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Distribution of Dengue and Zika Virus IgG Immunoglobulin

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Objective

Dengue Virus (DENV) and Zika Virus (ZIKV) are viruses that belong to the Flavivirus family. They are transmitted by the *Aedes aegypti* species of mosquitoes. Infection with DENV can result in no symptoms, mild symptoms which include fever, rash, and headache (dengue fever) or more severe symptoms which include hemorrhage, dengue hemorrhagic fever (DHF) and shock, dengue shock syndrome (DSS). ZIKV, until recently caused mild disease but an outbreak in Brazil was associated with fetal complications such as microcephaly or Guillain-Barré syndrome in adults. Due to the similarity between ZIKV and DENV, antibodies (Abs) generated in humans to these pathogens are of great interest. The focus of this honors project was to compile data that can be used in a comparison of the distribution of Abs in ZIKV and DENV immune donors.

Introduction

An antibody (Ab) is a protein used by the immune system to identify and neutralize foreign objects in the body. There are five major types of C regions which correspond to five different classes of antibodies which are IgM, IgA, IgD, IgE,or IgG. My project focused on IgG. This antibody class is responsible for resistance against many viruses, bacteria and bacterial toxins. There are four subclasses of IgG with distinct qualities.

IgG1 (~60-65% of total IgG)

The most abundant and most versatile subclass.

IgG2 (~20-25% of total IgG)

The second most abundant with an additional disulfide bond that reduces flexibility and proteolysis. Preferably made against repetitive carbohydrate antigens.

IgG3 (~5-10% of total IgG)

IgG3 is the best subclass at activating complement due to longest hinge region with greatest flexibility. This property also decreases the half-life of IgG3 in sera. This is due to the increased likelihood of cleavage by proteolysis.

IgG4 (only 4% of total IgG)

IgG4 is the least abundant subclasses and does not activate complement. IgG4 has anti-inflammatory effects and neutralization. It does possess the unique ability to exchange heavy and light chains. Therefore, the majority of IgG4 molecules are composed of two different heavy chains, two different light chains and two different antigen binding sites. Overall, the precise effects of IgG4 are still unknown.

Methods

We used an Enzyme-Linked Immunosorbent Assay (ELISA), to measure IgG subclass Abs (concentrations) in sera obtained from DENV and ZIKV immune donors from BEI Resources. The sera were assayed to measure ZIKV and DENV-specific Abs using well characterized isotype-specific secondary Abs (IgG, IgG1-Fc, IgG1-Hinge, IgG2, IgG3 and IgG4). The data was analyzed to compare antibody responses to multiple flavivirus antigens in the sera. The abridged Indirect ELISA protocol is described below.

1. Coat 96 well plates with diluted antigen or protein. Cover plates and store overnight.
2. Next day, remove protein from plate and add Block buffer. Discard block, wash 2 times with plate washer.
3. Add serum per 96 well and incubate. Follow with washing 2 times with plate washer.
4. Prepare secondary antibody at desired concentration with block solution and add to each well. Wash 2 times with plate washer.
5. Add substrate soluble TMB per 96 well and incubate.
6. Add 50 µl per 96 well 1N HCl to stop reaction.
7. Take O.D at 450 nm. Sample is considered positive if it is 2X over background. (no protein, coating buffer only)

Results

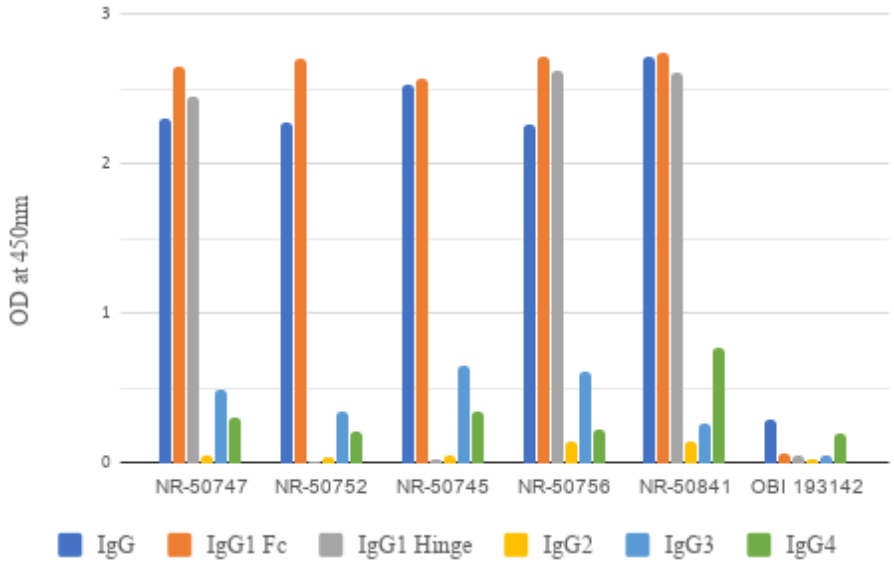

Fig.1 IgG Subclass ELISA for ZIKV Immune Sera. The plate was coated with a ZIKV VLP antigen. High levels of IgG and IgG1 were seen across immune donors with varying IgG2, IgG3 and IgG4 OD values. The OBI serum is ZIKV naive and was the negative control for this assay.

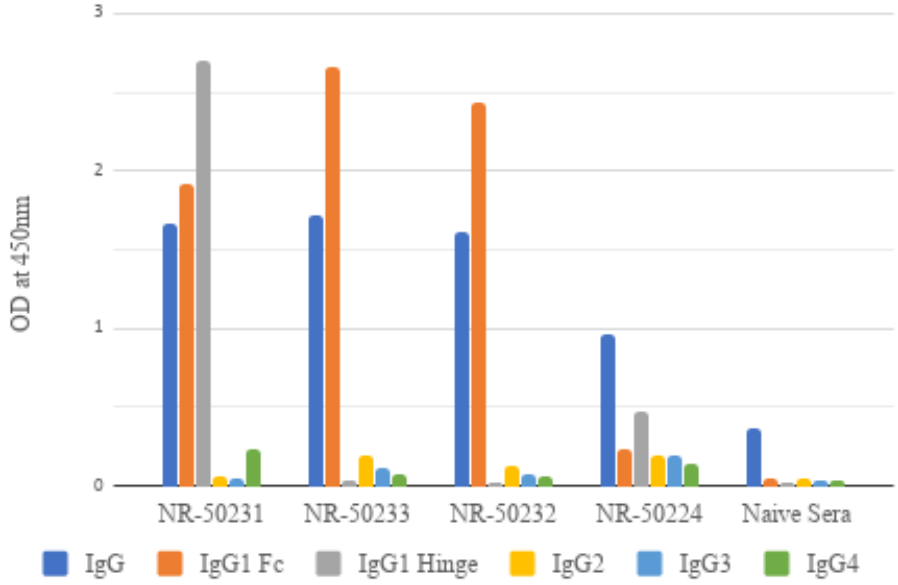

Fig. 2 IgG Subclass ELISA for DENV Immune Sera. In these assays, the plate was coated with a D1, D2, D3, and D4 VLP pool. The sera were tested in multiple rounds and the data seen above is a compilation of these assays with a naive serum used in each assay. The same trends are seen with a high response to IgG and IgG1 in the immune sera tested.

Discussion and Conclusion

Conclusions of data collected:

- Observed high IgG Abs to ZIKV virus like particles (VLPs) in sera from all donors
- IgG1 responses were dominant among subtypes as expected

The aim of my honors project was to study and compare the distribution of IgG subclasses in DENV and ZIKV immune sera. By the end of this project, I met my goal of applying previous research I have conducted to a ZIKV-specific assay. Furthermore, my knowledge on the mechanisms of ZIKV has also expanded. Through this honors project, I was able to conclude the research I have been conducting throughout my undergraduate career at I Cubed and generate data that has the potential to be used in future publications.

Acknowledgements

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