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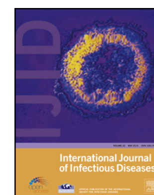

Short Communication

Infection of human uterine fibroblasts by Zika virus in vitro: implications for viral transmission in women

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Zika virus (ZIKV) is a single-stranded RNA arbovirus belonging to the *Flavivirus* genus.[1,2] Similar to other zoonotic arboviruses, ZIKV is transmitted by the Aedes mosquito. It causes Zika fever, a usually non-fatal condition with symptoms including headache, fever, malaise, maculopapular rash, joint pains, and conjunctivitis.[2] Recently, it was revealed that ZIKV may have additional pathogenic effects on pregnant women and their fetuses. Reports from South America suggest that ZIKV infection during pregnancy causes fetal microcephaly,[2] which has negative impacts up to and including death.[2,3] In addition to mosquito-borne transmission, it has been reported that ZIKV may be transmitted through heterosexual intercourse,[2] with concomitant implications for global women's health. This is the first example of a mosquito-borne arbovirus that may also utilize a sexual route of transmission. This knowledge has initiated a massive effort by the translational science community to develop more effective detection methods and treatment strategies.

Currently there are no approved vaccines or drugs for preventing or treating ZIKV infection or ZIKV-induced pathologies. Recent diagnostics development efforts have focused on ELISA and highly sensitive quantitative reverse transcription PCR (RT-qPCR)-based detection to identify affected individuals. Viral load assays can quantify ZIKV RNA extracted directly from patient specimens (e.g., blood) and utilize ZIKV-specific amplification primers that target conserved regions of the viral genome.[4,5] Another viral characterization strategy utilized by researchers involves infection of cells that are permissive to ZIKV infection before detection. Studies have shown that human dermal fibroblasts and placental macrophages can be directly infected by ZIKV.[6,7] By utilizing these in vitro diagnostic methods, it is possible to characterize the systemic infection to better inform vaccine or drug development. The objective of the current work was to determine whether uterine fibroblasts (UF), the primary histo-architectural cell of the uterus and endometrium, are susceptible to ZIKV infection in vitro.

Cryopreserved UF were revived as recommended by the American Type Culture Collection (ATCC, Manassas, VA, USA) and the cells cultured to confluency using methods reported previously.[8] Individual flasks of UF were then infected with either VR-84 or VR-1843 (ATCC). The inoculum was prepared in 1:100 and 1:1000 dilutions for each strain in low serum UF medium. Primary infection was conducted by removing growth media and then adding the diluted inoculum to each cell preparation. Cells were allowed to equilibrate with the virus for 3 h. Each flask of UF was then washed with phosphate buffered saline three times to ensure efficient removal of inoculum. Fresh media were added and the cells were cultured for 5 days, with aliquots of media removed at day 3 and day 5 to determine whether there was an incremental increase in viral load. At day 5, UF were trypsinized, pelleted, and frozen for RNA extraction on the QIAcube (Qiagen Inc., Valencia, CA, USA). To detect viral presence in these cultures, RT-qPCR with primer–probe sets that target the envelope region of the virus were utilized. The relative expression of viral load was analyzed using the threshold detection cycle (Ct) of each test sample normalized to NATTROL Zika Virus Ct (see Appendix 1), an inactivated, non-infectious molecular testing control material (ZeptoMetrix Corporation, Buffalo, NY, USA).

The results indicated that UF are susceptible to ZIKV infection (Figure 1). At day 3 and day 5, the conditioned medium exhibited a higher relative viral load compared to the inoculum for both strains of ZIKV, with the greatest relative increase observed for the 1:100 dilution of MR766 African strain at both time points. The relative

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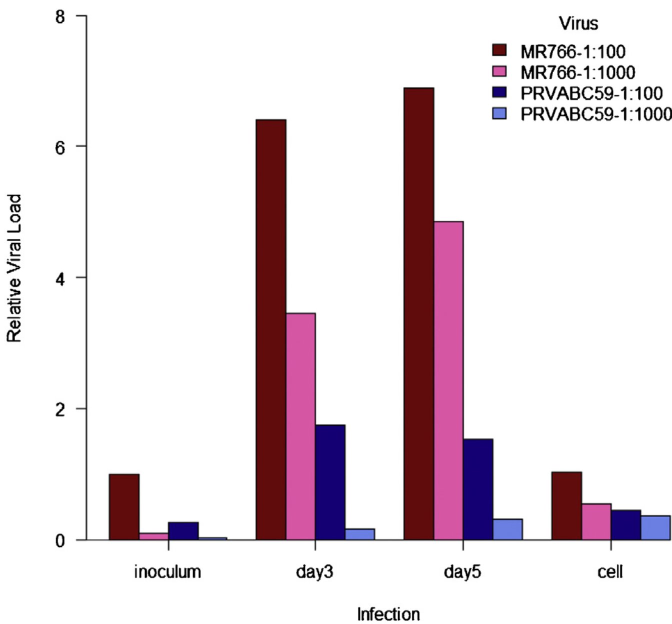

Figure 1. Relative expression of Zika virus in cultured uterine fibroblasts (UF) compared to negative control. The relative viral load is calculated utilizing the comparative cycle threshold (Ct) method. The relative levels of virus in the inoculum for 1:100 and 1:1000 dilutions of each strain are indicated. Relative levels of virus in the conditioned medium at day 3 and day 5 are shown. The relative expression in UF cells at day 5 is also reported. Uninfected cells and conditioned media were utilized as negative control.

quantitation results from pelleted cells after the removal of media were also higher than the initial inoculum. Moreover, it was observed that UF cells maintained viability after infection (95–99% viability at day 5), indicating that UF cells harbor and propagate ZIKV productively.

UF are derived from two main sources: endometrial stromal fibroblasts from the stratum functionalis, where attachment of the embryo and exposure to female reproductive tract components occurs, and the endometrial stratum basalis, where the systemic exchange of molecules with the uterus transpires.[9] That UF are susceptible to infection suggests that the uterus may act as a conduit for virus during heterosexual intercourse. Similar to studies in placenta, the uterus could also transfer virus from the maternal circulation to the developing fetus.

The authors acknowledge the limited scope of the experiment, and that the detection method used has only preliminary analytical performance parameters established. We are not aware of previous work describing the feasibility of this mode of infection in the female reproductive tract. These results provide support for additional investigations into this possible path to ZIKV infection. Furthermore, they inform the choice of cell type for infectivity assay development as a tool in achieving effective prevention and treatment strategies (763/800).

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Appendix 1

Infection of uterine fibroblasts with ZIKV strains

Both the African founder strain (MR766) and a more recently isolated Puerto Rico strain (PRVABC59) were tested. The inoculum was diluted to 1:100 and 1:1000 before adding to uterine fibroblast (UF) cultures. The description indicates the compartment sampled for RNA. The threshold detection cycle of real-time RT-qPCR (Ct) was utilized to determine the viral load. Controls included the positive control supplied by ZeptoMetrix (pos ctrl; the manufacturer did not indicate the strain used, but DNA sequencing was utilized to confirm homology to MR766), no template control (NTC, water), and uninfected cells. Primers were designed to the envelope region of the ZIKV MR766 African strain.

Sample	ATCC#	Strain	Dilution	Time	Description	Ct
Inoculum	VR-84	MR766	1:100	day 0	Inoculum	16.73
Inoculum	VR-84	MR766	1:1000	day 0	Inoculum	20.16
Inoculum	VR-1843	PRVABC59	1:100	day 0	Inoculum	18.67
Inoculum	VR-1843	PRVABC59	1:1000	day 0	Inoculum	21.65
UF cells	VR-84	MR766	1:100	day 3	Supernatant	14.05
UF cells	VR-84	MR766	1:1000	day 3	Supernatant	14.94
UF cells	VR-1843	PRVABC59	1:100	day 3	Supernatant	15.93
UF cells	VR-1843	PRVABC59	1:1000	day 3	Supernatant	19.38
UF cells	VR-84	MR766	1:100	day 5	Supernatant	12.89
UF cells	VR-84	MR766	1:1000	day 5	Supernatant	13.4
UF cells	VR-1843	PRVABC59	1:100	day 5	Supernatant	15.07
UF cells	VR-1843	PRVABC59	1:1000	day 5	Supernatant	17.34
UF cells	VR-84	MR766	1:100	day 5	Cells	15.65
UF cells	VR-84	MR766	1:1000	day 5	Cells	16.58
UF cells	VR-1843	PRVABC59	1:100	day 5	Cells	16.85
UF cells	VR-1843	PRVABC59	1:1000	day 5	Cells	17.13
Pos ctrl	ZKPC	ZeptoMetrix	1:1000	NA	NA	19.83
NTC	Water	NA	NA	NA	NA	No Ct
Uninfected	NA	NA	NA	NA	Cells	No Ct
Uninfected	NA	NA	NA	NA	Supernatant	No Ct

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