DISEASE WATCH

Zika virus — placental passage and permissivity for infection

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Animal models of human congenital infection are needed to understand when, how and why Zika virus (ZIKV) is transmitted to the fetus. The recent development of mouse models of ZIKV placental transmission, which results in high rates of pregnancy loss, alongside fetal brain and ocular malformations, have started to unravel the mysteries of placental infection and transmission.

Refers to Miner, J. J. *et al.* Zika virus infection during pregnancy in mice causes placental damage and fetal demise. *Cell* **165**, 1081–1091 (2016).

Zika virus (ZIKV) is an arbovirus that is transmitted to humans by the *Aedes* mosquito, but can be spread from one human to another, including fetal transmission during pregnancy. Infection of humans with ZIKV, an emerging mosquito-borne *Flavivirus*, has reached pandemic levels in the Americas, with at least 32 countries or territories reporting infection over the period May 2015 to April 2016 (REF. 1). Although previous outbreaks of ZIKV resembled that of dengue fever and chikungunya, its recent spread to the Americas has revealed an increase in the incidence of congenital microcephaly with brain and ocular malformations, as well as intrauterine growth restriction (IUGR) associated with placental insufficiency, and spontaneous pregnancy loss. As ZIKV RNA has been reported in amniotic fluid, the placenta and fetal neural tissue from women weeks to months after being infected early in gestation[2], understanding the role of the placenta in alternately facilitating or limiting vertical transmission of ZIKV is of paramount importance. In a recent study published in *Cell*, Miner and colleagues describe two mouse models of ZIKV infection during pregnancy that are associated with clinically relevant phenotypes in offspring, including growth restriction and placental damage[3].

Earlier this year, the Washington University team developed a mouse model of ZIKV infection and demonstrated that peripheral visceral replication of ZIKV is inhibited by the type I interferon response[4]. In mice lacking the IFN α/β receptor 1 (*Ifnar1*$^{-/-}$), ZIKV infection in adult, non-pregnant mice was associated with neurological destruction, accompanied by high levels of the virus in the brain and spinal cord, as well as in the spleen, testes and serum. The *Ifnar1*$^{-/-}$ mice succumbed to ZIKV infection, as did a triple knockout mouse strain that also lacked INFα and INFβ.

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In the current study[3], Miner and colleagues used a particularly creative and elegant approach to generate highly relevant animal models for studying ZIKV infection during pregnancy. Female *Ifnar1*$^{-/-}$ mice were crossed with wild-type male mice, generating heterozygous offspring with an intact type I interferon response (*Ifnar1*$^{-/+}$). The pregnant *Ifnar1*$^{-/-}$ mice were then inoculated with ZIKV on embryonic days 6.5 (E6.5) and E7.5, and pregnancy was allowed to progress until E13.5 or E15.5. The outcomes were devastating: the majority of fetuses were resorbed after *in utero* demise, similar to the observed pregnancy losses occurring in the current epidemic across the Americas. The remaining fetuses showed evidence of growth restriction and placental damage, including necrotic foci, but without evidence of microcephaly. However, in a second model of ZIKV infection in pregnancy, the investigators treated wild-type pregnant mice with an antibody targeting the IFN α/β receptor 1 1 day before infection with ZIKV at E6.5 and E7.5 and analysed phenotypes on E13.5 and E15.5. This second model was less severe than the first model, as prevalent fetal demise was not observed; however, the fetuses did exhibit IUGR.

Despite the 6–8 day interval between initial infection and delivery of the fetuses, a high placental viral load was persistently observed. The mouse placenta consists of three distinct layers, including the maternal layer (decidua), the junctional zone (where the placenta attaches to the uterus) and the labyrinth zone (where the placental villi are bathed in maternal blood and the site of maternal–fetal nutrient and oxygen exchange)[5]. Similar to the human placenta, the mouse placenta consists of highly specialized trophoblast cell types which have distinct roles in invading the maternal decidua, anchoring the placenta to the uterus and forming villi to facilitate nutrient transport. In humans, the placenta has been observed to largely protect against vertical transmission of viruses, although some viruses, such as hepatitis C (another *Flavivirus*) and cytomegalovirus, are able to replicate in the placenta[6]. In the case of cytomegalovirus, devastating fetal malformations are subsequently observed in many, but not all, cases[7].

The question of whether the placenta is permissive to infection with ZIKV has been challenging to resolve[2]. Prior to the work of Miner and colleagues[3,4], another team of investigators reported that although ZIKV is able to replicate in a log-fold manner in immortalized human trophoblast cells lines, replication was not as robust in primary human trophoblasts isolated from healthy, term placental donors compared

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with replication in human brain microvascular endothelial cells[8]. Although there are fundamental differences between the approach of the two groups (such as use of current endemic virus versus high passage historic strains, measurement of – strand RNA rather than + strand RNA, and the duration of both infectivity and culture), the findings are not necessarily conflicting. Existing data supports a model in which trophoblast production of type III interferons might inhibit ZIKV infection[9]. Using a low-passaged current endemic ZIKV strain, primary human trophoblasts were shown to be permissive to low levels of ZIKV replication[9]. However, this study used Hofbauer cells (macrophages present in the placental chorionic villi) that supported log-fold viral growth *ex vivo* relative to cytotrophoblasts. In this study, considerable donor-to-donor variability was observed.

As with any landmark study, many questions remain to be answered. Firstly, on the basis of Miner and colleagues *in vivo* experimentation, a considerably higher (1,000-fold) viral load was detected in the placenta than in maternal serum[3], which leads to the conclusion that ZIKV has an unusually high tropism for the placenta. Is this similarly true in humans? Secondly, unlike infection with dengue virus (a related mosquito-borne *Flavivirus*), following dengue virus infection, no phenotype was observed and no viral RNA was detected in the placenta of ZIKV-infected mice. Why does ZIKV but not dengue virus seem to replicate in the placenta? Using fluorescence *in situ* hybridization, direct visualization of ZIKV RNA was observed in several trophoblast cell types (glycogen and spongiotrophoblasts)[3]. Of potential clinical relevance to humans, placentas from ZIKV-infected pregnant mice notably show similar vascular injury, irregular shape, reduced number of fetal capillaries and evidence of apoptosis. This finding is clinically relevant as placental insufficiency in association with IUGR among women infected with ZIKV during multiple trimesters of pregnancy has been reported, and might occur independent of the more devastating microcephaly and brain malformations[10]. Although purely speculative, both variation in the severity of fetal infectivity and potentially natural mediation of the disease might exist.

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Competing interests statement
The authors declare no competing interests.