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## African and Asian Zika virus strains differentially induce early antiviral responses in primary human astrocytes

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### ABSTRACT

ZIKA virus (ZIKV) is a newly emerging arbovirus. Since its discovery 60 years ago in Uganda, it has spread throughout the Pacific, Latin America and the Caribbean, emphasizing the capacity of ZIKV to spread to non-endemic regions worldwide. Although infection with ZIKV often leads to mild disease, its recent emergence in the Americas has coincided with an increase in adults developing Guillain-Barré syndrome and neurological complications in new-borns, such as congenital microcephaly. Many questions remain unanswered regarding the complications caused by different primary isolates of ZIKV. Here, we report the permissiveness of primary human astrocytes for two clinically relevant, Asian and African ZIKV strains and show that both isolates strongly induce antiviral immune responses in these cells albeit with markedly different kinetics. This study describes for the first time the specific antiviral gene expression in infected primary human astrocytes, the major glial cells within the central nervous system.

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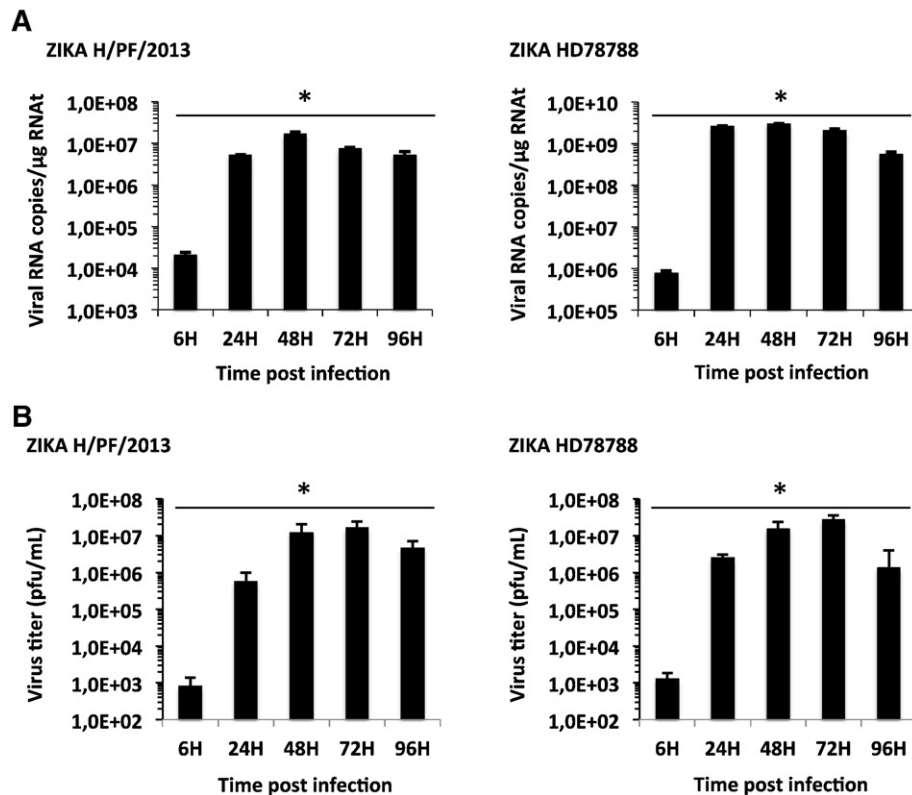
ZIKA virus (ZIKV) is a newly emerging arbovirus belonging to the *Flaviviridae* family that has caused several neurological disorders, including Guillain-Barré Syndrome and microcephaly, during recent French Polynesian and Latin American epidemics (Brasil et al., 2016; Cao-Lormeau et al., 2016; Mlakar et al., 2016). Strains implicated in these outbreaks have been traced to the Asian lineage (Enfissi et al., 2016). Although the virus has been circulating for decades in Sub-Saharan Africa and Asia, there has been, until recently, no evidence for significant human pathology associated with ZIKV infection. This might be due to deficient or inadequate surveillance systems or to the possibility that the virus may have evolved to become more neurotropic with increased replicative capacity. ZIKV has been shown to abrogate neurogenesis during human brain development (Garcez et al., 2016), thereby confirming the neural tropism of the virus (Qian et al., 2016). In this respect, it is important to note that astrocytes are among the first cells to respond to viral infection in the developing brain (Furr and Marriott, 2012). Indeed, this cell type was reported to be permissive for ZIKV infection (Retallack et al., 2016). However, notwithstanding the importance of astrocytes in potential viral transmission and neural pathogenesis, no studies

have addressed the antiviral response in these cells following ZIKV infection. Here, we have evaluated the effect of Asian (H/PF/2013) (Genbank KJ776791) and African (HD 78788) (Genbank KF383039) (Faye et al., 2014) clinical isolates of ZIKV on the innate antiviral response by infected human astrocytes (CliniSciences, France). Both virus strains were found to replicate in primary human astrocytes, as shown by a gradual increase in viral RNA levels of infected cells, detected by real-time PCR (Fig. 1A). Starting at 6 h postinfection (hpi), ZIKV RNA copy numbers increased with maximal expression levels detected at 48 hpi which were maintained up to 96 hpi during the course of infection. Next, we evaluated the ability of primary human astrocytes to produce viral progeny *in vitro* by determining viral titers in the supernatants of ZIKV-infected cells, using a standard plaque assay. The results show an increase in the production of viral particles over time, indicating active viral replication in the cells infected by the two viral strains (Fig. 1B). It is of note that although a 2 log difference in production of viral RNA between the two ZIKV strains was observed as early as 6 hpi, the viral production levels induced by both strains were comparable (Fig. 1).

The antiviral gene expression profile of ZIKV-infected astrocytes was determined at early time points post infection using qPCR array and compared with that of mock-infected cells (Fig. 2A, B and C). Unlike human fibroblasts (Hamel et al., 2015), ZIKV-infected astrocytes expressed several pattern recognition receptors (PRRs) involved in the innate immune

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**Fig. 1.** Primary human astrocytes are permissive to ZIKV infection. (A) Human astrocytes were infected with ZIKV strains H/PF/2013 or HD78788 at MOI (multiplicity of infection) 4 and harvested at different times postinfection. Expression of viral RNA was determined by real-time RT-PCR. (B) Viral replication was determined by plaque assay of culture supernatants of ZIKV-infected cells. Experiments were performed three times and error bar represent standard error of the mean. The *t*-test was employed to analyze the differences between sets of data. \*,  $P < 0.05$ .

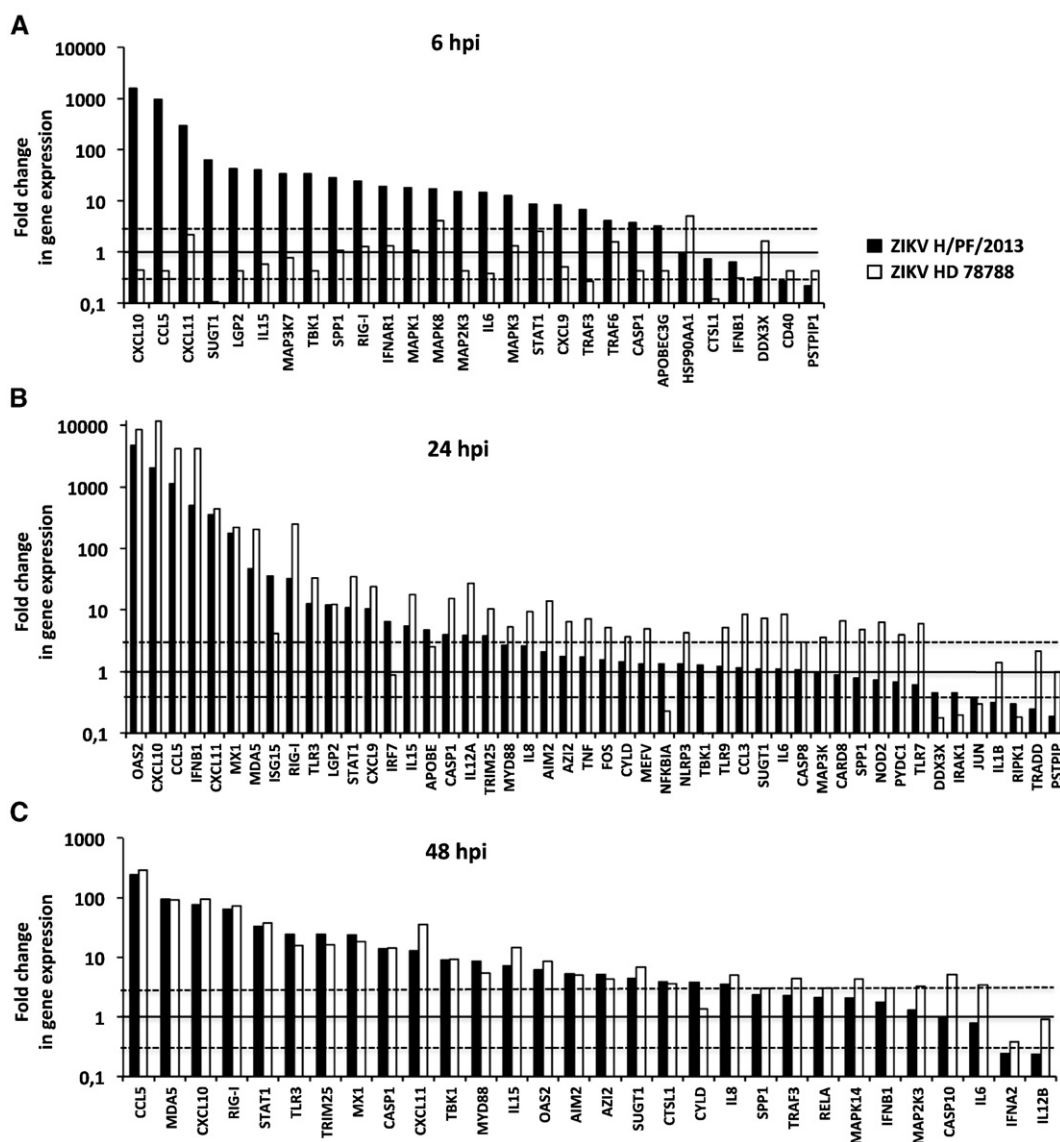
response, including TLRs (Toll Like Receptors), NLRs (NOD like Receptors) and RLRs (RIG-I Like Receptors), which was associated with the expression of not only IFN- $\beta$  and Interferon-stimulated genes (ISGs), but also chemokines and gene products involved in the activation of the inflammasome pathway (Fig. 2). Notably, the kinetics of induction of the antiviral responses by astrocytes differed significantly between the two ZIKV strains (Fig. 2A, B and C), whereas infection of astrocytes with the Asian strain lead to the expression of these innate immune response genes already at 6 hpi, their induction by the African strain was delayed and was observed around 24 hpi. On the other hand, the induction of several MAPKinases, as well as that of SPP1, TRAF3 and TBK1 genes, seemed to be upregulated to a greater extent by the Asian strain, as compared to its African counterpart (Fig. 2A). Moreover, the upregulation of the expression of the latter genes, involved in signaling events downstream of TLRs and RLRs, by the Asian strain is likely to be specific for astrocyte-mediated antiviral responses, because it was not observed in ZIKV-infected fibroblasts. Both strains were able to upregulate the expression of TLR3 in human astrocytes (Fig. 2B), confirming a similar observation in human fibroblasts (Hamel et al., 2015) and mouse neurospheres (Dang et al., 2016). In contrast to the Asian strain, the African strain also upregulated the expression of TLR7 and TLR9 transcripts (Fig. 2B). The two ZIKV strains were also able to modulate the NLR signaling pathway with some intrinsic differences at early time points following infection, such as the expression of transcripts for MEFV, NLRP3, and NOD2 by the African, but not Asian ZIKV strain-infected cells (Fig. 2B). However, at 48 hpi both strains induced a similar gene expression profile (Fig. 2C). Finally, infection of astrocytes with either strain lead to the induction of the IFN type I transcripts, with a slightly lower induction of ISGs by the African-, as compared

to the Asian strain. At present studies are ongoing in order to determine a possible link between the elevated accumulation of viral RNA in African ZIKV-infected astrocytes and the results of the transcriptome analysis. Nevertheless, it can be hypothesized that the delay in the induction of an antiviral response by the ZIKV strain of West-African origin brings about the replication of viral RNA, resulting a higher copy number of ZIKV transcripts in a time-dependent manner, as compared to that of the Asian strain. In addition, the antiviral response seems to mainly affect viral RNA replication step since the production of virus progeny is similar between the two ZIKV strains.

In conclusion, the results from this comparative analysis of ZIKV-infected human astrocytes show that both the Asian and the African strains induce the primary expression of genes, such as those belonging to the MAPKinase family (Kim and Choi, 2010) or those involved in ZIKV infection pathogenesis such as TBK1 (Onorati et al., 2016) and TLR3 (Dang et al., 2016), whose activity has been associated with neurodegenerative disease. Further investigation is however needed to establish a link between these observations and the physiopathological differences between both ZIKV strains.

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**Fig. 2.** Infected human astrocytes induce a differential antiviral response according to the viral strain. Primary human astrocytes were exposed to ZIKV H/PF/2013 or HD78788 at MOI 4. The modulation of antiviral gene expression was quantified by RT<sup>2</sup> Profiler PCR arrays for human antiviral response (PAHS-122Z format, Qiagen, Courtaboeuf, France) at 6 (A), 24 (B) and 48 (C) hours postinfection. Data were recorded on automatic datasheet for analysis. The fold change of gene expression was calculated in comparison to the values obtained from mock-infected cells. Statistically significant up-regulation and down-regulation in fold induction appear above the upper dotted line or below the down dotted line respectively. Statistical analysis was performed using the RT<sup>2</sup> profiler RT-PCR Array Data Analysis version 3.5.

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