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Viewpoint

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Zika Virus: Diagnosis, Therapeutics, and Vaccine

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ABSTRACT: The current explosive epidemic of Zika virus in South and Central America, as well as the Caribbean, poses a global public health emergency. Here we comment on the challenges on development of better diagnosis and potential therapeutics and vaccine for Zika virus.

The current explosive epidemic of Zika virus (ZIKV) in South and Central America, as well as the Caribbean, poses a global public health emergency. ZIKV was first identified in the Zika forest in Uganda in 1947.[1] Since its discovery, ZIKV has been detected predominantly in forests circulating between primates and mosquitoes and has caused sporadic, self-limited human infections in Asia and Africa. Genetically, ZIKV exists as two lineages: African and Asian. In 2007, the Asian lineage of ZIKV enkindled an epidemic on Yap Island, Micronesia; it then spread to French Polynesia and other regions of the South Pacific and caused large epidemics in 2013 and 2014; subsequently, ZIKV arrived in the Americas in 2015, causing an estimated 1.5 million clinical cases in Brazil alone in 2015.[2]

ZIKV belongs to the *Flavivirus* genus within the *Flaviviradae* family. Many flaviviruses are significant human pathogens that can cause life-threatening disease, including yellow fever virus (YFV), dengue virus (DENV), Japanese encephalitis virus (JEV), West Nile virus (WNV), and tick-borne encephalitis virus (TBEV). Flaviviruses have a positive-strand RNA genome of about 11 000 nucleotides. The viral genomic RNA encodes three structural proteins (capsid, premembrane/membrane, and envelope) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Similar to YFV, DENV, and chikungunya virus (an emerging alphavirus), ZIKV is transmitted by *Aedes* spp. mosquitoes. Recent studies suggest that ZIKV may also be sexually transmitted.[3]

■ DISEASE

Up to 80% of ZIKV infections may be asymptomatic.[4] Individuals with compromised immunity could be more susceptible to developing severe disease if infected with ZIKV. Disease symptoms and signs of ZIKV infection include fever, lethargy, eye pain, conjunctivitis, rash, muscle aches, and arthralgia. Many of these symptoms are similar to those produced by DENV or chikungunya virus infections, which share geographic ranges and vectors and often cocirculate with ZIKV. These symptomatic similarities have confounded clinical diagnosis to differentiate these viral infections. For severe cases, infections caused by ZIKV and, to a lesser extent, chikungunya virus are associated with Guillain-Barré syndrome (an autoimmune disease in which the immune system attacks peripheral nerves and damages their myelin insulation, leading to a rapid onset of muscle weakness and paralysis). Of greater concern is the link to microcephaly reported in Brazil and Colombia. An approximate 20-fold increase in incidence in microencephaly was noted from 2014 to 2015, which coincided both spatially and temporally with the arrival of ZIKV.[4] Microcephaly is a congenital neurodevelopmental disorder with unusual small head size in newborn babies. The linkage between ZIKV infection and microcephaly is supported by mounting evidence from clinical and epidemiological studies, although a definitive causal relationship remains to be strengthened through additional case control and cohort epidemiologic studies and has yet to be experimentally demonstrated.[4] The disease course of ZIKV infection remains to be well characterized. Animal models are urgently needed to study viral pathogenesis and to facilitate the development of vaccine and therapeutics.

■ DIAGNOSIS

There are two types of diagnosis for ZIKV. The first type involves the detection of the virus and/or viral components. RT-PCR, immunoassay, and virus isolation have been developed to detect ZIKV RNA, viral proteins (particularly NS1), and live virus, respectively.[5] These methods are used for both mosquito surveillance and for the diagnosis of patient specimens. Among them, RT-PCR is the most popular assay because of its sensitivity and specificity, while virus isolation remains the gold standard but requires more laboratory infrastructure for cell culture. Because up to 80% of infected individuals are asymptomatic, blood donors, blood bank samples, and organs for transplantation may have to be tested for ZIKV infection or contamination.

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The second type of diagnosis is based on the detection of antibodies elicited by ZIKV infection.[5] The major limitation of the current serological assays is the cross-reactivity of antibodies derived from different flavivirus infections. For example, 10 flaviviruses are found in Brazil, including ZIKV. In endemic regions, the specific type of flavivirus infection can often be determined only by virus neutralization assay, which requires skilled laboratory staff, reference reagents, laboratory containment, and a long turnaround time (at least a week), and in many cases, even neutralization can result in cross-reaction in individuals who have had multiple flavivirus infections. Therefore, a flavivirus type-specific serological assay is critically needed not only for patient diagnosis but also for seroprevalence surveys. The current serology assay is based on the detection of antibodies against flaviviral structural proteins. Antibodies against viral nonstructural proteins have not been systematically explored for serological assays. It was previously reported that a WNV NS5 protein-based immunoassay could (i) discriminate between WNV and DENV infections and (ii) differentiate between flavivirus vaccination (YFV and JEV) and natural WNV infection.[6] It remains to be determined if the NS5-based immunoassay could be applied to the diagnosis of ZIKV and other flaviviruses.

THERAPEUTICS

There is currently no clinically approved therapy for any flavivirus. Two strategies could be pursued for ZIKV antiviral development. The first is to repurpose existing clinical compounds, which have been previously developed for other disease indications, for potential ZIKV treatment. This approach has been attempted for other emerging viruses, such as Ebola.[7] Some of the repurposed compounds did show antiviral activity in cell culture (e.g., Ebola virus). A similar screening campaign should be performed for ZIKV. Once inhibitors have been identified, it will be critical to examine if the compound concentration required for anti-ZIKV efficacy could be reached in patients. The human pharmacokinetic data are usually available for these clinically approved drugs.

The second strategy is to develop bona fide inhibitors of ZIKV infection and replication. Both virus infection and viral enzyme assays could be used to identify inhibitors from compound libraries. These assays have been extensively employed for DENV drug discovery. Surprisingly, only the DENV infection-based screening campaign has led to inhibitors with in vivo efficacy,[8] whereas the viral enzyme-based screening effort has not yielded any compounds with in vivo antiviral activity.[9] In terms of chemical space, nucleoside/nucleotide inhibitors hold great potential for DENV antiviral development.[10] The experience gained from DENV drug discovery can be applied to ZIKV. However, caution should be taken when extrapolating DENV antiviral experience to ZIKV because these viruses are genetically distinct and the biology of the two viruses could be very different (potential differences remain to be characterized). Besides small molecule inhibitors, therapeutic antibodies could also been developed for treatment of ZIKV infection. However, antibodies for therapy should be carefully selected to minimize potential side effect of disease enhancement. One conceivable challenge for the clinical development of ZIKV antiviral is the highest-risk patient population of pregnant women. This challenge will require extra caution and time during the clinical development of ZIKV therapy.

VACCINES

Currently, there are licensed vaccines for four flavivirus diseases: YFV (live attenuated), TBEV (inactivated), JEV (both inactivated and live attenuated), and DENV (recombinant chimeric live attenuated).[11] Thus, there is extensive experience in developing flavivirus vaccines. It is conceivable that both inactivated and live attenuated virus vaccines could be applied to ZIKV. Other attractive vaccine approaches include subunit, DNA, and viral vector vaccine platforms, all of which contain or express ZIKV structural proteins. Each of the vaccine approaches has its advantages and disadvantages. Given the current urgency, all of the above approaches should be concurrently pursued to expedite the development of an effective vaccine. For instance, a subunit vaccine could be safer and could require a shorter development time prior to clinical testing when compared to a live-attenuated virus vaccine; however, the subunit vaccine will likely require multiple doses to induce protective immunity. In contrast, a live-attenuated virus vaccine may require only one dose and elicit a more rapid and robust immune response, leading to better, long-lived protection. The development timeline often depends on the nature of a vaccine candidate. Given sufficient resources, it is reasonable to estimate that candidate ZIKV vaccines could be available for clinical evaluation in 2017.

The impact of the current ZIKV epidemic goes beyond public health. ZIKV is affecting global security and the global economy. A coordinated effort is required by government, academia, industry, and funding agencies to efficiently study the virus, develop counter measurements, and halt the spread of this potentially devastating virus.

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Notes

The authors declare no competing financial interest.

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