Zika virus pathogenesis in macaques

Dave O’Connor
May 20, 2016
Key applications of macaque models of virus pathogenesis

- Longitudinal sampling
- Kinetics of virus replication and antiviral immunity
- Invasive tissue sampling
- Preclinical evaluation of interventions
- Rapid and cost-effective results
Why did we begin studying Zika virus in macaques?
Expected features of pre-zoonotic pathogens

- Microbial agents: RNA viruses
- Animal hosts: Primates
- Human exposure: Highly likely
  - High prevalence in natural hosts
  - High viral load in natural hosts
  - Frequent animal-human contact
- Genetic diversity: High
- History of cross-species transmission: Yes
ZIKA FOREST RESEARCH FIELD STATION.

UGANDA VIRUS RESEARCH INSTITUTE (UVRI)
P.O. BOX 49 ENTEBBE.
TEL: 0414-320631

examine cross-species pathogenesis of African primate viruses in Asian macaque monkeys
Are macaques appropriate models for Zika virus pathogenesis?
Key questions

• Can macaques be infected with Zika virus?
  - via physiologic routes and with physiologic doses of virus?
  - with strains similar to those circulating in the Americas?

• Do macaques develop disease similar to humans?
  - rash, asymptomatic infection in non-pregnant macaques?
    ✴ rare complications such as GBS hard to detect
  - fetal abnormalities in pregnant macaques?
Can macaques be infected with Zika virus?
13 Indian rhesus macaques challenged subcutaneously
All 13 animals had detectable virus in blood
Are Zika virus challenge doses physiologic?
$10^4$ PFU of Zika virus reliably establishes infection

$10^6$ PFU mosquitos transmit up to $10^4$ PFU per blood meal

$10^5$ PFU sexual transmission dose currently unknown

$10^4$ PFU
Do American Zika virus strains infect macaques?
Asian/American and African Zika virus infect macaques

<table>
<thead>
<tr>
<th></th>
<th>Asian/American</th>
<th>African</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^6$ PFU</td>
<td>![Monkey]</td>
<td>![Monkey]</td>
</tr>
<tr>
<td>$10^5$ PFU</td>
<td>![Monkey]</td>
<td>![Monkey]</td>
</tr>
<tr>
<td>$10^4$ PFU</td>
<td>![Monkey]</td>
<td>![Monkey]</td>
</tr>
<tr>
<td>$10^4$ PFU</td>
<td>![Monkey]</td>
<td>![Monkey]</td>
</tr>
</tbody>
</table>

Colleagues have infected macaques with Puerto Rican (Bioqual/Whitney/ONPRC) and Brazilian Zika virus (UC-Davis)
Do macaques develop disease similar to humans?
Infection of non-pregnant and pregnant macaques

<table>
<thead>
<tr>
<th></th>
<th>Asian/American</th>
<th>African</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>non-pregnant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^6$ PFU</td>
<td><img src="image" alt="Monkey" /></td>
<td><img src="image" alt="Monkey" /></td>
</tr>
<tr>
<td>$10^5$ PFU</td>
<td><img src="image" alt="Monkey" /></td>
<td><img src="image" alt="Monkey" /></td>
</tr>
<tr>
<td>$10^4$ PFU</td>
<td><img src="image" alt="Monkey" /></td>
<td><img src="image" alt="Monkey" /></td>
</tr>
<tr>
<td><strong>first trimester</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^4$ PFU</td>
<td><img src="image" alt="Monkey" /></td>
<td><img src="image" alt="Monkey" /></td>
</tr>
<tr>
<td><strong>third trimester</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^4$ PFU</td>
<td><img src="image" alt="Monkey" /></td>
<td><img src="image" alt="Monkey" /></td>
</tr>
</tbody>
</table>
Do we observe rash, asymptomatic infection in non-pregnant macaques?
Mild, asymptomatic infection in non-pregnant macaques

- animals appeared healthy throughout experiments
- mild rash observed at infection site in few WNPRC animals
- prominent descending rash noted in macaques infected with Puerto Rican Zika virus (ONPRC)
Are there fetal abnormalities in pregnant macaques?
Infection of non-pregnant and pregnant macaques

- First trimester: 10^4 PFU (74 dpi)
- Third trimester: 10^4 PFU (53 dpi)
- Second trimester: 10^4 PFU (25 dpi)
Infection of non-pregnant and pregnant macaques

- Mothers remained healthy throughout pregnancy.
- Head circumference ~ 2 standard deviations below normal; placental calcification at 42 dpi.

Diagram:
- 74 dpi: 10^4 PFU, first trimester.
- 25 dpi: 10^4 PFU, third trimester.
Both fetuses from 1st trimester infections are small

<table>
<thead>
<tr>
<th>Date</th>
<th>Biparietal Diameter</th>
<th>Head Circumference</th>
<th>Femur Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/23/16</td>
<td>1.3 SD below mean</td>
<td>1.8 SD below</td>
<td>---</td>
</tr>
<tr>
<td>3/30/16</td>
<td>1 SD below</td>
<td>1.7 SD below</td>
<td>0.6 SD below</td>
</tr>
<tr>
<td>4/12/16</td>
<td>0.7 SD below</td>
<td>1.7 SD below</td>
<td>0.5 SD below</td>
</tr>
<tr>
<td>4/19/16</td>
<td>1.2 SD below</td>
<td>2.4 SD below</td>
<td>0.5 SD below</td>
</tr>
<tr>
<td>4/26/16</td>
<td>0.7 SD below</td>
<td>1.3 SD below</td>
<td>0.4 SD below</td>
</tr>
<tr>
<td>5/3/16 (58 dpi)</td>
<td>0.7 SD below</td>
<td>1.4 SD below</td>
<td>0.2 SD below</td>
</tr>
</tbody>
</table>

4/19/16   | 1 SD below            | 2.3 SD below       | 1 SD below     |
| 4/26/16   | 3 SD below            | 2.4 SD below       | 1 SD below     |
| 5/3/16 (37 dpi) | 2.4 SD below  | 2.4 SD below      | 1 SD below     |

All values are shown as standard deviations (SD) below normal projected growth.
Need for caution

• fetuses are small, but are they pathogenically small due to ZIKV infection?
  - we do not know right now - more animals and more data will resolve more clearly

• head circumference and biparietal diameter would likely merit follow-up in pregnant women who have travelled to areas with high ZIKV prevalence
Macaques recapitulate key aspects of Zika virus pathogenesis
High priority questions in Zika virus research

- What is the natural history of Zika virus infection
  - when is viremia detected and cleared?
  - how much Zika virus is in different fluids throughout infection?
  - is there a difference between African and Asian strains?

- Do immune responses to Zika virus completely protect from re-infection?

- Does Zika virus infect or establish sanctuary in the nervous system?
When is viremia detected and cleared?
Plasma viremia lasts approximately 10 days in non-pregnant macaques.
Plasma viremia lasts approximately 10 days in non-pregnant macaques.
Plasma viremia is extended in pregnant macaques

copies vRNA / mL plasma

days since Zika virus infection
How much Zika virus is in different fluids throughout infection?
Zika virus is detectable in blood, saliva, urine, and CSF.

- Plasma
- Urine
- Saliva
- CSF

Also detected low levels in vaginal swabs; semen not yet tested.
Is there a difference between African and Asian strains?
Lower viral loads in macaques challenged with African virus

- 10^6 PFU
- 10^5 PFU
- 10^4 PFU

copies vRNA / mL plasma

days since Zika virus infection
Do immune responses to Zika virus completely protect from re-infection?
Robust pathogen sensing during primary infection

- Ki-67+ NK cells
- Ki-67+ CD8+ T cells
- Ki-67+ CD4+ T cells
Primary immunity completely protects against homologous rechallenge
From AIDS to ZIKV: maximizing macaque model utility
Key points

• Rapid results only matter if communicated quickly

• Experimental reproducibility requires careful coordination between investigators
  - selection and characterization of Zika virus strains for in vivo use

• Access and resources from National Primate Research Centers
Rapid results only matter if communicated quickly
http://zika.labkey.com

Enables stakeholders, scientists, and community to engage in experiments – leads to better, faster research
Zika virus real-time data sharing partners

- Wisconsin National Primate Research Center
- California National Primate Research Center
- Oregon National Primate Research Center
- Washington National Primate Research Center
- Sallie Permar, Duke University
- Amilcar Tanuri, Federal University Rio de Janeiro
Experimental reproducibility requires careful coordination between investigators.
“SIVmac251”

A: 251-RD.Consensus

B: 251-CM.Consensus

C: 251-DB.Consensus

Del Prete et al., J. Virol 2013
Seven different sequences are all called ZIKV
“MR766” (Uganda 1947) in Genbank
In vitro passage could impact biology — consider distribution of standardized “ready-to-use” viruses and challenge protocols for in vivo studies.
Access and resources from National Primate Research Centers
Coordinated management of NPRC resources will be essential

- HIV/AIDS investigators, reproductive biologists, and neurobiologists all interested in studying Zika virus

- Breeding needs directly conflict with studying Zika virus in pregnant macaques
  - importing macaques for pregnancy studies logistically difficult

- Outdoor colonies may be susceptible to locally acquired Zika virus infections
  - need for accurate, inexpensive reagents to identify Zika virus and dengue virus SPF animals
Macaque studies are an important component of the response to the Zika virus threat
Acknowledgements

**ZEST US:**
Dawn Dudley, Emma Mohr, Christina Newman, Mariel Mohns, Meghan Breitbach, Mustafa Rasheed, Michael Graham, Kristi Hall, Adam Ericsen, Adam Bailey, Tom Friedrich, Andrea Weiler, Gabrielle Lehrer-Brey, Jorge Osorio, Matt Aliota, Shelby O’Connor, Dane Gellerup, Sallie Permar, Tony Moody, Josh Eudailey, Buddy Capuano, Ted Golos, Nancy Schultz-Darken, Heather Simmons, Jen Post, Sandra Boehm, Tony Goldberg, Katie Antony

**Support:**
NIH NIAID  
WNPRC

**ZEST Brazil:**
Amilcar Tanuri, Renato Santana, Esper Kallas

**HIV colleagues:**
Matt Reynolds, Adrian McDermott, Todd Allen, Dave Evans, David Watkins, Rakai Health Sciences Program, Steve Reynolds, Kibale Ecohealth, Camilla Renato, Rodrigo Brindeiro

**Virus discovery collaborators:**
Jeffrey Rogers, Cristian Apetrei, Jens Kuhn, Peter Jahrling, Jane Phillips-Conroy, Cliff Jolly, Preston Marx, Jack Stapleton, David Hyeroba, Alex Tumukunde, Geoffrey Weny, Jera Pecotte, Nelson Freimer, Tony Goldberg, Sam Sibley