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Zika Virus Update II: Recent Development of Animal Models— Proofs of Association With Human Pathogenesis

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Abstract

Three recent studies in pregnant mice and one ongoing study in rhesus macaques evaluating the effect of ZIKV infection have provided important information about maternal-fetus transmission and ZIKV-related pathogenesis, confirming a causal role of ZIKV in neurological problems observed in humans. Here, we present an update of these works published in the past few weeks.

Keywords

Zika virus; animal model; pathology

Important papers evaluating how Zika virus (ZIKV) affects pregnant mice to model the pathogenesis in humans have been published since our recent publication of an update on ZIKV [Ramos da Silva and Gao, 2016]. The first animal study using a ZIKV strain isolated from Brazil (ZIKV^{BR}) has demonstrated that the association with the birth defects depends on the mouse strain [Cugola et al., 2016]. In this study, two different strains of pregnant mice, SJL and C57BL/6, were intravenously infected at around days 10–13 of gestation. Pups from C57BL/6 mice, which have functional responses of types I and II interferons, were not infected by ZIKV^{BR}, indicating that the virus did not cross the placenta barrier. These results are consistent with those of a recent study showing that AG129 mice were susceptible to ZIKV infection because they lack responses to types I and II interferons [Rossi et al., 2016]. In contrast, pups from SJL mice had intrauterine growth restriction (IUGR), which caused congenital malformations [Cugola et al., 2016]. Additionally, genes involved with autophagy and apoptosis were deregulated [Cugola et al., 2016].

In vitro experiments showed that the ZIKV infection phenotype varied according to virus strain, viral titer, and cell type. Production of infectious viral particles was observed in neural progenitor cells (NPCs) at multiplicity of infection (MOI) as low as one and in neurons only at 10 MOI [Cugola et al., 2016]. An increased number of cell death was only observed in infected NPC at MOI 10. There was a significant decrease in the sizes of neurospheres in 3D cell cultures infected with ZIKV^{BR} at 10 MOI compared with those

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infected with ZIKV⁷⁶⁶ or the uninfected control [Cugola et al., 2016]. However, this difference was not observed between the ZIKV strains at 1 MOI.

Human cerebral organoids infected with both ZIKV strains at MOI 0.1 showed a decrease of the CTIP2⁺ (deep cortical marker) and PAX-6⁺ (dorsal forebrain progenitor) cells while only ZIKV^{BR} caused a decrease of TBR1⁺ (deep cortical marker) cells and proliferating cells (Ki67⁺ and SOX2⁺ cells) in the ventricular zone [Cugola et al., 2016]. On the other hand, ZIKV^{BR} was not able to replicate in cerebral organoids derived from chimpanzee, and was unsuccessful to diminish CTIP2⁺ and TBR1⁺ cells.

A second study evaluated the effect of ZIKV in mouse fetuses [Li et al., 2016]. ZIKV from the Asian lineage (KU866423) was injected into the cerebroventricular space/lateral ventricle of the brain at embryonic day 13.5. Three to five days after infection, most of the ZIKV-infected cells were found at the ventricular and subventricular zones; a thinner cortical layer was detected; and smaller brains in the infected animals rather than in the uninfected littermates was observed. Additionally, cleaved-caspase-3 was detected in the intermediate zone and cortical plate. ZIKV preferentially infected NPCs and intermediate or basal progenitor cells (IPCs/BPCs), and inhibited the proliferation and differentiation of NPCs. RNA-seq analysis identified an upregulation of pathways associated with immune response and apoptosis, and downregulation of those related to cell proliferation and differentiation [Li et al., 2016].

A third study evaluated the effect of subcutaneous infection of an Asian ZIKV strain (H/PF/2013) at gestation day 6.5 and 7.5 of heterozygous fetuses (*Ifnar1*^{+/-}) derived from backcross of *Ifnar1*^{-/-} and C57BL/6 mice [Miner et al., 2016]. Infection of these fetuses, which have type I interferon response, resulted in fetal death and reabsorption in most of the fetuses while those that survived the infection had IUGR and growth impairment. ZIKV RNA detected in the placenta was significantly higher than in the maternal serum in these pregnant mice, suggesting active viral replication in the placenta [Miner et al., 2016]. In another experiment, mice with prior exposure to a blocking antibody against interferon- α receptor before ZIKV infection did not result in fetal demise but IUGR was also observed [Miner et al., 2016]. ZIKV was detected mainly in glucogen trophoblasts and spongiotrophoblast cells, and significant vascular alterations in the placenta was noted [Miner et al., 2016].

Although, all these animal studies have provided important contributions to the understanding of ZIKV infection and the associated pathogenesis in humans, a few limitations must be considered. First, the average gestational period of mice is much shorter than that of humans (21 vs. 280 days). Thus, it is difficult to analyze the impact of the virus in different times of infection in humans using a model that has a shorter period of pregnancy. Second, the virus strain and dose directly influences the infection results. In humans, most of microcephaly cases are associated with ZIKV strain from Brazil while in mice, both lineages (Asian and African) can induce ZIKV-related pathology. Furthermore, infection at a higher dose (10 MOI) led to dramatic differences in the fetus sizes and other pathologies among different strains of ZIKV; however, this was not observed at a lower dose (1 MOI). Third, the different time scale of development of the neurological system between

humans and mice might limit the understanding of the impact of the virus on the brain. Thus, a model with similar brain size and gestation period was in need. For this reason, a rhesus macaque model is being evaluated daily and the data from the study is posted online in a real time basis [Dudley et al., 2016a].

In this study, eight monkeys were used, two of which were pregnant. They were infected at gestation days 31 and 38 with an Asian ZIKV strain (H/PF/2013). ZIKV RNA was detected in all animals at day 1 post-infection, up to day 21 in six animals, and up to day 57 in the two pregnant monkeys. The RNA was detected in saliva, urine, and cerebrospinal fluid. All animals presented an increase in natural killers (NKs), CD8⁺ and CD4⁺ T cells, and plasmablasts. By day 21 post-infection, neutralizing antibodies against ZIKV was detected in all animals. At week 10 post-infection, a rechallenge of three non-pregnant monkeys did not show virus replication. The pregnant monkeys are expected to deliver around August and September of 2016. So far, samples obtained through amniocentesis have indicated that both fetuses (at days 43 and 36 post-infection) were negative for ZIKV RNA [Dudley et al., 2016a, b].

The results obtained from these recent studies are highly expected and have shed light on the mechanisms of maternal-fetus transmission and pathogenesis of ZIKV infection. Although they cannot be completely translated to humans, these results complement those obtained from the clinical that are continuing to reveal the differences among different ZIKV strains and whether the strains from Brazil are more associated with microcephaly while other strains are more associated with other neurological disorders such as Guillain–Barré syndrome.

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