Virology 518 (2018) 301–312

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Infection by Zika viruses requires the transmembrane protein AXL, endocytosis and low pH

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ARTICLE INFO

Keywords:
ZIKA
Entry
Low-pH
Endocytosis
Clathrin

ABSTRACT

The recent Zika virus (ZIKV) outbreak in Brazil has suggested associations of this virus infection with neurological disorders, including microcephaly in newborn infants and Guillian-Barré syndrome in adults. Previous reports have shown that AXL, a transmembrane receptor tyrosine kinase protein, is essential for ZIKV infection of mammalian cells, but this remains controversial. Here, we have assessed the involvement of AXL in the ability of ZIKV to infect mammalian cells, and also the requirement for endocytosis and acidic pH. We demonstrated that AXL is essential for ZIKV infection of human fibroblast cell line HT1080 as the targeted deletion of the gene for AXL in HT1080 cells made them no longer susceptible to ZIKV infection. Our results also showed that infection was prevented by lysosomotropic agents such as ammonium chloride, chloroquine and bafilomycin A1, which neutralize the normally acidic pH of endosomal compartments. Infection by ZIKV was also blocked by chlorpromazine, indicating a requirement for clathrin-mediated endocytosis. Taken together, our findings suggest that AXL most likely serves as an attachment factor for ZIKV on the cell surface, and that productive infection requires endocytosis and delivery of the virus to acidified intracellular compartments.

1. Introduction

The recent outbreak of Zika Virus (ZIKV) in Brazil has raised important public health issues, particularly due to possible associations with neurological disorders including microcephaly and Guillian-Barré syndrome. ZIKV is a mosquito-transmitted flavivirus closely related to Dengue virus (DENV), West Nile virus (WNV), and yellow fever virus (YFV). Although ZIKV is transmitted by mosquitos, recent reports indicate the potential for male-to-female sexual transmission of the virus (Abushouk et al., 2016; Russell et al., 2017; Tang et al., 2016).

Similar to other flaviviruses, ZIKV contains a positive single-stranded genomic RNA encoding a polyprotein that is processed into three structural proteins [capsid (C), precursor of membrane (prM) and envelope (E)] and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) (da Fonseca et al., 2017). ZIKV is a membrane enveloped virus that requires fusion of the viral membrane with host cell membranes in order to cause infection. Entry of ZIKV to target cells is coordinated by the E protein arrayed on the surface of the virion. Once the virion components have reached the cytoplasm, the NS proteins form a replication complex that synthesizes a negative-sense RNA, which subsequently serves as a template for the positive-sense RNA. The newly synthesized RNA is encapsidated, transported by the host secretory pathway, and released from the infected cell by exocytosis.

The entry of ZIKV into mammalian cells is poorly understood, although evidence suggests that the membrane protein AXL contributes to this process by serving an attachment factor at the cell surface (Hamel et al., 2015; Liu et al., 2016; Meertens et al., 2017; Savidis et al., 2016). However, entry of ZIKV has been observed in human and mouse cells that do not express AXL (Miner et al., 2016; Rausch et al., 2017; Wells et al., 2016), suggesting that there may be additional factors that can perform the required functions for ZIKV entry on at least some types of cells. A recent study suggested that the interaction of the ZIKV virion with AXL is mediated by the AXL ligand, Gas6 (Meertens et al., 2017), which has also been shown to be involved in the entry of other viruses (Meertens et al., 2012; Morizono and Chen, 2014). In this scenario, Gas6 interacts with both the surface-exposed phosphatidylserine on the ZIKV virion and AXL on the surface of the cell, bridging the interaction of the ZIKV virion with AXL. These findings open the possibility that other proteins with the ability to bind to phosphatidylserine are contributing to the entry of ZIKV into different cell types, including those that do not express AXL.

The current study was carried out to further explore the role of AXL

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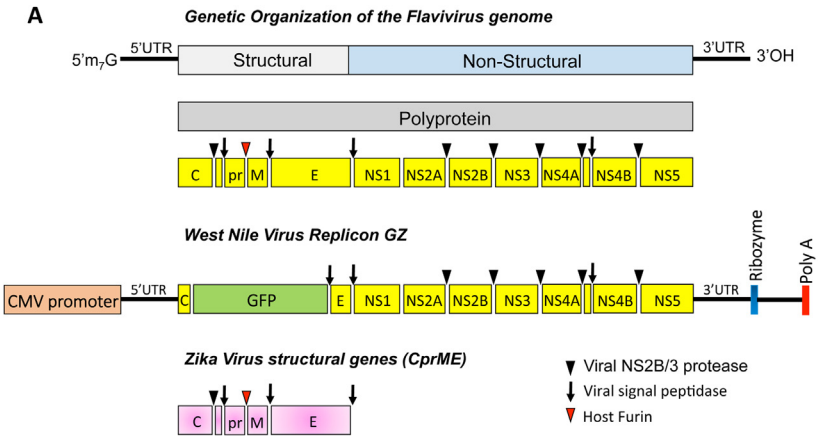
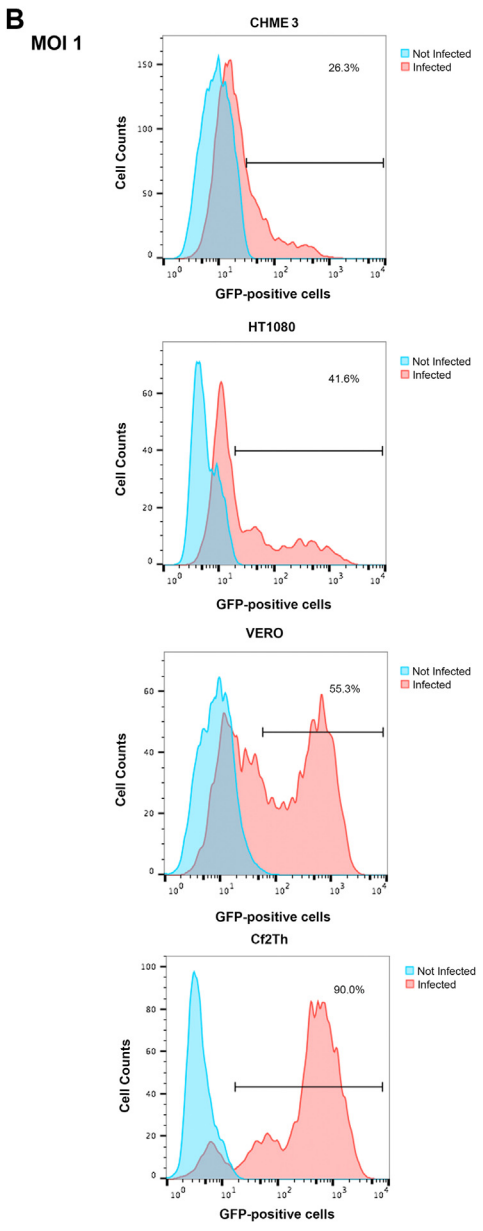
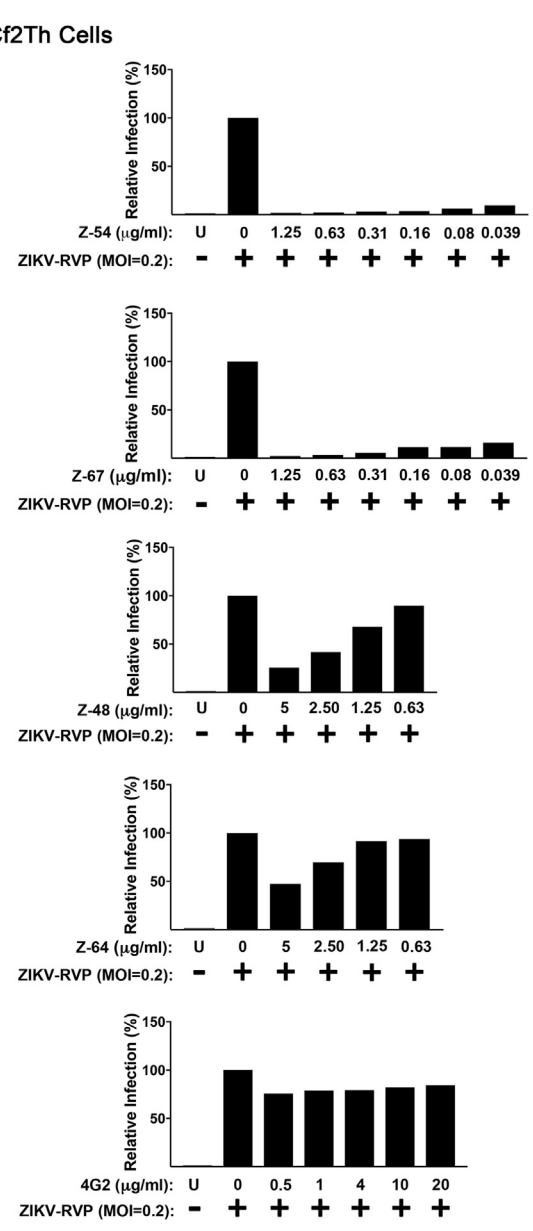
https://doi.org/10.1016/j.virol.2018.03.009
Received 6 March 2018; Accepted 10 March 2018
0042-6822/







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