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Consequences of congenital Zika virus infection

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Abstract

The 2015 Zika virus (ZIKV) epidemic in the Americas led to the discovery that ZIKV causes congenital abnormalities including microcephaly, intrauterine growth restriction, and eye disease that can result in blindness. Studies in animal models and human organoid cultures, together with human epidemiological studies, have shown that ZIKV crosses the placenta and subsequently replicates within fetal tissues including the developing brain. Preferential infection of neural cell precursors causes damage to the developing fetal brain. However, a majority of congenitally infected humans do not develop microcephaly or other overt congenital abnormalities, so longitudinal epidemiological studies are necessary to more completely define the long-term consequences of *in utero* ZIKV infection.

INTRODUCTION

The *Flaviviridae* family is comprised of a diverse group of RNA viruses, including more than 70 flaviviruses that are transmitted by mosquitos (e.g., Zika (ZIKV), Dengue (DENV), West Nile (WNV), St. Louis encephalitis (SLEV), Yellow Fever (YFV), Japanese encephalitis (JEV) viruses) or ticks (e.g., Powassan (POWV) and Tick-borne encephalitis (TBEV) viruses). Many of these viruses are neurotropic and have the capacity to invade the brain and cause encephalitis (e.g., WNV, ZIKV, SLEV, JEV, YFV, POWV, TBEV). By contrast, DENV is less neuroinvasive, but causes large epidemics of fever in humans, including life-threatening hemorrhagic fever, with nearly 400 million infections per year [1]. Thus, flavivirus infections represent a major global public health concern that is being addressed with even greater urgency since the discovery that ZIKV causes congenital

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infection resulting in microcephaly, blindness, intrauterine growth restriction (IUGR), and fetal demise [2–5]. New animal models of transplacental ZIKV infection have yielded insights into potential consequences of this emerging infectious disease [6–8]. Additionally, recent human epidemiological studies are beginning to define the impact of congenital ZIKV infection in humans.

Transplacental flavivirus transmission

Long before the discovery that ZIKV causes congenital abnormalities, there were numerous case reports and animal studies describing transplacental infection by other flaviviruses [9–23]. However, the clinical relevance of congenital flavivirus infection was not well defined, perhaps due to the fact that some flaviviruses cause relatively smaller or geographically confined outbreaks [24]. The enormity of the ZIKV epidemic in the Americas likely made the phenomenon of congenital flavivirus infection more discoverable than it had been in previous outbreaks [25]. For example, an analysis of the French Polynesian ZIKV outbreak also demonstrated an association with microcephaly, which was uncovered only in retrospect [26,27].

The spread of flaviviruses is largely influenced by host-vector interactions [28]. Humans are incidental dead-end hosts for many of the encephalitic flaviviruses including WNV, JEV, SLEV, POWV and TBEV, which depend on amplification by transmission between non-human hosts and arthropod vectors [29–31]. By contrast, DENV, YFV, and ZIKV can perpetuate transmission between humans and mosquitos [1,31–33]. Many flaviviruses (e.g., SLEV and POWV) cause only small outbreaks in humans, whereas other flaviviruses (e.g., ZIKV, TBEV, YFV, and DENV) have caused larger outbreaks [1,34]. Additionally, ZIKV has the capacity for human-to-human sexual and non-sexual transmission [35–37], which likely has implications for the ability of this virus to spread intercontinentally.

Since the 1970s, the biomedical research community has produced numerous reports of transplacental infection by a variety of flaviviruses, which are summarized in Table 1, although most of these studies received little attention [9–23,38]. For example, there are some reports of congenital JEV and WNV infection in humans [15,21], resulting in a variety of disease phenotypes including fetal demise and stillbirth during a JEV outbreak [15], and retinal damage and blindness in a case of congenital WNV infection [21]. Congenital WNV infection also has been reported to cause spontaneous abortion in other mammals [39,40]. Transplacental transmission of YFV in humans also occurred after inoculation with an attenuated YFV vaccine [9]. Similarly, mouse model studies have demonstrated transplacental SLEV and WNV transmission, which resulted in either severe neurological abnormalities (due to SLEV) or diminished litter size (due to WNV) [11,12,22,23]. Several studies have shown congenital JEV infection in mice, swine, and bats [16–18,41–43]. However, case reports of congenital infections with flaviviruses had been sporadic prior to the ZIKV epidemic. Unlike the relatively smaller outbreaks of WNV and JEV in humans, the 2015 ZIKV epidemic resulted in more than 1 million infections [34]. Thus, it is intriguing to consider whether other flaviviruses may cause congenital infection in humans on a more limited basis than ZIKV. Furthermore, prior ZIKV outbreaks in the developing

world also might have caused congenital infection without being noticed by the medical community.

The discovery and emergence of ZIKV

ZIKV was first isolated from a febrile rhesus macaque at a yellow fever research station in the Zika Forest in Uganda in 1947 [44]. At the time, many local inhabitants were found to be seropositive for antibodies against ZIKV [45], but no clinical symptoms were directly linked to the virus until much later. There had been some speculation that clinical ZIKV infection may cause jaundice, in part because ZIKV is related to YFV [46]. In an attempt to define clinical manifestations of ZIKV infection, Dr. William Bearcroft injected the virus into his own arm in 1956 [47]. He reported having only mild clinical symptoms (e.g., fever and headache), which resolved within a few days [47]. Thus, the clinical consequences of ZIKV infection were thought to be very mild until the virus began to emerge over the last decade.

From the time of its discovery in 1947 until the 2015 ZIKV epidemic in the Americas, only three animal model studies of ZIKV pathogenesis had been described [48–50], the most recent of which was published in 1976 [48]. In 2007, ZIKV began to spread to the Islands of the Pacific, including a large outbreak on the Island of Yap, Micronesia [51]. A 2013 outbreak in French Polynesia revealed an association between ZIKV infection and Guillain-Barre syndrome [52], a neurological autoimmune disease characterized by peripheral demyelination resulting in ascending paralysis and respiratory failure requiring mechanical ventilation. When ZIKV spread to the Americas in 2015, millions of individuals were infected and an epidemiological association was made for the first time between ZIKV infection and microcephaly [53].

Clinical manifestations of congenital ZIKV infection

There are numerous unanswered questions about congenital ZIKV infection, including: (1) How common is transplacental ZIKV infection? (2) What is the range of clinical outcomes resulting from congenital infection? and (3) What are the long-term clinical consequences of congenital infection? To begin to address these questions, clinical researchers began conducting epidemiological studies in 2016 to define the consequences of ZIKV infection in human neonates. In parallel, scientists rapidly developed mouse and non-human primate models of congenital ZIKV infection. Other scientists have studied ZIKV infection using *ex vivo* organoid culture systems, which also have yielded new insight into ZIKV tropism and mechanisms of pathogenesis. Collectively, these model systems are demonstrating what may be possible in humans, while also providing a platform for testing of vaccines and treatments.

Microcephaly and fetal demise—In early 2016, Brasil et al. monitored longitudinally a group of women with PCR-confirmed ZIKV infection during pregnancy [54]. Ultrasonographic examination demonstrated congenital abnormalities in 42% of fetuses, with a wide range of clinical phenotypes including IUGR, cerebellar atrophy, ventriculomegaly, diminished cerebral artery flow, anhydramnios, cerebral calcifications, microcephaly, and fetal demise [54]. A subsequent study utilized magnetic resonance and computed tomography imaging of congenitally infected neonates to describe a variety of

abnormalities within the developing human brain, including ventriculomegaly, hypoplasia of the cerebellum and brainstem, defects in the corpus callosum, calcifications, cortical malformations, and other abnormalities of the neonatal brain [55]. Thus, although microcephaly has been the most publicized ZIKV-related congenital malformation, the clinical consequences of congenital infection in humans are quite varied. Human brain organoid cultures and other cell culture studies have shown that ZIKV preferentially infects and causes apoptosis of neural progenitor cells [56–58], although ZIKV also replicates to a lesser extent in mature neurons [56]. ZIKV-induced apoptosis of progenitor cells likely contributes to development of microcephaly and other neurodevelopmental outcomes in humans [59]. In addition, infection of the placenta itself and the resulting damage and immune response within the placenta also may contribute to IUGR or play a role in the development of other fetal abnormalities [6,60].

Animal models of transplacental ZIKV infection have experimentally demonstrated a range of clinical phenotypes, as well as the effect of gestational age on clinical outcomes. However, modeling congenital ZIKV infection in mice is a challenge due to the fact that ZIKV does not replicate efficiently in rodents [61]. The limited capacity of ZIKV to replicate in mice results from the fact that mice are not a natural host for the virus, and ZIKV lacks the capacity to efficiently antagonize the type I interferon (IFN) response in these animals [62]. Thus, in order to develop mouse and other rodent models of transplacental ZIKV infection, some scientists have modeled congenital ZIKV infection in immunodeficient animals [6,63,64]. The most commonly used mouse model of congenital ZIKV infection involves subcutaneous or intravaginal inoculation of ZIKV in pregnant dams lacking the type I IFN receptor (*Ifnar1*^{−/−} mice) crossed to immunocompetent wild-type (WT) sires. This allows efficient ZIKV replication in the pregnant dam to facilitate infection of heterozygous *Ifnar1*^{+/-} fetuses that have an intact type I IFN response [6,63,65]. In this *Ifnar1*^{−/−} mouse model of congenital infection, intravaginal or subcutaneous inoculation at early gestational dates (e.g., embryonic day (E) 6.5) results in high viral burden in the fetus and placenta, which leads to IUGR and fetal demise [6,65]. After infection at early gestational dates, IUGR and fetal demise likely result from damage and insufficiency of the placenta, leading to fetal death [6]. By contrast, infection at later gestational dates (E9.5) resulted in diminished viral burden in the placenta and fetus, which caused microcephaly but not fetal demise [65]. Finally, infection of mice at even later gestational time points (E11.5) resulted in even lower viral burden in the fetus without any appreciable clinical effect in the fetus or the placenta [65]. Therefore, gestational age influences outcome during congenital ZIKV infection in mice, a phenomenon that also has been suggested by epidemiological studies in humans [66]. A second approach to modeling ZIKV infection in mice has been to inoculate immunocompetent SJL mice intravenously with up to 4 x 10¹⁰ plaque forming units (PFU) of ZIKV, a biologically unnatural amount of virus [67]. This resulted in diminished size of the congenitally infected fetuses. Consistent with ZIKV infection of the fetal brain, histological analysis revealed thinning of the cerebral cortex in the infected mouse fetuses [67]. In another model using direct intra-amniotic inoculation of ZIKV, Cui et al. showed that congenitally infected mice exhibited neurological impairment including hindlimb paralysis as adults [68]. Thus, multiple animal models have shown ZIKV to be teratogenic in mice, and that ZIKV has the capacity to cause a range of congenital

abnormalities, similar to clinical observations in congenitally infected humans [54]. In particular, the immunodeficient mouse models of congenital infection have served as an effective platform for testing the efficacy of vaccines and therapies. For example, using two separate vaccine platforms (mRNA and live-attenuated virus), Richner et al. utilized this model to demonstrate protection from congenital infection and fetal demise in mice [69]. However, it should be taken into consideration that there are major anatomical, molecular, and functional differences between the mouse and human placenta that may influence the translatability of mouse models of congenital ZIKV infection [70].

In non-human primate models, congenital ZIKV infection causes fetal brain lesions [7], but has not yet been linked to fetal demise or microcephaly, despite the fact that transplacental infection in macaques occurs with high efficiency [8]. Thus, there may be species-specific or even environmental contributors to risk of ZIKV-induced fetal malformations. Alternatively, transplacental infection may occur frequently, whereas microcephaly and severe congenital malformations may be a relatively less common phenomenon in primates (including humans), especially in asymptomatic cases.

Congenital eye malformations—ZIKV infection in adult humans causes nonpurulent conjunctivitis in up to 55% of cases [51]. Less frequently, ocular ZIKV infection leads to uveitis [71–73], a form of severe eye inflammation that can cause permanent eye damage and blindness. Chorioretinal lesions were also reported in one case [74]. Aspiration of fluid from the anterior chamber of the eye of one patient with ZIKV-associated uveitis demonstrated persistence of ZIKV RNA within the eye [71]. Congenital ZIKV infection in humans also causes a variety of ocular abnormalities including chorioretinal atrophy, optic neuritis, retinal hemorrhages, lens subluxation, retinal mottling, and bilateral iris coloboma [2–4,75,76]. Since ZIKV infects the fetal eye in mouse and non-human primate models [8,77], it seems plausible that ocular malformations are a direct consequence of ZIKV infection of the developing eye.

In mouse models of ZIKV pathogenesis, subcutaneous inoculation with ZIKV results in infection of the eye that clinically manifests as conjunctivitis and uveitis with shedding of virus in tears [77]. A subsequent study in humans revealed that infectious ZIKV can be cultured from human conjunctival swabs [78]. Another report detected viral RNA in the vitreous of an asymptomatic cornea donor [79], which may have implications for potential transmissibility in the context of cornea transplantation. In contrast, congenital infection models in mice and in non-human primates have not revealed substantive evidence of ocular abnormalities [6], suggesting that eye pathology may occur with low frequency in congenitally infected mammals.

Other implications of congenital ZIKV infection—The long-term consequences of *in utero* ZIKV infection are not known, especially in normal-appearing, congenitally infected humans. Answering this question will require large epidemiological studies that include neurological and neurocognitive testing in cohorts of congenitally infected humans. In the meantime, studies of congenitally infected animals will help to define what is possible in terms of the subtle neurodevelopmental effects of congenital ZIKV infection, including potential effects on behavior, learning, and memory. Prior reports have shown that other

teratogenic pathogens that did not cause congenital disease in neonates were linked to adverse outcomes that were observed postnatally [80,81]. Similarly, infants who tested positive for ZIKV infection but displayed no noticeable congenital defects at birth later experienced slow postnatal head growth that develop into microcephaly in some cases [82]. Thus, the absence of microcephaly and other congenital abnormalities at birth does not exclude the possibility that some ZIKV-induced teratogenic effects may arise postnatally.

Congenital ZIKV infection also may impact reproductive health later in life. Since ZIKV infects and damages the testes in animal models [83,84], longitudinal studies in humans are necessary to determine whether congenital ZIKV infection may affect fertility and/or hormone production in congenitally infected humans.

Summary

Over the past two years, widespread outbreaks have caused ZIKV to emerge from obscurity to become a virus of global concern because of severe congenital abnormalities resulting from transplacental infection (Figure 1). Clinical outcomes due to congenital infection include microcephaly, IUGR, fetal demise, and ocular abnormalities that can cause blindness. These outcomes appear to be strongly influenced by gestational age at the time of infection [65,66]. Although some ZIKV-related flaviviruses may share the capacity to cause transplacental infection and fetal demise in mammals (Table 1), ZIKV is by far the most clinically relevant flavivirus in terms of the epidemiological impact resulting from congenital infection.

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Figure 1. Consequences of transplacental ZIKV infection

Maternal infection may be acquired either by mosquito or via sexual transmission. Maternal viremia leads to transplacental infection. Placental damage by ZIKV may contribute to placental insufficiency, IUGR, and fetal demise. Severe infection of fetal tissues likely causes microcephaly, blindness, and also fetal demise. Whether placental factors also contribute to microcephaly is not well established. Animal models of congenital ZIKV infection, along with human epidemiological studies, suggest that clinical outcomes are strongly influenced by gestational age at the time of infection [65,66].

Table 1**Consequences of congenital flavivirus infection in mammals**

Congenital flavivirus infection has been described in a variety of mammals in nature (columns 2–4) and in animal models (columns 5–6). Consequences of congenital infection with ZIKV resemble the consequences of infection with other flaviviruses, including spontaneous abortion, ocular abnormalities, and neurological malformations.

Virus	Congenital infection in humans	Congenital infection in other mammals (in nature)	Consequences (in nature)	Congenital infection (in animal models)	Consequences (in animal models)
JEV	Yes [15]	Swine [42]	Human: Spontaneous abortion [15]; Swine: Stillbirth, neonatal death [42]	Bat [43]; Mouse [16]	Mouse: Fetal and neonatal death [16]
SLEV	No	Not described	N/A	Bat [43]; Mouse [11,12]	Mouse: Delayed postnatal growth, encephaloceles, hydrocephalus, neonatal death [12]
WNV	Yes [21]	Horse [39], Sheep [40]	Human: Chorioretinal changes and brain abnormalities on MRI [21] Horse: Spontaneous abortion [39] Sheep: Spontaneous abortion, stillbirth, neonatal death [40]	Mouse [22,23]	Mouse: Stillbirth, neonatal death [22]
YFV	Yes [9]	Not described	N/A	Not described	N/A
ZIKV	Yes [5,53,54]	Not described	Human: Fetal demise, IUGR, microcephaly, brain abnormalities on MRI, ocular abnormalities and blindness [2,3,5,54,75,76]	Macaque [7,8]; Mouse [6,63,65,67]; Hamster [64]	Macaque: Fetal brain lesions [7]; Mouse: Fetal demise, IUGR, microcephaly [6,63,65,67]