Human neuron infection with Zika virus and hybrid IgG antibody response to *Aedes aegypti* salivary proteins

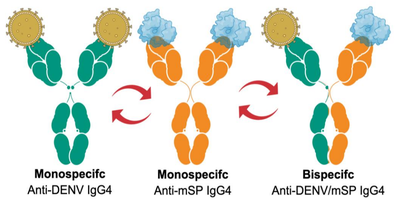
Shanice Harris[1,2] and Berlin Londono-Renteria[1]

[1]Vector Biology Laboratory, Department of Entomology, College of Agriculture, Kansas State University

[2]College of Veterinary Medicine, Kansas State University

Background Information

Zika virus (ZIKV) and Dengue virus (DENV) are flaviviruses carried by the mosquito vector *Aedes aegypti*. Most ZIKV infections are mild. However, the virus has been linked to many neurological complications, including microcephaly in newborns and Guilliain-Barré syndrome in adults. DENV is the leading virus in tropical and subtropical climates with Colombia being one of the most affected endemic countries.



Mosquito salivary proteins (mSPs) injected during bite transmission produce a humoral immune response that favors IgG4 antibody (Ab) production. About 30% of IgG4 Abs undergo Fab arm exchange to form bispecific molecules that can recognize both DENV and mSPs. Bispecific IgG4 Abs may decrease DENV pathogenesis by blocking both the virus and the mSPs and activating an anti-inflammatory response. However, antibody-dependent enhancement (ADE) can occur when sub-neutralizing levels of DENV Abs are present and facilitate viral entry into target cells through Fc receptors. It is believed that pre-existing DENV Abs in humans in endemic areas can enhance ZIKV infection through this mechanism.

Objectives

1. Determine the effect of chronic mSP exposure in DENV endemic areas through characterization of *Ae. aegypti* salivary proteins inducing mSP/DENV bispecific antibodies
2. Evaluate Zika neutralization potential of IgG4 bispecific antibodies in human neuron cells *in vitro* to observe anti-inflammatory vs. ADE effect

ELISA Results

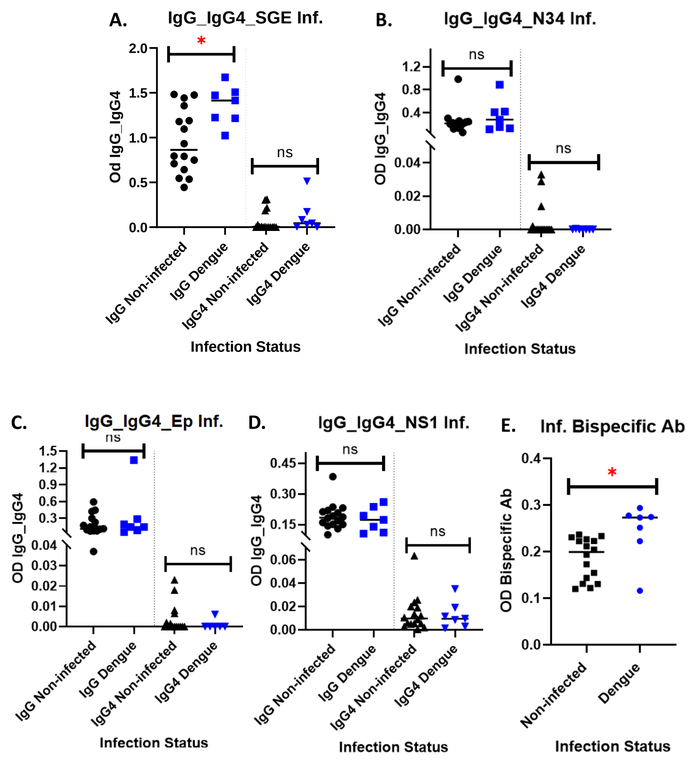


Figure 1. Total IgG and IgG4 Ab levels in DENV positive versus non-infected individuals against total *Ae. aegypti* salivary gland extract (**A**), N34 portion of 34kDa mSP (**B**), and DENV envelope (**C**) and NS1 proteins (**D**). Bispecific κ/λ Ab concentrations among these groups are also shown (**E**).

Neuron Infection

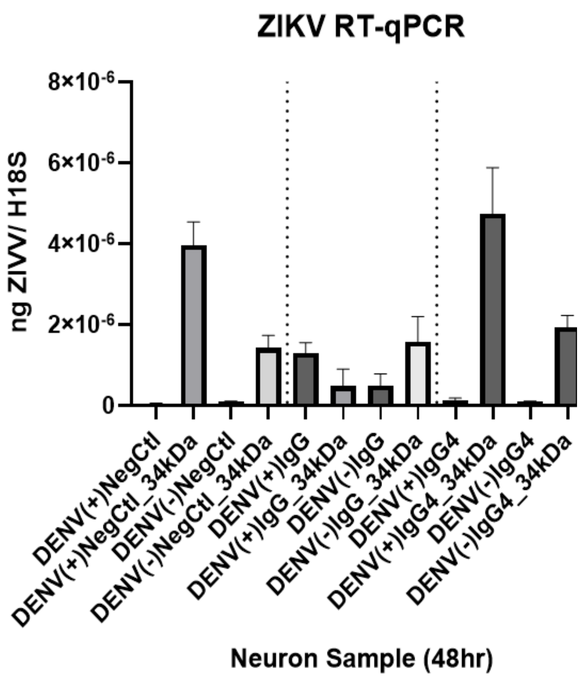


Figure 2. RT-qPCR results showing the amount of ZIKV RNA detected in human neuron cultures infected with ZIKV in the presence or absence of 34kDa protein and purified total IgG and IgG4 from DENV+ and non-infected serum samples. Negative control infections contained ZIKV only and ZIKV + 34kDa protein.

Methodology

Samples from residents of non-endemic areas n = 9

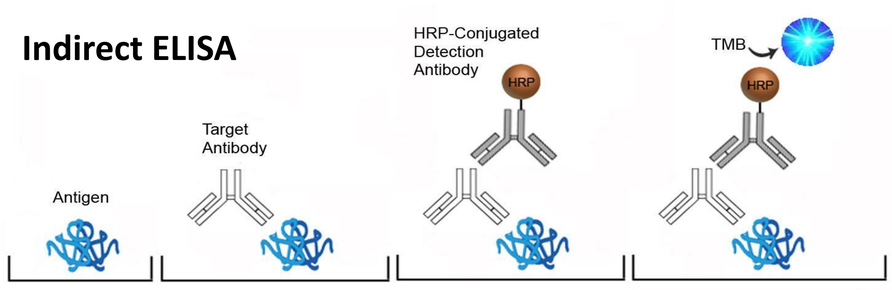
Total IgG, IgG4, κ/λ bispecific

Samples from endemic areas (Colombia) Exposed/non-infected = 7 Dengue positive = 7

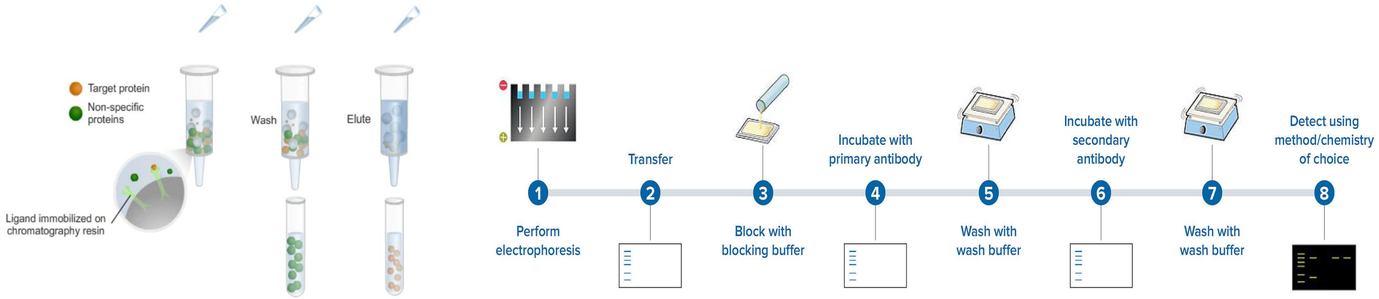
Salivary proteins (SGE, N34, D7)

DENV proteins (NS1, Ep)

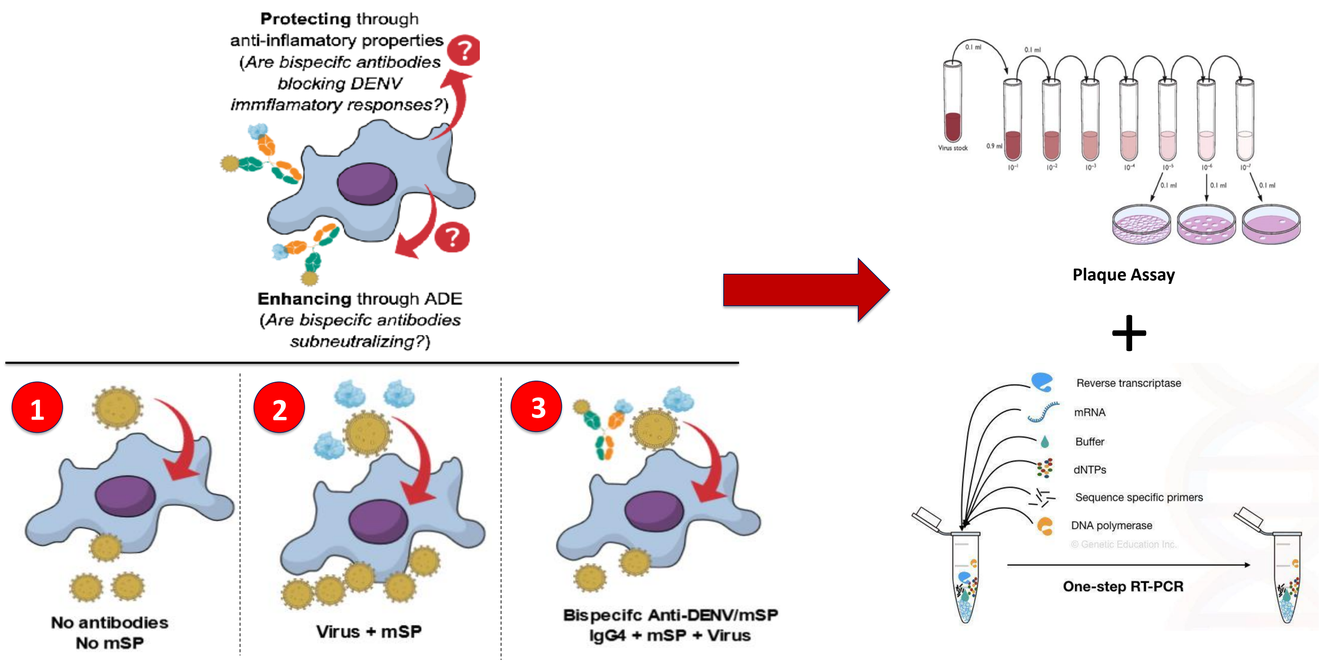
Indirect ELISA



Antibody Purification and Western Blot



ZIKV Neuron Infection and Analysis



Discussion

Based on our ELISA results, we found that IgG levels against total SGE were significantly higher in DENV infected versus non-infected samples. While differences for the other proteins tested between these two groups were not significant, slight variations were observed that correlate with previous studies. The 34kDa protein has been shown to recruit DENV target cells to the bite site to increase viral replication, and our results display higher concentrations of IgG4 anti-N-term34kDa peptide in non-infected samples, possibly indicating a blocking effect against the protein. IgG4 Abs against DENV Ep and NS1 were also detected in all samples, and tests for bispecific Abs showed that samples infected with DENV exhibit higher levels than non-infected samples. This contributes to our hypothesis that chronic exposure to *Aedes* salivary proteins in Dengue endemic areas leads to hybrid Ab production.

ZIKV neuronal infection RT-qPCR showed that negative control infections containing ZIKV alone resulted in lower levels of viral RNA when compared to the cells cultured along with the 34kDa protein, which aligns with previous observations that this protein has a replication-enhancing effect. Infection with ZIKV + total IgG from the DENV positive sample suggests an increase in ZIKV entry into the neurons, which may occur through ADE. This elevation was also observed in cells containing total IgG from the non-infected sample, indicating the possibility that these individuals may have pre-existing Abs to other flaviviruses, such as West Nile Virus, and these Abs may be acting similarly to those in the DENV positive individual. Also, IgG4 Abs in the presence of 34kDa protein increased ZIKV infection even further, supporting our hypothesis that bispecific DENV/mSP antibodies can result in an ADE effect and increase viral replication.

Future Directions

- Design a bispecific Ab kinetic model using HP-SEC and FRET microscopy to aid our understanding in the dynamic of bispecific Ab formation, which can be used to monitor human-vector interaction and disease risk after implementation of vector control interventions.
- Evaluate the *in vivo* therapeutic and preventative effect of synthetic hybrid mSP/DENV bispecific IgG4 Abs using a NOD scid gamma humanized mouse model infected with DENV serotypes 1-4.

Acknowledgements

Thank you to the KSU Veterinary Research Scholars Program and to Boehringer Ingelheim Veterinary Scholars Program for their generous scholar support and to Dr. Londono-Renteria for mentoring me throughout this project. Funding was provided by the NIH-P20GM103638 (COBRE – University of Kansas) grant.

References

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2. Londono-Renteria, B. (2020). The Biology of Hybrid IgG4 antibodies in Viral Emerging and Re-emerging Diseases.