The purpose for the experiment is to test the immunogenicity of recombinant Zika virus (ZIKV) E protein in BALB/c mice and to attempt to generate monoclonal antibodies (MAbs) against the antigen. ZIKV is a member of the family Flaviviridae and genus Flavivirus and was first isolated in 1947 in the Zika forest in Uganda. It has been shown to be transmitted by infected mosquitoes of various Aedes species. Since 2015 it has been implicated in causing birth defects such as microcephaly in babies born to mothers infected during the early stages of pregnancy. Generating hybridoma cell lines secreting monoclonal antibodies specific to ZIKV E protein can aid in biomedical research to understand viral epidemiology, but also with the development of diagnostics or potentially even future immunotherapeutics. Detailed study of the generated MAbs can also be used to help further the understanding of the importance for cross-reactivity with other flaviviruses such as West Nile (WNV) or dengue viruses (DENV).

**ABSTRACT**

In recent years there has been a surge in interest by the US Government after confirmed ZIKV outbreaks in the Americas, African countries & some Pacific countries have been shown to cause birth defects such as microcephaly. The first case confirmed in the Americas was in May 2015 in Brazil. This started a surge towards making vaccines and antibodies against ZIKV. Previous to 2007, ZIKV wasn’t widely thought of when diagnosing patients (only 14 confirmed cases) because ZIKV’s symptoms are quite similar to those of other Flavivirus such as WNV & DENV. The symptoms can be varied from mild to severe and can include joint pain, fever, red eyes & rash; however these symptoms can be mild enough not to ever cause suspicion of any major disease. So visiting a medical center may never occur. This is the reason why ZIKV infection often remains un-diagnosed. If symptoms occur, they typically last only several days up to a week. Severe disease and fatalities are rare, but transmission of ZIKV can occur via several different routes. In addition to the traditional route of an infected person being bitten and passing the infection to a mosquito vector, a mother can also pass on the virus to her child in the womb, and sexual transmission has also been seen. Since currently there is no treatment for ZIKV it is recommended that caretakers of infected persons take extra precautions until symptoms subside. CDC’s recommendations are for patients to keep well hydrated, get plenty of rest, and take acetaminophen to alleviate fever/pain.

**INTRODUCTION**

Administration of recombinant, highly purified E Protein to BALB/c mice should generate antibody responses specific to ZIKV. The mouse B cells can be used to create hybridomas stably producing MAbs.

- Generate hybridoma cell lines using immune mouse splenocytes and screen clones for antibody production
- Test resulting clones for specificity to ZIKV to demonstrate the utility for future laboratory assays and research

**EXPERIMENTAL DESIGN**

(1) Recombinant Antigen Expression

- Transfect and express in Drosophila melanogaster S2 cells
- Purify the E Protein using protein-specific immunofluorescent chromatography

(2) Immunizations of BALB/c Mice

Doses of 10μg of purified ZIKV E Protein adsorbed to Alhydrogel were injected into 5 BALB/c mice over the course of 31 days. Each serum collection was assayed using ELISA to determine the IgG titer of each mouse and select the two highest responders. After the final booster, two of the mice were euthanized and spleenocytes used for the fusion to create the hybridoma cell lines.

**RESULTS**

Monoclonal Antibody Production against Zika virus Envelope Protein

**REFERENCES**

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