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REVIEW

Insights into the molecular roles of Zika virus in human reproductive complications and congenital neuropathologies

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Summary

The recent upsurge in the association of congenital neurological disorders and infection by the Zika virus (ZIKV) has resulted in increased research focus on the biology of this flavivirus. Studies in animal models indicate that ZIKV can breach the placental barrier and selectively infect and deplete neuroprogenitor cells (NPCs) of the developing fetus, resulting in changes of brain structures, reminiscent of human microcephaly. *In vitro* and *ex vivo* studies using human cells and tissues showed that human NPCs and placental cells are targeted by ZIKV. Also of concern is the impact of ZIKV on human reproductive structures, with the potential to cause infertility, as the virus appears to remain in the genital tract for extended periods of time. This review discusses the putative roles of ZIKV on human reproductive complications and congenital neuropathologies.

Key words: Zika virus; neuropathology; fetus; placenta; microcephaly; congenital.

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INTRODUCTION

Zika virus (ZIKV), a positive-strand RNA virus, is an arbovirus that belongs to the *Flaviviridae* family and emerged as a mosquito-borne virus originally identified in Uganda in 1947.¹ Early studies indicated that ZIKV was infectious and pathogenic to humans,^{2–4} resulting in headache, malaise, fever, cutaneous rash, arthralgia, and conjunctivitis in about 20% of patients and with 80% of infections being asymptomatic.⁵ Thus, ZIKV was not considered a major public health concern; however, the recent explosion in ZIKV infection and its association with severe congenital neurological disorders in South America (mainly Brazil) in 2015–2016 prompted the World Health Organization (WHO) to declare the situation as a Public Health Emergency of International Concern.⁶ Similar outbreaks and associated consequences occurred in the Pacific (specifically the island of Yap) in 2007⁷ and in French Polynesia in 2012–2014.⁷ The ZIKV-associated neurological disorders mainly include microcephaly in newborns,⁸ chorioretinopathy in microcephalic infants,^{9,10} and severe

paralytic neuropathic symptoms of Guillain–Barré syndrome (GBS) in adults.^{11–13}

Primary microcephaly is a paediatric neurological disorder characterised by significant reduction in head circumference, commonly associated with varying degrees of impaired sensory, motor, and cognitive functions.^{14,15} Primary congenital microcephaly is caused by decreased neurogenesis in the brain *in utero*, resulting from depletion of cortical neural progenitor cells (NPCs) (a consequence of either apoptosis or reduced proliferation or a combination of these factors). The impaired cellular processes that limit neurogenesis in microcephaly arise from deregulated expression of a number of genes mostly associated with the expression of centrosomal proteins (reviewed by Tang¹⁶ and Barbelanne and Tsang¹⁷). Congenital microcephaly can also arise from infections caused by *Toxoplasma*, rubella virus, cytomegalovirus, herpes virus and the syphilis bacteria (dubbed the TORCHS factors).^{18–22}

An epidemiological association between ZIKV infection and microcephaly became obvious during the recent epidemic, mainly due to the unprecedented increase in infants born with this devastating neurological disorder.^{23–26} Despite warning of an epidemic in February 2015 by WHO, there were still concerns about whether ZIKV was the cause of the sudden rise of microcephaly in infants. However, increased research quickly established ZIKV as a potential teratogenic agent, resulting from an initial infection. Whilst the principal mode of ZIKV transmission is by infected *Aedes* mosquitoes (*Ae. aegypti*, *Ae. albopictus*, *Ae. africanus*, and *Ae. luteocephalus*) (reviewed in Hayes),²⁷ other reported means of transmission include sexual contact,²⁸ the prenatal route from mother to fetus,²⁹ and through blood transfusion.³⁰ Although viral particles have been detected in other body fluids such as saliva,^{31–33} urine,³⁴ and breastmilk,³⁵ there are no reports substantiating that ZIKV is spread by these fluids. Evidence suggests that ZIKV can breach the placental barrier in early gestation and disrupt neurogenesis, potentially resulting in severe congenital malformations, as well as giving rise to intra-uterine growth restriction (IUGR). In addition, emerging studies indicated that ZIKV could remain in the human genital tract for months, long after the virus was cleared from other body fluids,^{36,37} and in mice ZIKV infection could lead to severe reproductive pathologies.

This review discusses the putative molecular roles of ZIKV in human reproductive complication and congenital neuropathologies.

EPIDEMIOLOGICAL AND CLINICAL ASSOCIATION WITH NEUROCONGENITAL DEFECTS

ZIKV infection and associated neurological defects have now been reported for a number of countries in South America³⁸ and among pregnant travellers to ZIKV-affected areas.³⁹ Evidence of congenital infection is based on detection of ZIKV RNA in tissues, amniotic fluid and fetal brain tissue^{29,40–42} and anti-ZIKV IgM antibodies in the cerebrospinal fluid (CSF) of infants born with microcephaly.⁴³ Several studies, including population-based analyses, revealed that there is a greater risk of congenital microcephaly during the first trimester of pregnancy in ZIKV-infected mothers.^{44–46} However, ZIKV infection at the 36th week (third trimester) of pregnancy can still result in fetal brain injury, characterised by subependymal cysts and lenticulostriate vasculopathy in an otherwise normal sized brain.⁴⁷ Other clinical aspects associated with ZIKV infection include brainstem and cerebellar hypoplasia, delayed myelination/demyelination, severe ventriculomegaly due to loss of brain tissue, gross calcification of the brain parenchyma, and some cases of lissencephaly (absence or reduced neurocortical gyration).^{48–51} Studies also reported the prevalence of sensorineural hearing loss in babies with ZIKV-associated microcephaly.⁵² In adults, as stated, ZIKV infection is associated with GBS,^{11,12} an autoimmune disease characterised by demyelination of peripheral motor axons, leading to muscle weakness and paralysis, and death.^{11,53} Other flaviviruses are also associated with GBS.⁵⁴

CELLULAR MECHANISMS OF ZIKV TROPISM: RECEPTOR-MEDIATED AND RECEPTOR-INDEPENDENT

It is now established that ZIKV tropism is mediated by cell surface receptors DC-SIGN, AXL, heat shock proteins, TYRO3, and TIM-1.^{55,56} AXL is a phosphatidylserine protein that belongs to the TAM receptor family of phagocytic receptors⁵⁷ and it appears to play an important role in ZIKV infection of a wide range of cells of organ systems targeted by this virus, as discussed below. However, ZIKV may also use a combination of different cell surface/adhesion molecules to gain cellular entry.

Some studies have indicated that ZIKV can infect host cells in the absence of AXL expression. Genetic ablation of AXL failed to prevent ZIKV infection of NPCs and cerebral organoid.⁵⁸ In Axl^{-/-} knockout mice, retinal cells were not protected against ZIKV infection *in vivo* and ZIKV RNA levels in the brains of infected Axl^{-/-} mice were comparable to that of wild-type animals.⁵⁹ These studies suggest that cell surface receptor(s) other than AXL may be needed for ZIKV attachment to human cells. One such candidate is TYRO3. Hamel *et al.* showed that overexpression of TYRO3 in HEK293 cells, which are normally resistant to this flavivirus, resulted in ZIKV infection.⁵⁵ The co-expression of AXL and TYRO3 by human NPCs⁶⁰ may explain the extreme susceptibility of these cells to ZIKV. Therefore, inhibition of AXL in the presence of TYRO3 can still lead to ZIKV infection. However, induced pluripotent stem cells (iPSCs) recalcitrant to ZIKV infection express high levels of TYRO3 (>50 TPM) RNA,⁵⁸ leading to concerns about the role of this

receptor in viral attachment and cellular entry. Similar concerns may also exist for placental cells targeted by ZIKV, although more research is needed. However, it is possible that other unknown or undiscovered receptors mediate cellular entry of ZIKV.

ZIKV may also invade target cells by receptor-independent mechanisms, which may be related to exosomes and extracellular vesicles. Exosomes are cell-derived, membrane-bound, nano-sized vesicles, used in cell-cell communication. Exosome-derived cargo, which may include DNA, RNA, proteins, and lipids, are capable of influencing cellular responses of target cells. Studies showed that cells infected by viruses, including the Dengue virus which is closely related to ZIKV, released exosomes that shuttled viral RNA and proteins and other genetic elements and regulatory factors to target cells, effectively spreading infection.^{61–64} Several molecular players (syndecan-1, ALIX, syntenin) involved in exosome biogenesis^{65,66} are expressed by subsets of cellular components of the placenta where they may be involved in immune-modulation.^{67,68} *In vitro* studies have shown that trophoblast-derived exosomes deliver pro-autophagic microRNA (miRNA) to bystander cells, thereby conferring resistance to viral infection.⁶⁹ However, it remains to be determined whether ZIKV utilises a similar mechanism for viral infection of cells of placental tissues. In the nervous system, neural stem cell-derived exosomes significantly enhanced cellular entry of adenovirus type 5 (Ad5) into Cocksackie virus and adenovirus receptor (CAR)-deficient cells.⁷⁰ The uptake of exosomes may be facilitated by either interaction with the membrane lipid raft or through mechanisms that do not involve TAM receptors.

Upon cellular entry, ZIKV triggers the upregulation of Toll-like receptor 3 (TLR3), RIG-I, and MDA5, and genes involved in innate immune response as well as several interferon-stimulated genes, including OAS2, ISG15, and MX1.⁵⁵ Most studies have shown that ZIKV limits cell growth. ZIKV infection of human neuroepithelia stem cells resulted in translocation of centrosomal and cytosolic protein pTBK1 (phosphorylated TANK binding kinase 1) to the mitochondria leading to deregulated mitosis and increased cell death.⁶⁰ Increased cell death may also be a result of ZIKV-induced upregulation of the tumor suppressor gene TP53⁷¹ and of TLR3,⁷² and suppression of Akt-mTOR,⁷³ which results in autophagy (Fig 1).

PUTATIVE MECHANISM(S) OF ZIKV SUPPRESSION OF CELLULAR DEFENSES

It has been speculated that once ZIKV infection is fully established, the virus becomes resistant to interferon treatment, suggesting that the virus develops a set of counter-measures against the host cell defenses.⁷⁴ STAT2, which is the transcriptional activator of interferon responses,⁷⁵ appears to be targeted by the ZIKV proteins.⁷⁶ ZIKV NS5 protein binds to STAT2, leading to proteosomal degradation of STAT2.⁷⁴ Also, ZIKV NS1, NS4A, and NS5 proteins inhibit the induction of type-I IFNs by suppressing IRF3 expression and NF- κ B anti-viral signalling.⁷⁴ The loss of critical antiviral defense measures (e.g., proteosomal degradation of STAT2) can contribute to the detriment of the host cells.

Fig. 1 Putative receptor-mediated ZIKV infections. ZIKV preferentially targets cells that predominantly express TAM receptors (AXL, Tyro3) and DC-SIGN. Upon endocytosis, ZIKV (1) triggers translocation of pTBK1 from cytosol to mitochondria; (2) activates TP53 and TLR3 signalling; (3) activates autophagy by suppression of Akt-mTOR, leading to cell death. ZIKV also induces cell cycle arrest and reduces cell division (not indicated). These mechanisms are especially involved in death of NPCs, resulting in cortical defects seen in microcephaly.

PUTATIVE MECHANISM(S) OF MOTHER-FETUS TRANSMISSION

ZIKV-associated disruption of fetal brain growth requires viral passage across the placental barrier. Placental tropism of ZIKV is suspected because of the detection of ZIKV particles and RNA in the placental tissue, amniotic fluid and fetal brain tissues^{29,40,77,78} of infected pregnant mothers. However, the mechanism(s) of mother-to-fetal transmission of ZIKV are not completely understood. The human placentation displays unique trophoblast invasion into the maternal *decidua basalis* (decidua). Of importance is the establishment of immune-privileged fetal-maternal interfaces where embryonic and fetal tissues are in close contact with the mother's immune system. The two main interfaces are the decidua that serves as the anchoring point for the placenta and the intervillous space where maternal blood is in contact with placental villi.^{78–80} Placental villi are covered by the superficial multinuclear syncytiotrophoblast layer (STBs) formed from the fusion of cells of the inner mononuclear cytotrophoblast layer (CTBs). At the base of the anchoring villi, proliferative CTBs differentiate into extravillous trophoblasts (EVTs), invade the decidua and remodel uterine arteries to facilitate blood flow into the intervillous space.^{78–80} It has been proposed that viral infection of placental tissue may occur by direct and contiguous infection of the cellular layers, virion passage through a breach, or by cell-mediated transport. Placental-permissive viruses include rubella, cytomegalovirus, herpes simplex, HIV-1, hepatitis B and C virus, and parvovirus B19, avian influenza H5N1, coxsackie virus, and Japanese encephalitis virus,^{81–84} some of which have been implicated in congenital neurological defects.^{85,86} A recent *in vitro* study showed that type III interferon IFN- λ confers resistance to trophoblast and non-trophoblast cells to ZIKV exposure, presumably by autocrine and paracrine modes.⁸⁷ However, in humans, considerable placental damage is associated with ZIKV infection,⁴² indicating possible viral access to the fetus. A number of investigators exploited mouse models with

defects in interferon- α/β receptor (IFNAR).^{88–92} Exposure of female mice lacking the ability to produce interferon type 1 (IFN)– *Ifnar1*^{−/−} to ZIKV, resulted in high levels of systemic ZIKV infection.⁹² The authors then crossed the *Ifnar1*^{−/−} with wild type (WT) to produce heterozygous mice (*Ifnar1*^{+/-}) which are supposed to carry functional type I interferon. However, most of the *Ifnar1*^{+/-} fetuses, upon exposure to ZIKV, became infected.⁹² The ZIKV-infected *Ifnar1*^{+/-} fetuses died *in utero*, leaving only placental remnants.⁹² The remaining intact *Ifnar1*^{+/-} fetuses showed significant IUGR.⁹² In a second model of ZIKV infection during pregnancy, WT mice treated with MAR1-5A3 (a blocking anti-fnarn monoclonal antibody) and inoculated with ZIKV, did not result in death *in utero*, although IUGR was detected.⁹² These studies suggest that high load of ZIKV in immune-compromised (impaired interferon response) pregnant mice can lead to mother-to-fetal transmission of this virus. In addition, co-infection of pregnant C57BL/6 mice with herpes simplex virus-2 (HSV-2) and ZIKV resulted in enhanced placental sensitivity to ZIKV, presumably resulting from increased expression of TAM receptors.⁹³ This study suggests that fetuses of patients with HSV-2 infection may be at higher risk for the detrimental effects of ZIKV. However, laboratory tests revealed ZIKV-associated cases in Brazil are HSV-2 negative.²⁹ Studies on human placental cells and tissues from various gestational periods revealed potential damage to this transient organ. ZIKV-infection of placental explants obtained from first-trimester pregnancy (7–12 weeks of gestation) of healthy women undergoing vaginal elective termination of pregnancy, with the Brazilian strain of ZIKV (ZIKV^{BR}), revealed widespread damage to the maternal decidua, fetal placenta, and umbilical cord.⁸⁰ The virus replicated differentially in a wide range of maternal and fetal cells, including dFibroblasts (decidual fibroblasts), dM ϕ (decidual macrophages), trophoblasts, and Hofbauer cells,⁸⁰ suggesting that trans-placental passage of the virus may occur through succession of adjacent cellular targets from the *decidua basalis* to the anchoring villi. A study conducted on a 21-week gestation fetus terminated during pregnancy, to assess the effects of ZIKV on the placental barrier *in vivo*, showed enlarged, hydropic (sign of cellular damage) chorionic villi with hyperplasia and focal proliferation of Hofbauer cells.⁹⁴

Tabata *et al.* showed that primary placental cells (cytotrophoblasts, endothelial cells, fibroblasts, and Hofbauer cells in chorionic villi and amniotic epithelial cells and trophoblast progenitors in amniochorionic membranes) of mid to late gestation, from 20 healthy humans, were vulnerable to ZIKV infection, presumably owing to their expression of various combination of co-factors/receptors that facilitate ZIKV binding and cellular entry.⁹⁵ Although this study indicates that it is possible that late-pregnancy ZIKV infection can lead to congenital neurological problems because of placental expression of entry receptors during late gestational stages, the fetal immune response may be well developed to minimise or prevent neuroinvasion of the brain. In addition, the fetal brain during late gestation may be advanced enough that ZIKV infection can inflict harm to only a few neurons and glial cells, and therefore do not produce severe detrimental neurological effects. However, late-pregnancy ZIKV-associated lesions can still occur via receptor-independent mechanism(s) as discussed later.

POTENTIAL EFFECTS OF ZIKV ON REPRODUCTIVE STRUCTURES

There has been a small but growing number of reports on the presence of ZIKV in body fluids associated with the reproductive system, implying potential for sexual transmission of the virus and potential for adverse reproductive functions. Indeed, sexual transmission of ZIKV has been suspected in a number of cases.⁹⁶ ZIKV particles and RNA have been observed in human sperm and in seminal fluid for extended periods of time, well after it has been cleared from other fluids such as blood and urine.⁹⁷ Although spermatozoa lack AXL expression,⁵⁵ suggesting potential resistance to cellular entry by ZIKV, Sertoli cells showed strong expression of this receptor, which also plays essential roles in mammalian spermatogenesis.^{98,99} Therefore, it has been speculated that the Sertoli cells might be involved in ZIKV transmission to spermatozoa,⁹⁷ perhaps by a mechanism that involves cytoplasmic bridges between Sertoli cells and developing spermatocytes. Given the cytotoxic effects of ZIKV, it is not clear what the impact of this flavivirus on human spermatozoa and Sertoli cells might be. However, studies in mice catalogued widespread damages to male reproductive structures. Separate studies using a mouse-adapted African ZIKV strain (Dakar 41519) and a contemporary ZIKV strain (ZIKV-SMGC-1) showed viral infection of spermatogonia, primary spermatocytes, and Sertoli cells, Leydig, and epididymal epithelial cells, resulting in cell death and destruction of the seminiferous tubules.^{100,101} Other associated effects included reduced levels of testosterone and inhibin B¹⁰¹ and stimulation of pro-inflammatory cytokines/chemokines production and infertility.^{100,101} *In vitro* and *in vivo* studies showing the effects of ZIKV on human testicular structures have not been carried out as yet. In addition, it is not clear whether the immune-privileged nature of human testes will allow ZIKV to persist in this tissue following cellular invasion by the virus. Should this occur, such a reservoir can potentially initiate new transmission cycles from seemingly healthy individuals.¹⁰² However, a longitudinal study should provide greater insights into the impact of ZIKV on human male reproductive structures. Expected impact in humans could be male infertility and sterility.

ZIKV may also persist in the female genital tract. Experiments in a murine model of ZIKV infection by intravaginal deposition of viral particles, demonstrated that ZIKV replicated within the genital mucosa, persisted post-infection, and was detected in the fetal brain of the mice.¹⁰³ In humans, ZIKV has been detected in the genital tract of females and may remain there for a prolonged period of time,^{104–106} mirroring reports of the virus in the male genital tract. However, it is not clear whether ZIKV lodged in the female vaginal tract can spread to the follicular fluids and potentially disrupt oocyte viability and embryonic development.

DISRUPTED NEUROCORTICOGENESIS AND MICROCEPHALY: SELECTIVE ERADICATION OF NEURONAL PRECURSORS AND NEUROCORTICAL THINNING

An early study by Bell *et al.* revealed replication of ZIKV in neurons and astroglia in the brain of newborn and 5-week old mice intracerebrally inoculated with the virus,¹⁰⁷ indicating neurovirulence of this flavivirus. A recent study showed the

overexpression of AXL in developing human brain cells, including radial glia, astrocytes, endothelial cells, and microglia,¹⁰⁸ rendering these cells particularly vulnerable to ZIKV infection. This vulnerability is reflected in *in vivo*, *ex vivo*, and *in vitro* studies. The processes of radial glial cells form tissue scaffold along which neuronal precursors migrate to the final destination of outer cortical layers. These consequences may partly be a result of ZIKV-induced deregulation of genes involved in immune response (as indicated above), cell cycle, and differentiation, and apoptosis of NPCs of infected embryonic mice.^{109,110} The ZIKV-associated cortical malformations are reminiscent of human microcephaly: thinner cortex, reduced gyration, and smaller brain (Fig 2). In addition, ZIKV infection eradicated NPCs from a cerebral organoid grown from first-trimester human brain embryonic stem cells, resulting in restricted growth of the organoid.⁷² Reduction in organoid size may be the result of deregulation of genes involved in neurogenesis, differentiation and apoptosis of ZIKV-infected NPCs.⁷² Restricted neurogenesis may also arise from autophagy resulting from suppression of the Akt-mTOR pathway by ZIKV non-structural proteins NS4A and NS4B, in viral-infected human fetal stem cells.⁷³

Studies have shown that some aspects of ZIKV infection of NPCs mirror molecular, chromosomal, and subcellular changes that occur in non-ZIKV-associated, inherited homozygous recessive microcephaly. Underlying genetic causes of 'primary' developmental microcephaly are linked to defects in centrosomal proteins controlling the mitotic spindle, thereby assuring normal cell proliferation during mitosis.¹¹¹ Disruption of centrosomal proteins may be associated with restricted cell growth resulting from ZIKV infection. Gene expression analyses revealed that genes (including *Microcephalin*, *CDK5RAP2*, *CASC5*, *ASPM*, *CENPJ*, *STIL*) mapped to the microcephaly loci were downregulated to varying degrees in ZIKV-infected fetal brain samples.¹¹² ZIKV-infected SOX2⁺ NPCs showed significant increase in mitosis abnormalities, including multipolar spindle, chromosome laggards, micronuclei and death

Fig. 2 ZIKV infection reduces neurogenesis. In normal neurogenesis, neuronal precursors migrate along radial glia processes, from the ventricular zone to form the outer layers of the cerebral cortex, in an inside-out manner. However, ZIKV infection is likely to eradicate large numbers of radial glia and neuronal precursors, thereby limiting the number of cortical layers (if any) formed. This mechanism is suspected to contribute to ZIKV-associated microcephaly.

of progeny after cell division, and large number of cells with supernumerary centrosomes.¹¹³ Further analyses by fluorescent *in situ* hybridisation (FISH) showed increased frequencies of aneuploidy, such as monosomy, trisomy and polyploidy on chromosomes 12 and 17.¹¹³ Supernumerary centrosomes, structural disorganisation, and mitotic abnormalities in part may be a consequence of sequestration of the centrosomal and cytosolic protein pTBK1 (phosphorylated TANK binding kinase 1) to mitochondria as described for ZIKV-infected human neuroepithelial stem and radial glial cells.⁶⁰ In addition, an analysis of predicted epitopes and human antigens indicated putative peptide sharing between ZIKV polyprotein and human proteins which are associated with neuropathologies, including microcephaly and brain calcification.¹¹⁴ Such proteins include centriolar and centrosomal components implicated in microcephaly, i.e., C2CD3, CASC5, CP131, GCP4, KIF2A, STIL, and TBG.¹¹⁴

Disrupted neurogenesis may also be driven by ZIKV-induced sustained production of proinflammatory cytokines via paracrine mechanisms. During early development, cranial or cephalic neural crest cells (CNCCs), which form most of the cranial bones, exert paracrine effects on the developing brain. An *in vitro* study has shown that ZIKV-infected CNCCs display limited apoptosis but secrete significant levels of cytokines that can effectively be detrimental to NPCs.¹¹⁵ This paracrine effect may contribute to the development of microcephaly in babies born to ZIKV-infected mothers, months after birth.

CONGENITAL NEUROLOGICAL DEFECTS OF LATE STAGE PREGNANCY ZIKV INFECTION

As indicated, late-pregnancy exposure to ZIKV can still produce congenital neurological deficits. ZIKV infection in the 36th week of pregnancy resulted in fetuses which showed preserved head circumference at birth but developed subependymal cysts and lenticulostriate vasculopathy.⁴⁷ The preserved head circumference is consistent with normal cranial growth, which takes place up to 30 weeks. In a separate case, a mother who reported ZIKV syndrome during third trimester, presumably after sexual intercourse with her ZIKV-infected husband, gave birth to a baby with microcephaly.¹¹⁶ The sensitivity of primary placental cells and placental tissue explants from early to late gestation to ZIKV infection appears to be consistent with the vulnerability of fetal brain structures at these stages of gestation. However, the risk for congenital neurological defects is still greater at early gestation.

LATE POST-NATAL NEUROLOGICAL COMPLICATIONS FOLLOWING IN UTERO ZIKV INFECTION

It has emerged that a subset of babies born to ZIKV pregnancies with normal head circumference preserved, showed severe post-natal brain growth restriction, reminiscent of microcephaly.¹¹⁷ Neuroimaging showed cortical malformations predominantly in the anterior brain, and calcifications predominantly in the subcortical region.¹¹⁷ Some of the infants developed epilepsy, and all had significant motor disabilities.¹¹⁷ However, the CSF for infants investigated was negative for ZIKV, although ZIKV-specific IgM antibodies were detected.¹¹⁷

The underlying mechanism that results in post-natal development of microcephaly is not completely understood. However, development of cortical malformation typical of microcephaly may be related to eradication of NPCs and astroglia (both of which are required to complete neurocortogenesis), persistent inflammation, or residual infection in neural cells, due to ZIKV infection. Hopefully a large-scale follow-up study will reveal the extent of the devastating post-natal impact of ZIKV infection.

ZIKV INVOLVEMENT IN OTHER NEUROPATHOLOGIES

ZIKV can also infect cells beyond the placenta, reproductive structures, and the nervous system. In mice, ZIKV infection was found in the optic nerve, retina, iris, and cornea, and also triggered panuveitis, conjunctivitis, and neuroretinitis.⁵⁹ In humans, ZIKV invasion of ophthalmic tissues is associated with optic neuritis, chorioretinal atrophy, and blindness in neonates, and conjunctivitis and uveitis in adults.^{59,118} In humans, cells of the outer margin of the neural retina and cells of the ciliary marginal zone adjacent to neural retina express AXL,^{108,119} making these cells susceptible to ZIKV infection, potentially triggering macular atrophy (leading to blindness) in babies born to ZIKV-infected mothers. In the developing human fetus, congenital ocular disease triggered by ZIKV could be due to the direct invasion of fetal ocular cells by the virus.

In addition, association of ZIKV and Guillain-Barré syndrome, a severe neurological disease characterised by an immune-mediated progressive muscle weakness that can result in flaccid paralysis and respiratory failure, has been noted.^{12,13,120} Also, a single study revealed that ZIKV may be associated with meningoencephalitis in an adult patient.¹²¹

CONCLUSION

It is now evident that ZIKV is a teratogenic agent, resulting in the increased incidence of primary, congenital microcephaly and other associated neurodevelopmental disorders. Cells that are most vulnerable to the destructive effects of ZIKV (principally NPCs and astroglia and a subset of placental cells of early pregnancy) are those that express the presumed cell surface receptors that facilitate cellular entry of the virus. Upon infection, ZIKV triggers reduced cellular proliferation and activates apoptosis and autophagy. These effects may produce decreased cortical layers in the cerebral cortex. To access the fetus, ZIKV has to breach the integrity of the placental barrier. Although the *in vitro* and *in vivo* evidence suggests vulnerability of placental cells to ZIKV, only a subset of infants born to mothers infected with the virus showed malformation at birth, while others with normal head circumference at birth developed microcephaly months after birth. In addition, the ability of ZIKV to infect AXL-deficient cells indicates that the virus can exploit other receptors not yet identified, or a receptor-independent route, for cellular invasion. Hopefully additional research will uncover other receptors used by ZIKV. Because exosomes and extracellular vesicles are widely used for intercellular communication, they may be involved in the spread of ZIKV infection. In addition, additional large-scale research will be required to assess the long-term effect of ZIKV on human reproductive organs. Much remains to be known about the biology of ZIKV.

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