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MINIREVIEW

Zika Virus: New Clinical Syndromes and Its Emergence in the Western Hemisphere

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Zika virus (ZIKV) had remained a relatively obscure flavivirus until a recent series of outbreaks accompanied by unexpectedly severe clinical complications brought this virus into the spotlight as causing an infection of global public health concern. In this review, we discuss the history and epidemiology of ZIKV infection, recent outbreaks in Oceania and the emergence of ZIKV in the Western Hemisphere, newly ascribed complications of ZIKV infection, including Guillain-Barré syndrome and microcephaly, potential interactions between ZIKV and dengue virus, and the prospects for the development of antiviral agents and vaccines.

Zika virus (ZIKV) is a member of the *Flavivirus* genus of the *Flaviviridae* family, which includes other globally relevant human pathogens such as dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), Japanese encephalitis virus (JEV), and tick-borne encephalitis virus (TBEV) (1, 2). ZIKV is an enveloped virus with an approximately 10.7-kb positive-sense RNA genome. Similarly to other flaviviruses, the ZIKV genome encodes a single polyprotein that is cleaved posttranslationally by host and viral proteases into three structural proteins (capsid [C], premembrane [prM], and envelope [E]) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (3, 4). C binds to the viral RNA to form a nucleocapsid, prM prevents premature fusion with host membranes, and E mediates cellular attachment, entry, and fusion (5). The viral nonstructural proteins regulate viral transcription and replication and also attenuate host antiviral responses (1, 6, 7). ZIKV is a member of the Spondweni virus group within the mosquito-borne clade of flaviviruses (Fig. 1) and is closely related to the four serotypes of DENV, with approximately 43% amino acid identity across the viral polyprotein as well as in the ectodomain of E.

ZIKV was first isolated in 1947 from a febrile sentinel rhesus monkey in the Zika forest, a research station of the East African Virus Research Institute (now the Uganda Virus Research Institute) in Entebbe, Uganda (8, 9). The virus was isolated subsequently from *Aedes africanus* mosquitoes in the same forest (9–11), and multiple monkey species in the Zika forest were found to be seropositive for ZIKV (11). Small mammals in the Zika forest (including squirrels, tree rats, giant pouched rats, and civets) did not show serological evidence of ZIKV infection, consistent with a model where primates (both humans and monkeys) are the primary vertebrate hosts for ZIKV (10). Multiple species of *Aedes* mosquitoes contribute to enzootic maintenance of ZIKV, but likely only a subset of these transmit the virus to humans (12, 13). There is evidence of high rates of ZIKV seroprevalence in Africa and Asia (9, 14–17), although the specificity of such assays is uncertain, given the significant serological cross-reactivity between ZIKV and other flaviviruses (see below). In the decades following its discovery, ZIKV was isolated from human patients sporadically during outbreaks in Africa and Southeast Asia (15, 18) but remained obscure due to the fairly benign nature of the infection (which generally manifests as a self-limiting febrile illness; see below).

ZIKV came to global attention in 2007, when it caused an explosive outbreak in Micronesia (18–21). It is estimated that approximately 75% of the population of the island of Yap became infected during a 4-month period (19). In the ensuing years, ZIKV spread throughout Oceania (22–25) and then was detected in Brazil in early 2015 (26, 27). Although the precise means by which ZIKV was introduced to the Western Hemisphere is unknown, the presumption is that the virus came to Brazil from Polynesia via a viremic traveler or an infected mosquito (2, 26, 28, 29). The *Aedes aegypti* mosquito, which can transmit ZIKV, is abundant in Brazil, and autochthonous transmission was established. The outbreak initially was concentrated in northeastern Brazil. However, the virus rapidly spread throughout Latin America and the Caribbean, such that within 1 year most countries in the region reported local transmission (30–32). Further spread of the virus is anticipated, and imported cases already have been reported in the United States, Europe, and elsewhere in travelers returning from Latin America and the Caribbean during the current outbreak (30, 33–35). The rate at which ZIKV has spread through Latin America and the Caribbean since its introduction appears comparable to that seen with chikungunya virus (CHIKV) after its introduction to the Western Hemisphere in late 2013, suggesting that it reflects the abundance and competence of the *Aedes aegypti* mosquitoes that are used as vectors by both viruses, as well as the availability of a susceptible host population (36). ZIKV genome sequences from Polynesia and South America are highly similar (2, 26, 29) (approximately 99% nucleotide identity across the viral genome), but there are genetic differences, for example, 6 amino acid changes

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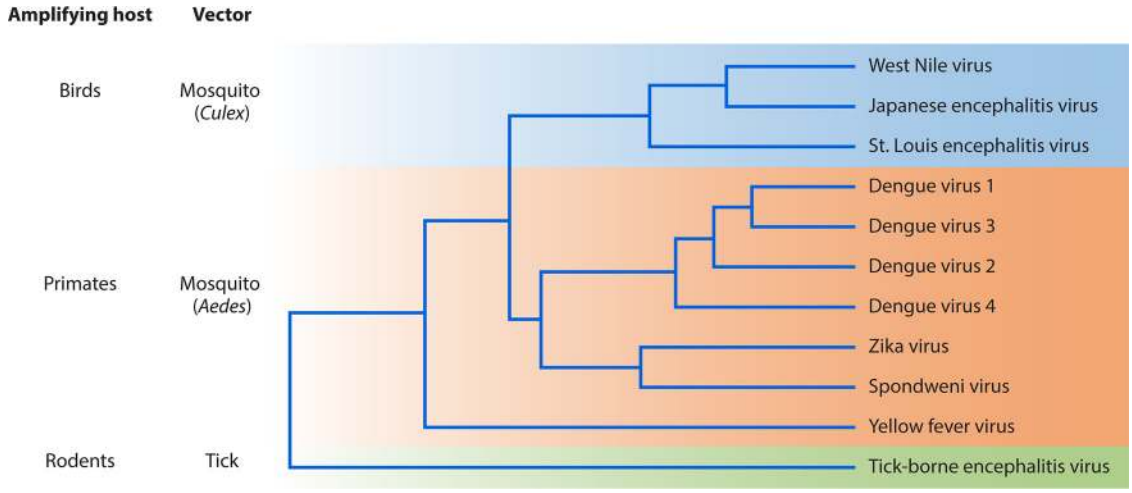

FIG 1 Schematic phylogeny illustrating the genetic relationships between selected flaviviruses that are human pathogens. The dendrogram (145) is based on the amino acid sequence of the complete polyprotein.

between the H/PF/2013 strain from French Polynesia and the SPH2015 strain from Brazil (GenBank accession numbers KJ776791.1 and KU321639.1) (37). Future studies are needed to determine whether such changes impact disease pathogenesis, tropism, or vector competence. The ability of changes in viral sequence to impact the epidemic potential of arboviruses was seen previously with CHIKV, where a small number of mutations, including a A226V change in the E1 glycoprotein, enabled the virus to use *Aedes albopictus* mosquitoes as vectors, which have an expanded geographic range compared to *Aedes aegypti*, facilitating epidemic spread into new areas (37–39).

MODES OF TRANSMISSION

Vector-borne transmission. ZIKV is a mosquito-transmitted virus (Fig. 2). ZIKV has been isolated from many species of *Aedes* mosquito, but only a subset of these (including *Ae. aegypti*, *Ae. albopictus*, *Ae. hensilii*, and *Ae. polynesiensis*) are competent vectors for transmission (9–13, 18, 21, 40–42). *Aedes aegypti* is thought to be the principal vector spreading ZIKV during the current outbreak in Latin America and the Caribbean, likely due to the urban abundance and anthropophilic nature of this mosquito (43). Monkeys are presumed to serve as reservoir hosts for ZIKV, although the primary species has not been identified (11, 18). It is unclear whether ZIKV will become endemic in New World monkeys and establish a sylvatic transmission cycle in Latin America analogous to that seen with YFV or will be maintained exclusively through urban transmission cycles with no New World sylvatic cycle, similarly to DENV (44). Humans are amplifying hosts for ZIKV, and urban cycles of transmission between humans and mosquitoes sustain and cause epidemics. Indeed, the island of Yap in Micronesia experienced an extensive ZIKV outbreak, and yet there are no nonhuman primates on this island (19). There currently is no evidence that animals other than humans and nonhuman primates serve as amplifying hosts for ZIKV, suggesting a mode of transmission similar to those of DENV, YFV, and CHIKV. While mosquito-borne transmission clearly is the main cause of ZIKV outbreaks, other modes of transmission have been reported.

Blood-borne transmission. As is the case for other blood-borne infections, a ZIKV viremic donor could potentially contaminate the blood supply (45, 46), and cases of ZIKV transmission through transfusions of donated blood, although not yet published, have been reported in Brazil. In many areas, including the United States, Canada, and Europe, the blood supply is already screened by nucleic acid amplification tests to detect WNV (47–50). The same approach, once a screening test becomes available, could be used to detect ZIKV, and plans exist in several countries to screen the blood supply for ZIKV or to defer blood donation from those who have traveled to countries where ZIKV is circulating. In the absence of an approved diagnostic assay to detect ZIKV contamination, strategies are available to inactivate infectious agents in the blood supply (46, 51).

Sexual transmission. There is evidence of sexual transmission of ZIKV (34, 52, 53), and ZIKV RNA has been detected in semen (54, 55). To date, all reported sexually transmitted cases of ZIKV infection have been from infected men to their female partners. Although some of these cases were accompanied by hematospermia, infectious ZIKV was detectable in semen even after viremia had cleared (undetectable ZIKV RNA in serum), arguing against blood-borne transmission (54). Moreover, while other sexually transmitted infections cause hematospermia (56), this has not been a common presentation of ZIKV infection, nor has it been evident in all cases of sexually transmitted ZIKV (34). Recent reports of infectious ZIKV in urine, along with the detection of ZIKV RNA in urine even after viremia has cleared (57), could be consistent with ZIKV replication in urogenital tissues. ZIKV RNA has been detected in saliva (58), and infectious ZIKV in saliva was recently reported. Due to the highly correlated nature of behaviors, sexual and salivary transmission can be difficult to distinguish. Indeed, Kaposi's sarcoma-associated herpesvirus initially was thought to be sexually transmitted, but subsequent findings indicated that the primary mode of transmission was through saliva (59). Also, pigs can develop high viral loads in the tonsils and transmit JEV through oronasal secretions, which demonstrates that this is a possible transmission route for flaviviruses (60). Although sexual transmission is unlikely to be a major cause of ZIKV outbreaks, the presence of virus in semen warrants investigation, especially given recent evidence that Ebola virus RNA can be de-

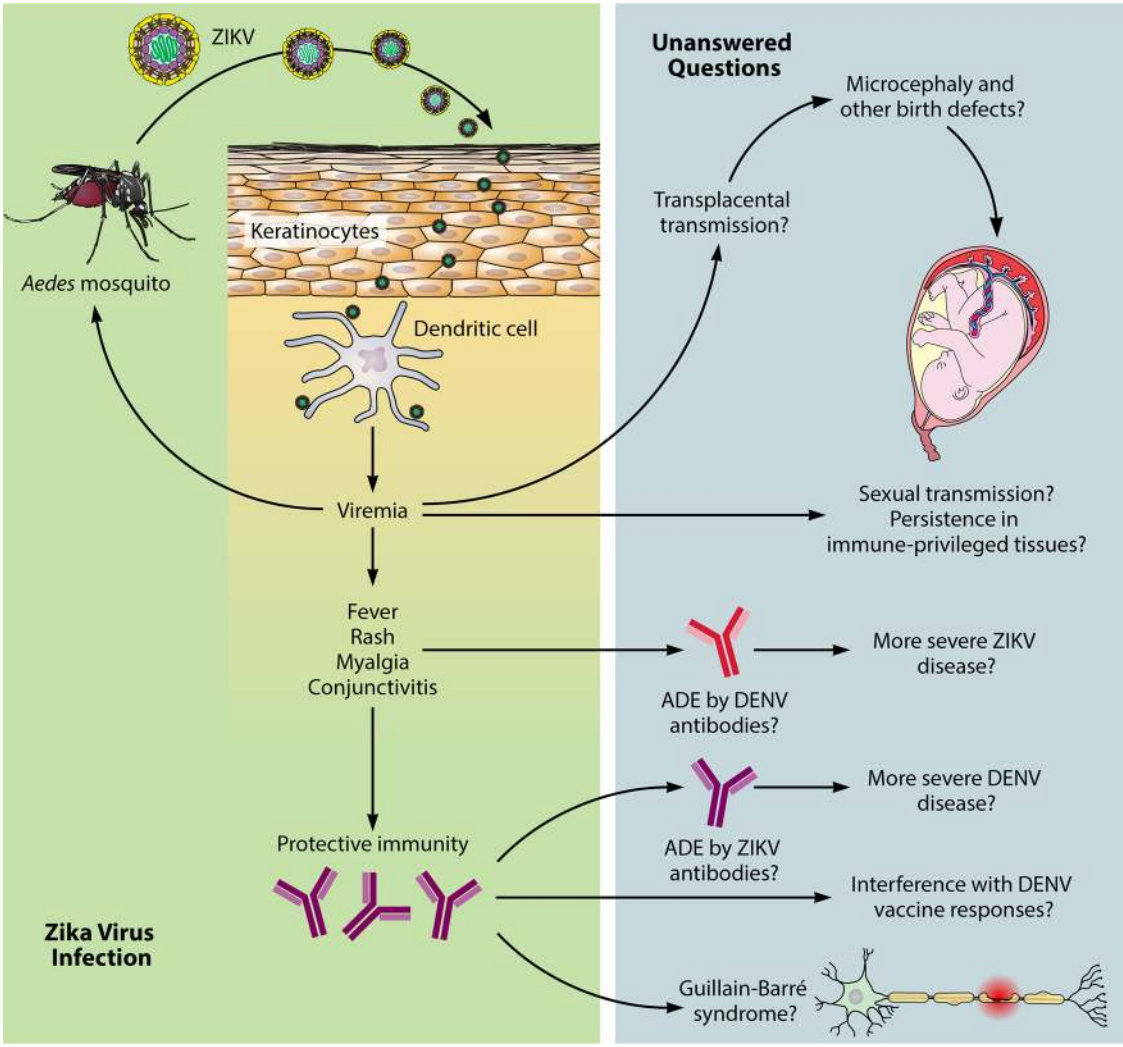

FIG 2 ZIKV pathogenesis. The typical course of ZIKV infection is illustrated (green background), with potential severe effects requiring further investigation indicated (blue background). DENV, dengue virus; ADE, antibody-dependent enhancement.

tected in the semen of survivors for months after the acute infection has cleared. Similarly, ZIKV RNA was detected in semen 62 days after the onset of febrile symptoms (55). The immune-privileged nature of the testes may allow ZIKV to persist in this tissue. Such reservoirs have the potential to initiate new transmission cycles from seemingly healthy individuals (61, 62). The growing number of imported ZIKV cases in areas of the United States and Europe where local mosquito transmission is less likely provides an opportunity to detect and determine the significance of alternative transmission mechanisms (34).

Maternal transmission. ZIKV RNA has been detected in breast milk (63). As this route of transmission has been documented for other flaviviruses (64–66), ZIKV-infected mothers may be able to pass the virus to nursing children. However, it is not known whether infectious ZIKV is present in breast milk or what its possible duration is relative to that of acute infection, and ZIKV-infected mothers are still encouraged to breastfeed their infants (67). Perinatal transmission of ZIKV was documented in French Polynesia (63), but it is unknown whether this represented transmission in breast milk, blood-borne transmission during delivery, or *in utero* transmission.

The question of *in utero* transmission has gained urgency as the emergence of ZIKV in Brazil has coincided with an alarming increase in the number of cases of microcephaly, with the northeastern states reporting >4,000 cases over approximately 4 months, a more than 20-fold increase from prior years (68–71). Microcephaly is a congenital abnormality in which the fetal brain is underdeveloped (72, 73). There is not a standard definition of microcephaly, as definitions range from a newborn head circumference of ≤32 cm to a circumference of 33 cm and from ≥2 to 3 standard deviations below the mean for gestational age (69). Many factors can cause microcephaly during pregnancy, including other viral infections (e.g., human cytomegalovirus, rubella virus, and varicella-zoster virus), exposure to toxins (e.g., drugs or alcohol), and genetic mutations. Microcephaly can be asymmetric, meaning a small head on an otherwise normally proportioned body, or symmetric, meaning that the small head is proportional to a small overall body size; the type of microcephaly can be characteristic of its etiology. Microcephaly can be diagnosed by prenatal ultrasound, but generally not until the late second trimester, and many cases are not evident until after birth. The long-term effects of microcephaly can vary widely, from virtu-

ally no defects to cognitive deficits and severe physical disability (73).

It is important to note that the majority of the microcephaly cases reported during the current outbreak have yet to be confirmed or linked directly to ZIKV; in ongoing follow-up studies, approximately one-third of reported microcephaly cases had been corroborated, and presumably some of these will be shown to be attributable to causes other than ZIKV infection (68, 70, 71). Further complicating the analysis, the case definition for microcephaly has changed over the course of the current outbreak: in December 2015, the Brazilian Ministry of Health adopted a newborn head circumference measurement of ≤32 cm as the case definition, compared to the less stringent ≤33-cm cutoff used previously (69). Clearly, better data are required to assess the potential connection between ZIKV infection and microcephaly; epidemiological studies, including case-control and prospective cohort studies, are under way and should bring clarity to this issue in time. Nonetheless, accumulating evidence strongly suggests a causal role for ZIKV in the development of microcephaly. In addition to the timing and geographic distribution of microcephaly cases relative to ZIKV infections, data supporting transplacental infection include the following: (i) detection of ZIKV RNA and sequencing of full-length viral genomes from the amniotic fluid of fetuses diagnosed with microcephaly by ultrasound in mothers who reported previous ZIKV infection but were not viremic at the time of amniocentesis; (ii) detection of ZIKV RNA and/or antigen in the tissues of three microcephalic infants who died shortly after birth; (iii) detection of ZIKV RNA in the placenta from a microcephalic fetus after miscarriage; (iv) detection of a partial sequence of a ZIKV genome and viral antigen in four fetal brain tissue samples recovered from miscarriages and neonatal death; (v) sequencing of a full-length ZIKV RNA genome and visualization of ZIKV-like particles by electron microscopy in a fetal brain from a terminated pregnancy (74–81). A recent report of anti-ZIKV IgM in the cerebral spinal fluid of 12 infants with microcephaly also supports the hypothesis of *in utero* infection with ZIKV.

Although other viruses can cross the placenta and cause microcephaly in humans and/or animals, this presentation has never previously been associated with flaviviruses (82–86). *In utero* infection with WNV has been studied, with no clear evidence of an association with microcephaly (87–89). Furthermore, there are an estimated >390 million DENV infections occurring annually (including an estimated ~25 million in Brazil [90]), so even a very low rate of DENV-induced microcephaly would have been observed. While the mechanisms by which ZIKV may cause microcephaly are unknown, the preliminary evidence and the severity of the disease have prompted the U.S. Centers for Disease Control and Prevention (CDC), Public Health Agency of Canada, Australian Department of Foreign Affairs and Trade, and Public Health England, among other agencies, to recommend that women who are pregnant or planning to become pregnant avoid travel to areas where ZIKV is circulating (in effect, nearly all of Latin America and the Caribbean, among other locations) (74, 75, 80, 91, 92). Such travel advisories have a significant economic impact on the affected countries, especially with the approach of the 2016 Olympic Games in Rio de Janeiro. Furthermore, in response to the potential for sexual transmission of ZIKV, CDC has cautioned pregnant women against unprotected sex with partners who have had potential ZIKV exposure (34, 91, 93). Remarkably, health officials in several Latin American and Caribbean countries have recommended that women postpone pregnancy in response to the ZIKV outbreak. In the United States, pregnant women have become infected while traveling to areas with active ZIKV transmission or by sexual contact with ZIKV-infected male partners. The outcomes of these ZIKV-exposed pregnancies have been variable, including early pregnancy loss, elective termination, delivery of an infant with severe microcephaly, and seemingly unaffected infants (80). Many unanswered questions remain concerning *in utero* transmission of ZIKV infection and the development of microcephaly, as further discussed below.

CLINICAL FEATURES OF ZIKA VIRUS INFECTION

Historically, ZIKV infection caused a variable clinical syndrome in humans ranging from no signs or symptoms to an influenza-like viral illness that appeared similar in the early stages to those caused by other epidemic arboviruses, including DENV and CHIKV. For ZIKV, approximately 20% of individuals who become infected progress to a clinically apparent febrile illness, although hospitalization is rare (18, 19). Signs and symptoms associated with ZIKV infection occur on average within 3 to 7 days of mosquito inoculation and include an abrupt onset of fever accompanied by headache, arthralgia, myalgia, conjunctivitis, vomiting, fatigue, and/or maculopapular rash (94) (Fig. 2). For many years, ZIKV infection was considered self-limiting with no long-term sequelae, but more severe complications have become apparent during the more recent ZIKV outbreaks in the South Pacific and Latin America, possibly because the greater number of infections has facilitated detection and reporting of rare outcomes (though other factors may also contribute to increased ZIKV pathogenesis). Although ZIKV infection has not been reported to cause the plasma leakage and hemorrhage associated with severe DENV disease, ZIKV has caused thrombocytopenia and hematospermia (52, 54, 95). There are no reported fatal cases of ZIKV in otherwise healthy people. However, ZIKV-associated mortality has been described in patients with comorbidities, including sickle cell disease (96), and congenital ZIKV infection and post-ZIKV Guillain-Barré syndrome (GBS) can be fatal.

During the 2013–2014 ZIKV outbreak in French Polynesia, neurological disorders were linked to ZIKV infection, as there was an increase in the incidence of GBS, a postinfection autoimmune neuropathy that can result in weakness, paralysis, and death (92, 97–99). A case-control study of the outbreak found that GBS patients were more likely to have evidence of past ZIKV infection than controls, with 0.24 cases of GBS per 1,000 ZIKV infections (98). Patients with post-ZIKV GBS had atypically low levels of anti-ganglioside antibodies compared to patients with GBS of other etiologies, suggesting that ZIKV may induce GBS by mechanisms different from those of other causes (98). Cases of a diffuse demyelinating disorder consistent with GBS that are temporally associated with ZIKV infection have also been reported in Brazil, El Salvador, Colombia, and Venezuela (75, 92). More studies are needed to understand the linkage between ZIKV infection and GBS, particularly the pathophysiological mechanisms at play. Possible mechanisms include (i) immunopathology due to viral antigen mimicry with a host protein; (ii) virus sequence changes resulting in enhanced tropism for the peripheral nervous system; and (iii) an association with prior or concurrent immune responses to DENV (97–100).

Most concerning is the sharp increase in the number of cases of microcephaly in newborns in the northeastern region of Brazil

that is associated with ZIKV infection of pregnant women (101). Several cases of presumed intrauterine ZIKV infection resulted in coarse cerebral calcifications in different brain regions of newborn infants or fetuses *in utero* (76). A recent study of a fetus with microcephaly recovered after elective termination at 32 weeks of gestation also revealed numerous calcifications in the cortical and subcortical regions of the frontal, parietal, and occipital lobes of the cerebral cortex (77). Hydrops fetalis and hydranencephaly were noted in a fetus with microcephaly, which was followed by fetal demise (81). The reported microcephaly cases may represent only the severe end of the spectrum, such that newborns with less-severe infection could still have long-term cognitive or functional sequelae (76). Indeed, ocular findings in infants with presumed ZIKV-associated microcephaly were described recently. Approximately 30% of children with suspected ZIKV infection *in utero* had evidence of significant retinal and optic nerve abnormalities (102).

PATHOGENESIS OF ZIKV INFECTION

Although no recent ZIKV pathogenesis studies have been performed to explain the possible microcephaly observed in Brazil, experiments in mice that were performed 40 and 60 years ago suggest that under certain circumstances ZIKV has a tropism for cells in the brain. The original ZIKV strain (MR 766) was isolated by George Dick and colleagues in 1947 from the brain of a 5- to 6-week-old Swiss mouse after it was inoculated via an intracerebral route with the serum of a febrile sentinel rhesus macaque (9). The same group showed subsequently that passaged ZIKV strains caused signs of central nervous system (CNS) disease, including motor weakness and paralysis, after intracerebral inoculation in mice of different ages (8). Mice under 7 days of age were susceptible to lethal ZIKV infection when inoculated by an intraperitoneal route, whereas adult mice were less sensitive (103). In mice, the pathological manifestations of disease were restricted to CNS tissues. Neuronal degeneration and cellular infiltration were observed in regions of the spinal cord and brain with evidence of Cowdry type A inclusion bodies (8), which also are described as occurring after neuronal infection by herpesviruses. Evidence of neuronal injury also was observed in the pathological evaluation of a human fetus infected *in utero* with ZIKV. In this case, diffuse astrogliosis and activation of microglia were present, and damage extended to the brain stem and spinal cord, with Wallerian degeneration of the descending corticospinal tracts noted (77). Beyond the CNS, no other tissue, including the kidney, lung, spleen, and liver, supported significant ZIKV infection. In comparison, other animals, including cotton rats, guinea pigs, rabbits, and rhesus monkeys, did not develop CNS disease, even after intracerebral inoculation (8). More recent studies using a ZIKV isolate from French Polynesia demonstrated infection of human keratinocytes, dermal fibroblasts, and skin biopsy specimens, consistent with the skin being the initial site of ZIKV replication after mosquito inoculation, similarly to WNV and DENV infections (104–107). Similarly to DENV, ZIKV can use DC-SIGN and the TAM receptors Axl and Tyro3 as attachment factors (104). Also similarly to other flaviviruses, ZIKV infected human dendritic cells in culture and its activity was restricted by the antiviral effects of type I and type II interferon (104).

Some ZIKV strains have one N-linked glycosylation site in their envelope (E) protein (N154), whereas others lack predicted glycosylation sites (108). This pattern contrasts with the pattern seen with DENV, which has two N-linked glycosylation sites (N67 and N154), and is similar to the patterns seen with the E proteins of more distantly related flaviviruses, including WNV and TBEV (N154) (109–111). Although N-linked glycosylation on E is associated with enhanced mosquito transmission and/or increased virulence in mammals for some flaviviruses, including WNV, TBEV, and others (112–118), it remains unknown whether differential glycosylation between ZIKV strains determines or even correlates with pathogenicity.

DIAGNOSIS OF ZIKV INFECTION

Because ZIKV causes a nonspecific influenza-like illness without pathognomonic features, it is challenging clinically to distinguish it from other viral illnesses. This is especially true because ZIKV cocirculates and shares mosquito vectors with DENV and CHIKV, which present similarly, with fever, rash, arthralgia, and myalgia (25, 119). In addition to cocirculation, recent reports have described coinfection of multiple arboviruses, including ZIKV and DENV (24).

Given the challenges in clinical diagnosis, a laboratory-based diagnosis of ZIKV is the gold standard (120). Beyond direct virus isolation, which can be difficult outside highly specialized laboratories, the most definitive current diagnostic tool is a reverse transcription-PCR (RT-PCR)-based assay that detects ZIKV RNA and can distinguish ZIKV infections from DENV, CHIKV, and other viral infections (120). Because ZIKV viremia in humans lasts for a short duration of 3 to 5 days (20, 121), serum RT-PCR assays, while highly specific, have low sensitivity rates. Urine and saliva samples may have greater utility for diagnosing ZIKV infection by RT-PCR, as viral RNA is detectable at a higher load and with a longer duration in these body fluids than in serum (57, 58). In one study in French Polynesia, 19.2% of tests were positive for ZIKV RNA in saliva but negative in blood. The use of saliva samples increased the rate of molecular detection of ZIKV and was of particular interest in groups (e.g., children and newborns) where blood was difficult to collect (58). Viral detection in urine and saliva is not unique to ZIKV, as DENV RNA has been detected in both fluids, whereas infectious WNV and WNV RNA have been detected in urine (122–124).

Serology-based diagnosis of ZIKV infection, which is critical to surveillance, epidemiologic analyses, and acute diagnoses, poses a challenge even to experienced laboratory personnel due to the extensive cross-reactivity of antibodies against related flaviviruses (e.g., YFV, DENV, and JEV) that are derived from natural infection or vaccination (19, 20, 120). As an example, results for ZIKV-infected patients can be positive in an IgM assay for DENV, particularly if ZIKV occurs as a secondary flavivirus infection. Cross-reactivity was observed more frequently with DENV than with YFV, JEV, or WNV, although further studies are needed, as small numbers of samples were tested. In comparison, in cases in which ZIKV is the first flavivirus encountered, the extent of cross-reactivity is lower (20). Anti-ZIKV IgM was detectable as early as 3 days after onset of illness, with most samples having it present by day 8. Neutralizing antibody developed as early as 5 days after illness onset, but, again, assays may still yield substantial cross-reactivity in the setting of prior flavivirus infection or vaccination. The use of paired acute-phase and convalescent-phase sera and a greater than 4-fold rise in ZIKV antibody titers specifically may increase the accuracy of serological testing.

Thus, if ZIKV epidemics occur in populations with DENV or

other flavivirus vaccines or natural immunity, extensive cross-reactivity in the IgM and neutralization assays can occur, which could lead to an incorrect diagnosis. This is particularly problematic as ZIKV epidemics spread through Latin America and the Caribbean, where DENV prevalence is high. Ideally, a serological assay that minimizes cross-reactivity of other flaviviruses is needed to increase the specificity of IgM and IgG assays. Based on published studies performed with related flaviviruses (125–127), the development of diagnostic assays with ZIKV NS1 proteins or ZIKV E proteins and subviral particles encoding mutations in the highly cross-reactive fusion loop in domain II might enhance the specificity of serological tests substantially.

UNANSWERED QUESTIONS

***In utero* transmission and teratogenic effects.** While the introduction of a pathogen into a new environment often brings epidemiological and diagnostic challenges, at the outset of the ZIKV outbreak in Brazil, there was no reason to expect a unique presentation; indeed, Zika fever is typically milder than dengue fever. The association between ZIKV and microcephaly was unexpected, as this presentation has not been associated with flaviviruses, and congenital abnormalities are not characteristic of flavivirus infection. Accumulating evidence indicates a role for maternal ZIKV infection as an explanation for the increase in microcephaly cases in Brazil, although further assessment of reported and historical cases is necessary to determine the magnitude of the increase and the attack rate (68, 70, 71). Many questions remain regarding the mechanisms by which ZIKV might cause congenital defects, including microcephaly. The simplest mechanism would be an inherent ability of ZIKV to cross the placenta, followed by direct infection of nervous tissue in the developing fetus. This idea is supported by the detection of ZIKV RNA, complete genomes, antigen, and viral particles in fetal tissues, placenta, and amniotic fluid from pregnancies with microcephaly (74, 76–78, 80, 81, 92) and by prior studies in mice that suggested a tropism for central nervous system tissues (8). If ZIKV is neurotropic and neurovirulent in the developing fetus, its effects seem unlikely to manifest only as microcephaly. While microcephaly may be the most apparent congenital abnormality from ZIKV infection, it remains possible that the virus can cause a spectrum of neurological effects, some of which may not be evident for months or years. The association between ZIKV and microcephaly also could be a consequence of its introduction into a ZIKV-naive population or, alternatively, into a population with unique patterns of flavivirus immunity, with prior immunity to DENV or other flaviviruses modulating ZIKV pathogenesis.

As the placenta generally is an effective barrier preventing microorganisms in the maternal circulation from accessing the developing fetus, it will be important to determine what mechanisms ZIKV uses to circumvent this barrier. For example, can ZIKV infect placental trophoblast cells directly, or does it employ some other method to access the fetal compartment? For other congenital infections, the risk of fetal infection varies at different stages of pregnancy (82, 83), and the most extensively described cases of ZIKV-associated microcephaly have all involved infection during the first trimester (76–78, 80). It will be important to determine the temporal risk of congenital ZIKV infection, in order to make informed recommendations to pregnant women about the risks of exposure to ZIKV (74, 91).

A growing body of evidence indicates that ZIKV can cross the placenta, infect the fetus, and damage the developing brain (74, 76–80, 92). However, demonstrating a direct causal role for congenital ZIKV infection in the development of microcephaly will require more extensive clinical and epidemiological studies, many of which are now in progress. The existing data do not demonstrate that ZIKV infection is sufficient to cause microcephaly, and other factors may potentiate the teratogenic effects of ZIKV, including coinfections, environmental factors, viral strain differences, or host genetics. It is noteworthy that to date, ZIKV-associated microcephaly has been observed only in Brazil and not in previous outbreaks or in other countries. This may reflect the large number of ZIKV infections in Brazil (>1.5 million estimated) and the timing of the outbreak, with Brazil experiencing the earliest effects. However, if microcephaly remains exclusive to women who are in Brazil or who were infected with the virus while traveling there, it will be important to consider cofactors that may impact *in utero* infection by ZIKV.

Interactions between ZIKV and DENV. One of the characteristic features of DENV pathogenesis is that whereas infection with one serotype provides durable immunity to that same serotype, antibodies to one DENV serotype can exacerbate infection with different serotypes via antibody-dependent enhancement (ADE) (128–130). ADE occurs when cross-reactive nonneutralizing antibodies bind to a heterologous DENV serotype. Antibody-opsonized but nonneutralized virus can infect myeloid cells (e.g., monocytes or macrophages) expressing Fc-gamma receptors at a higher rate, allowing enhanced infection and yield. Because of this, secondary DENV infections (or primary infections in infants with circulating maternal antibodies) can produce severe disease manifestations, including plasma leakage, hemorrhage, and circulatory collapse. ADE can be demonstrated for many flaviviruses in cell culture, but the phenomenon appears to be biologically relevant only in the context of DENV, possibly due to the degree of antigenic relatedness between different DENV serotypes or because of the unique biology of the DENV NS1 protein (131, 132). Given the relatedness between DENV and ZIKV, and the high cross-reactivity demonstrated in serological assays, ADE between DENV and ZIKV and altered disease pathogenesis warrant further evaluation. The severity of disease seen in recent outbreaks of ZIKV has been greater than that seen in earlier outbreaks. While explanations for this include changes in the virus and an enhanced ability to detect rare presentations in larger outbreaks, one feature that distinguishes the most recent ZIKV outbreaks is that they occurred in regions of DENV hyperendemicity, where multiple strains of DENV cocirculate and where most people have been infected previously by one or more DENV serotypes. This raises the possibility that ZIKV infection in individuals immune to DENV could result in more severe disease presentations. While the natural history of recent outbreaks has been of ZIKV introduction into regions with high DENV prevalence, as ZIKV becomes endemic in the Western Hemisphere it also will be important to monitor reciprocally how ZIKV immunity impacts DENV pathogenesis. If prior DENV immunity impacts ZIKV pathogenesis, we might expect an even greater burden of ZIKV disease if outbreaks emerge in areas of Southeast Asia where the burden of DENV infection is even greater than in Latin America (90).

Vaccine development. Successful vaccination programs have reduced the global health burden of many flavivirus infections. More than 500 million doses of vaccine to prevent YFV infection have been administered since the vaccine was developed in 1937,

and effective vaccines have blunted the impact of JEV and TBEV. Recently, after decades of study, the first live-attenuated tetravalent DENV vaccine (Dengvaxia) completed phase III human trials and is being deployed in Brazil, Philippines, and Mexico.

As no ZIKV vaccines have been tested even at the preclinical stage, we are likely years away from the introduction of a ZIKV vaccine. It is expected that at least some groups with existing flavivirus vaccine platforms (e.g., chimeric live attenuated strains, passaged or genetically engineered live attenuated strains, E protein subunit, subviral particles, inactivated virions, or DNA plasmid) will apply these strategies toward ZIKV vaccine development in an expedited manner. A major issue remains as to whether it will be easy or difficult to generate an immunogenic and safe vaccine against ZIKV. Given the relatively low variation between ZIKV strains (2, 26, 29, 108) (approximately 94% amino acid identity across the viral genome) and the lack of different genotypes or serotypes, it is plausible that an effective vaccine against one strain would function broadly against all circulating ZIKV strains. ZIKV outbreaks are occurring in areas with high seroprevalence rates for DENV infection and vaccination with YFV. Thus, at least some fraction of candidates for ZIKV vaccines would have preexisting cross-reactive antibodies derived from natural or vaccine-induced flavivirus immunity. This could impact ZIKV responses in one of three ways: (i) by boosting cross-reactivity immunity, conferring protection against ZIKV; (ii) by boosting cross-reactive immunity at the expense of generating protective ZIKV-specific responses ("original antigenic sin"); (iii) by neutralizing live-attenuated ZIKV without appreciably affecting cross-reactive immunity (sterilizing immunity).

Development of therapeutics. Given that vaccines against ZIKV may be years away, the development of immediate measures to control or limit ZIKV disease should be a priority. To date, no drug screening studies have been published with ZIKV. Because DENV infections are so frequent worldwide, effort over the past decade has been made in evaluating inhibitors of specific steps in the DENV life cycle. Such drugs, were they to advance through clinical trial, might have inhibitory activity against other flaviviruses, including ZIKV. Indeed, antiviral drug discovery screens have been performed to identify inhibitors of the fusogenic activity of E protein, the protease and helicase activity of NS3, and the RNA-dependent RNA polymerase and methyltransferase activities of NS5, with ongoing further preclinical development (133). Additional strategies being considered include the repurposing of drug screens, including the testing of FDA-approved or well-studied "orphan" drugs against ZIKV infection. Because drugs against flavivirus proteins could select rapidly for resistant variants, the concept of targeting host molecules required for DENV infectivity (134) or viral proteins that require oligomerization (135) has emerged as a possible strategy. Drugs that target steps in flavivirus infection or cell-intrinsic immunity also could be considered. Finally, passive transfer or antibody-based therapeutics against ZIKV as prophylaxis or treatment may be possible, once strongly neutralizing human monoclonal antibodies are isolated, in analogy to studies performed with other flaviviruses (136, 137). Regardless of the approach, one obstacle to developing ZIKV therapeutics is that a key target population would be pregnant women; the design and implementation of trials to test new drugs in pregnant women will be challenging.

Animal models of ZIKV pathogenesis. Development of vaccines and therapeutics would be expedited by the development of animal models of the different manifestations of ZIKV disease. There are few available data in nonhuman primates apart from the original isolation of ZIKV from the serum of a febrile rhesus monkey (9) and a study recently initiated to assess ZIKV infection dynamics in three rhesus macaques (https://dholk.primate.wisc.edu/project/dho/public/Zika/public/ZIKV-001-public/begin.view?). There are also few available data in mice, as only three papers have reported on ZIKV infection in mice and nothing has been published in almost 40 years (8, 103, 138). Although these studies suggested that ZIKV can replicate and cause injury in cells of the central nervous system, whether this pathogenesis is or is not related to the current linkages to GBS or microcephaly remains uncertain and requires further study. A systematic analysis of ZIKV disease resulting from infection through multiple routes (e.g., intradermal, subcutaneous, and intravenous) in different strains of mice at different ages is needed. Such studies might include panels of genetically diverse mice, such as Collaborative Cross mice (139), to identify genetic susceptibility loci that could be related to human disease or to develop infection models for therapeutic and vaccine testing (140, 141). In addition to direct infection of newborn, juvenile, adult, and old mice, studies in which pregnant dams are inoculated with ZIKV and the effects on fecundity, neonatal infection, and brain development are evaluated could address the presumed linkage to microcephaly in humans.

Public health considerations. The association between ZIKV infection and neurological complications such as microcephaly and GBS prompted the World Health Organization to declare on 1 February 2016 a Public Health Emergency of International Concern surrounding the current ZIKV epidemic in Latin America and the Caribbean (142). The sudden surge of public health, clinical, and basic science interest in ZIKV will increase our understanding of this virus that had remained an obscure viral curiosity until quite recently.

In analogy to the introduction of WNV into the United States in 1999 and the arrival of CHIKV in the Caribbean in 2013, the emergence of ZIKV in Brazil represents another example of an arbovirus introduction into the Western Hemisphere with significant impacts on human health and ecology (143). The appearance of new, more severe clinical presentations in recent ZIKV outbreaks also highlights that familiar infections can produce new phenotypes when introduced to new ecological and host systems. The abundance of *Aedes aegypti* mosquitoes in Latin America and the Caribbean suggests that ZIKV may become endemic in the region. Autochthonous transmission also is a possibility in the southern United States, where *Aedes aegypti* mosquitos are common, and perhaps farther north, where *Aedes albopictus* may serve as a vector. However, the presence of cultural and economic factors such as air conditioning, window screens, indoor lifestyles, and vector control measures, as well as a temperate climate, may prevent widespread ZIKV outbreaks in the United States, much as DENV and CHIKV have not caused epidemics here. Nonetheless, imported cases from travelers are likely to increase in the United States, Europe, and elsewhere (30, 33–35). Indeed, ZIKV infection is now a nationally reportable disease in the United States.

The lack of specific antiviral measures to combat ZIKV emphasizes the importance of vector control strategies for combatting arbovirus disease. Such approaches (removing sources of standing water that serve as breeding sites, larvicide and insecticide application, behavioral modifications to avoid mosquito exposure, and

possibly the controlled introduction of genetically modified or sterile mosquitoes into an epidemic site) also will protect against DENV, CHIKV, and other mosquito-transmitted diseases (144). The unexpected linkage between ZIKV and microcephaly and the lack of specific measures to prevent or treat ZIKV disease in pregnant women, as well as a lack of information to assess the risks posed by ZIKV infection during pregnancy, have prompted public health authorities in some countries to issue highly unusual recommendations regarding pregnancy, including postponement. In the United States, the CDC has recommended enhanced prenatal surveillance of pregnant women who have traveled to areas with ZIKV circulation (74, 80, 91). Such recommendations are framed as representing an "abundance of caution" but must be considered in light of the reality of implementation. Access to contraceptives, prenatal care, and safe abortion services should be components of any public health response to ZIKV.

CONCLUSIONS

ZIKV emergence in the Western Hemisphere has followed what has become a familiar script, in which a previously obscure vector-borne disease is introduced into a new ecological system and host population and then spreads rapidly with significant implications for human health. In the case of ZIKV, this most recent outbreak has been associated with unexpected clinical presentations, and it has been difficult to evaluate the risks and severity of ZIKV infection due to an absence of specific diagnostic reagents and of a basic understanding of the molecular virology and pathogenic mechanisms of this virus.

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