Potential Mechanisms for Enhanced Zika Epidemic and Disease

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ABSTRACT: A number of mechanisms have driven the explosive epidemics and severe diseases of Zika virus since 2007. Here, we comment on how herd immunity, heterologous flavivirus preimmunity, and viral mutations could enhance the epidemic potential and disease severity of Zika virus in humans.

Zika virus (ZIKV) belongs to the flavivirus genus of the Flaviviridae family. Many flaviviruses are transmitted by mosquitoes or ticks, including ZIKV, yellow fever virus (YFV), dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV), and tick-borne encephalitis virus (TBEV). These flaviviruses cause frequent epidemics and outbreaks, posing significant public health threat. The genome of flavivirus is a single-stranded RNA of positive polarity of about 11,000 nucleotides. The single open-reading-frame of the flaviviral genome encodes ten viral proteins. Three structural proteins (capsid, premembrane/membrane, and envelope), together with the viral genome, form virion particles. Seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) are responsible for viral replication, modulation of virion assembly, and evasion of innate immune response.

**EPIDEMIC MECHANISMS**

What has triggered the explosive epidemic and severe diseases of ZIKV infection in the past few years? Like ZIKV, many arboviruses frequently emerge and re-emerge to cause global outbreaks and epidemics. Understanding the mechanisms that drive the epidemic potential of these viruses is critical for countermeasure development. So far, at least three non-exclusive mechanisms have been supported by epidemiological and experimental evidence.

**Herd Immunity.** Herd immunity is a form of indirect protection from infectious disease that occurs when a large percentage of a population has become immune, thereby providing protection for individuals who are not immune. In the case of recent ZIKV epidemics, stochastic introduction of the virus into a naïve population in the Pacific and Americas that lacks herd immunity could lead to high susceptibility to ZIKV infection and efficient mosquito transmission. A large number of human infections allow sufficient statistical power to detect rare clinical syndromes such as CZS and GBS.

In support of this mechanism, a correlation between the recent increase in seroprevalence and the decrease in ZIKV cases (https://www.cdc.gov/zika/reporting/2017-case-counts.html) was observed in the Americas in 2017. However, the minimal seroprevalence rate required for herd protection against infection and transmission remains to be determined.

**Heterologous Flavivirus Preimmunity.** Previous DENV infection may exacerbate ZIKV disease. Since ZIKV and DENV share approximately 43% amino acid identity, antibodies produced from DENV infection could cross-react with ZIKV. At subneutralizing concentrations, such cross-reactive DENV antibodies could enhance ZIKV infection in...
Fc-receptor-bearing cells and in mice, leading to higher levels of virus production.\textsuperscript{13–15} However, it is noteworthy that all mouse experiments showing antibody enhancement were performed through passive transfer of DENV antibodies into animals followed by ZIKV infection. So far, no enhancement has been observed when mice are sequentially infected with DENV followed by ZIKV. The later evidence is critically needed to support the hypothesis of “DENV enhancement of ZIKV infection” because the immune response to whole viral infection is completely different from the simple antibody transfer. In support of this idea, DENV immunity elicited through whole viral infection (not antibody transfer) did not cause severer ZIKV disease in rhesus macaques.\textsuperscript{16} Furthermore, neither CZS nor GBS has been frequently observed in Southeast Asia where both DENV and ZIKV cocirculate. Nevertheless, epidemiology studies are urgently needed to determine whether preimmunity could cross enhance infections of different flaviviruses in humans. The answer to this question will guide ZIKV vaccine design and development.

**Viral Mutations.** Virus–host interactions determine an infection outcome. RNA viruses replicate at a high mutation rate due to the lack of proofreading capability of viral polymerase. This is particularly important for arboviruses to complete their transmission cycle between vertebrate and mosquito hosts whose cellular environment and immunity are drastically different. Acquisition of genetic mutations often propels the emergence and re-emergence of arboviruses. Indeed, three studies have provided in vivo evidence to support the hypothesis that ZIKV has acquired mutations that increase its ability to infect mosquitoes and humans through three distinct mechanisms (Figure 1).

\begin{figure}[h]
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\caption{ZIKV mutations enhance mosquito transmission, microcephaly, and evasion of innate immunity. Since 2012, the Asian lineage of ZIKV has acquired mutations that may increase the epidemic potential and disease severity through three distinct mechanisms: increased mosquito infection/transmission, enhanced neurovirulence/microcephaly, and augmented evasion of type-I interferon (IFN) production. See text for details.}
\end{figure}

(i) ***Mosquito Infection and Transmission.*** The first hypothesis is that ZIKV has accumulated mutation(s) that enhance mosquito infection, leading to more efficient transmission and therefore increased number of human infections.\textsuperscript{17} Flavivirus NS1 protein facilitates mosquitoes to acquire viral infection.\textsuperscript{18} Compared with pre-epidemic strains from Asian lineage, the epidemic strains have acquired an Ala-to-Val mutation at amino acid position 188 (A188V) of NS1 protein since 2012. This mutation enables ZIKV to secrete NS1 protein to a higher concentration in the blood of infected AG6 mice [deficient in type-I and -II interferon (IFN) receptors]. The higher level of NS1 antigenaemia promotes ZIKV infectivity and prevalence in mosquitoes and subsequently viral transmission to people (Figure 1). Since the study used Rockefeller laboratory A. aegypti mosquito strain, it is important to recapitulate the observation using field mosquitoes. In addition, the molecular mechanism of how the mutation modulates NS1 secretion into extracellular milieu remains to be defined.

(ii) ***Microcephaly in Mouse Fetus.*** The second hypothesis is that ZIKV has adapted to produce higher viremia in humans, leading to increased cross-placental infection and CZS.\textsuperscript{19,20} In support of this hypothesis, a Ser-to-Asn mutation at amino acid position 17 (S17N) of prM protein had occurred in the epidemic strains before the 2013 outbreak in French Polynesia; this mutation has been stably maintained during subsequent spread to the Americas. The prM S17N mutation significantly increases ZIKV infectivity of neural progenitor cells, neurovirulence in neonatal mice, and microcephaly when virus was directly injected into the fetal brain in pregnant mice (Figure 1).\textsuperscript{21} It should be noted that microcephaly was not achieved through infection of pregnant mice but rather through direct injection of virus into the lateral ventricle of fetal brain. Since ZIKV could not efficiently replicate in immune-competent ICR mice used in the study, a better mouse pregnancy model or nonhuman primate pregnancy model is needed to strengthen the findings.\textsuperscript{21} In addition, the molecular details of how the prM S17N mutation enhances the infectivity of neural progenitor cells and neurovirulence remain to be determined.

(iii) ***Evasion of Innate Immunity.*** The third hypothesis is that ZIKV has acquired mutation(s) that augment its ability to evade host immune response; the dampened immune response allows robust viral infections and consequently severe disease. Interestingly, the NS1 A188V mutation that enhances mosquito transmission was found to confer NS1 to inhibit type-I interferon induction (Figure 1).\textsuperscript{22} This mutation enables NS1 binding to TBK1 and reduces TBK1 phosphorylation, a component from the RIG-I pathway essential for type-I IFN production. Compared with the pre-epidemic virus, ZIKV containing the NS1 A188V mutation replicated to higher levels in both cell culture and mice.

Notably, African strains of ZIKV also contain the NS1 188V residue.\textsuperscript{27} Conceivably, an NS1 V188A substitution occurred when ZIKV spread from Africa to Asia decades ago. In 2013 (before the virus had arrived in the Americas), the NS1 V188A substitution reverted back to its original African sequence of 188V. If the NS1 188A residue has a selective disadvantage for transmission, the A188V change could have occurred through a founder effect when a single infected traveler introduced ZIKV to Asia.

It remains to be determined whether other mutations accumulated in the recent ZIKV strains could also contribute to the epidemic potential and disease outcome. Besides the mechanisms of mosquito transmission, microcephaly in mouse fetus, and evasion of innate immunity, environmental factors as well as genetic predisposition in certain ethnic populations may also account for disease susceptibility and outcome; however, the latter two hypotheses require more epidemiological and genetic evidence. Finally, it should be noted that all results summarized here were obtained from ZIKV strains from the Asian lineage. Unexpectedly, African strains were shown to have higher fitness and virulence in mosquitoes and mice than the Asian strains.\textsuperscript{23–27} Why do the African strains not cause
notable epidemic and CZS disease? Is the unnoticeable disease in Africa due to lack of ZIKV diagnostics and surveillance? More field and clinical studies are needed to understand the epidemiology and vector transmission of ZIKV in Africa.

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Notes
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