Zika Virus

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Introduction

The scientific data collected, and the lessons learned following Zika virus (ZIKV) introduction into the Western Hemisphere, first in Brazil and then spreading very rapidly in the Americas, provided a huge amount of unexpected information and have been crucial in informing a better understanding on several aspects related to the transmission of the virus, its clinical manifestations, neurological complications and particularly the risk of microcephaly and other neurological malformations in fetuses born to mothers infected with ZIKV during pregnancy.[1] In this context the development of potential therapeutic interventions and preventive strategies, including vaccines, are of paramount importance.

This chapter summarizes the current knowledge on the ZIKV infection in humans and provides a perspective on the issues and challenges related to the development of a safe and efficacious vaccine against ZIKV.

Etiology

ZIKV is an emerging arthropod-borne, single-stranded RNA virus, member of the *Spondweni* serocomplex (genus *Flavivirus*, family *Flaviviridae*) and related to other mosquito-borne viruses that cause yellow fever, dengue, West Nile disease, St. Louis encephalitis, and Japanese encephalitis. Two major lineages, African and Asian, have been identified through phylogenetic analyses.[2]

Epidemiology

After initial identification in 1947 from a sentinel rhesus monkey (i.e., monkeys held captive with the purpose of identifying yellow fever activity) in a forest in Uganda,[3] ZIKV was associated only with few sporadic cases in humans in Africa and Asia over the next 60 years.[4] However, since 2007, when the first outbreak of ZIKV outside Africa and Asia was reported in the Federated States of Micronesia (Yap), it has been identified in subsequent outbreaks in French Polynesia and other Pacific islands.[4] In May 2015, the Ministry of Health in Brazil confirmed autochthonous transmission of ZIKV associated with an outbreak of "dengue-like syndrome" cases in Northeastern Brazil. The ZIKV outbreak continued to evolve, spreading geographically very rapidly.[5]

Since then, in the Americas, 49 countries and territories reported local transmission, 24 countries and territories have reported microcephaly and/or central nervous system (CNS) malformation cases potentially associated with ZIKV infection and 15 countries and territories have reported Guillain-Barré syndrome (GBS) potentially associated with ZIKV infection. As of 14 April 2018, Uruguay is the only country in the Americas with evidence of established competent vector, but no known documented past or current transmission of ZIKV (Figure 1).[5]

Figure 1. Areas With Risk of Zika in Latin America and the Caribbean

Brazil was the most affected country in the Americas, reporting 216,207 probable cases in 2016, 17,594 cases in 2017 and only 1,174 cases by week 10 in 2018.[6] Since 2015, the Ministry of Health in Brazil confirmed 3,071 cases of microcephaly and/or CNS malformation associated with ZIKV infection, with the majority (60%) occurring in the Northeast, followed by the Southeast (24%) and Central-West (7%) region.[7]

In 2018, a substantial decline in cases of Zika virus infection has been reported in most affected countries in the Americas, probably because of "herd immunity" of the population that became immune after being infected in previous years, reducing the number of susceptible, naive subjects, and thus, limiting the transmission of the virus in the community.

Transmission and Incubation Period

ZIKV is transmitted to humans primarily by *Aedes aegypti* mosquitoes (and less commonly by other *Aedes* species, like *Aedes polynesiensis, Aedes hensilli, Aedes africanus*, and *Aedes albopictus),* the same vector that can transmit dengue, chikungunya, and yellow fever viruses.[8] ZIKV has already been isolated from other non-*Aedes* mosquitoes. However, it is important to emphasize that the isolation of ZIKV from a mosquito is not evidence that transmission is feasible by this mosquito. Human and nonhuman primates are the main reservoirs of the virus, with humans acting as the primary host.

Additionally, non-vector modes of transmission have been identified, including: perinatal, *in utero*, sexual, blood transfusion, and laboratory exposure.[8] Although ZIKV RNA has been detected in breast milk, transmission through breastfeeding has not yet been demonstrated, reinforcing the current recommendations that mothers with ZIKV infection should continue to breastfeed their infants.[8]

Intrauterine transmission of ZIKV was confirmed in Brazil by the detection of virus genome, by reverse transcriptase-polymerase chain reaction (RT-PCR), in amniotic fluid samples of women with symptoms of ZIKV infection during the first trimester of pregnancy whose fetuses have been diagnosed with microcephaly, in placental tissues from early miscarriages, and also in the blood and brain tissue of infants with congenital neurologic anomalies, including microcephaly.[8,9]

Reports of cases with ZIKV possibly transmitted by blood transfusion are being investigated in Brazil. Interestingly, during the French Polynesian outbreak, 2.8% of blood donors tested positive for ZIKV by RT-PCR, with all of them asymptomatic at the moment of blood donation.[10]

The incubation period in humans prior to onset of symptoms is thought to be between 3 to 14 days after the bite of an infected mosquito. Infected people, both symptomatic and asymptomatic, can transmit ZIKV to mosquitoes throughout the viremic period that usually ranges from a few days to one week.[8]

Diagnosis

Clinical diagnosis is limited by the non-specific signs and symptoms of ZIKV infection which are similar to other arboviral infections (e.g., chikungunya and dengue) common in endemic areas. Abnormal laboratory findings, including mild thrombocytopenia, leukopenia, and elevations in acute-phase markers of inflammation, serum lactate dehydrogenase, or liver transaminases have been observed in symptomatic patients.[8]

ZIKV specific diagnosis in nonpregnant symptomatic individuals is primarily based on the detection of ZIKV RNA by RT-PCR performed on serum and/or urine specimens collected <14 days after onset of symptoms.[8] ZIKV-specific immunoglobulin M (IgM) and neutralizing antibodies can be detected by enzyme-linked immunosorbent (ELISA) assays in serum specimens collected by the end of the first week of illness and up to 12 weeks post onset of symptoms. As the immune response develops, IgM titres rise in peripheral blood and the level of viral RNA generally declines. Serum IgM antibody testing should be performed if the RT-PCR result is negative or when ≥14 days have passed since illness onset. IgG antibodies develop within days after IgM and can be detected for months to years. However, false-positive results due to cross-reaction with related flaviviruses (e.g., dengue and yellow fever viruses) are commonly observed. During the outbreak of ZIKV infection in the Yap state of Micronesia, the presence of low levels of cross-reactive IgM was demonstrated in all patients with secondary flavivirus infection.[11]

Positive results in primary *flavivirus* infections should be confirmed with a four-fold increase in the titer of neutralizing antibodies to ZIKV with plaque reduction neutralization test (PRNT). In endemic areas, where a great proportion of the population may have been previously infected with other flaviviruses or vaccinated against a related *flavivirus* (i.e., secondary *flavivirus* infection), neutralizing antibodies might still yield cross-reactive results in these individuals.[8]

Clinical Manifestations

It is estimated that approximately 80% of persons infected with ZIKV are asymptomatic. When symptomatic, the infection is considered to be associated with a mild, self-limited disease, lasting few days and characterized by low fever, pruritic rash, edema of extremities, conjunctivitis, headache, and myalgia. Less common manifestations include gastrointestinal symptoms, retro-orbital pain and lymphadenopathy.[1,8,12] Clinical manifestations in infants and children with acquired infection are similar to the findings observed in adults with ZIKV infection. The presence of arthralgia in infants and young children is difficult to detect and can manifest as irritability, limited moving or refusing to move an extremity. During outbreaks of ZIKV, cases were reported in all age groups with higher incidence rates in adults compared to children.[1,8,12] The World Health Organization (WHO) developed interim case definitions with the purpose of providing global standardization for classification and reporting of Zika virus cases: patient with rash and/or fever with at least one of the following signs and symptoms: arthralgia; arthritis or conjunctivitis (non-purulent conjunctival hyperemia). A confirmed case is a suspected case with laboratory confirmation of recent Zika virus infection: presence of Zika virus RNA or antigen in serum or other samples – e.g., saliva, tissues, urine, whole blood; or IgM antibody against ZIKV positive and $PRNT_{90}$ for ZIKV with titer ≥20 and ZIKV $PRNT_{90}$ titer ratio ≥ 4 compared to other flaviviruses; and exclusion of other flaviviruses.[13]

Neurological and Autoimmune Complications

Neurological complications, such as Guillain-Barré syndrome (GBS), meningitis, acute disseminated encephalomyelitis and myelitis have been reported following ZIKV infection, mainly in adults. French Polynesia, Brazil, Colombia, Venezuela and several other countries from Central America and the Caribbean reported an increase in the rates of GBS during the recent ZIKV outbreak.[14] The reported incidence of GBS was higher among males and consistently increased with age, with males over 60 years having the highest rates, findings that are in line with previous reports on the epidemiology of GBS. This epidemiological situation reinforces the hypothesis of a link between ZIKV infection and the occurrence of GBS, highlighting that ZIKV should now be included in the list of potential infectious pathogens that can trigger the development of GBS.[14-16]

Congenital Syndrome

The most striking finding during the ZIKV outbreak in Brazil, however, was the strong cumulative evidence that provided the basis to establish a relationship between ZIKV infection during pregnancy and congenital abnormalities. A wide range of congenital malformations was described, characterized predominantly by CNS alterations and associated symptoms: microcephaly (with significant cranium-facial disproportion), spasticity, convulsions, marked irritability, and brainstem dysfunction including feeding difficulties. The results of neuroimaging studies suggest that intrauterine ZIKV infection is associated with severe brain anomalies, such as cerebral calcifications, hydrocephalus, lissencephaly with agenesis of the corpus callosum, pachygyria, cerebellar dysplasia, and white-matter abnormalities.[1,8,16-18] The severity of the neurological alterations appears to be related to the period of gestation when the women are infected, i.e., the earlier the infection during pregnancy, the more severe the neurologic outcomes to the fetus. Arthrogryposis, microphthalmia, funduscopic alterations in the macular region, as well as optic nerve abnormalities were also described in infants with suspected congenital ZIKV syndrome.[1,8,16-18]

The true burden of the congenital disease associated with ZIKV is probably underestimated assuming that it is likely that a significant proportion of the affected newborns have subclinical manifestations at birth, without microcephaly, preventing these infants from being diagnosed by the current ascertainment methods, at least until later stages of childhood/adolescence when cognitive, developmental, and/or visual limitations can be detected.

The unique characteristics of the ZIKV outbreak in Brazil, where the population was completely susceptible (naïve) to the virus, affecting highly populated urban areas with high density of *Aedes aegypti*, and the established surveillance reporting system, are possible reasons to explain why the role of ZIKV as a potential cause of congenital disease has only been recognized after circulation in Brazil. Furthermore, if ZIKV infection is associated with life-long immunity, it is expected that in endemic places in Africa and Asia, where the virus is circulating for years, a proportion of the women in childbearing age is likely to be previously infected, limiting the number of susceptible women.

It is also possible that the more severe outcomes of ZIKV infection observed in Brazil and other countries may be related to mutation in virulence characteristics of the ZIKV circulating strain or even immune interaction between consecutive *Flavivirus* infections. Interestingly, after the reports from Brazil[17-19] raised a causal relationship between ZIKV infection in pregnancy and microcephaly and other congenital malformations, a retrospective study performed in French Polynesia found an association between ZIKV and microcephaly.[20]

Treatment

We currently do not have any available specific antiviral treatment for patients with ZIKV disease. Only supportive care is indicated, including rest, fluids and symptomatic treatment (acetaminophen to relieve fever and antihistamines to treat pruritus). Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) should be avoided to reduce the risk of hemorrhagic complications. One recent study showed that chloroquine exhibited antiviral activity against ZIKV in VERO cells, human brain microvascular endothelial and neural stem cells. In this study, the authors were able to demonstrate that chloroquine reduced, *in vitro,* the number of ZIKV-infected cells, virus production and cell death promoted by ZIKV infection without cytotoxic effects.[21]

Vaccines

Preliminary studies identified a single ZIKV serotype and suggested that immune response after ZIKV infection induces broadly neutralizing antibodies against multiple strains (South American, Asian, and early African ZIKV strains proved to be similarly sensitive to neutralization by ZIKV convalescent human serum), paving the way for the development of an effective vaccine.[22] Similarly to other flaviviruses, neutralizing antibodies appears to play a critical role in protection against infection.

Several ZIKV vaccine candidates using different technologies, based on plasmid DNA, modified mRNA, purified inactivated virus, recombinant live attenuated vaccines and viral vectored vaccines, showed promising results in mouse and non-human primate studies and are now advancing to clinical trials in humans.[23,24] Taking in account the need to protect women at childbearing age, vaccination strategies should be prioritized to target individuals of both sexes of reproductive age (to prevent sexual transmission), nine years of age or older. The recent results with the live-attenuated chimeric dengue vaccine, showing increased risk of severe dengue among dengue-naïve subjects vaccinated compared to the unvaccinated control group,[25] highlights the importance of long-term safety surveillance, to evaluate the duration of the protective immune responses of the current candidate ZIKV vaccines.[26] It will be also critical, when planning future vaccine trials, to have a better knowledge on the immune responses after subsequent infections with these flaviviruses. It is still unknown whether a previous infection with other flaviviruses, like dengue, or the presence of antibodies against yellow fever in populations where vaccination is routinely recommended, will increase the risk of severe disease, neurological complications like Guillain-Barré syndrome, or congenital disease in pregnant women.

Inovio Pharmaceuticals is developing a synthetic DNA plasmid vaccine against the ZIKV virus, which encodes for the premembrane-membrane and envelope regions of the virus, currently in Phase I trials, assessing the safety, tolerability, and immunogenicity of the candidate vaccine in adults 18-65 years of age.[27]

The National Institute of Allergy and Infectious Diseases (NIAID) has a candidate ZIKV DNA vaccine that is currently in Phase II trials. Previous studies demonstrated that the vaccine was safe and elicited a neutralizing antibody response against Zika virus.[28] The current Phase II/IIb trial, started in 2016, is divided in two parts: a safety and immunogenicity study and an efficacy study, enrolling at least 2,490 healthy participants in areas of confirmed or potential active mosquito-transmitted Zika infection, including: the United States and Puerto Rico, Brazil, Costa Rica, Mexico, Panama, and Peru. The U.S. National Institutes of Health (NIH) has also a partnership with the Butantan Institute in Brazil to develop a live attenuated vaccine against Zika, currently in early phase trials.

Although DNA vaccines, as well as subunit vaccines are safe and potentially easily manufactured, they have limited immunogenicity compared to other vaccine types such as live-attenuated vaccines.[29]

Prevention and control currently relies on personal strategies to avoid mosquito bites and community-level programs to reduce vector densities in endemic areas. Personal measures include using insect repellent containing DEET, picaridin, oil of lemon eucalyptus, or IR3535. Permethrin-treated clothing and gear can repel mosquitoes.[8]

Future Research and Challenges

Despite the advances that were recently achieved on the understanding of several aspects related to ZIKV infection, it is important to acknowledge that we still have many research gaps and unanswered questions about ZIKV. Crucial areas of future research include the need for a better understanding of the full spectrum of fetal outcomes resulting from fetal ZIKV infection; evaluation of potential risk factors for vertical transmission (viral load, co-infections, timing, virulence of the circulating strain); development of more specific diagnostic tests; the role, if any, of non-*Aedes* mosquitoes in the transmission, as well as other potential modes of non-vector transmission; the pathogenesis of neurological and auto-immune complications following ZIKV infections.

Finally, novel methods of vector control, and the development of specific antivirals drugs and vaccines will be of paramount importance to control the disease and decrease the burden of ZIKV infection.

Conflict of Interest Statement

The authors have no funding or conflicts of interests to disclose.

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