What is the role of animal models in studying protective titres and the need for establishing surrogates/correlates of protection?

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Overview

1. Definition of correlates/surrogates of protection
2. Studies with licensed flavivirus vaccines
3. Studies with ZIKV vaccine candidates
4. Are animal models informative?
Correlates of Protection

• “A correlate of protection is a marker of immune function ……that correlates with protection after vaccination.

• Correlates of protection have generally been inferred from studies:
  - Passive antibody administration,
  - Analysis of immune responses in protected and unprotected subjects in nature, and in efficacy trials,
  - Observations of immunosuppressed humans or animals,
  - Human challenge studies, and
  - Extrapolation from the results of challenges in animals.

Plotkin & Gilbert, CID 2012
Surrogate of Protection

• “A Surrogate of Protection is an immune marker that can substitute for the clinical end point and …….can be used to reliably predict vaccine efficacy”.

Plotkin & Gilbert, CID 2012
Disclaimers!

- The definition of “protection” is critical.
- There is no correlate of protection for any flavivirus vaccine.
- There are **surrogates of protection** for licensed flavivirus vaccines.
- It is very unlikely that a correlate of protection will be identified for a Zika vaccine in the near future.

- Therefore, we will be discussing potential surrogates of protection in animal models.

- Until clinical data are available, the applicability of the potential surrogates of protection in animals to humans is unknown. Interpretation needs to be done with great care!
Correlates of Protection

How do we define protection?

• Neutralizing antibody
• Lack of clinical disease
• Reduced/lack of viremia
• Memory?
Correlates of Protection

How do we define protection?

- Neutralizing antibody – surrogate for licensed flavivirus vaccines
- Lack of clinical disease - ?
- Reduced/lack of viremia - ?
- Memory? Important for live vaccines…. YF 17D vaccine appears to give life-long protective immunity after one dose.
Mechanism of protective immunity of licensed flavivirus vaccines in humans is poorly understood

so we tend to use neutralizing antibodies as a surrogate of protection
Cell mediated immune (CMI) responses

- Role in protection not clear; remains experimental, qualitative.
- Indirect evidence for role of CMI in protective immunity → secondary criterion?
- However, it is clear that CMI is important for memory responses.
- Important to evaluate role of CMI in protective immunity.
- Choice of antigen to measure CMI is critical.
- CMI not used in licensing of current flavivirus vaccines.
- How would you validate and standardize an assay?
## Surrogate of protection for licensed flavivirus vaccines

<table>
<thead>
<tr>
<th>Flavivirus</th>
<th>Live, subunit or inactivated?</th>
<th>Serotypes (Genotypes)</th>
<th>Test</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese encephalitis</td>
<td>Live and inactivated</td>
<td>1 (5)</td>
<td>PRNT/neutralization</td>
<td>1 in 10#</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>Live</td>
<td>1 (7)</td>
<td>Log neutralization index</td>
<td>0.7^</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>1 in 10-50^</td>
</tr>
<tr>
<td>Tick-borne encephalitis</td>
<td>Inactivated</td>
<td>1? (?)</td>
<td>PRNT/neutralization</td>
<td>1 in 10*</td>
</tr>
<tr>
<td>dengue</td>
<td>Live</td>
<td>4 (4-6)</td>
<td>PRNT/neutralization?</td>
<td>??????</td>
</tr>
<tr>
<td>Zika</td>
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<td>1 (3?)</td>
<td>“Neutralization”?</td>
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* During the vaccine licensure procedure titers of $\geq 1:2$ were accepted as a correlate of immunity
# Live SA14-14-2 had titer of 1 in 5 accepted initially
^ The level of antibody considered to be protective was an $\log_{10}$ neutralization index of 0.7 originally based on studies in nonhuman primates
^ Seroprotective levels of neutralizing antibodies, measured by PRNT, have not been determined
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<td>a. Empirical live attenuated</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>b. Recombinant chimeric live attenuated</td>
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<td>c. Inactivated</td>
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⁺ The level of antibody considered to be protective was an log₁₀ neutralization index of 0.7 originally based on studies in nonhuman primates

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Zika is more complex than other flaviviruses as it has multiple tissue tropisms.

Figure 1. ZIKV Tissue and Cell Tropism. Human studies and animal models (mice and non-human primates) have detected ZIKV in cells of the placenta, including Hofbauer cells (in vitro and in explanted human placental tissue), trophoblasts (mice, non-human primates...)

Jonathan J. Miner, Michael S. Diamond Zika virus pathogenesis and tissue tropism. Cell Host & Microbe 21; 134-142 (2017)
Animal Models

• Mice
  ➢ Immunocompetent (with/without anti-IFNAR1 mab treatment)
  ➢ Interferon αβ-receptor knock-out
  ➢ Interferon αβγ-receptor knock-out

• Hamster
  ➢ Immunocompetent
  ➢ STAT2 knock-out

• Guinea pig

• Non-human primates
  ➢ Rhesus macaque
  ➢ Pigtail macaque
  ➢ Cynomolgus macaque
Natural history studies

- Different animal models (mostly mice and NHP)
- Different strains of animal
- Different routes of inoculation
- Different doses of virus
- Different ZIKV strains
- Different passage histories of ZIKV strains
- Different neutralization assays
- Variety of other immunological readouts
- Most studies use small numbers of animals

- Comparison of different studies difficult.
- Need a standardized animal model system (Research vs. Development)
What is the biomarker for “protection” in animal model?

- Standardized virus strain?
- Standardized animal model/age/sex
- A particular neutralizing antibody titer is predictive of “protection”?
  - Limited information on neutralizing antibody titers over time
- How do we define “protection”?
  - Protection from death (immunocompromised mice)
  - No clinical signs of disease
  - No detectable viremia
  - No detectable viruria
  - No detectable viral RNA in one or more selected anatomical sites/tissues.... testes, ovaries?
  - Sterilizing immunity (no anamnestic immune response following virus challenge)
Preclinical development…. Based on licensed Flavivirus vaccines…

*Candidate vaccine induces undetectable viremia (as detected by viral RNA) in animal model would be a “signal” to proceed to clinical evaluation*
Current “state-of-the-art with ZIKV vaccine studies in mice and NHPs

- ZIKV is one serotype and multiple genotypes; vaccines based on one ZIKV strain give protection against challenge by heterologous strains.
- Vaccine-induced neutralizing antibody titers between 1 in 100 and 1 in 10,000 in animal models are “protective” BUT no standardized assays.
- Vaccine-induced protection often defined as lack of detectable viremia (viral RNA) post-challenge. No standardized RT-PCR assay.
- Sterilizing immunity based on lack of anamnestic neutralizing antibody response?
- Passive protection in mouse and NHP models with sera from immunized mice and NHPs.
- Purified IgG/neutralizing antibodies mediate passive protection.
- Adaptive immune response critical in controlling ZIKV infection
- Depletion of CD4+ and CD8+ T lymphocytes did not abrogate protection.
- (As expected) results qualitatively similar to that for licensed flavivirus vaccines
• Persistence of ZIKV in CNS and lymph nodes in NHPs
• Viremia cleared quickly by day 10
• Viral RNA detected in CSF up to day 42
• Viral RNA detected in lymph nodes up to day 72
• Neutralization titer: 1 in 1000-10,000
Zika virus infection damages the testes in mice

Jennifer Govero1,2, Prabagaran Esakkyn2, Suzanne M. Scheaffer2, Estefania Fernandez2, Andrea Drury2, Derek J. Platt4, Matthew J. Gorman3, Justin M. Richner1, Elizabeth A. Caine1, Vanessa Salazar1, Kelle H. Moley2,5 & Michael S. Diamond1,3,4,6

Zika Virus Causes Testis Damage and Leads to Male Infertility in Mice

Wenjiang Ma,1,2,4 Shihua Li,2,3,4 Shuoqian Ma,1,3,4 Lina Jia,1,3,4 Fuchun Zhang,3 Yong Zhang,1,4 Jingyuan Zhang,1,4 Gary Wong,2 Shanshan Zhang,1 Xuancheng Lu,3 Mei Liu,5 Jinghua Yan,2 Wei Li,6 Chuan Qin,7 Daishu Han,8 Chengfei Qin,3,10 Na Wang,3 Xiandong Li,3,11 and George Fu Guo2,3,4,7,8,15

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- High virus titers in testis and epididymis
- Anti-IFNAR1 mab treated or IFNAR−/− mice
- ZIKV given by SQ/IP routes
- Variability in effects by ZIKV strain
- Need to “standardize” model
Neutralizing human antibodies prevent Zika virus replication and fetal disease in mice

Gopal Sapparapu1,2#, Estefania Fernandez3#, Nurgun Kose2, Bin Cao4, Julie M. Fox5, Robin G. Bombardi2, Haiyan Zhao3, Christopher A. Nelson3, Aubrey L. Bryan6, Trevor Barnes6, Edgar Davidson6, Indira U. Mysorekar3,4, Daved H. Fremont3, Benjamin J. Doranz5, Michael S. Diamond3,5,7,8 & James E. Crowe Jr1,2,9

- Neutralizing human mabs can protect against maternal-fetal transmission, infection and disease
- “Protection” not 100%
A live-attenuated Zika virus vaccine candidate induces sterilizing immunity in mouse models

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Zika virus (ZIKV) infection of pregnant women can cause a wide range of congenital abnormalities, including microcephaly, in the infant, a condition now collectively known as congenital ZIKV syndrome.1 A vaccine to prevent or significantly attenuate viremia in pregnant women who are residents of or travelers to epidemic or endemic regions is needed to avert congenital ZIKV syndrome, and might also help to suppress epidemic transmission. Here we report on a live-attenuated vaccine candidate that contains a 10-nucleotide deletion in the 3' untranslated region of the ZIKV genome (10-del ZIKV). The 10-del ZIKV is highly attenuated, immunogenic, and protective in type 1 interferon receptor-deficient A129 mice. Crucially, a single dose of 10-del ZIKV induced sterilizing immunity with a saturated neutralizing antibody titer, which no longer increased after challenge with an epidemic ZIKV, and completely prevented viremia. The immunized mice also developed a robust T cell response. Intracranial inoculation of 1-d-old immunocompetent CD-1 mice with 1 x 10^4 infectious focus units (IFU) of 10-del ZIKV caused no mortality, whereas infections with 10 IFU of wild-type ZIKV were lethal. Mechanistically, the attenuated virulence of 10-del ZIKV may be due to decreased viral RNA synthesis and increased sensitivity to type-1-interferon inhibition. The attenuated 10-del ZIKV was incapable of infecting mosquitoes after oral feeding of spiked-blood meals, representing an additional safety feature. Collectively, the safety and efficacy results suggest that further development of this promising, live-attenuated ZIKV vaccine candidate is warranted.
Neutralizing antibodies as a surrogate of protection for ZIKV?
“Correlates of Protection” for ZIKV based on preclinical studies in mice and NHPs

How do we define protection?

• Neutralizing antibody
• Lack of clinical disease
• Reduced/lack of viremia
• Memory? -
Correlates of Protection for ZIKV based on preclinical studies in mice and NHPs

How do we define protection post vaccination in animal models?

• Neutralizing antibody – Yes, but neutralization titers needed for protection are higher than those required for currently licensed JE, TBE and YF vaccines.

• Lack of clinical disease – Yes, but limited clinical disease in animal models

• Reduced/lack of viremia - Yes, undetectable viremia by qRT-PCR, but mouse & NHP natural history studies show evidence of persistence of “virus” in anatomical sites in presence of adaptive immune response.

• Memory? – Yes, evidence from NHP and mouse studies

• Lack of detectable viremia ≠ “protection” from clinical syndromes

• Some candidate vaccines appear to give sterilizing immunity in animal models
“Are animal models informative?”

- Are animal models informative ….. Yes
- Non-human primates – Mild disease
- Mice – immunocompetent (with/without treatment with anti-IFNAR mab) and immunocompromised.
- Do animal models accurately recapitulate human disease? No.
- Relatively large number of different clinical diseases in humans…. No one animal model recapitulates all human clinical diseases
- Absence of viremia does not mean absence of infection…. Presence of virus/viral RNA in various anatomical locations in animals (and humans)
- Are animal models predictive of what happens in humans… unknown
Moving forward

- **Can you translate animal studies to humans?**
  - Animal models are very useful in helping to define a surrogate of protection.
  - However, there is no substitute for vaccine studies in human volunteers.
- Will serum from human vaccinees elicit passive protection in animal models? Particular neutralization titer?
- Will neutralizing antibodies induced by vaccinees be the surrogate of protection? …. How would we measure a Zika-specific neutralizing antibody response in vaccinees?
- Will candidate vaccines induce sterilizing immunity in volunteers?
Questions?