Evaluation of Viral Entry and Cellular Passage of Zika Virus Immune Complexes in a Tissue Culture Model of the Maternal-Fetal Interface

Momben Abolfath.S; *Xu.Y; *He.Y; *Eller.N; *Norton.M; *Struble.EB

***Plasma Derivatives Branch, Division of Plasma Protein Therapeutics, Office of Tissues and Advanced Therapies, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration**

Abstract

When acquired during pregnancy, Zika virus (ZIKV), a member of the Flaviviridae family of RNA viruses, is associated with fetal microcephaly and other birth defects of the newborn. Treatment with anti-ZIKV polyclonal antibodies has been proposed as a potential therapy, benefiting both mother and baby. Unlike most biologics, maternal immunoglobulin G (mIgG) passes the placenta, playing a crucial role in protecting the fetus and future newborn from viral infections. This process is mediated by the neonatal Fc receptor (FcRn), widely expressed in placental cells. The potential exists that mIgG may facilitate the transfer of ZIKV pathogen across placenta leading to the enhancement of fetal infection. This process is analogous to antibody-dependent enhancement (ADE) of viral disease, which has been shown to correlate with enhanced viremia and disease severity in other viruses. Thus, before anti-ZIKV IgG therapy can be tested in clinical trials, there is a need in determining whether it could enhance Zika replication and exacerbate disease pathogenesis, especially in the fetus. To evaluate this potential, we developed an in vitro model using mammalian placenta cells and cells overexpressing human FcRn to evaluate the role of ADE in viral infection using anti-Zika antibody. We found that ZIKV can enter and be transferred across placental and epithelial cells expressing FcRn. The viral entry in FcRn+ cells was depended on the IgG concentration in a bimodal way: it was reduced at the lowest (0.3-3 ng/mL) and highest (3 µg/mL) concentrations, yet it was increased at intermediate concentrations. Enhancement of viral entry was also seen at intermediate IgG concentrations in placental cells. On the other hand, anti-ZIKV antibodies degraded at a faster rate in the presence of ZIKV immunogen. Of the two monoclonal antibodies tested, the preparation with higher aggregation exhibited higher degradation. In conclusion, Zika virus has the potential to be transferred across the placenta and epithelial cells expressing FcRn. Blockage or enhancement of viral entry depends on the anti-ZIKV IgG antibody concentration. Our in vitro model could be used as a screening tool to assess the prophylactic and therapeutic antibody treatments against Zika infection.

Introduction

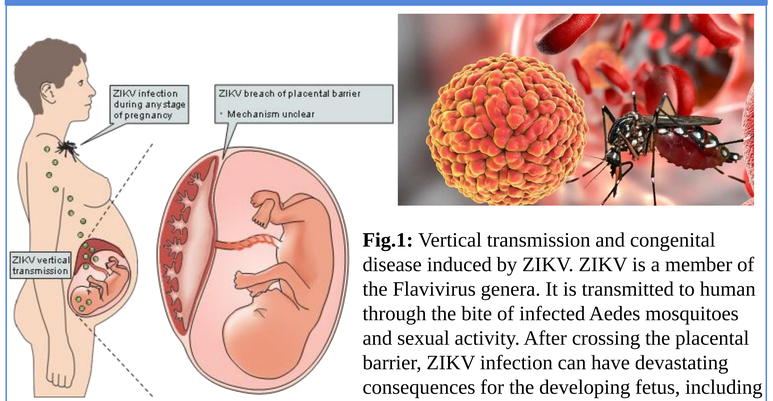

Fig.1: Vertical transmission and congenital disease induced by ZIKV. ZIKV is a member of the Flavivirus genera. It is transmitted to human through the bite of infected Aedes mosquitoes and sexual activity. After crossing the placental barrier, ZIKV infection can have devastating consequences for the developing fetus, including microcephaly.

Coyne.CB, Nat Rev Microbiol.. 2016 Nov;14(11):707-715. doi: 10.1038/nrmicro.2016.125.

Introduction

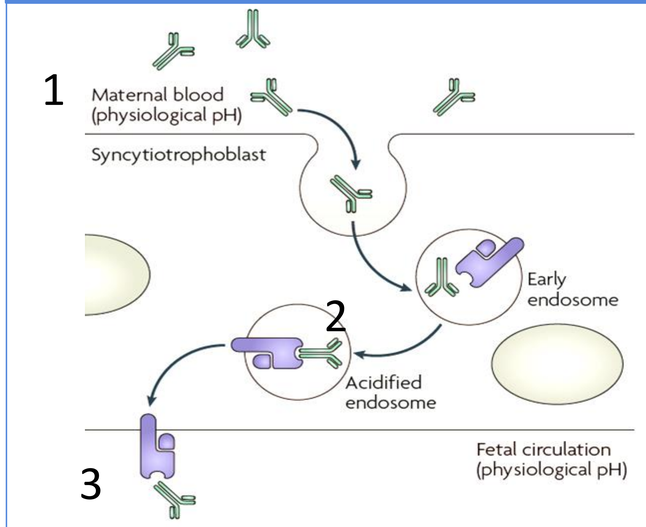

Fig.2: FcRn mediates the perinatal transfer of IgG**.** Syncytiotrophoblasts are bathed in maternal blood and internalize serum containing maternal IgG. The neonatal Fc receptor for IgG (FcRn) is expressed in the endosomes of the syncytiotrophoblast. Upon acidification in the endosome, FcRn binds to maternal IgG and transcytoses it to the fetal circulation where it is released at physiological pH.

Roopenian and Akilesh, Nature Rev Immunol. 2007 Sep;7(9):715-25. doi: 10.1038/nri2155. Epub 2007 Aug 17

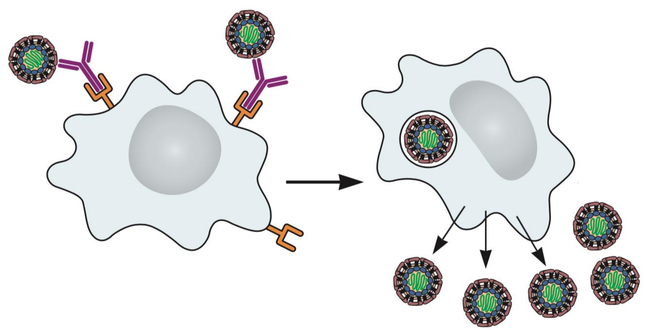

Fig.3: Schematic diagram of antibody-dependent enhancement (ADE) of infection in the Fc receptor-positive cells. Flaviviruses are unusual because antibodies that cross-react with different species and serotypes can enhance infection and disease. This property called ADE, has been documented to occur among the four serotypes of dengue virus. It has implications for infection with or vaccination against Zika virus or dengue virus.

https://www.virology.ws/2016/07/28/antibodies-aid-dengue-and-zika-virus-infection/

Objectives

- Develop a model of the maternal-fetal interface to study the vertical transmission of Zika virus and its immune complexes during pregnancy.
- Use the model to assess the role that IgG antibodies play in blocking fetal infection.

Material and Method

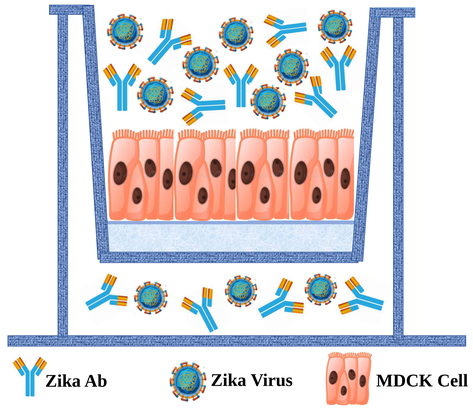

Fig.4: Schematic of the experimental set up. MDCK cells that overexpress human FcRn and BeWo cells were grown on a semipermeable membrane; immune complexes (IC) were added on the apical chamber and assessed in the basolateral chamber.

Results and Discussion

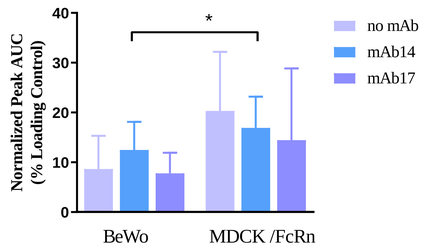

Fig.5: Quantification of band intensity from Western blots analysis of the basolateral chamber of transcytosis experiments with MDCK/FcRn or BeWo cells, blotting for Zika gpE. For both cell lines and both antibodies tested, the addition of IgG in the input chamber did not significantly change the amount of gpE in the output buffer. Compared to MDCK/FcRn cells, transcytosis of gpE was significantly lower in BeWo cells

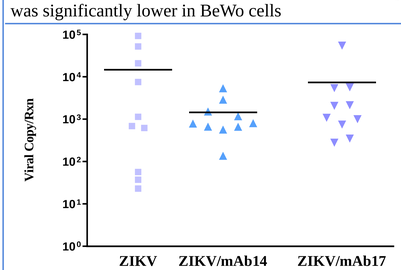

Fig.6: Transcytosis of intact Zika virus in MDCK/FcRn and BeWo cell monolayers occurs for the virus alone or in the presence of mAb. For both cell lines and both antibodies tested, the addition of IgG in the input chamber did not significantly change the amount of Zika virus in the output buffer. Similar high variability but lower group averages was seen in BeWo cells.

Results and Discussion

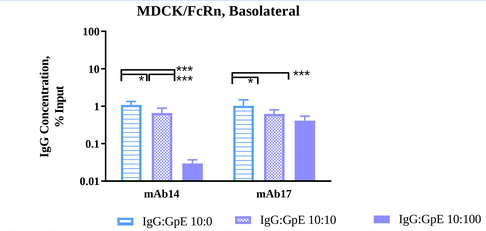

Fig.7: Transcytosis of IgG-Zika gpE immune complexes(IC), assaying for IgG. IC were formed by mixing IgG and gpE at nominal w:w ratios of 10:0, 10:10 and 10:100 µg/mL in the apical chamber of transwells containing monolayers of MDCK/FcRn or BeWo cells. Less transcytosis levels of IgG were seen with increasing amounts of antigen in the basolateral and apical chambers, respectively (data from experiments with MDCK/FcRn cells, analogous results were obtained from BeWo cells).

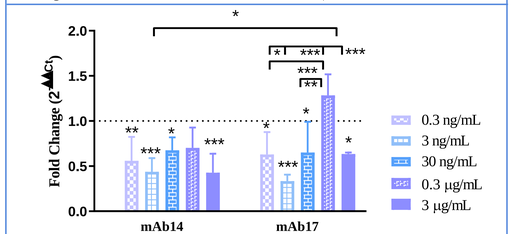

Fig.8: Cell entry of ZIKV in MDCK/FcRn cells in the presence of anti-ZIKV mAb14 and mAb17 showed a bimodal behavior with two local minima at 3 ng/mL and 3 µg/mL

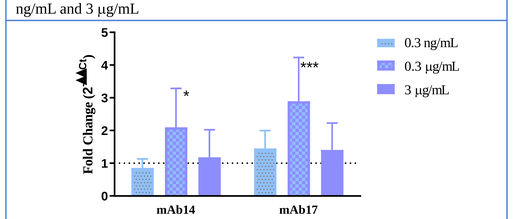

Fig.9: In BeWo cells an intermediate concentration (0.3 µg/mL) anti-ZIKV mAb14 and mAb17 significantly enhanced viral entry; no change was seen at the lowest and highest concentrations tested.

Conclusions

- Zika virus has the potential to be transferred across the placenta and epithelial cells expressing FcRn.
- Blockage or enhancement of viral entry depends on the anti-ZIKV IgG antibody concentration.

Regulatory Implications

The tissue culture model of maternal-internal face could be used as a screening tool to assess the prophylactic and therapeutic antibody treatments against Zika infection.

Acknowledgment

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