



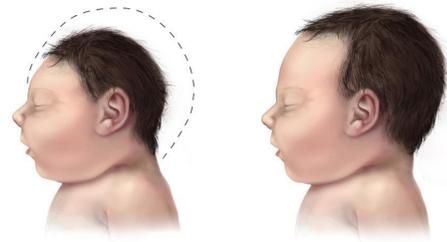
The Effects of Autophagy Induction on ZIKV Parthogenesis

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Introduction

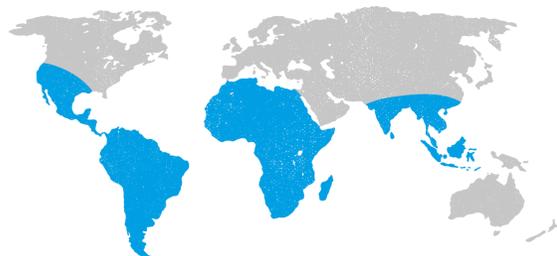
- Zika virus (ZIKV) is an emerging flavivirus (1)
 - Spread primarily by the bite of the female *Aedes aegypti* mosquito (2)
 - Endemic in the continents of South America and Africa as well as various other tropical and equatorial locations.
 - Also spread via other means. (3)
 - Can be transmitted sexually or through blood transfusions, though the latter is comparatively rare due to advanced screening of donated blood before a transfusion can take place (4)
- Zika fever is caused by ZIKV (5)
 - Generally presents with symptoms similar to mild forms of other flaviviruses such as dengue (4)
 - Muscle and joint pain
 - Generalized malaise
 - Fever
 - Rash
 - In the majority of cases, Zika fever does not require hospitalization or drastic measures such as government-enforced quarantine, generally subsiding after approximately one week (5)
- Causes microcephaly in developing fetuses when it infects a pregnant woman (6)
 - Approximately 42% of births, excluding spontaneous abortions, from ZIKV-infected mothers resulted in some degree of microcephaly (6)
 - a condition in which the fetus is born with an abnormally small and disproportionate cranium in comparison to its body size and age
 - Causes a variety of neurological issues (7)
- Autophagy (8)
 - The process by which cells destroy themselves when they can no longer properly function
 - Regulated by the ATG12-ATG5 complex (9)
 - Beneficial to the bactericidal process
 - Potentially harmful in the viricidal process (9)



<https://upload.wikimedia.org/wikipedia/commons/e/ee/Microcephaly-comparison-500px.jpg>

Literature Review

- A new virus had been isolated from rhesus monkey serum in the Zika forest, and it bore little resemblance to previously known viruses and therefore was new.
 - Dick, G., et al. (1953)
- There are two strains of Zika hailing from Africa and America with 90% genomic similarity.
 - Baronti, et al. (2014)
- Zika could potentially transmit through blood transfusion.
 - Musso, D., et al. (2014)
- Zika's primary transmission method was via mosquitoes.
 - Bogoch, I. et al. (2016)
- Zika had the potential for sexual transmission.
 - Foy, B. D. et al. (2016).
- Zika was a possible implicating factor in the development of microcephaly.
 - Talan, J. et al (2016)
- Zika was declared a public health emergency by the Centers for Disease Control and Prevention.
 - Gulland, A. (2016).
- Zika consisted of a 10 protein genome with 3 structural proteins.
 - Enfissi, A., et al.(2016)



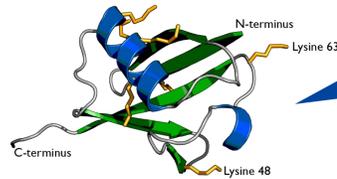
<https://www.nomadtravel.co.uk/travel-vaccines/attachment/zika-virus-disease-map>

Hypothesis

Removing the ATG12 protein will decrease the overall viral replication rate and viral load.

The knockout culture will exhibit a lower viral load at the 24-hour mark.

Gap in the Research



The mechanisms which drive the replication of ZIKV are currently not well understood.

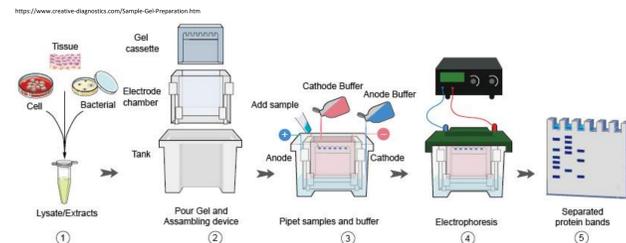
Methodology

Cell Culture

Trophoblasts incubated at 37c in a CO2 incubator and grown in RPMI medium treated with 10% of FBS and pen-strep antibiotic
Trophoblast knockout generated and cultured in similar conditions to the wild type

Vero cells cultured in DMEM medium treated with 10% FBS and pen-strep antibiotic

Western Blot



Performed to ensure that the gene had been effectively knocked out

ZIKV Infection

- The wildtype trophoblast cells and the ATG12 knockout were cultured to approximately 90% confluence
- Cells were treated with cold PBS for one minute before being dislodged by the appliance of fresh RPMI medium
- Cells were seeded in a 24-well plate and incubated until reaching confluence.
- Medium was then removed, and the cells were treated with Zika virus in all wells for 3 hours

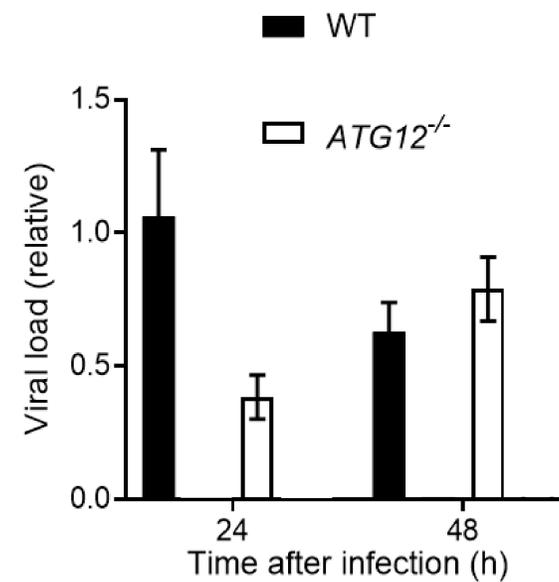
RNA extraction

- The Total RNA Kit I by OMEGA was used to extract RNA samples from both the wild type and ATG12 knockout samples
- Extracted RNA samples were subjected to RT-PCR to generate cDNA
- cDNA samples were then subjected to QPCR analysis
 - The target for the cDNA samples were ZIKV, IFNBI, and Actin.



<https://www.huck.psu.edu/institutes-and-centers/center-for-ma-molecular-biology>

Results



Discussion

- Significantly decreased relative viral load in the ATG12 knockout cell like compared to the wild type cell line
 - Indicates that the presence of the ATG12 protein increases ZIKV replication
 - Supports the predicted hypothesis
- At the 48-hour-timepoint, the two cell lines had approximately similar viral loads
 - Indicates that viral load in wild-type cells decreases over time following the initial 24-hours of infection
 - Could be attributed to immune response from the cells themselves
- Potential applications include preventing viral replication during gestation
 - Potentially limit the development of microcephaly or eliminate it entirely
 - Reduce severe symptoms and other side effects of ZIKV infection
 - Guillain-Barré syndrome



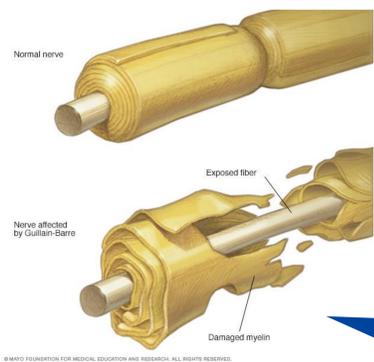
<https://www.theverge.com/2016/4/13/11424476/zika-microcephaly-link-confirmed-cdc>

Conclusion

- The data observed during this study supports the hypothesis that removal of the ATG12 protein decreases viral replication rate
- Viral load decreases 48 hours after the initial infection
 - Attributed to cellular defense mechanisms
- Results were statistically significant
- Applications in preventing microcephaly
 - Decreasing microcephaly rates also decrease instances of epilepsy and learning disabilities
- Data can also be utilized in the prevention of neurological side effects like Guillain-Barré syndrome
 - Prevents paralysis and chronic nerve pain

Future Research

- Future research will include studies that determine the applications of this data
 - How viral replication rates can be decreased during pregnancy
 - Whether the chances of developing microcephaly decrease if viral replication rate is lower
 - Reducing viral replication in mosquitoes
 - Vectors of ZIKV
- Research will focus primarily on preventing the development of microcephaly and other severe side effects of ZIKV infection



- Prevention of neurological syndromes like Guillain-Barré syndrome will decrease rates of paralysis and epilepsy
- Reduction of harmful symptoms such as high fevers or hematospermia
- Prevent infertility and reproductive issues

Nerve damage in Guillain-Barré syndrome

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