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The South Pacific epidemic strain of Zika virus replicates efficiently in human epithelial A549 cells leading to IFN-β production and apoptosis induction

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ABSTRACT

Zika virus (ZIKV) is an emerging flavivirus since the first epidemics in South Pacific in 2007. The recent finding that ZIKV is now circulating in Western Hemisphere and can be associated to severe human diseases, warrants the need for its study. Here we evaluate the susceptibility of human lung epithelial A549 cells to South Pacific epidemic strain of ZIKV isolated in 2013. We showed that ZIKV growth in A549 cells is greatly efficient. ZIKV infection resulted in the secretion of IFN-β followed by the expression of pro-inflammatory cytokines such as IL-1β, and transcriptional activity of *IFIT* genes. At the maximum of virus progeny production, ZIKV triggers mitochondrial apoptosis through activation of caspases-3 and -9. Whereas at early infection times, the rapid release of IFN-β which exerts an antiviral effect against ZIKV might delay apoptosis in infected cells.

1. Introduction

The mosquito-borne Zika virus (ZIKV) is a newly emerging arbovirus that belongs to the *flavivirus* genus of the *Flaviviridae* family. Mosquito *Aedes* (*Ae.*) species with mainly *Ae. aegypti* are the vectors of ZIKV transmission (Li et al., 2012; Wong et al., 2013). Serological evidence points to a widespread distribution of epizoonotic ZIKV in the northern half of the African continent, as well as in many countries in South-East Asia (Alera et al., 2015; Berthet et al., 2014; Buathong et al., 2015; Diallo et al., 2014; Grard et al., 2014; Hayes, 2009). For decades, human ZIKV infections have remained sporadic till 2007 when a large outbreak of ZIKA fever was reported on Yap Island (Micronesia) (Duffy et al., 2009). ZIKV has recently gained a medical importance following the large-scale epidemics in the South Pacific especially in French Polynesia in 2013 (Hancock et al., 2014). The expansion of ZIKV in the South and Central America has been recently documented emphasizing the remarkable capacity of ZIKV to spread to non-endemic regions worldwide (Campos et al., 2015; Enfissi et al., 2016; Zanluca et al., 2015). Classically, human disease known as Zika fever is characterized by fever, maculopapular rash, headache, conjunctivitis, arthralgia and myalgia. Evidence of perinatal transmission of ZIKV has been initially documented during 2013/14 epidemic of ZIKV in French Polynesia (Besnard et al., 2014). High incidence of congenital microcephaly has been recently associated to ZIKV in Brazil (Dyer, 2015). Neurological complications such as Guillain-Barré syndrome were also documented during the major outbreak of ZIKV in French Polynesia (Oehler et al., 2014). No strategies for disease control are available to date. The finding that ZIKV is newly emerging has

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highlighted the need for an in-depth characterization of circulating strains responsible for the epidemics of Zika fever.

The purpose of our study was to improve our knowledge on the replication of ZIKV in human cells and the host-cell responses to viral infection. The clinical isolate PF-25013-18 of ZIKV was isolated from a patient during the epidemic of Zika fever in French Polynesia in 2013. Recently, Hamel et al. (2015) reported that primary human fibroblasts and immature dendritic cells are permissive to the PF-25013-18 strain of ZIKV. The enhanced expression of pattern recognition receptors RIG-I, MDA5 and TLR3 as well as Interferon-Stimulated Genes (ISGs) OAS2, ISG15 and MX1, were detected in human fibroblasts infected with ZIKV. Also, expression of pro-inflammatory cytokines such as IL-1β was induced by ZIKV. Lastly, the efficiency of ZIKV growth was associated with the induction of autophagy in human fibroblasts. Flaviviruses have been shown to infect epithelial cells (Suthar et al., 2012; Umareddy et al., 2007) and the permissiveness of human lung epithelial A549 cells to ZIKV has been recently reported (Hamel et al., 2015). In the present study, we evaluated the ability of ZIKV strain PF-25013-18 to replicate in A549 cells. The growth of ZIKV strain in A549 cells was highly efficient and stimulated the production of Type-I interferons (IFNs), ISGs, and pro-inflammatory cytokines. Importantly, we showed that ZIKV replication resulted in a rather delayed mitochondrial apoptosis in human epithelial cells.

2. Materials and methods

2.1. Reagents and antibodies

Recombinant interferon-β (IFN-β) and TNF-α were purchased from Peprotech. The *pan*-caspase inhibitor, Z-VAD-fmk was purchased from Promega. The autophagy inhibitor 3-methyladenine (3-MA), the autophagy inducer rapamycin and the anti-β-tubulin antibody TUB 2.1 were purchased from Sigma-Aldrich. Anti-*pan* flavivirus E monoclonal antibody 4G2 was purchased from RD

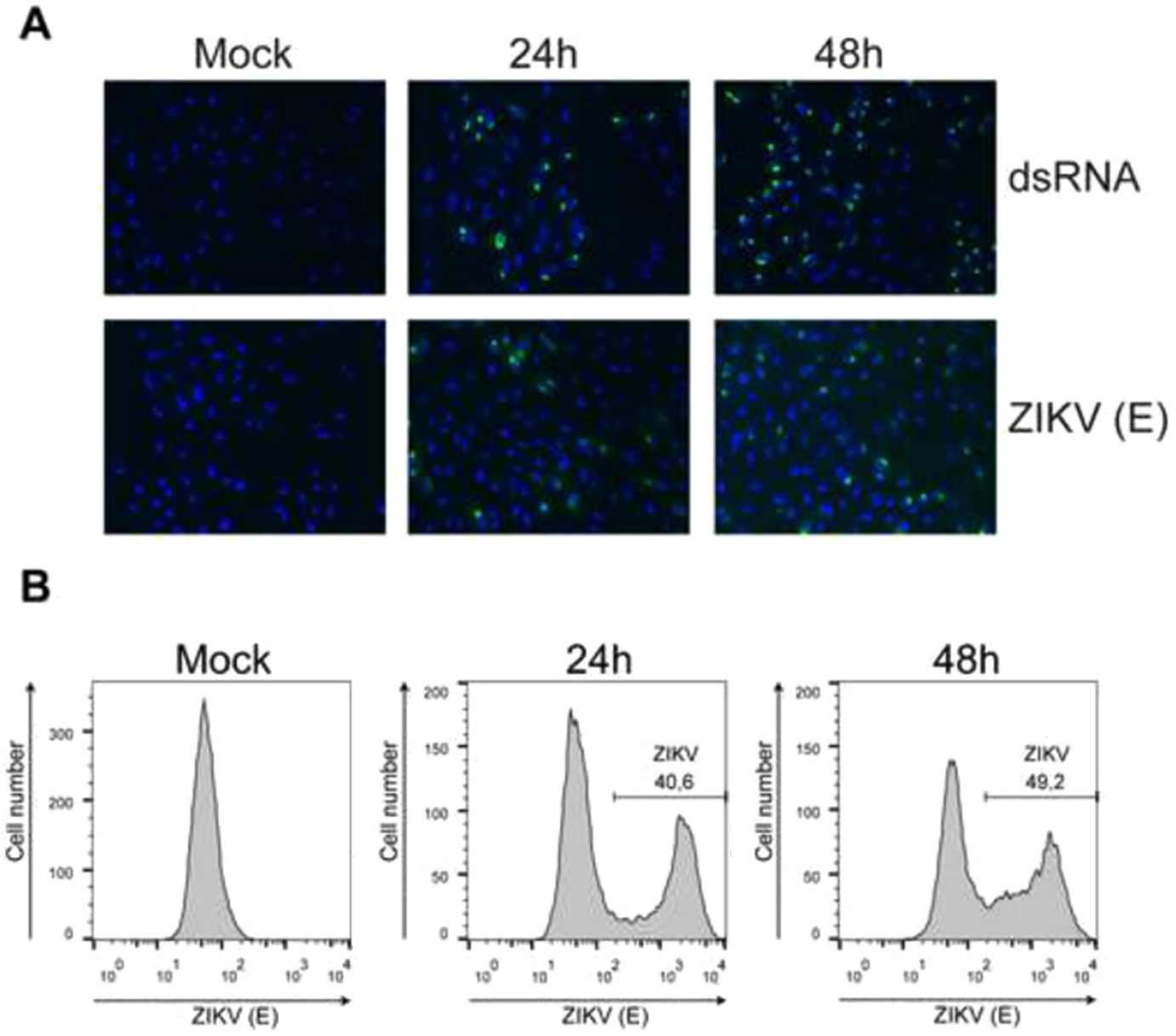

Fig. 1. Replication ZIKV strain PF-25013-18 in A549 cells. A549 cells were infected 24 h and 48 h with ZIKV strain PF-25013-18 at MOI of 5 or mock-infected. In (A), cells were immunostained with anti-dsRNA antibody J2 (dsRNA) or anti-*pan* flavivirus E protein monoclonal antibody 4G2 [ZIKV (E)]. Nuclei were stained with DAPI. In (B), expression of ZIKV E protein was analyzed by flow cytometry assay using 4G2 antibody. Percentages of positive cells were gated. Data are representative of three individual experiments.