Flaviviruses – key immunological considerations for vaccine development

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Flaviviruses

- Family *Flaviviridae*
- Four genera: *Flavivirus*, *Pegivirus*, *Pestivirus*, *Hepacivirus*
- *Flavivirus* genus contains approximately 70 viruses
- “Arboviruses”
  - 50% Mosquito-borne
  - 25% tick-borne
  - 25% non-vector-borne
- Yellow fever virus is the prototypical member of the *Flavivirus* genus

Current flaviviruses of major medical importance

Mosquito-borne

- Dengue viruses (DENV)
- Japanese encephalitis virus (JEV)
- West Nile virus (WNV)
- Yellow fever virus (YFV)
- Zika virus (ZIKV)

Tick-borne

- Tick-borne encephalitis virus (TBEV)
- Omsk hemorrhagic fever virus (OHFV)
- Kyasanur Forest disease virus (KFDV)

Manifestations of flavivirus infection

Febrile illnesses

DENV (dengue fever)

Encephalitic disease

JEV

TBEV

Hemorrhagic fever

YFV

DENV (dengue hemorrhagic fever)

OHFV

KFDV

Dengue

Flavivirus serologic-/genetic-groups

Mammalian tick-borne
Seabird tick-borne
Yellow fever
Dengue
Spondweni
Aroa
Japanese encephalitis
Ntaya
Kokobera
Rio Bravo
Modoc
Entebbe bat

Dengue-1
Dengue-2
Dengue-3
Dengue-4

The complexity of developing a dengue vaccine

Need to develop not just one immunogen but four immunogens that will give a balanced immune response whereby a protective immune response is induced against all four viruses simultaneously, i.e., the vaccine has to be tetravalent.

Mechanism of protective immunity against DEN infection is poorly understood. It is assumed that neutralizing antibodies are the main effector of protection against DEN infection.

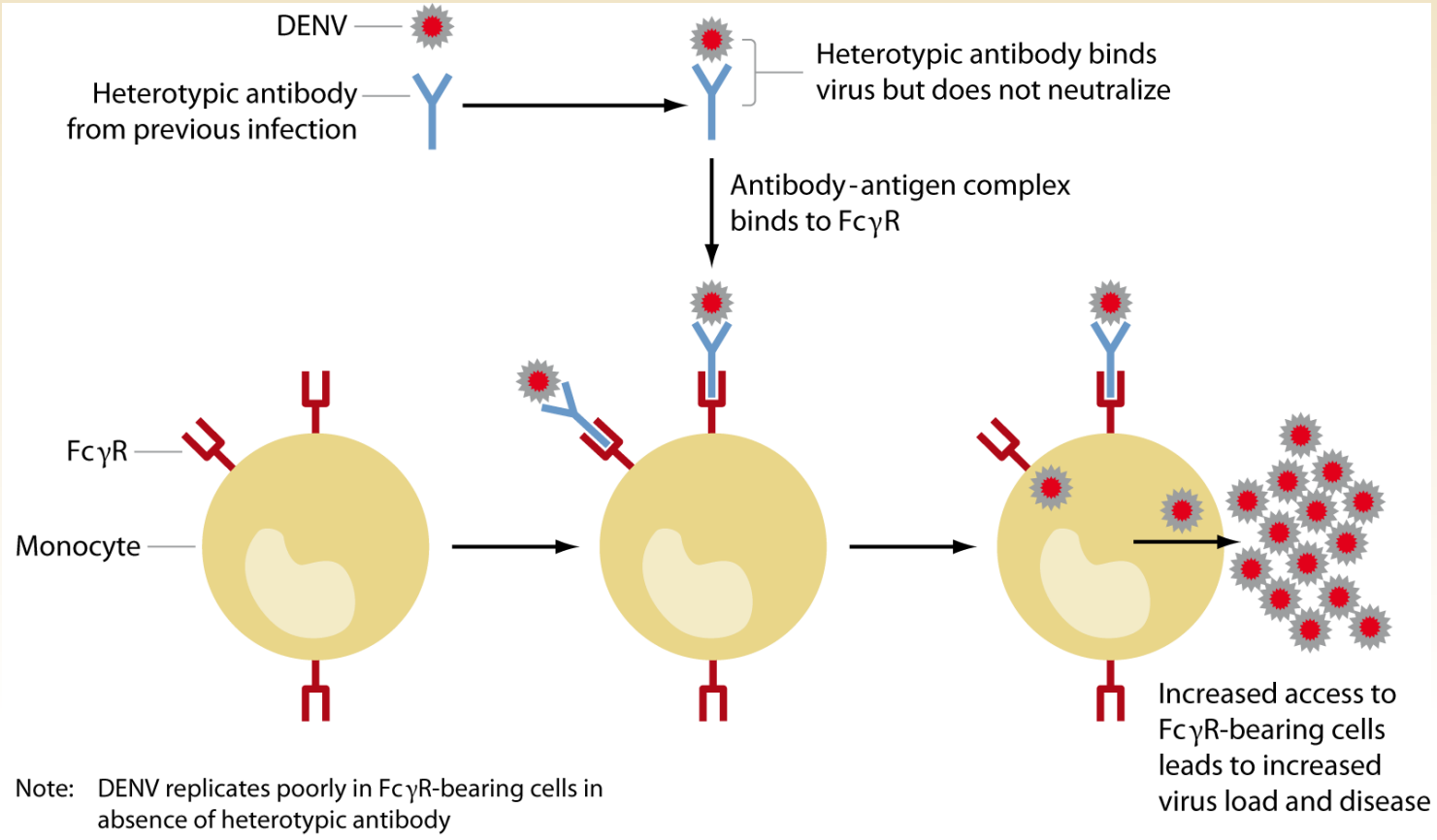
Lack of a suitable animal model with which to evaluate candidate vaccines. This severely hindered progress on identifying determinants of attenuation, virulence and immunogenicity of DEN viruses that can be applied to vaccine development.

Immune enhancement, including antibody dependent enhancement.

Interference between vaccine components

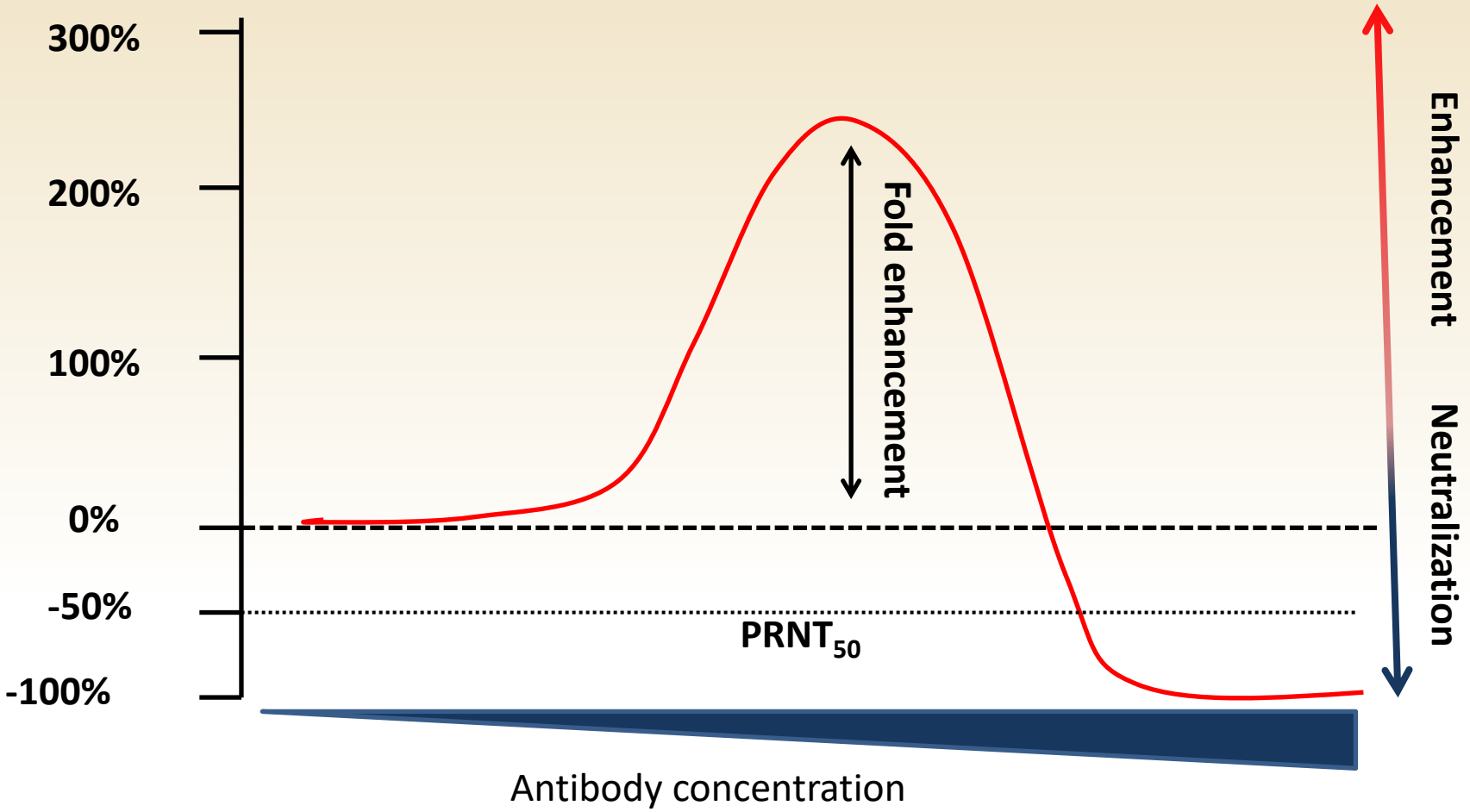
Antibody dependent enhancement (ADE)

Model of antibody-dependent enhancement (ADE) of dengue virus (DENV) replication and disease.



Murphy BR, Whitehead SS. 2011.
Annu. Rev. Immunol. 29:587–619

Antibody-virus interactions on monocytes/macrophages



Enhancement of dengue disease

Enhancing antibodies associated with disease severity

- What are the role(s) of humoral immunity after vaccination in protecting/decreasing the severity of dengue disease?
- Evidence that wild-type DEN infection after Dengvaxia live attenuated vaccine vaccination can lead to increased disease in dengue immunes
- Broadly cross-reactive dengue neutralizing antibodies wane and non-neutralizing antibodies persist.

T cells associated with disease severity?

Interference?

- Monovalent DEN vaccines induce good neutralizing antibody titers.
- Live attenuated vaccine tetravalent formulations do not induce the same neutralization titers as the four individual monovalent vaccines.
- One or more components give good neutralization titers while one or more components give reduced neutralization titers compared to the monovalent vaccine.
- This is termed interference. The mechanism is unknown.
- BUT we do not know what level of neutralizing antibodies is protective…. So reduced neutralization titers may still be protective

Do other flaviviruses mediate ADE?

- Maybe.
- All flavivirus sera, even yellow fever 17D vaccine, will mediate ADE in cell culture
- Multiple animal models for different flaviviruses show ADE… but does it happen in natural infections?
- Some evidence that ZIKV infection induces antibodies that mediate ADE of subsequent DENV infection

Current Licensed Flavivirus Vaccines

- Dengue
- Kyasanur Forest disease
- Japanese encephalitis
- Tick-borne encephalitis
- Yellow fever

No licensed vaccines

- West Nile
- Zika

- No antiviral agents available for any flavivirus disease

Licensed flavivirus vaccines

- Dengue
  - recombinant live attenuated
- Japanese encephalitis – boosters doses needed
  - Recombinant live attenuated
  - Empirically-derived live attenuated
  - Inactivated
- Tick-borne encephalitis – boosters doses needed
  - Inactivated
- Kyasanur Forest disease – boosters doses needed
  - Inactivated
- Yellow fever
  - Empirically-derived live attenuated – one dose gives life-long immunity
- Wesselsbron - veterinary
  - Empirically-derived live attenuated
- West Nile – veterinary – booster doses needed
  - Inactivated, canarypoxvirus vector

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

Surrogate of protection for licensed flavivirus vaccines

Flavivirus	Live, subunit or inactivated?	Serotypes (Genotypes)	Test	Quantity
Japanese encephalitis	Live and inactivated	1 (5)	PRNT/neutralization	1 in 10[#]
Yellow fever	Live	1 (7)	Log neutralization index PRNT/neutralization	0.7[+] 1 in 10-40^
Tick-borne encephalitis	Inactivated	1? (?)	PRNT/neutralization	1 in 10*
dengue	Live	4 (4-6)	PRNT/neutralization?	?????
Zika	????	1 (2?)	"Neutralization"?	1 in 100 ?

* During the vaccine licensure procedure titers of ≥ 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

Surrogate of protection for licensed flavivirus vaccines

Flavivirus	Live, subunit or inactivated?	Serotypes (genotypes)	Test	Quantity
Japanese encephalitis	a. Empirical live attenuated b. Recombinant chimeric live attenuated c. Inactivated	1 (5 – up to 10% amino acid divergence)	PRNT/neutralization	1 in 10
Yellow fever	Live	1	Log neutralization index PRNT/neutralization	0.7+ 1 in 10-50^
Tick-borne encephalitis	Inactivated	1?	PRNT/neutralization	1 in 10*
dengue	Live	4	PRNT/neutralization?	????
Zika	????	1?	PRNT/neutralization?	????

Overview of licensed flavivirus vaccines

- All vaccines monovalent (except dengue)
- Neutralizing antibodies are surrogate of protection
- Vaccines do not induce sterilizing immunity (?)
- Animal models based on mice and NHPs; NHP not good model for JEV/TBEV; only good model in some NHP species for YFV
- Vaccine-induced immunity not the same as that induced by natural infection
- Formalin inactivation “removes” some conformational epitopes on E protein (TBE vaccine)

Extrapolation to ZIKV

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

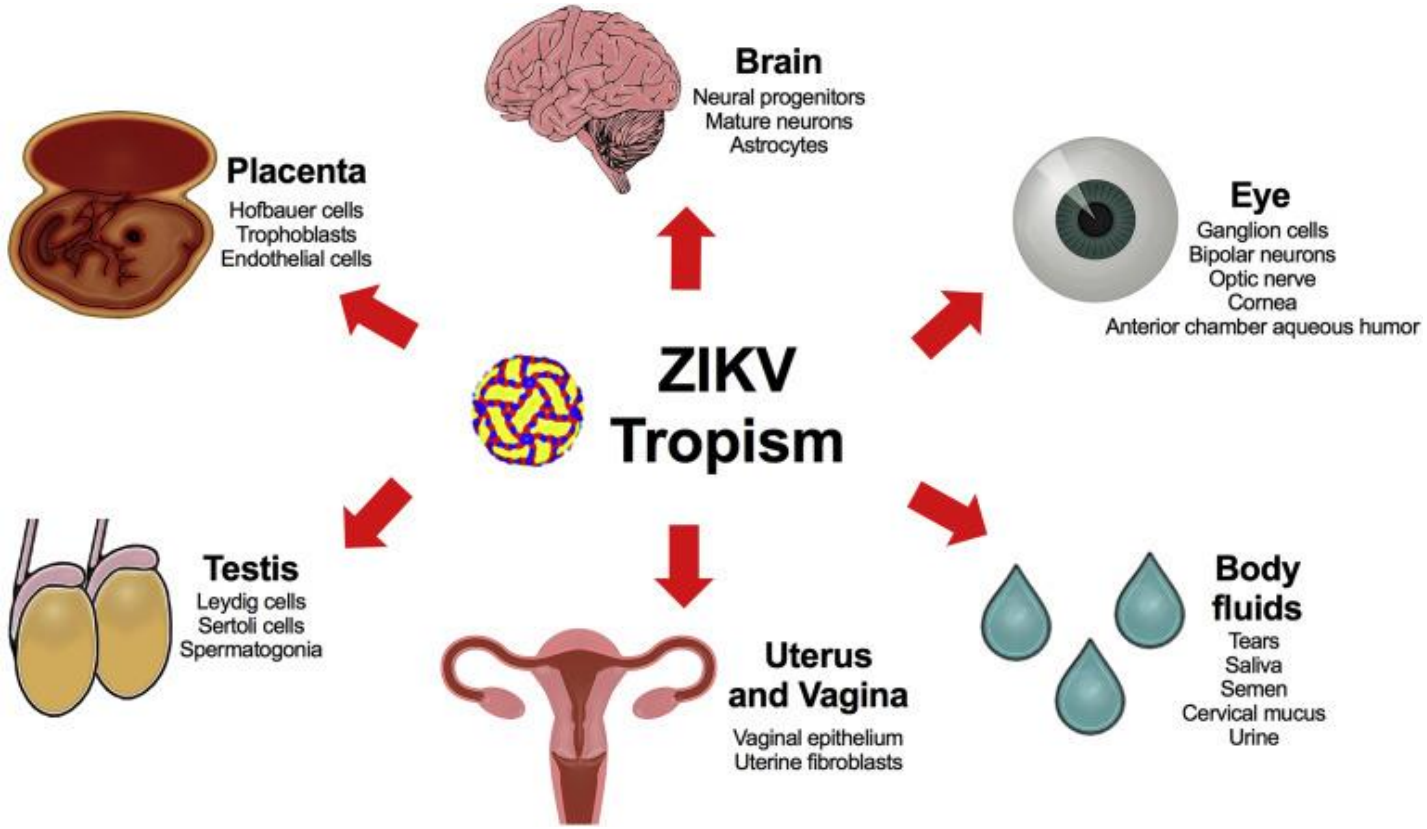


Figure 1. ZIKV Tissue and Cell Tropism. Human studies and animal models (mice and non-human primates) have detected ZIKV in cells of the placenta, including Hofbauer cells (in vitro and in explanted human placental tissue), trophoblasts (mice, non-human primates...

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

Surrogate of protection for licensed flavivirus vaccines

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Tick-borne encephalitis	Inactivated	1? (?)	PRNT/neutralization	1 in 10*
dengue	Live	4 (4-6)	PRNT/neutralization?	?????
Zika	Various	1 (2/3?)	"neutralization"?	1 in 100 ?

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Neutralizing antibodies as a surrogate of protection for ZIKV?

(As expected) results qualitatively similar to that for licensed flavivirus vaccines

Diagnostics

Complexities of evaluating flavivirus immune responses

- Flavivirus serology is a “minefield” due to antigenic cross-reactivity. Hard to serologically identify an infection as due to a particular flavivirus unless the individual is flavivirus-naïve.
- Karl Johnson called flaviviruses the “*Hall of Mirrors*”
- Challenging to assess and interpret immunological data due to cross-reactivity.

Need standards!

Thank you very much!