

Zika virus as a causative agent for primary microencephaly: the evidence so far

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Abstract Zika virus (ZIKV) infection has been associated with congenital microcephaly and peripheral neuropathy. The ongoing epidemic has triggered swift responses in the scientific community, and a number of recent reports have now confirmed a causal relationship between ZIKV infection and birth defect. In particular, ZIKV has been shown to be capable of compromising and crossing the placental barrier and infect the developing fetal brain, resulting in the demise and functional impairment of neuroprogenitor cells critical for fetal cortex development. Here, the evidence for ZIKV as a teratogenic agent that causes microcephaly is reviewed, and its association with other disorders is discussed.

Keywords Microcephaly · Neuroprogenitor cells · Zika virus

Abbreviations

GBS Guillain–Barré syndrome
NPCs Neuroprogenitor cells
ZIKV Zika virus

Introduction

The recent global emergence of Zika virus (ZIKV) (Musso and Gubler 2016; Bharucha and Breuer 2016; Ramos da Silva and Gao 2016) has received unprecedented attention from the health and biomedical sciences community. First isolated and identified in 1948 (Dick 1952), infections by this (+)strand RNA virus of the family *Flaviviridae* and genus *Flavivirus* may be asymptomatic, or non-febrile, and in the case of a febrile disease result in relatively mild symptoms which include fever, headache, conjunctivitis, arthralgia and a maculopapular rash (Hamel et al. 2016; Ramos da Silva and Gao 2016). The reason why it was declared a public health emergency of international concern by the World Health Organization (WHO) in February 2016 (Heymann et al. 2016) is largely down to its association with congenital defects in the form of primary or congenital microcephaly (Vargas et al. 2001), as well as paralytic neuropathy symptoms of Guillain–Barré syndrome (GBS) (Willison et al. 2016).

Primary microencephaly is presented with a significant degree of reduction in the head circumference of the newborn and is associated with varying degrees of impairment in motor, sensory and cognitive functions (Woods et al. 2005). Microcephaly is manifested by reduced production of neurons during brain development in utero, resulting from the demise, or diminished proliferation of cortical progenitor cells, or a reduction in its number of cell divisions. The primary etiological cause for microcephaly ranges from mutations in a number of recognized microcephaly genes, many of which encode centrosomal proteins (Tang 2006; Chae and Walsh 2007), to various in utero insults, including microbial infection. For the latter, fetal exposure to *Toxoplasma*, Rubella virus, Cytomegalovirus, Herpes virus and the Syphilis bacteria (termed the

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TORCHS factors) represents the main congenital infections that could cause microcephaly (Neu et al. 2015). The Flavivirus genus does not lack members that are neurotrophic and could cause rare but documented cases of brain infection in infants or pediatric subjects (Turtle et al. 2012), such as West Nile virus (Tyler 2014), Japanese Encephalitis virus (Li et al. 2015) and Dengue virus (Rao et al. 2013). However, ZIKV is perhaps the first Flavivirus to be so strongly associated with congenital teratogenesis.

Epidemiological association between ZIKV infection and microcephaly first became obvious during the current epidemic, particularly with the increase in microcephaly cases in Brazil (Schuler-Faccini et al. 2016; Ribeiro et al. 2016). Although a strong association between microcephaly and ZIKV infection was already noted with the WHO announcement in February 2016, there were cautious reservations with regard to whether the virus infection was causative of the congenital defect. Recent work by several groups has, however, established causation of the congenital disorder by the virus. In the following paragraphs, I summarize the evidence thus far for ZIKV infection, particularly of pregnant mothers in the first trimester, resulting in birth defects of the newborn. The recently reported experiments with human neuroprogenitor cells (NPCs), brain organoids and animal models of maternal–fetal transmission as well as fetal intracerebral viral inoculation shall be discussed. I shall also ponder briefly on the risk of adult neurological diseases resulting from ZIKV infection.

Epidemiological and clinical association between ZIKV infection and congenital defects

Reports of an unusual increase in the number of infants born with microcephaly in ZIKV-affected areas began to emerge in the third quarter of 2015, and the epidemiological association became apparent with an investigation by the Brazilian Ministry of Health task force (Schuler-Faccini et al. 2016; Kleber de Oliveira et al. 2016). ZIKV-linked birth defects were also reported elsewhere in South America (Butler 2016) and among pregnant travelers to affected areas (Meaney-Delman et al. 2016). Congenital infection was observed with the detection of viral RNA in placental tissue, amniotic fluid and fetal brain tissues (Martines et al. 2016; Calvet et al. 2016; Mlakar et al. 2016; Driggers et al. 2016), and anti-ZIKV IgM in the cerebrospinal fluid in neonates with microcephaly (Cordeiro et al. 2016). Population-level data analyses have indicated a strong association between ZIKV infection in the first trimester of pregnancy and microcephaly risk based on cases from Bahia (Johansson et al. 2016) and a retrospective study of the cases in the earlier French Polynesia outbreak (Cauchemez et al. 2016). The percentage of microcephaly cases in these infected populations are close to 1 % at their

lower boundaries, which is about 20-fold higher than the percentage of baseline, sporadic occurrence of microcephaly in the general population. Other than microcephaly, some of the affected infants were also presented with varying degrees of macular atrophy (Ventura et al. 2016a; Jampol and Goldstein 2016; de Paula Freitas et al. 2016). The risk of ocular defects appears to be associated with maternal ZIKV infection in the first trimester and smaller cephalic diameter at birth (Ventura et al. 2016b). The urgency in determining experimentally whether ZIKV could result in placental–fetal infection was therefore obvious.

ZIKV infection of human tissues and placental–fetal infection

Congenital defects resulting from maternal infection could be due to a placental inflammatory response that affects fetal development, or direct viral infection of the fetus through intrauterine, transplacental transfer, or both (Mor 2016; Adibi et al. 2016). The placenta defends the fetus from infections, serving as a physical barrier and also through the innate and adaptive immune response elicited by trophoblastic tissues. Recent findings indicated that trophoblasts constitutively produce the type III interferon IFN- λ 1, which functions in an autocrine/paracrine manner to protect both trophoblast and non-trophoblast cells from ZIKV infection (Bayer et al. 2016). However, recent evidence appears to indicate that ZIKV infection of the pregnant mother could result in appreciable placental damage and viral access to the fetus. A number of mouse models with defective interferon- α/β receptor (IFNAR) and type I interferon signaling for studying ZIKV infection has now been reported (Aliota et al. 2016; Lazear et al. 2016; Rossi et al. 2016; Miner et al. 2016). Miner and colleagues have very recently investigated ZIKV infection during pregnancy with two such models (Miner et al. 2016). The first is based on the infection of *Ifnar1* $-/-$ females, which resulted in high levels of systemic ZIKV replication. Fetuses from crosses between *Ifnar1* $-/-$ females with wild-type males will have an *Ifnar1* $+/-$ genotype and, presumably, a largely functional type I interferon (IFN) response. A majority of these fetuses nonetheless became ZIKV infected and underwent fetal demise, displaying only placental remnants, and even the more intact ones exhibited at least some degree of intrauterine growth restriction (IUGR). In a second model, ZIKV-infected pregnant wild-type females were treated with an IFNAR-blocking monoclonal antibody MAR1-5A3. In this case, fetal demise was not observed, but fetuses in antibody treated females also exhibited a significantly higher degree of IUGR compared to controls and mocks, and these have an elevated viral RNA level that was dose dependent upon the amount of

antibody administered. From the first model, it appears that an immune-compromised pregnant mother mouse with a massive viral load could effectively result in vertical placental–fetal infection. An obvious caveat is whether such high loads could be attained in immune-competent animals or humans, and whether the mice fetuses heterozygous for IFNAR are equally competent in terms of IFN response compared to wild-type animals.

Placental damage from ZIKV infection has also been reported for human placental tissues from infected mothers (Noronha et al. 2016). Human placental macrophages and cytotrophoblasts could be productively infected by ZIKV in culture, which elicited a type I interferon response and the production of proinflammatory cytokines (Quicke et al. 2016). Such infections would likely compromise placental integrity and allow viral access to the fetus. Indeed, vertical transmission in immune-competent pregnant mice was demonstrated by infection of the pregnant mother leading to IUGR (Cugola et al. 2016) or by intraperitoneal injection of the virus leading to CNS infection of the developing fetus (Wu et al. 2016), as discussed below. Ongoing experiments with pregnant macaques posted online (<https://zika.labkey.com/project/OConnor/begin.view>) have also reported the detection of viral RNA in amniotic fluid. In the section below, ZIKV infection and the cellular pathology elicited in brain cell/tissue types in vitro and in vivo shall be discussed.

ZIKV infection of fetal neuroprogenitor cells and associated pathology

That ZIKV is neurotrophic and could infect the CNS has been shown earlier in mice long before the current epidemic (Bell et al. 1971). ZIKV readily and productively infects dermal fibroblasts and keratinocytes at the site of mosquito vector inoculation (Hamel et al. 2015) through a bunch of known entry factors that is also utilized by other Flaviviruses. The C-type lectin Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN)/CD209 and members of Tyro-3, AXL, and Mer (TAM) family of receptor tyrosine kinases (Lemke and Rothlin 2008) appear to be prominent in this regard (Hamel et al. 2015). ZIKV infection induces a typical cellular innate immune response, with the elevation in the transcription of pattern recognition receptors such as Toll-like receptor 3 (TLR3), retinoic acid-inducible gene 1 (RIG-I), melanoma differentiation-associated protein 5 (MDA5), as well as β -interferon production with the elevation of a host of interferon-stimulated genes (Hamel et al. 2015). Both type I and type II interferons appear to protect against viral proliferation (Hamel et al. 2016). ZIKV infection of skin fibroblasts also induces autophagy, which appears to

enhance viral replication (Hamel et al. 2015), as previously observed for some other Flaviviruses such as Dengue virus and Modoc virus (Lee et al. 2008; McLean et al. 2011).

A flurry of recent reports have now confirmed ZIKV's targeted infection of developing mouse and human brain, particularly neural progenitor cells (NPCs), with pathological consequences (see summary in Table 1). In a report involving the use of two mice models, infection of the pregnant mother resulted in pups that exhibit clear delay in body growth and IUGR in Swiss Jim Lambert (SJL) mice, but not in C57BL/6 mice (Cugola et al. 2016). Viral RNA was detectable in several tissues of newborn littermates, and the levels were particularly high in the brain. Careful examination revealed discernible cortical malformations in the surviving newborns, with reducing cell number, thickness, as well as apparent ocular abnormalities. ZIKV infection induced apoptotic cell death and autophagy in the fetal brains, which corresponded to changes in a host of apoptosis/autophagy-associated gene expressions. The reason why maternal–fetal transmission was not observed in C57BL/6 mice in this report was unclear, although it is possible that these mice may have a more robust type I/II IFN response, or a different entry receptor profile from the SJL mice.

In two other studies, intraperitoneal injection of virus into pregnant mothers (Wu et al. 2016) as well as intracerebral injection into the developing fetal brain (Li et al. 2016; Wu et al. 2016) also resulted in prominent infection of fetal brain tissues. Wu et al. (2016) introduced ZIKV by peritoneal inoculation of pregnant C57 mice at embryonic day (E) 13.5, as well as direct injection of the virus into the lateral ventricle of fetal mice, which allowed observation of fetal brain infection at a critical stage of embryonic cortical development. The authors found that ZIKV targets the radial glial cells of fetuses. Another particularly important group among the infected cells were those of the dorsal ventricular zone of the developing cortex, where NPCs that give rise to cells that will eventually migrate and populate the cerebral cortex originate (Bystron et al. 2008; Tang 2016). ZIKV infection markedly reduced the numbers of these progenitor cells and, consequentially resulted in a quantifiably smaller cortical area (Wu et al. 2016). Li and colleagues observed that intracranially introduced ZIKV infection of fetal NPCs caused cell-cycle arrest, apoptosis, and inhibition of NPC differentiation (Li et al. 2016), which would conceivably lead to microcephaly. Actual measurement of the cranial size of newborns from the fetuses infected by intracranial injection of ZIKV proved difficult as the pups were too weak and fell victim to maternal cannibalism (Li et al. 2016).

ZIKV is likewise shown to readily infect human NPCs in culture (Tang et al. 2016; Cugola et al. 2016) as well as those present in developing human cerebral organoids (Qian et al. 2016; Cugola et al. 2016; Garcez et al. 2016;

Table 1 A summary of reports on ZIKV infection of cells/tissues of the developing mouse and human brain

Model system	ZIKV strain and dose	Main/notable findings and pathological observations	References
<i>Mouse models</i>			
1. Infection of <i>Iftar</i> $-/-$ pregnant female mice conceived by mating with wild-type males, thus carrying heterozygous <i>Iftar</i> $+/-$ fetuses	French Polynesia isolate H/PPF/2013 10 ⁸ focus forming units (FFU)	ZIKV infection detected in fetuses, which exhibited fetal demise or intrauterine growth restriction (IUGR)	Miner et al. (2016)
2. ZIKV-infected wild-type pregnant female mice treated with an IFNAR-blocking antibody	Brazilian ZIKV strain 10 ⁷ –10 ¹² plaque forming units (PFU)	ZIKV infection detected in newborns, with cortical malformation in littermates of the SJL strain	Cugola et al. (2016)
Infection of pregnant SJL and C57BL/6 mice	Asian ZIKV strain SZ01 1. 9 × 10 ⁴ PFU for i.p. 2. 450 PFU for intracranial injection	ZIKV infection of fetal radial glial cells. Infected fetus has reduced cavity of lateral ventricles and decreased cortical surface area	Wu et al. (2016)
1. Intraperitoneal (i.p.) injection of virus into C57 pregnant mice	Asian ZIKV strain SZ01	ZIKV infection of NPCs, causing cell death, dysregulation of cell cycle and differentiation, and dysregulation of microcephaly-associated genes	Li et al. (2016)
2. In utero injection into lateral ventricles of developing fetus	450 PFU for intracranial injection		
Intracranial injection of virus into brain ventricle of E13.5 ICR mice fetuses			
<i>Human models</i>			
Human NPCs differentiated from iPSCs	African ZIKV strain MR766 Multiplicity of infection (MOI): <1	Cell death, cell-cycle dysregulation, alterations in gene expression profile	Tang et al. (2016)
Human iPSC-derived organoids	African ZIKV strain MR766 Asian strain FSS13025 Low MOI as in Tang et al. above. Exact titer used not specified	Reduced proliferation, cell death and decrease neuronal cell layer volume	Qian et al. (2016)
Human iPSC-derived NPCs, neurospheres and cerebral organoids	Brazilian ZIKV strain MOI: 0.1–10	Cell death, reduction in size of neurospheres and defective organoid development	Cugola et al. (2016)
Human iPSC-derived neural stem cells and organoids	African ZIKV strain MR766 MOI: 0.0025–0.25	Growth impairment of neurospheres and organoids, cell apoptosis	Garcez et al. (2016)
Human embryonic stem cell-derived cerebral organoids	African ZIKV strain MR766 MOI: 1	Reduction of organoid growth via induction of TLR3	Dang et al. (2016)
Human brain tissues and organoids	NA	Analysis of putative ZIKV entry receptors and highlighting AXL as a key viral entry receptor expressed in radial glia, astrocytes and endothelial cells	Nowakowski et al. (2016)

Dang et al. 2016). These experimental infections by and large resulted in dysregulation of NPC proliferation, cell cycle and differentiation, and caused cell death. There are two major conceivable ways whereby ZIKV infection of the developing fetal brain could affect cortical development. The first is simply by the killing of NPCs or by dysregulating their number of replications. Other than acute stress and demise of the NPCs and other fetal brain cells, upregulation of TLR3 drastically changes the expression profile of genes related to cerebral development (Dang et al. 2016), which likely contributed to the gross microcephaly phenotype in human fetuses. Secondly, ZIKV infection could also affect the construction of the cerebral architecture during the critical period of corticogenesis. In this regard, a particularly important cell type in the fetal brain that is targeted by ZIKV is the radial glial cells, which are key regulators of cerebral cortex development (Borrell and Götz 2014). Other than being progenitor cells, these cells serve as a tissue scaffold for the migration of cortical neurons from the ventricular/subventricular zone to the cortical surface in the generation of the laminar structure of the mammalian cortex (Rakic 2007; Molnár and Clowry 2012; Tang 2016). A recent report has highlighted the prominent expression of a putative ZIKV entry receptor AXL receptor tyrosine kinase (Corno et al. 2016) in radial glia cells, as well as in astrocytes and NPCs of multiple mammalian species (Nowakowski et al. 2016). ZIKV infection and impairment of function of these cells would fundamentally affect human fetal corticogenesis. Interestingly, AXL is also found to be expressed in the outer margin of human neural retina, as well as its adjacent ciliary marginal zone (Nowakowski et al. 2016), which could potentially explain the occurrence of macular atrophy reported in ZIKV-associated microcephalus infants.

ZIKV infection and the risk of neurological diseases

ZIKV infection in adults has been known to present only mild symptoms. However, given its neurotrophic capacity and tendency to infect NPCs, could ZIKV also infect the brain and results in neurological disorders in adults? Brain infection and neurological symptoms have been observed in ZIKV infection of mice lacking type I and/or type II interferon response (Rossi et al. 2016; Lazear et al. 2016; Aliota et al. 2016; Dowall et al. 2016; Zmurko et al. 2016). Acute myelitis with the detection of the virus in the CSF has been recently reported in a 15-year-old patient (Mécharles et al. 2016). It is therefore plausible that ZIKV infections that might result in unusually widespread systemic infection, or prolonged viremia in immune-compromised adult individual, could culminate in CNS infection. A previous report

has shown that TLR-3 stimulation in West Nile virus infections could compromise the blood–brain barrier (BBB) and enhance CNS infection in mice (Wang et al. 2004). Given that TLR-3 is also specifically elevated in ZIKV infection (Hamel et al. 2015; Dang et al. 2016), it is again plausible that weakening of the BBB could lead to CNS infection in adult subjects.

ZIKV outbreaks in French Polynesia and Brazil have both been associated with increased cases of paralytic neuropathy characteristic of GBS (Beckham et al. 2016; Lucchese and Kanduc 2016; Brasil et al. 2016). A case–control analysis of the French Polynesia cases showed a clear association between patients with GBS and a recent infection, as well as neutralizing antibodies against ZIKV (Cao-Lormeau et al. 2016). Interestingly, a scan of ZIKV protein polyprotein peptide sequence indicates an unexpectedly high degree of peptide sharing with GBS autoantigens known for demyelinating and axonal neuropathy (Lucchese and Kanduc 2016). This finding, although preliminary, is suggestive of an autoimmunity precipitated GBS in ZIKV-infected patients. Further work would be required to confirm this notion.

Epilogue

In this brief review, I provided an update of reports demonstrating causality between ZIKV infection and congenital microcephaly. It is now clear that transplacental infection of the NPCs in the developing fetus, particularly in the first trimester, is the most likely cause for the ZIKV infection-associated congenital disorder. ZIKV productively infects human fetal brain cells and organoids *in vitro*. Mouse models have also recapitulated critical aspects of human ZIKV pathology pertaining to fetal demise, fetal brain infection and cortical development *in vivo*. It is likely that the macular atrophy observed with some of the microcephalic newborns is also a result of ZIKV infection of the developing fetal retinal structures, as this was also seen in infected mice newborns, but the extent to which this occurs in humans remains to be confirmed. There is some evidence for the capability of ZIKV to target the adult human CNS, but any confirmation of such neurotropism would require the analysis of a larger number of cases. The association between ZIKV and GBS is likewise tentative, and further evidence for ZIKV antibodies targeting autoantigenic epitopes is needed for a causal association.

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Compliance with ethical standards

Conflict of interest The author declares no conflict of interest.

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