.

• Long-lasting neurological sequelae (headaches, muscle wasting and memory problems) have been documented in 50% of survivors

• Some infections may cause only febrile illness or be asymptomatic
Maximum clade credibility tree of the tick-borne flaviviruses.
• POWV is maintained in a cycle between ticks and small-to-medium-sized rodents.

• In North America, three main enzootic cycles occur: *Ixodes cookei* and woodchucks, *Ixodes marxi* and squirrels, and *Ixodes scapularis* and white-footed mice. *Ixodes cookei* and *Ixodes marxi* rarely bite humans. *Ixodes scapularis* often bite humans.
Human cases have also been documented in Canada (Quebec, New Brunswick, Ontario) and Russia (far-eastern Primorski region).

http://www.ccwhc.ca/wildlife_health_topics
Distribution of *Ixodes scapularis*

*IXODES SCAPULARIS* (a.k.a. the deer tick or blacklegged tick) is a competent vector for POWV. (Costero and Grayson, 1996)

http://www.cdc.gov/ticks/geographic_distribution.html
Distribution of *Ixodes scapularis* and POWV cases

http://www.ccwhc.ca/wildlife_health_topics/arbovirus/arbopow.php


Tick-virus-host interface

- Host immune system
- Salivary factors
- Immune evasion
- Pathogen (POWV)

Nidus of arbovirus transmission
Skin: the interface for tick-pathogen-host interactions.

- Skin serves as a physical barrier meant to protect the host from injury and infection.
- Skin is the first host organ that POWV and tick saliva encounter as a POWV-infected tick initiates feeding.
- Tick saliva modulates various innate immune cell functions.
The importance of tick saliva

• Infectious agents transmitted by ticks are delivered to the vertebrate host together with saliva at the bite site.

• Tick salivary glands produce complex cocktails of bioactive molecules that facilitate blood feeding and pathogen transmission by modulating: host hemostasis, pain/itch responses, wound healing, innate and adaptive immunity.

• Saliva-activated transmission (Labuda M et al 1993, Zeidner NS et al., 2002; Sukumaran B et al. 2006; Krocova Z et al. 2003)

• Significant morphological changes occur in salivary glands during tick attachment and feeding on the host.

• Salivation is not a continuous process during blood feeding. The repertoire of saliva proteins changes during feeding.
Tick saliva enhances infectious agent transmission

**Powassan virus, *Ixodes scapularis***

**Thogoto virus, *Rhipicephalus appendiculatus***

**Theileria parva, *Rhipicephalus appendiculatus***

**Tick-borne encephalitis virus, *Ixodes ricinus***
Labuda *et al.* 1993. Medical and Veterinary Entomology 7: 193-196

**Vesicular stomatitis virus, *Dermacentor reticulatus***
Hajnicka *et al.* 2000. Parasite Immunology 22: 201-206

**Borrelia burgdorferi, *Ixodes scapularis***

**Francisella tularensis, *Ixodes ricinus***

**Borrelia burgdorferi, *Ixodes ricinus***
Part 1

Tick-virus-host interface: *Immunomodulation at the site of Powassan virus transmission.*

The *rationale* for this study is that by gaining an understanding of how tick feeding/saliva immunomodulates the tick-host interface in the presence of POWV, we will build a foundation towards the future development of salivary protein immunogens that have the potential to block tick-borne virus transmission and dissemination.
Timeline of tick-borne pathogen transmission

• POWV is delivered from the infected salivary glands of an *I. scapularis* nymph to the skin of the host by 3 hours post-tick infestation. (Hermance & Thangamani, 2014) (Ebel & Kramer, 2004)

• During early feeding time points, the viral load of *Tick-borne encephalitis virus* (TBEV) in the tick salivary glands increases. (Alekseev & Chunikhin, 1990)

• TBEV is transmitted as early as one hour post-attachment and initiation of tick feeding. (Alekseev *et al.*, 1996) (Thangamani *et al.*, unpublished).
Overview of host gene modulation at 3 and 6 hours post POWV-infected tick feeding

Changes in mouse gene expression in response to POWV-infected vs. uninfected tick feeding

Hermance and Thangamani, 2014
The host’s induced immune response to POWV-infected tick feeding does not have a defined Th1 or Th2 profile.

After POWV-infected tick feeding, a complex pro-inflammatory environment exists at the feeding site. This includes increased granulocyte recruitment, migration, and accumulation, especially at 3hpi.

Our data suggests that POWV-infected tick feeding recruits immune cells much earlier than the uninfected tick feeding.

Immunophenotyping immune cells at the site of POWV transmission

- Take 5μm sections for analysis
- Trim (20μm) until cement is visualized

- **H&E staining**
- **IFA immune cell detection:** mononuclear cells, fibroblasts, neutrophils, etc.
Example of an optimal skin / tick section
Infiltration of immune cells at the site of POWV transmission
Immunophenotyping POWV susceptible cells

Merged
POWV
Macrophages

POWV-infected section - 3 hpi

Uninfected section - 3 hpi
Immunophenotyping POWV susceptible cells

Merged
POWV
Fibroblasts

POWV-infected section - 3 hpi

Uninfected section - 3 hpi

10 um

Thangamani Lab
This is the first report of what cell types are infected by POWV:
- POWV-infected **macrophages** and **fibroblasts** were detected by IFA.
- No POWV-infected neutrophils were detected.

The most distinct difference between the uninfected versus POWV-infected tick feeding site was observed at 3 hpi.
Immunophenotyping immune cells at the site of POWV transmission

Uninfected tick feeding site:

POWV-infected tick feeding site:

Adapted from: Hermance and Thangamani, 2014; Hermance et al 2016)
Part 1: Conclusions

• The cellular infiltrates observed at the infected 3 hpi feeding site were greater than those observed at the 6 hpi feeding sites.
  o This correlates to the gene expression analysis where a very pro-inflammatory was detected at the 3 hpi feeding site.

• At 3 hpi in the presence of POWV more inflammatory cells infiltrate the feeding lesion, possibly contributing to the early dissemination of POWV.

• Overall this phenomenon could be attributed to:
  o POWV infection
  o Changes in POWV-infected tick saliva secretion
  o Additive effect of both
The central hypothesis for this study is that the presence of tick saliva during POWV infection creates an immunologically privileged micro-environment in the host’s skin that influences the disease course and pathogenesis of POWV infection.
Survival curves

N = 5 mice per time point and infection condition. P<0.05 for $10^6$ PFU versus $10^6$ PFU+2SGE comparison is represented by [*], and for $10^3$ PFU versus $10^3$ PFU+2SGE comparison is represented by [#]. Limit of detection is 10 PFU.

Popliteal lymph nodes were harvested from mice at the indicated time points. The sizes of lymph nodes were compared for the treatment group that received $10^3$ PFU of POWV versus the group that received $10^3$ PFU of POWV + 2 SGE.

POWV infection in brain

- 4 dpi
- 5 dpi
- 6 dpi
- 7 dpi
- 8 dpi

= Lowly infected areas
= Highly infected areas

# = Number of mice with detectable POWV in a specific region of the brain
X = Time points at which all mice succumbed to disease

MY = medulla; P = pons; MB = midbrain; CBX = cerebellum; TH = thalamus; HY = hypothalamus; HPF = hippocampal formation; CTX = cortex; MOB = main olfactory bulb

Santos R, Hermance M, Thangamani S. Manuscript In-preparation
POWV detection in the brain

CD11b detection in the brain

Control

10^3 PFU POWV

10^3 PFU POWV + 2 SGE
POWV detection in the brain

<table>
<thead>
<tr>
<th>Clinical Manifestation</th>
<th>10^6 PFU</th>
<th>10^6 PFU + SGE</th>
<th>10^3 PFU</th>
<th>10^3 PFU + SGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningitis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Meningo-encephalitis</td>
<td>Meningo-encephalitis</td>
<td>Meningo-encephalitis</td>
<td>Meningitis</td>
<td>Meningo-encephalitis</td>
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</table>

In the presence of SGE, low-dose infected mice presented a scattered pattern of POWV staining. The progression of infection was abrupt and occurred almost simultaneously throughout the brain by 6 dpi.

Our study demonstrated that at low doses of POWV, the presence of tick saliva impacts the neuropathogenesis of POWV.
Part 2: Conclusions

• Mice infected with $10^3$ PFU POWV plus SGE succumbed by 8 dpi. Neuroinvasion was demonstrated, and all mice displayed paralysis. Meningoencephalitis was observed.

• All mice infected with $10^3$ PFU only (no SGE) survived the infection and appeared completely healthy. Meningitis was observed.

• Overall: At low doses of POWV, tick saliva facilitates infection and influences disease outcome of BALB/c mice.
Tick-virus-host interface: Overall Summary

- Early immunologic response at the tick-POWV-mammalian host interface are pro-inflammatory with a marked increase in immune cell infiltrates at the tick feeding foci.

- **Adjuvant effect of tick saliva:** At low doses of POWV, tick saliva facilitates infection and influences disease outcome of BALB/c mice.
Other tick-borne diseases research in my Lab

- Impact of Co-infection on the clinical outcome of Lyme disease and Powassan encephalitis.
- Comparative neuropathogenesis DTV and POWV.
- Development of a tick transmission model for Heartland virus and Bourbon virus pathogenesis.
- Ticks and tick-borne pathogen surveillance in Texas.
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