Zika Virus Animal Models
Suitability for Vaccine Testing

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Desired Properties of Zika Animal Model

• Infection results in measurable pathogenesis

• For vaccine testing, pathogenesis should be apparent in older animals due to amount of time needed to prime, boost, and challenge

• Animal is capable of mounting immune response to vaccine antigen

• Pathogenesis and immune responses resemble what is seen in humans
Zika Animal Models

• Mouse
  • Wild type (immune competent) strains
  • Strains lacking components of innate immunity

• Hamster (Morrey, Julander, Utah State Univ.)
  • STAT2 KO

• Non-human Primate (David O’Connor, U. of Wisconsin, Madison)
  • Rhesus Macaques
Mouse Models

• Immune competent strains

  • CD1, C57Bl6 do not exhibit Zika virus induced pathogenesis (unless infected at 1 week of age)
    • Do not show fetal abnormalities when infected during pregnancy
      (Rossi et al., 2016; Lazear et al., 2016; Cugola et al., 2016)

  • SJL (Cugola et al., 2016; Kurtz et al., 1995), Brazilian isolate of Zika highly pathogenic in SJL mice, virus infected placenta and fetus. Need evaluation in older animals

  • Unpublished data from D. Barouch: viremia observed in adult Balb/c mice
Robust ZIKV Replication in Wildtype Balb/c Mice (Brazil Stock > Puerto Rico Stock)

Balb/c
Mean peak 5.42 log copies/ml
N=10

C57BL/6

ZIKV-BR (Brazil)
N=5

ZIKV-PR (Puerto Rico)
N=5

Mean peak 4.96 log copies/ml
N=5

Days Following Challenge
Larocca et al., 2016
Zika infection of pregnant, immune competent SJL mice results in IUGR and cortical malformations in the brain

Cugola et al., 2016
Mouse Models

Immunocompromised mice lacking components of innate immunity

• A129 (Rossi et al., 2016; Lazear et al., 2016)
  
  • Lack receptor for type 1 IFN (IFNα/β), virus titers in brain, spinal cord, and testes, mice up to 6 months old susceptible, need evaluation in pregnancy model

• Type I IFN-blocking monoclonal antibody (Lazear et al., 2016): in adult mice viral replication is seen, but no clinical signs are observed. In pregnant mice, infection of fetus occurred, but was not severe. No fetal demise

• Irf3−/− Irf5−/− Irf7−/− triple knock out, produce little type I IFN (Lazear et al., 2016), more susceptible to Zika than A129 mice, needs further evaluation esp. in pregnancy model and in older animals
Mouse Models

Immunocompromised strains lacking components of innate immunity

• AG129 (Aliota et al. 2016; Rossi et al., 2016; Zmurko, et al.; 2016; Julander et al., unpublished data; review by Sarathy et al. 2015 (Dengue))
  - Lack receptors for types I and II IFN (IFNα/β and IFNγ)
  - Significant pathogenesis and clinical signs evident in both young and older (2 month old) mice (Aliota et al., 2016)
  - Evaluation of Zika infection needed in pregnant dams and in older (≥3 month pregnant dams) to account for time needed to prime, boost and challenge

• A129 females crossed with wild type males produce heterozygote fetuses with largely intact Type I IFN response (Miner et al., 2016)
  - Zika infects pregnant dams and placenta and results in damage to placental barrier and infection of fetus, severe cases lead to fetal demise
ZIKV AG129 Mouse Model

- Neurologic disease
- Conjunctivitis
- Hunching, lethargy, and excitability @ late stage
- Measurable viremia
- Hindlimb paralysis

Appropriate for antiviral and vaccine studies except evaluation of interferon pathway agents
Fig 1. ZIKV causes mortality and morbidity in young and adult AG129 mice

http://journals.plos.org/plosnatsdis/article?id=info:doi/10.1371/journal.pntd.0004682
Fig 2. Young and adult mice have high serum viremia early during infection.

http://journals.plos.org/plosntds/article?id=info:doi/10.1371/journal.pntd.0004682
Fig 3. Young and adult mice have high tissue viral loads 7 days post infection.


http://journals.plos.org/plosntds/article?id=info:doi/10.1371/journal.pntd.0004682
Zika/AG129 Mouse Model

- Pathogenesis more severe than that experienced in humans
- Virus infects fetus resulting in fetal abnormalities
- No significant differences in pathogenesis between young (4 week) and adult (8 week) animals
- Mice that survive exposure to virus are completely protected from re-challenge
- Caveat: AG129 mice lack receptors for Type 1 and Type II IFN therefore not appropriate for testing vaccines that rely on intact IFN pathways
A129 (IFNAR -/-) Mice Develop Neurological Disease and Succumb to Infection

Mouse Model of Zika Virus Pathogenesis

*Ifnar*^−/−^ mice

Zika virus

Viremia
Systemic spread

Understanding Zika virus disease

High viral loads in brain, spinal cord, testes

Evaluating vaccines and antivirals

Neurological disease
Death

Lazear Cell Host Microbe 2016
Zika virus can cause lethal disease in adult mice lacking type I Interferon immunity (but not in WT mice)

Ifnar1^{-/-} and Irf3^{-/-} Irf5^{-/-} Irf7^{-/-} C57BL/6 mice

Strains: MR 766 (Uganda 1947)  
H/PF/2013 (French Polynesia 2013)

Causes paralysis and encephalitis
Older A129 (IFNAR1 -/-) Mice Remain Susceptible to Infection
Creating a model of *in utero* transmission of Zika virus infection

**Pregnant mice**

- Infect with Zika virus
- Different days E6, E7, E10

**Analyze**

- newborn or fetal IFNAR+/- mice
  (young mice susceptible)

**Readouts:** survival, pathology, virology, immune infiltrates, mother/fetus/neonate

*A129 IFNAR KO
♂
♀
X
WT
♂
♀

*Miner Cell 2016*
Zika virus infection of pregnant dams results in fetal resorption

A

<table>
<thead>
<tr>
<th>WT sire x Ifnar1-/- dam</th>
<th>WT sire x WT dam + anti-Ifnar mAb</th>
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Infect pregnant dam on E6.5 or E7.5 with ZIKV (1000 FFU)

E13.5 or E15.5

Sacrifice and harvest

Measure fetal size

Viral titers

Histology

B

WT (ZIKV)  Ifnar1 het (ZIKV)

E13.5

WT (ZIKV)  Ifnar1 het (Mock)  Ifnar1 het (ZIKV)

E15.5

Infect E7.5, Harvest E15.5

Miner Cell 2016
Infect E12, Harvest E18.5

Infect later in pregnancy
- No fetal demise
- +++ Intrauterine growth retardation

Ifnar1 het - MOCK  Ifnar1 het - ZIKV

Miner Cell 2016
Rhesus macaques model of Zika
Dave O’Connor-U. Wisconsin

• 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?
All challenged animals had detectable virus in blood
Zika virus is detectable in blood, saliva, urine, and CSF.

Also detected low levels in vaginal swabs; semen not yet tested.
Mild, asymptomatic infection in non-pregnant macaques

- animals appeared healthy throughout experiments
- mild rash observed at infection site in few WNPRC animals
Plasma viremia lasts approximately 10 days in non-pregnant macaques
Infection of pregnant macaques

- mothers remained healthy throughout pregnancy
- placental calcification in one macaque at 42 dpi
Plasma viremia is extended in pregnant macaques infected in the first trimester and variable when infected in third trimester.
Fetuses from 1st trimester infections are smaller than average.
Primary immunity completely protects against homologous rechallenge

Challenged with French Polynesia Zika, rechallenged 10 weeks later
Primary immunity apparently protects against heterologous rechallenge

Initially challenged with African lineage Zika, 10 weeks later rechallenged with Asian lineage
Progress towards a Zika virus vaccine using monkeys

- Macaque monkeys are susceptible to infection with Zika virus
- Pregnant monkeys are infected for an unusually long time
- A vaccine should work because monkeys resist reinfection
Enables stakeholders, scientists, and community to engage in experiments – leads to better, faster research

http://zika.labkey.com

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Zika Animal Models

- Zika/NHP model recapitulates human disease very well
- Effects of viral infection on fetuses will be known in the coming weeks
- Macaques exposed to virus are protected from re-challenge
- Promising model for vaccine testing

Range from no disease (C57BL6) to highly susceptible (SJL)

Sensitive to infection, but lacks IFN signaling to prime the innate and adaptive immune response