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Zika virus: An emergent neuropathological agent

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ABSTRACT

The emergence of Zika virus in the Americas has followed a pattern that is familiar with earlier epidemics of other viruses, where a new disease is introduced into a human population and then spreads rapidly with important public health consequences. In the case of Zika virus, an accumulating body of recent evidence implicates the virus in the etiology of serious pathologies of the human nervous system, i.e., the occurrence of microcephaly in neonates and Guillain-Barré syndrome in adults. Zika virus is an arbovirus (arthropod-borne virus) and a member of the family Flaviviridae, genus Flavivirus. Zika virions are enveloped, icosahedral and contain a nonsegmented, single-stranded, positive-sense RNA genome, which encodes 3 structural and 7 nonstructural proteins that are expressed as a single polyprotein that undergoes cleavage. Zika genomic RNA replicates in the cytoplasm of infected host cells. Zika virus was first detected in1947 in the blood of a febrile monkey in Uganda's Zika forest and in crushed suspensions of the *Aedes* mosquito, which is one of the vectors for Zika virus. The virus remained obscure with few human cases confined to Africa and Asia. Two lineages of the Zika virus: African and Asian with the Asian strain causing outbreaks in Micronesia in 2007 and French Polynesia in 2013-2014. From here, the virus spread to Brazil with the first report of autochthonous Zika transmission in the Americas in March 2015. The rapid advance of the virus in the Americas and its likely association with microcephaly and Guillain-Barré syndrome makes Zika an urgent public health concern.

INTRODUCTION

Once a rare virus found in the rhesus monkey in the Zika forest in Uganda, the Zika virus has become an urgent public health concern in many countries and has been associated with microcephaly in neonates and Guillain-Barré syndrome in adults (1-5). Globalization of the Zika virus epidemic follows upon that of West Nile, Ebola, Dengue, and Chikungunya viruses. The extent of the ultimate spread of Zika is hard to predict but preventive measures such as mosquito control may help towards its restriction. Major challenges in responding to Zika include halting the spread of Zika, or if not, finding and developing diagnostics, devices, antivirals, vaccines and a better understanding of the disease and its epidemiology so that progression can be reduced (6, 7, 8). In this review, we will briefly discuss the history of Zika and its spread, Zika virology, molecular biology, epidemiology and transmission, the emerging evidence for its involvement in neonatal microcephaly and Guillain-Barré syndrome and prospects for stopping its progress.

HISTORY AND EPIDEMIOLOGY

The discovery of Zika virus was made by Scottish virologist George W.A. Dick and his coworkers in April 1947 in the blood of a febrile monkey in Uganda's Zika forest and was first reported in humans in 1952 (3, 9). The same virus was also later found in pulverized suspensions of *Aedes africanus* mosquito from the same area (3, 10, 11). Other monkey species in the Zika forest tested seropositive for Zika (11) but small mammals in the forest showed no serological evidence of infection, consistent with monkeys and humans being the vertebrate hosts for Zika infection (10). The virus remained obscure with few human cases confined to Africa and Asia (12) until the Asian strain caused Zika outbreaks in Micronesia in 2007 (13) & French Polynesia in 2013-2014 (14).

From French Polynesia, the outbreak then spread to other Pacific Islands: the Cook Islands, Easter Island, New Caledonia, the Solomon Islands and Vanuatu (15). Zika virus then spread to Brazil by an unknown means of transmission but phylogenetic studies indicated a close relationship between the strain that emerged in Brazil and samples from French Polynesia and the Pacific Islands, indicating that this was the likely source (15, 16). The first report of the autochthonous transmission of Zika in the Americas was in March 2015 in the state of Rio Grande do Norte, Northeast Brazil (17, 18). The epidemic has spread in Brazil with now ~1,300,000 suspected cases in late 2015 (17, 19). Already Zika virus has begun to spread beyond Brazil and further spread of the virus is anticipated with imported cases already been reported in the US, Europe and other countries where travelers are returning after visiting Latin America and the Caribbean (17 , 20). The spread of Zika virus around the world is illustrated schematically in Figure 1.

Ominously, it now appears that the virus may be able to be transmitted by means other than the *Aedes* mosquito (reviewed in 4). Firstly, since Zika is a bloodborne pathogen, it is possible that a Zika-infected blood donor could contaminate the blood supply and unpublished cases of Zika transmission through transfusion have been reported in Brazil (reviewed in 4). The efficiency of the transmission of Zika virus by transfusions is still unknown and additional studies are needed (21-23). Screening of donated blood by PCR-based tests, as is done for West Nile Virus, would prevent this possibility if such tests become available. If not, application of strategies for inactivation of the virus should be applied (24, 25). Secondly, Zika can be transmitted sexually (20, 26, 27, 28) and in these cases, virus was transmitted from infected men to their female partners. Accordingly, Zika viral RNA can be detected in semen (27, 28) and in one report, the RNA virus load was about 100,000 times that of matched blood or urine samples at a time of more than 2 weeks after the onset of symptoms (29). Musso et al (27) reported a patient whose semen and urine samples tested positive for Zika virus by PCR and

genome sequencing ten weeks after the onset of Zika-like symptoms although two sequential blood samples tested negative. Lastly, perinatal transmission of Zika has been reported but it is not known if this occurred *in utero*, via breast milk or by a bloodborne route (30). This may be particularly important given the association of Zika with neonatal abnormalities such as microcephaly as described below. Modes of transmission of Zika virus is illustrated schematically in Figure 2.

While other arboviruses such as Chikungunya and West Nile viruses have spread globally and caused epidemics, the public health significance of Zika virus lies in the growing evidence that it is associated with microcephaly and other birth defects in neonates and Guillain-Barré syndrome in adults (5, 31-33). Another potential issue with the current Zika epidemic is the possibility that the virus might mutate. Indeed, comparative genomic analysis of strains of Zika virus found pre-epidemic and those of the epidemic strains has revealed differences between the 1947 pre-epidemic prototype strain and the current epidemic strains. Since mutations in other flaviviruses have been reported to be associated with changes in replication efficiency, antigenic epitopes, host tropism and virulence, more research is needed to determine the biological significance of these Zika mutations (34).

VIROLOGY AND MOLECULAR BIOLOGY

Zika virus is a mosquito-borne flavivirus related to yellow fever virus, dengue virus and West Nile virus (35). It is a single-stranded positive RNA virus with a 10,794 nucleotide genome that is most closely related to the Spondweni virus (36) and is transmitted by many *Aedes spp.* mosquitoes, including *Ae. africanus*, *Ae. aegypti, Ae. hensilli* and *Ae. luteocephalus*, (6). The virus was identified in rhesus monkeys during sylvatic surveillance for yellow fever in the Zika Forest in Uganda in 1947 (3). Zika is an arbovirus, arthropod-borne virus, and a member of the

family *Flaviviridae* and the genus *Flavivirus* with an enveloped, icosahedral virion of 40-50 nm in diameter containing the nonsegmented, single-stranded, positive-sense RNA genome (37).

Like other flaviviruses, the genome is about 11 Kb in length and expresses seven nonstructural proteins and three structural proteins that are encoded as a single polyprotein in a unique long open reading frame containing all of the structural protein genes at the 5′ portion of the genome and the nonstructural (NS) protein genes at the 3′ portion. The genome organization of flaviviruses, concerning the protein expression order is:

5′-C-prM-E-NS1-NS2a-NS2b-NS3-NS4a-NS4b-NS5-3′

(38, 39). The capsid protein (C) is 13 kDa in size, highly basic and complexes with the viral RNA in the nucleocapsid while the outer membrane of the virion is a lipid bilayer containing the viral membrane protein (M) and envelope protein (E). The M protein is expressed as a larger glycosylated precursor protein (prM) while the E protein may or may not be glycosylated and this is a determinant of neuroinvasion, acting to increase both axonal and trans-epithelial transportation (40). The genomic RNA of flaviviruses lacks a poly-A tail at the 3' end (41) and has an $m^7gpppAmpN_2$ at the 5' end (42). Several regions within the genome of flaviviruses have a highly conserved structure including a 90-120 nucleotide stretch near the 3' end, which is thought to form a stable hairpin loop (43). Mutational analysis of this region in Dengue virus revealed that it has an essential role in viral replication (44).

Flavivirus particles bind to the surface of target cells by interactions between viral surface glycoproteins and cellular cell surface receptors. Virions undergo receptor-mediated endocytosis and are internalized into clathrin-coated pits (45). Uncoating of the virus envelope releases the viral RNA into the cytoplasm and also activates the host cell innate response followed by complex interplay between virus and host where virus co-opts the host cytoplasmic membranes for replication of its genome and the host attempts to control infection with several responses including interferon release, the unfolded protein/endoplasmic reticulum response,

autophagy and apoptosis (46). Translation of viral proteins from the viral RNA occurs from the long open reading frame to produce a large polyprotein that is cleaved co- and posttranslationally into the individual viral proteins and leads to replication of the viral genome.

The viral RNA, structural and non-structural proteins and some host proteins are involved in the assembly of the viral replication complex in vesicle packages in the cytoplasm of infected cells (38). Replication initiates with the synthesis of a negative-strand RNA, which then serves as a template for the synthesis of copies of the positive-strand genomic RNA in an asymmetric fashion such that there is 10- to 100-fold excess of positive strands over negative strands (47). Replication requires the activities of several of the viral nonstructural (NS) proteins. Zika NS3 protein has a serine protease domain at the N-terminus and a helicase domain at the C-terminus. The activity of the NS3 protease activity requires NS2B as a cofactor and cleaves the Zika viral polyprotein at several positions between the NS proteins as shown in Figure 3. The helicase domain of NS3 has a number of activities including RNA helicase, RNA-stimulated nucleoside triphosphate hydrolase and 5′-RNA triphosphatase. The helicase activity is required for unwinding the double-stranded RNA intermediate formed during synthesis of the genome. The 5′-RNA triphosphatase activity is required for formation of the 5′-terminal RNA cap. NS5 contains a C-terminal RNA-dependent RNA polymerase (RdRp) activity that is involved in viral genome replication and carries out both (−) and (+) strand RNA synthesis (48). Virus particles assemble by budding into the endoplasmic reticulum and nascent virus particles traverse the host secretory pathway, where virion maturation occurs followed by release from the cell (38). Zika virus can be cultured in suckling mice and also grows well in Vero cells (49). In infections in vivo, flaviviruses can target a variety of cell types including dendritic cells, macrophages, endothelial cells and neuronal cells (30, 50-51).

RELATED VIRUSES: SIMILARITIES AND DIFFERENCES

Zika virus is related to other human flaviviruses that cause significant pathology including yellow fever, dengue, tick-borne encephalitis, Saint Louis encephalitis, Japanese encephalitis and West Nile viruses and is most closely related to Spondweni virus (36). Sequence alignments between Zika viral proteins and the sequences of homologous structures from other flaviviruses can be used to map amino acid changes seen between Zika virus isolates in a structural context. For example, Faria et al (52) examined seven Zika viral genomes from Brazil, including four self-limited cases, a blood donor, a fatal adult case, and a neonate with microcephaly. Phylogenetic analysis indicated a single introduction of Zika into the Americas in late 2013 and mapping of mutations onto the structural models revealed the context of mutations found in the present outbreak. None of the mutations were predicted to have major effects on the biochemical properties of the proteins. Of note, no common mutations were found that are shared between the genomes of the three Zika virus isolates that have so far been sequenced from microcephaly cases (52).

While Zika virus shares significant sequence similarity with the other human flaviviruses, it shows significant differences in pathology. Yellow fever is the prototypical viral hemorrhagic fever and is an acute disease characterized by symptoms including fever, chills, nausea, muscle pains and headaches that may progress to liver damage begins causing yellow skin, bleeding and kidney problems. From the 1600s to the early 1900s, yellow fever caused numerous epidemics throughout the world with case fatality rates that could exceed 20% (53). Yellow fever virus was the first human virus to be isolated (54-57). Live attenuated vaccines of these strains were developed in the 1930s, which together with vector control measures has resulted in the virtual eradication of yellow fever in North America and Europe. However, there are no approved antiviral therapies for yellow fever at the present time and yellow fever remains

endemic in many parts of Africa and South America where it circulates with other flaviviruses including dengue virus (57, 58). Moreover, a decline in vaccination rates and the discontinuation of vector control have led to the resurgence of periodic epidemics of yellow fever in Africa, e.g., during 2011-2012, epidemics of yellow fever occurred in Sudan and Uganda, with additional cases reported from Cameroon, Chad and Cote d'Ivoire (60).

The dengue virus is an *Aedes* mosquito-borne Flavivirus that causes dengue fever, which is an emerging threat with a four-fold increase in incidence over the last 20 years and major public health challenge (61). Mainly present in South and South-East Asia, it has recently exploded in other parts of Asia, Latin America, and the Caribbean. Dengue presents as a variety of clinical phenotypes ranging from mild to a severe disease with capillary leak, coagulopathy, and organ impairment, which may be part immune mediated. Treatment relies on supportive measures and no approved licensed therapeutics are presently available (61).

Tick-borne encephalitis is a viral infectious disease involving the central nervous system that has been increasing in number of reported cases in most countries (62). It is most common in Central and Eastern Europe, and Northern Asia. The tick-borne encephalitis virus infects many hosts including ruminants, birds, rodents, carnivores, horses and, like Lyme disease, is one of the many tick-borne diseases that can infect humans. The virus can infect the brain causing encephalitis, the meninges causing meningitis or both causing meningoencephalitis and has a mortality of 1% to 2% (63). Saint Louis encephalitis virus is borne by the *Mansonia pseudotitillans* mosquito and the disease mainly affects the United States. Japanese encephalitis virus is mosquito-borne and is closely related to West Nile and St. Louis encephalitis viruses. It is transmitted in an enzootic cycle involving birds, particularly wading ardeids, and typically, kills 20-30% of patients and about half of the survivors have severe neuropsychiatric sequelae (64). West Nile virus is a mosquito-borne zoonotic flavivirus found in temperate and tropical regions of the world and was first identified in East Africa in 1937 but is

now one of the most widely distributed arboviruses worldwide (65). As of January 12, 2016, a total of 2,060 cases of West Nile virus disease in people were reported to CDC and 1,360 (66%) of these were classified as being neuroinvasive, e.g., meningitis or encephalitis and 700 (34%) were classified as being non-neuroinvasive (http://www.cdc.gov/westnile/statsMaps/index.html, accessed March 18, 2016). A review of the long-term sequelae of West Nile virus-related illness concluded that muscle weakness, memory loss, and difficulties with everyday activities were the most common sequelae but some population groups were at greater risk of severe neurological disease or death, i.e., older men with underlying illnesses such as cardiovascular disease or cancer (66). Finally, Spondweni virus, which is the most closely related flavivirus to Zika virus phylogenetically, is transmitted by mosquitoes and is responsible for Spondweni fever. This is characterized by symptoms that include fever, nausea, headaches, malaise and nosebleeds and is found in sub-Saharan Africa and Papua New Guinea (67).

Zika-related viruses pose technical difficulties for both the sensitivity and specificity of diagnostic assays for Zika virus (8). Hemagglutinin inhibition and plaque reduction neutralization assays are “gold standards” for anti-Flavivirus serological differentiation but require highly specialized laboratories and are expensive, so ELISA is often used but has the problem of cross-reaction with other flaviviruses (8). However, the Euroimmun anti-ZIKV IgG and IgM ELISA tests have demonstrated high specificity (68) Clinical features of Zika fever may be diagnostic but care must be exercised in ascribing a clinical diagnosis in areas where more than one pathogen is common. Real-time PCR can be used for molecular detection of ZIKV (8). It is particularly challenging to diagnose patients in the acute phase of Zika infection, which appears to be short, because serological testing is complicated by cross-reactivity, vaccination status and the scarce availability of specific ZIKV tests (69).

In addition to the flaviviruses Zika and tick-borne encephalitis virus, there are two other viruses that are emerging as important arboviruses in North America that are not flaviviruses

(70). Jamestown Canyon virus is an orthobunyavirus and was first identified in Jamestown Canyon, Colorado in 1961. It is transmitted by various mosquito species and infections, which may present as a nonspecific febrile illness or meningencephalitis, have been reported throughout the US (71). Chikungunya virus is an alphavirus in the Togavirus family, which was first identified in Tanzania and is also transmitted by mosquitoes. From 2013 on, Chikunguya has spread throughout tropical and subtropical areas of North and South America and the virus causes a febrile illness characterized by polyarthralgia (72).

NEUROLOGICAL SEQUELAE OF ZIKA VIRUS: MICROCEPHALY/GUILLAIN-BARRÉ SYNDROME

The public health significance of Zika virus lies in the expanding body of evidence linking it to microcephaly in neonates and Guillain-Barré syndrome in adults. In September 2015, researchers reported a substantial increase in the number of cases of neonatal microcephaly among women giving birth in northeastern Brazil (73, 74) and subsequently an increase was also reported in southeast Brazil (75). Zika virus has been isolated from the amniotic fluid of women who were pregnant with infants with confirmed microcephaly (73, 75, 76) and from the brain of a fetus with abnormalities of the central nervous system (77). Perhaps the strongest evidence to date linking Zika to microcephaly comes from a study by Brasil et al (31) of the Fundação Oswaldo Cruz who followed patients in Rio de Janeiro with regard to clinical manifestations of acute Zika virus infection in mothers and the consequences in fetuses. Pregnant women were enrolled in whom a rash indicative of possible Zika infection had developed within the preceding 5 days and specimens of blood and urine were taken and tested for the Zika virus by RT-PCR assays. The women were followed prospectively and clinical and ultrasonographic data collected. Eight-eight women were examined, and of these 72 (82%) tested positive for the Zika virus in blood, urine or both. The timing of acute viral infection fell in

a range from 5-38 weeks of gestation. Forty-two Zika-positive women (58%) and all Zika-negative women were examined using fetal ultrasonography. Twelve of the 42 Zika-positive women (29%) and none of the 16 Zika virus-negative women had fetal abnormalities that were detectable by Doppler ultrasonography. Adverse findings included 2 fetal deaths occurring at 36 and 38 weeks of gestation, 5 fetuses showed in utero growth restriction occurring with or without microcephaly, 7 fetuses had ventricular calcifications or other lesions of the central nervous system and 7 fetuses had an abnormal amniotic fluid volume or cerebral or umbilical artery flow. Cordeiro et al (78) collected blood and cerebrospinal fluid (CSF) samples from 31 neonates with microcephaly in the state of Pernambuco, Brazil. Zika-specific IgM was detected in 30 (97%) of 31 CSF samples and in 28 (90%) of 31 serum samples. Guillemette-Artur et al (79) performed prenatal brain MRI of fetuses with Zika virus infection and observed severe cerebral damage with indirect findings that suggested the germinal matrix is the principal target for Zika virus with lesions resembling to severe forms of congenital cytomegalovirus and lymphocytic choriomeningitis virus infections. In another study, Cavalheiro et al (80) observed decreased brain parenchymal volume associated with lissencephaly, ventriculomegaly secondary to lack of brain tissue and calcifications while de Fatima Vasco Aragao et al (81) reported severe cerebral damage with brain calcifications in the junction between cortical and subcortical white matter and malformations of cortical development, Brasil et al (31) concluded that Zika virus infection during pregnancy is associated with several kinds of serious outcomes, including fetal death, fetal growth restriction, placental insufficiency and injury to the fetal central nervous system. In another study, Cauchemez et al (82) reported a lower incidence of microcephaly of about 1% and suggested that the biggest risk is in the first trimester based on the outbreak in French Polynesia, 2013–15. In April 2016, the Centers for Disease Control and Prevention concluded that a causal relationship exists between prenatal Zika virus infection and microcephaly and other serious brain anomalies (83).

Zika virus infection has also been linked to the development of Guillain-Barré syndrome in adults. Guillain-Barré syndrome is a rapid-onset muscle weakness caused by the immune system damaging the peripheral nervous system (84). Coincident with the largest Zika virus outbreak ever described at that time, 42 patients presented at a hospital in French Polynesia with Guillain-Barré syndrome between November, 2013 and February, 2014, compared to reports of five, ten, three, and three, in 2009, 2010, 2011, and 2012, respectively (85). In a case-control study by Cao-Lormeau (32), patients with Guillain-Barré syndrome diagnosed at the Centre Hospitalier de Polynésie Française (Papeete, Tahiti, French Polynesia) during the outbreak period were compared to controls who were age-matched, sex-matched, and residence-matched patients who presented at the hospital with a non-febrile illness. In this study, virological investigations included RT-PCR for Zika virus and both microsphere immunofluorescent and seroneutralisation assays for Zika and dengue viruses. Forty-two patients were diagnosed with Guillain-Barré syndrome during the study period and 41 (98%) patients with Guillain-Barré syndrome had anti-Zika virus IgM or IgG, and all (100%) had neutralising antibodies against Zika virus compared with 54 (56%) of 98 in control group 1 ($p<0{\cdot}0001$). 39 (93%) patients with Guillain-Barré syndrome had Zika virus IgM and 37 (88%) had experienced a transient illness in a median of 6 days (IQR 4–10) before the onset of neurological symptoms, suggesting recent Zika virus infection. Patients with Guillain-Barré syndrome had electrophysiological findings compatible with acute motor axonal neuropathy and showed a rapid evolution of disease. Twelve (29%) patients required respiratory assistance. No patients died. This study by Cao-Lormeau (32) was the first study to provide evidence for Zika virus infection causing Guillain-Barré syndrome. Fontes et al (86) reported magnetic resonance imaging findings in Guillain-Barré syndrome caused by Zika virus infection. The features described were attributed to demyelination, ischemia, inflammation and breakdown of the blood-brain barrier, as occurs in autoimmune polyneuropathy. Recently, Paploski et al (87) investigated temporal correlations and time lags between outbreaks of acute exanthematous

illness (AEI) attributed to Zika virus, Guillain-Barré syndrome and microcephaly occurred in 2015 in Salvador, Brazil. Number of Guillain-Barré syndrome cases peaked after a lag of 5-9 weeks from the AEI peak while the number of cases of microcephaly peaked after a lag of 30-33 weeks from the AEI peak. This corresponds to a time of potential infection during the first trimester and these findings support association of Guillain-Barré syndrome and microcephaly with Zika virus infection (87). As well as Guillain-Barré syndrome, Zika virus has also been detected in cerebrospinal fluid from patients with encephalopathy (88) and meningoencephalitis (89).

The suspected link between Zika virus infection and microcephaly and Guillain-Barré syndrome is an urgent global health concern and has prompted new laboratory research into the neurotropic and neuropathic potential of the Zika virus. It has been known since the 1950s that Zika virus will grow in mouse brain when injected intracranially (3, 9). Bell et al (90) infected newborn mice with Zika virus injected intracranially and observed necrosis in hippocampal neurons, inflammation and active replication of virus. Of particular note were the remarkably prominent enlarged astrocytes with extended processes and containing cytoplasmic virus factories throughout the cortex of the infected mouse brains. Interestingly, astrocyte pathology is also observed in post-mortem analysis of neonatal brain from Zika virus-associated microcephaly where diffuse astrogliosis was present with focal astrocytic outburst into the subarachnoid space (77). It was also reported that Zika virus can cause disease and mortality in mice lacking the interferon (IFN) alpha receptor, which might a potential model system to test antivirals and vaccines (91). Recently, Lazear et al (92) described a mouse model for Zika virus infection in which mice lack interferon α/β signaling and develop neurological disease succumbing to infection with high viral loads in the CNS, spinal cord and testes, consistent with severe Zika disease in humans. Similarly, Dowall et al (93) the effect of Zika virus infection in type-I interferon receptor deficient mice using subcutaneous challenge in the lower leg to mimic

a mosquito bite and severe symptoms with striking histological changes and widespread Zika viral RNA detection in the blood, brain, spleen, liver and ovaries of these animals.

Recently, Tang et al (94) reported that Zika virus directly infects human cortical neural progenitor cells with a high efficiency resulting in stunted cell growth and transcriptional dysregulation. Zika virus was found to efficiently infect neural progenitor cells derived from induced pluripotent stem cells and release infectious progeny Zika virus. Zika virus infection increased cell death, dysregulated cell-cycle progression and attenuated growth (94). Analysis of global gene expression revealed transcriptional dysregulation especially of genes related to of cell-cycle pathways (94). These data identify neural progenitor cells as a direct Zika virus target and importantly establish cultures of these cells to be a tractable experimental model system to investigate the impact and mechanism of Zika virus on human brain development. Cultured neural progenitor cells also constitute a potential platform to screen therapeutic compounds against Zika virus. Another recently developed alternative is to use human neurospheres and brain organoids, which support Zika virus infection and show reduced viability and growth resembling microcephaly (95, 96).

CONCLUSIONS AND FUTURE DIRECTIONS

Zika virus has emerged of in the Americas as an epidemic that could spread rapidly with important public health consequences. Accumulating recent evidence from different sources implicates Zika in neonatal microcephaly in neonates and Guillain-Barré syndrome in adults. Zika virus is an arbovirus and a Flavivirus, spread by mosquitoes but also by blood transfusions and sexual intercourse. Virologically, Zika is an enveloped virus with a nonsegmented, positive-sense RNA genome, which replicates in the cytoplasm. The virus was originally discovered in 1947 in a monkey in the Zika forest of Uganda and for many years remained obscure until it

spread to cause outbreaks in Micronesia in 2007 and French Polynesia in 2013-2014 and thence to Brazil where autochthonous transmission was first reported in March 2015.

The rapid advance of the virus and the reported high rates of microcephaly and Guillain-Barré syndrome associated with Zika infection in Polynesia and Brazil have raised concerns that it represents an evolving neuropathic and teratogenic public health threat. The Pan American Health Organization predicts that Zika virus will spread to eventually reach all areas where *Aedes* mosquitoes are endemic (6). There are no licensed vaccines, therapeutic or preventive drugs available for Zika virus and hence the development and deployment of countermeasures are urgently needed.

Zika is spreading rapidly in the Americas and it is important to keep up with this outbreak by surveillance and outbreak tracking, threat analysis, case reporting and vector and reservoir animal sampling (17). Since the *Aedes* mosquito is the main vector of transmission, mosquito control and avoidance is of primary importance including avoiding mosquito bites (long-sleeved clothes, repellent, screens on windows), elimination or protection of containers of standing water such as large tanks used to store household water or used tires, and intensifying measures to combat and eradicate or reduce *Aedes* (97).

Better diagnostic assays for Zika are needed owing to the nonspecific clinical presentation of the disease and serological cross-reactivity of Zika, which may allow it to be easily missed or misdiagnosed as dengue fever (98). Another important objective is the development of a vaccine for Zika. A number of approaches have been applied to successfully produce efficacious vaccines to flaviviruses. Clinically approved vaccines are available for four flaviviruses: Yellow fever virus vaccine, which is a live attenuated virus used since 1937; Japanese encephalitis virus for which both inactivated-virus and live attenuated virus vaccines are available; an inactivated tick-borne encephalitis vaccine; and a chimeric virus of yellow fever virus with the dengue structural membrane and envelope genes, which was recently approved

for clinical use for dengue virus (99). These approaches could be adopted for the Zika virus. In addition, subunit vaccines representing Zika virus proteins, DNA vaccines expressing Zika viral proteins and other viral vectors expressing Zika antigens could be explored. Compared to live attenuated virus vaccines, subunit vaccines generally have a better safety and shorter development time, while a live attenuated virus vaccine might be expected to induce more robust cellular and humoral immune responses and better protection.

No clinically approved therapy is currently available for the treatment of Zika or indeed any other flavivirus infection (100). Over the past decade, significant effort has been made towards dengue drug discovery. Due to the similarity between Zika virus and dengue virus, it is possible that knowledge from dengue drug discovery could be applied to Zika virus. Several approaches are possible, e.g., high-throughput screening using virus replication assays or viral enzyme assays, structure-based in silico docking and rational design strategies and repurposing hepatitis C virus inhibitors for Zika. The development of antivirals should focus on distinctive features of Zika molecular biology that can be exploited. For example, Zika NS3 protein has a protease activity that is necessary for the viral life cycle and this may be a viable target for small molecule antiviral inhibitors. In this regard, the inhibitors of the NS3/4A protease of Hepatitis C, telaprevir and boceprevir, revolutionize the management of hepatitis C genotype 1 patients (101). NS3 also has a 5′-RNA triphosphatase activity required for 5′-RNA cap formation and NS5 contains a C-terminal RNA-dependent RNA polymerase (RdRp) activity as described above and these are also potential targets for the development of small molecule antiviral inhibitors (100, 102).

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AUTHOR CONTRIBUTIONS

Author(s) who contributed to the concept and design of this review: KK, HW, KT, DB

Author(s) who collected references and analyzed them: KK, MKW, HW

Author(s) who wrote the bulk of the text and prepared the figures: KK, MKW, KT, DB

CONFLICT OF INTEREST

Dr. Khalili is a scientific advisor and holds equity in Excision Biotherapeutics, a biotech start-up who has licensed the viral gene editing technology from Temple University for commercial development and clinical trials. In addition, Dr. Khalili has a patent Compositions for eradicating flavivirus infections in subjects that is pending.

Dr. White has nothing to disclose

Dr. Beckham has nothing to disclose

Dr. Tyler has nothing to disclose

Dr. Wollebo has nothing to disclose

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FIGURE LEGENDS

Figure 1. *Spread of Zika virus around the world.* The spread of Zika virus around different parts of the world is shown (103). Countries are grouped by geographical location and a date of the first reported Zika infection is given. The first group is in Africa where the virus originated with infections with dates for each country ranging from 1947-1981. Next, virus spread to the Far East, with dates between 1966-2012 and thence to the Pacific Islands starting with an outbreak on Yap Island in 2007. More recently, Zika has spread to Central and South America between 2014-2016. Miscellaneous cases are grouped in North America first reported in 2007 and European countries first reported in 1972: these may be spread by air travel, laboratory infections and other events.

Figure 2. *Transmission of Zika virus.* Zika virus was originally transmitted in a sylvatic cycle between monkeys and *Aedes* mosquitoes in Africa. Zika was then spread by reciprocal infection of man and mosquitoes as shown, which is the major mode of transmission today. Zika virus can also be spread by sexual transmission from men to women via semen and can also be spread by blood transfusions.

Figure 3. *Life cycle of Zika virus.* Zika virus has a single-stranded positive RNA genome, which is about 11 Kb in length and is complexed with the viral capsid protein (C) within the nucleocapsid while the outer membrane of the virion is a lipid bilayer containing the viral membrane protein (M), and envelope protein (E), as shown on the left. Virions attach to the surface of a host cell by interactions between viral surface glycoproteins and cell surface receptors and subsequently enter the cell by receptor-mediated endocytosis and are internalized into clathrin-coated pits ①. Acidification of the endosomal vesicle triggers conformational changes in the virion, fusion of the viral and cell membranes, particle disassembly and the genome is released into the cytoplasm ②. The positive-sense genomic

RNA is translated into a single polyprotein ③ that is processed co-translationally and post-translationally by cellular and viral proteases ④. This cleavage makes a total of 10 proteins, 3 structural proteins: C, prM (glycosylated precursor to M), and E; and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The structural proteins are located at the N-terminus of the polyprotein and the non-structural proteins are found at the C-terminus (shown inset). Genome replication occurs on intracellular membranes known as vesicle packages which facilitate the assembly of the viral replication complex containing viral RNA and cellular and viral proteins, e.g., NS3 and NS5 ⑤. Replication initiates with negative-strand RNA synthesis and this then serves as a template for the synthesis of multiple copies of the positive-strand genomic RNA ⑥. Virus assembly occurs on the surface of the endoplasmic reticulum ⑦ by budding and nascent virus particles travel along the host secretory pathway through the trans-Golgi network ⑧ where virion maturation occurs followed by release from the cell by exocytosis ⑨.

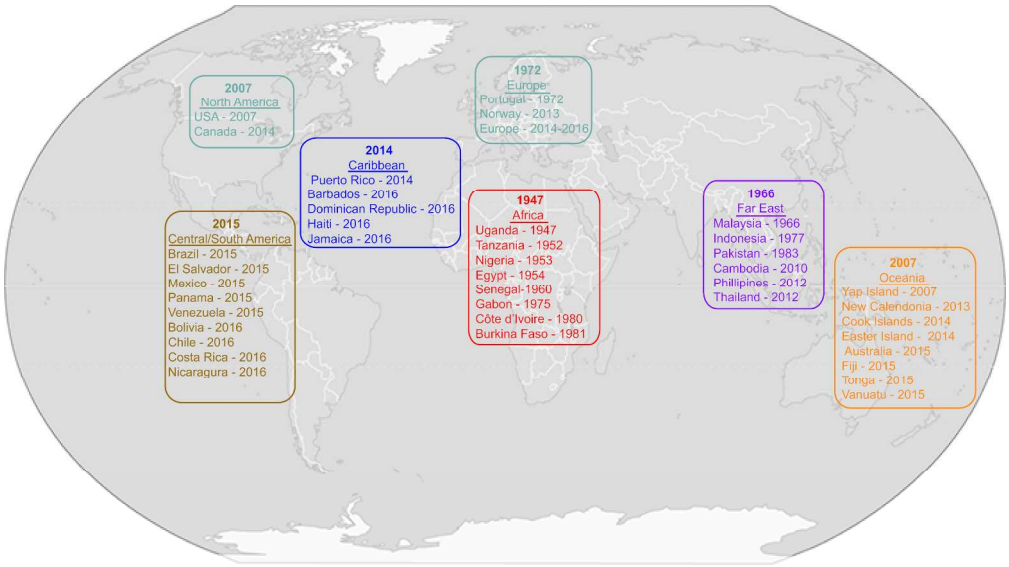

Figure 1

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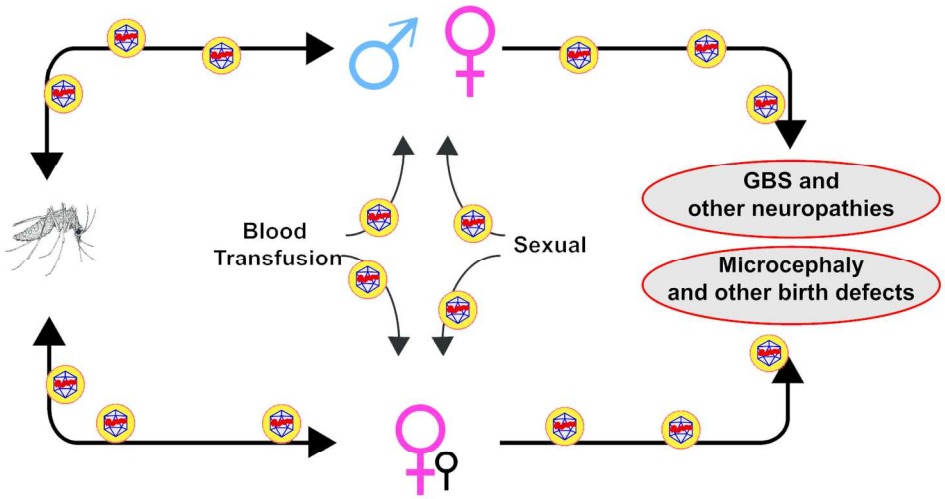

Figure 2

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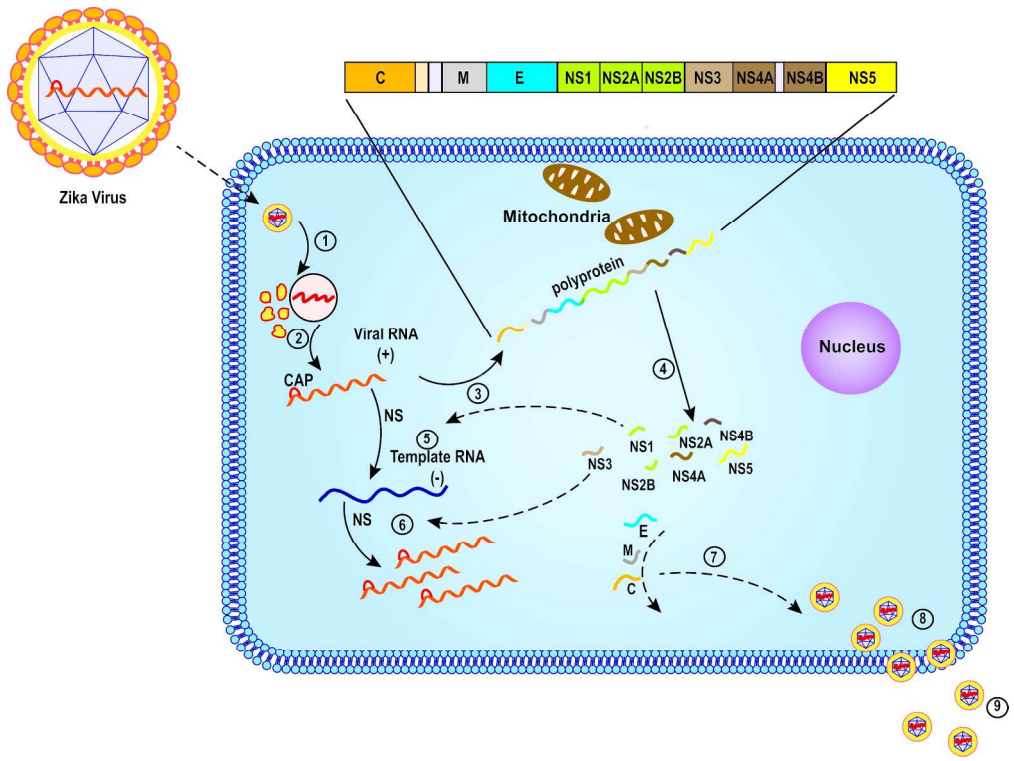

Figure 3

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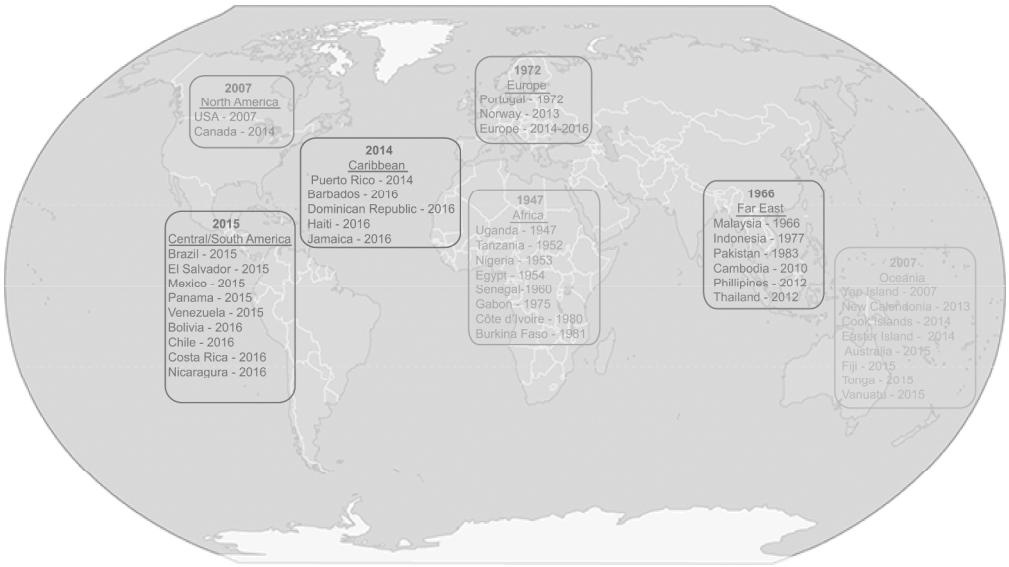

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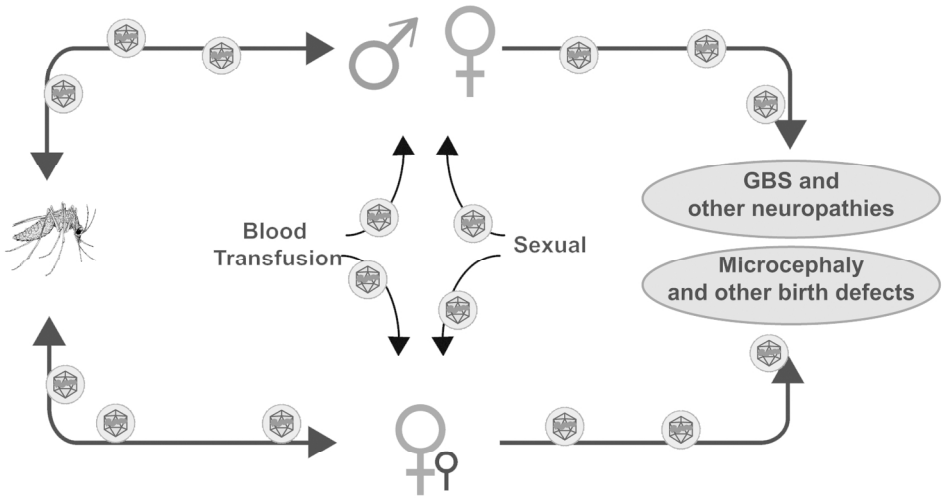

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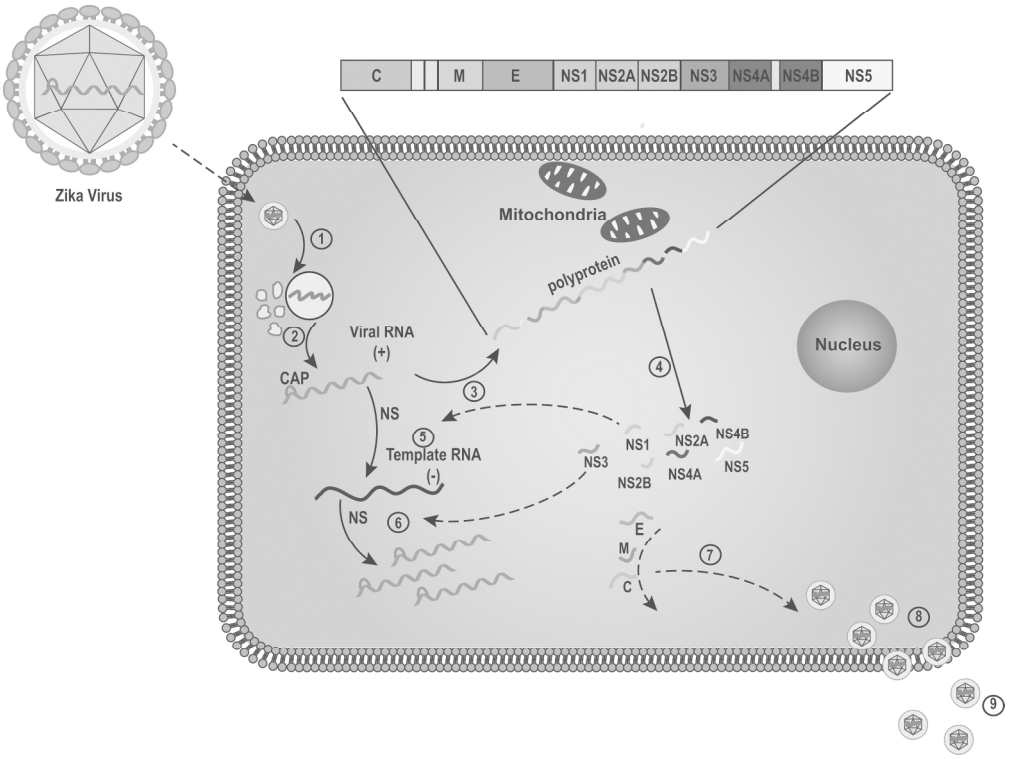

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