Aedes aegypti Mosquito transmission and the development of STAT2 KO hamster animal model for Zika Virus

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Background

- Mosquito-borne Zika virus (ZIKV) is responsible for the recent outbreak of febrile illness that appears to be asymptomatic in many cases. According to WHO, 65 countries and territories have reported evidence of mosquito-borne Zika virus transmission since 2007. Latin America and the Caribbean are currently experiencing an overwhelming number of ZIKV diseases and congenital infections. Additionally, the CDC reports approximately 5200 cases throughout the United States and Territories.
- While many cases fail to present with clinical manifestations, the neurological implications that result in microcephaly and other aberrant neurological developments in neonates as well as associated Guillain–Barré syndrome in select individuals have raised concern regarding the spread of the virus. Information concerning disease transmission, immune response to infection and efficacy of antiviral components in early gestation remain unclear.
- To address these unknowns, an appropriate and effective animal model is necessary to study the pathogenesis and pathology of the virus.

Aim

To develop an animal model that recapitulates ZIKV infection cycle using both natural route by Aedes aegypti vector and/or an intra-dermal route that mimics human infection. By identifying the viral-induced immune response and pathology in the STAT2 KO hamsters, we expect that this will be a suitable in vivo model to further study ZIKV transmission and pathogenesis.

Growth characteristics of Zika virus strain MR-766

- Figure 4: 6 days after Vero cells were infected with ZIKV MR-766 in a 96-well cell, cells were stained with 0.2% crystal violet in 30% Formaldehyde and 20% ethanol. a) Vero cells with marked CPE, as indicated by loss of cell structure (positive). b) Vero cells showing no CPE remade and intense staining. Images were taken from dissecting microscope.
- Figure 5: Three cell cultures infected with ZIKV MR-766 (positive) from larval tumour, which harbors human papillomavirus (HPV) gene sequences. Zein from kidney of an African green monkey, C6/36 from larvae of Aedes aegypti, and Vero cells were infected with ZIKV MR-766 at a multiplicity of infection of 0.03. Cell monolayers were inoculated with 30 μl of stock virus mixed with 0.5 ml of growth medium (DMEM) and 10% FBS, incubated for 4 h at 37°C and gently swirled every 15 min. 5 μl of growth media was added to the cells at 2 h. After 72 hrs, when ~80-100% cells exhibit CPE, infected cells were collected from the supernatant to give the ZIKV viral stocks and maintained at -80°C until later use.

Competency of mosquito strain, Aedes aegypti HWE strain, for ZIKV MR-766

- Figure 6: 7 DPI ZIKV stained midgut and carcass of 17 infected mosquitoes. The presence of ZIKV in the carcasses demonstrates that there is not a midgut escape barrier for the virus, suggesting the possible competency of Aedes aegypti as a ZIKV MR-766 vector.

Experimental Procedures

Part 1: Prior to infecting hamsters

- Propagating ZIKV MR-766 virus on Vero cells: Vero cells were seeded in T25 flasks with 0.5 x 10⁶ cells/ml. When confluency was determined to be ~90% cells/ml after 3 days, cells were infected with ZIKV MR-766 (African lineage), at a multiplicity of infection of 0.03. Cell monolayers were inoculated with 30 μl of stock virus mixed with 0.5 ml of growth medium (DMEM) and 10% FBS, incubated for 4 h at 37°C and gently swirled every 15 min. 5 μl of growth media was added to the cells at 2 h. After 72 hrs, when ~80-100% cells exhibit CPE, infected cells were collected from the supernatant to give the ZIKV viral stocks and maintained at -80°C until later use.

- Feeding and infecting mosquitoes

Aedes aegypti, HHE strain, are maintained on raisins and water until adulthood, before being fed defibrinated sheep’s blood, supplemented with ZIKV MR-766 infected cell culture (1:1) and ATP as a phage-stimulant (figure 2). After one hour of feeding, blood is removed and fully engorged females (figure 1) are separated from un-fed mosquitoes.

- Determining viability of viral strain and competence of mosquito

At 14 days post blood feeding, mid-guts and carcasses are collected from infected mosquitoes. To analyze ZIKV titers, tissue culture infectious dose 50 assay (TCID50) was performed. Vero cells in 24 well plates were inoculated with 150 μl of sterile-filtered saliva sample (diluted in DMEM+10%FBS). After 7 days of incubation, media was removed and 100μl of 0.2% crystal violet in 10% Formaldehyde and 20% ethanol was added to stain for 10-15 minutes. Wells were washed and scored for CPE effects. This was repeated daily for 3-4 days. The virus titer was calculated based on 50% end points using the Reed and Muench algorithm and expressed as a log10 TCID50/mL.

Part 2: Infecting the hamsters

To compare WT and STAT2 KO hamsters with ZIKV, Syrian Golden hamsters (Mesocricetus auratus) (figure 3) will each be anesthetized and infected by mosquito bites or by intra-dermal injections, to best recapitate the natural route of infection. A 2cm x 2cm patch will be shaved on the ventrum of the hamsters. There will be three groups, with pairs of WT and STAT2 KO hamsters. Group 1, natural route (n=6): infected mosquitoes from part 1 of the experiment will be exposed to anesthetized hamsters for 30 mins. 3 pairs of WT and KO, with 40, 10, 10 mosquitoes to allow different amounts of exposure and levels of infection.

Group 2, intra-dermal route (n=6): 30ul, 10⁷-10⁸ pfu/mL, to be injected into the dermis at multiple sites of anesthetized hamsters.

Group 3 (n=2): WBIC counts and antibody to be assessed following determination of infection results of Group 1 and 2.

Once infected, hamsters will be monitored daily for signs of illness and disease progression for at least 14 days post infection. Blood will be collected to assess viral titers and peak viremia. Whole blood will also be analyzed by RT-PCR for ZIKV RNA, which would be used to inoculate cell cultures to isolate infectious virus. At a later time, this will allow the study of uninfected mosquitoes to feed on infected hamster and assess whether or not the mosquitoes can be infected from an infected host. Liver, spleen, neurological tissues and testes/ovaries would be collected for virus isolation and histopathology.

Additional information and pitfalls

On STAT2 KO Syrian Golden Hamsters

- Advantage over other rodents: greater metastasis and physiological similarities to humans.
- STAT2 is a crucial element of Type I and III IFN signal transduction pathway.
- Type I IFN pathway is disrupted in STAT2 KO hamsters; cannot up-regulate the expression of ISGs, which makes them more susceptible to viral infections. 1, 2, 3

Pitfalls: Polyomavirus in Hamsters

- Hamster 1: found dead and autolyzed
- Hamster 2: moribund and euthanized: positive HaPV: gastric mucosal necrosis, hemangiosarcoma (figure 9)

On Hamster Polyomavirus

DNA oncogenic virus that induces necrotizing or proliferating lesions mainly in lungs and liver. The virus uncommonly induces two neoplastic syndromes, depending on age of animal when infected. In young, virus is shed and transmitted in urine and multicentric lymphoma involving mesenteric, lymphatic and abdominal vessels is common. In adults, virus is shed and transmitted in infected epithelial cells and skin neoplasms (trichoepitheliomas) is common. Endothelial cells and other components of the vascular wall are the main target cells for oncogenic activity of the polyoma virus.

Conclusions at this time

Aedes aegypti mosquitoes were infected with ZIKV MR-766 propagated on Vero cell culture. Viable ZIKV MR-766 virus was detected in mid-gut, carcass and saliva, suggestive of their ability to be competent vectors. Once STAT2 KO hamsters are infected by the mosquitoes or intra-dermally injected from the viral stocks, immune response and pathogenicity can be assessed.