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VACCINES IN PREGNANT WOMEN & INFANTS (D SCHWARTZ AND C KRUBINER, SECTION EDITORS)

Effects of Flavivirus Cross-Reactivity (Zika and Dengue) on the Development of Vaccines for Use in Pregnancy

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Abstract

Purpose of Review The aim of this article is to discuss the implications of immunological cross-reactivity with other prevalent flaviviruses, such as DENV, for the development of a safe and effective vaccine against ZIKV.

Recent Findings The severe clinical manifestations of ZIKV can be due to antibody-dependent enhancement (ADE). This indicates that immunity against DENV and other flaviviruses influences ZIKV disease pathogenesis and the development of vaccines against ZIKV and DENV.

Summary Zika is a re-emerging disease caused by the ZIKV with an unusual clinical presentation characterized by severe manifestations such as Guillain-Barré syndrome. In pregnancy, it can lead to abortion or congenital Zika syndrome (CZS). Currently, no specific treatment or licensed vaccine for this virus is available; therefore, there is an urgent need for the development of a vaccine that can be used during pregnancy.

Conclusion Studies of vaccines against ZIKV are progressing positively, and in their designs, modifications of the antigens are being considered so that they do not cause cross reactions with other flaviviruses that can cause complications in people previously exposed to other flaviviruses

Keywords Flavivirus · Cross-reactivity · Dengue · Zika · Vaccines · Pregnancy

Introduction

Flaviviruses include many medically important viruses, such as Dengue virus (DENV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), West Nile virus (WNV), yellow fever virus (YFV), and Zika virus (ZIKV). Currently, there are approved vaccines against DENV, JEV, TBEV, and YFV for use in humans, yet none are available for WNV or ZIKV [1•]. Similar to DENV, ZIKV is transmitted by the bite of female mosquitos of the genus *Aedes*. ZIKV is an enveloped positive-stranded RNA virus that belongs to the family *Flaviviridae* and the genus *Flavivirus*. ZIKV and DENV belong to the same family and, therefore, share several characteristics due to their phylogenetic closeness. Both viruses have a ~ 11 Kb genome, which encodes a polyprotein that divides into three structural proteins (capsid, pre-membrane/membrane [prM], and envelope [Env]) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). However, unlike DENV, which has four serotypes, ZIKV has a single serotype with two lineages: African and Asian [2••, 3].This virus has become a public health threat, in particular, due to its association with severe congenital birth defects [4]. ZIKV has recently re-emerged as a threatening human disease, yet no licensed vaccine or treatment exists for ZIKV infections. For this reason, the development of a safe, efficient, and affordable vaccine for use in pregnant women is a public health priority. These women are one of the main risk groups so vaccination should aim to prevent the terrible consequences of intrauterine infection. ZIKV is one of the infections responsible for TORCH syndrome since the

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vertical transmission of this virus can infect the fetus and cause a unique pattern of birth defects known as congenital Zika syndrome (CZS) [5, 6]. However, there are several biological, technical, and bioethical obstacles to overcome in developing an efficient, cost-effective, and safe vaccine for use in a mother-baby setting. This is mainly due to cross-reactivity of the specific immune response (humoral and cellular) between DENV and ZIKV since the former virus is highly prevalent in locations where ZIKV has emerged and re-emerged in recent years. Therefore, to avoid the risk of antibody-dependent enhancement (ADE), a safe anti-DENV vaccine should confer protective immunity against the four serotypes and also protect against ZIKV, given the co-circulation of this virus [7]. As a result, the adaptive immune response to flaviviruses (DENV/ZIKV) can be both protective as well as pathogenic, an issue that challenges the development of a vaccine for use in pregnant women and is discussed in this article.

Worldwide epidemiology of ZIKV

Infections caused by arboviruses are a rising public health problem worldwide. Studies on the seroprevalence of dengue, chikungunya, and Zika have shown a rapid expansion of these infections around the world in recent years [8]. The current situation is complex since there are no vaccines against Zika and chikungunya or specific treatment for arboviruses [9]. The first significant ZIKV outbreak in humans outside of its endemic areas occurred on the island of Yap in Micronesia in 2007. After, in 2013, the virus caused a greater epidemic in French Polynesia and, until then, the symptomatic ZIKV infections were mainly associated with a slight disease state that included fever, skin eruption, myalgia, arthralgia, and conjunctivitis. However, during the French Polynesia outbreak, many ZIKV patients presented severe clinical manifestations, including Guillain-Barré syndrome. In 2015, ZIKV had extended to Brazil and initiated the greatest epidemic of this virus known to date.

After its appearance in Brazil, there have been reports of autochthonous ZIKV transmission cases in nearly 50 countries and additional territories in the Western hemisphere, including the USA. The ZIKV infections in the Brazilian outbreak were associated with pregnancy complications as well as severe ocular and neurological deformities, including microcephaly, in newborns of ZIKV-infected mothers. In addition to the marked increase in the incidence of microcephaly simultaneous to the ZIKV outbreak, the presence of ZIKV in the brain tissue of aborted fetuses with microcephaly and the amniotic liquid of pregnant women with microcephalic fetuses demonstrated the causal relationship between ZIKV infection and this devastating developmental defect [10•].

Physiopathological findings in DENV and ZIKV infections

The physiopathology of severe dengue is multifactorial due to the complex interaction between the virus and the host-mediated immune response. Persons with primary infection by DENV induce a lifelong protective immunity against the infecting serotype, accompanied by a short-term cross-protective immunity against the other serotypes for approximately 4 months. However, a subsequent infection with a different DENV serotype is likely to cause dengue with warning signs or severe dengue (DWWS/SD). The epidemiological data suggest a higher risk of DWWS/SD in persons with pre-existing heterotypic antibodies against dengue, which has led research in dengue pathogenesis to focus on subsequent infections by other serotypes and the greater risk of developing severe dengue. Accordingly, the antibodies against a given serotype can cross-react with the three other serotypes during secondary infections, yet do not confer cross-protection [11].

Several immunopathological mechanisms, such as antibody-dependent enhancement (ADE), have been proposed to contribute to the increased risk of developing DWWS/SD. It is widely acknowledged that these cross-reactive antibodies can promote the entry of the heterologous virus into the host cells. This, in turn, results in a hyperimmune reaction that increases vascular permeability and leads to a potentially fatal hypovolemic shock [12–15]. According to the ADE hypothesis, primary infection with DENV produces an insufficient amount of antibodies or antibodies with low avidity incapable of neutralizing a secondary infection by a different DENV serotype. These sub-neutralizing antibodies can promote infection and higher viral loads by facilitating Fcγ receptor-mediated viral entry into myeloid cells such as monocytes and macrophages (i.e., the main replication site of DENV) [7, 16].

A primary infection induces antigen-specific memory T lymphocyte responses against the serotype as well as cross-reactivity against other serotypes, which is activated by viral epitopes expressed on infected cells during a secondary infection by a heterologous DENV. This prompts the production of pro-inflammatory cytokines (TNFα, IL6, IL8, IL10, IL12, macrophage migration inhibitory factor, HMGB1, MCP-1, and matrix metalloproteinases) by T cells, monocytes, macrophages, and mastocytes, which leads to plasma loss from the vascular endothelium and subsequent hypovolemic shock. In turn, the secreted NS1 protein along with anti-NS1 antibodies and complement activation can stimulate the production of pro-inflammatory cytokines associated with DWWS/SD involved in DENV-induced vascular drainage [17].

The clinical manifestations of severe ZIKV are believed to be due to ADE. Also, primary infection with DENV or other flaviviruses is thought to induce cross-reactive antibodies against the antigens of a "secondary" infection with ZIKV,

leading to a higher viral load and a cascade of immunological and clinical events similar to those described for DENV. It is also suggested that anti-ZIKV antibodies can affect a subsequent infection by DENV through an analog mechanism (yet, currently, there is no definite data regarding this issue in humans) [16]. Dejnirattisai et al. used a panel of anti-dengue human monoclonal antibodies to demonstrate that most antibodies that react to the DENV E protein also react to ZIKV. Although the cross-reactive antibodies bound to ZIKV, these did not neutralize the virus but rather promoted ADE. These findings indicate that immunity against DENV could lead to greater ZIKV replication, which has clear implications on disease pathogenesis and the development of vaccines against ZIKV and DENV [18].

Clinical findings of DENV and ZIKV in pregnancy

Pregnancy is a known risk factor for higher maternal mortality and the presentation of complications, such as abortion or severe congenital birth defects, associated with several vertically transmitted infectious diseases. Therefore, a rigorous control program during pregnancy and early ultrasounds and other tests are required to detect plasma loss, the presence of ascites or pleural effusion in pregnant women infected with DENV [19]. The concern about the risk of DENV or ZIKV infection during pregnancy increases as the number of epidemics and the geographical range of disease transmission also increase. Previous studies suggest that dengue infection during pregnancy is associated with a higher risk of severe dengue and adverse pregnancy outcomes. Accordingly, women of or before a fertile age who live in endemic areas are an important target group for DENV and ZIKV vaccination [20, 21••].

The most relevant complications by ZIKV occur when pregnant women become infected at the beginning of pregnancy since the vertical transmission of ZIKV can infect the fetus and cause a unique pattern of birth defects, namely congenital Zika syndrome (CZS). This syndrome is characterized by placental insufficiency, fetal growth restriction, oligohydramnios, fetal brain development disruption, including microcephaly, ventricular calcifications, migration defects, cerebellar hypoplasia, cranial bone collapse; ocular pathology including ocular disorders such as macular scarring and focal pigmentary retinal mottling, auditory deficiencies; and articular illness that includes congenital contractures, neonatal hypertonia with extrapyramidal symptoms, and other anomalies. A study conducted by the CDC of the USA showed that one in seven children born from mothers infected by ZIKV during pregnancy has a birth defect, a neurological anomaly, or both. Likewise, other studies estimate that the risk of CZS after an infection acquired during the first trimester of pregnancy ranges from 0.88 to 22%. In addition to presenting malformations, the incidence of neurological problems such as epilepsy, loss of vision, and neurological development retardation has been shown to increase with age in children with CZS [5, 6, 22–25].

Development of vaccines against ZIKV and DENV for use in pregnant women

Vaccines against ZIKV are a critical weapon in the arsenal against ZIKV outbreaks in the short-term. An important aim of any anti-ZIKV vaccine is to prevent the infection and/or neurological deterioration of the fetus; however, the vaccination of pregnant women to prevent congenital anomalies caused by ZIKV infection faces several challenges. (1) To protect the fetus, protective immunity must be achieved during the first trimester of pregnancy or at the beginning of the second trimester since the highest vulnerability occurs during this timeframe. (2) Many women are unaware that they are pregnant until late in the first trimester. (3) Although live attenuated vaccines can confer protection after a single dose, these are generally unsuitable for administration during pregnancy. (4) Vaccines based on inactivated recombinant subunits and other non-replicative vaccines that are more suited for use during pregnancy generally require multiple doses to achieve protective immunity, which delays the establishment of effective immunity and exceeds the window of the maximum vulnerability of the fetus [26].

There are several knowledge gaps regarding ZIKV, which raise various questions to be answered before achieving full control over the disease. These questions are: how, why, and when do ZIKV outbreaks occur; what are the risk factors for the presentation of the different clinical manifestations of the disease; what are the effects of co-infection with other arboviruses (e.g., antibody-dependent enhancement (ADE)), the details on the routes of transmission, the moment of greatest risk during pregnancy, the importance of age, race, gender, and genetics on disease susceptibility and the clinical outcome; what role does cross-reactivity of the vaccine strains play in protection or immunopathogenesis; and should animal models be developed that accurately portray the behavior of the disease in humans, among others [27, 28].

It is essential to appropriately address the singularities of ZIKV infection in pregnant women since pregnancy can alter the immunological response and the fetal immune response can also change during gestation [21••]. The administration of any vaccine against ZIKV will be more beneficial before pregnancy or during the first weeks of pregnancy. The WHO and UNICEF expect that

the development of a vaccine against ZIKV will prevent clinical diseases in individuals over 9 years old in 70–80% of the population, assuming that a reduction in viremia will be associated with the prevention of the clinical disease and infection of the fetus during at least 1 year after the administration of the vaccine. For this, a systematic approach is needed to assess the vaccine candidates based on the following criteria: safety (even during pregnancy), specificity, speed and duration of the protective immunity, vaccination scheme (administration route, volume, and number of doses), interactions with other flaviviruses and available vaccines against other flaviviruses, combined administration with other vaccines to maximize coverage even during periods of low incidence of ZIKV infection, key attributes of the vaccine (e.g., storage and stability), assessment of protection and immunological risk, and validation in clinical models. The knowledge gaps on the use and safety of vaccines in early pregnancy constitute a challenge for the development and recommendation of an effective vaccine against ZIKV for use during pregnancy since maternal immunization represents the "next step" in vaccinology [21••, 29–34].

When facing these issues in the development of a vaccine against ZIKV, it is important to critically assess the safety of the vaccine during pregnancy in terms of determining the substantial risk of infection for the mother and her fetus in the absence of immunization, as well as the possible immunopathological complications caused by ADE that can arise from cross-reactivity of ZIKV antigens with DENV. Currently, several candidate vaccines are being developed, including traditional inactivated vaccines and live attenuated, as well as DNA, mRNA, and protein subunit vaccines. These experimental vaccines are in different preclinical and clinical stages and some have advanced to phase I and II clinical trials [22] (see Table 1).

The routine administration of live vaccines in pregnant women has generally been contraindicated, due to concerns about fetal damage derived from attenuated infectious agents. The concern is greater for live vaccines that replicate systemically and could potentially cross the placental barrier. Despite the involuntary exposure during pregnancy to several types of live vaccines (e.g., vaccines against rubella, yellow fever, and smallpox) in hundreds or thousands of women, there is convincing evidence of fetal damage only for the vaccine against smallpox (a small increase in the risk of birth defects [2.4% compared with 1.5%] among women vaccinated in the first trimester). For this reason, vaccines against yellow fever and smallpox are only recommended to pregnant women with a high risk of infection since, in this case, the potential benefit greatly exceeds the risks. To guarantee that pregnant women have access to vaccines with reassuring safety data, the vaccine candidates with the highest probabilities of administration during pregnancy must be assessed [21••]. Despite the precaution of not administrating live vaccines in pregnant women, there is safety data regarding the administration of some attenuated vaccines against flaviviruses to pregnant women who were unaware of their pregnancy during very early stages. Further, these women received the vaccines in clinical studies and, therefore, represent an important source of information for future studies on the safety and immunogenicity of the use of the ZIKV vaccine on pregnant women. For example, although CYD-TDV is contraindicated during pregnancy due to a supposed risk for the fetus, the preclinical data has not revealed any teratogenic effects on the offspring of animals vaccinated with CYD-TDV during pregnancy. Despite the precautions taken in CYD-TDV clinical trials, a small number of women were unknowingly vaccinated during pregnancy (most of these women a few days before or after conceiving) and there is no evidence of an increase in adverse pregnancy outcomes of these women vaccinated against CYD-TDV during early pregnancy compared with the control group [20].

Implications of cross-reactivity of flaviviruses for the development of a vaccine against ZIKV

Currently, there is much pressure to produce a vaccine against ZIKV and, because of this, the presence of serological cross-reactivity between DENV and ZIKV must be considered. The vaccine will likely be used in areas with a high DENV seroprevalence and the increase of de novo antibody responses against ZIKV in this context represents a challenge. Similarly, the vaccination against ZIKV in individuals not immune to DENV could lead to ADE of DENV infection, while the vaccination against DENV can also promote ADE of ZIKV infection. Consequently, cross-reactivity of ZIKV antibodies with DENV and ADE of the infection can occur due to the similarities between the two viruses, although ZIKV and DENV differ by 41–46% in their global protein sequence identity. Although the sequence of the E protein (i.e., the main target of neutralizing antibodies against flaviviruses) between ZIKV and DENV is different, the two structures are very similar [35]. Various studies have shown that antibodies isolated from patients infected with DENV or ZIKV show high cross-neutralization or cross-protection in vitro and in vivo [36]. Overall, ZIKV can be considered a fifth member of the DENV serocomplex, a factor that should be accounted for in the vaccine development approaches of the two viruses [16] (Fig. 1).

Since the underlying mechanisms of pathogenesis of the ZIKV infection are largely unknown, several research

Table 1 General aspects of vaccines Zika virus

Vaccine candidate	Sponsor	Platform	Immunogen	Phase	Status
VRC-ZKADNA085-00-VP	NIAID	DNA	prME	1	Ongoing, not recruiting
VRC-ZKADNA090-00-VP	NIAID	DNA	prME	1	Ongoing, not recruiting
VRC-ZKADNA090-00-VP	NIAID	DNA	prME	2	Open, recruiting
GLS-5700	GeneOne Life Science/Inovio Pharmaceuticals	DNA	prME	1	Ongoing, not recruiting
GLS-5700	GeneOne Life Science/Inovio Pharmaceuticals	DNA	prME	1	Ongoing, not recruiting
MV-Zika	Themis Bioscience	Recombinant viral vector	prME	1	Ongoing, not recruiting
mRNA-1325	Moderna Therapeutics	mRNA	prME	2	Open, recruiting
ZIKV PIV	NIAID	Inactivated whole target organism	Whole virus	1	Ongoing, not recruiting

NIAID, National Institute of Allergy and Infectious Diseases; *PIV*, purified inactivated vaccine; *prME*, pre-membrane and envelope proteins
Adapted from Makhluf et al. [3]

studies have focused on the potential role of cross-reactive antibodies of flaviviruses in enhancing the infection (ADE) by ZIKV. ADE is especially concerning due to the structural similarities between ZIKV and other flaviviruses, such as DENV, that co-circulate in the areas affected by ZIKV [38, 39]. Furthermore, little is known about the cross-protection between ZIKV and other flaviviruses.

In 2017, Lima et al. conducted a study on non-human primates infected with ZIKV, finding that pre-existing immunity to DENV or YFV leads to greater activation of T-CD4 cells and higher titers of anti-ZIKV IgG. This indicates that the specific responses of ZIKV benefit from the pre-existing immune memory for other flaviviruses. However, this pre-immunity against flaviviruses did not provide cross-protection against infection by ZIKV [40]. Thus, the strategy of combining vaccines against ZIKV with other flavivirus vaccines could improve the immune response and increase the efficacy of the vaccine by taking advantaging of cross-reactivity. Besides, the vaccination of individuals previously infected by other flaviviruses could also benefit from the cross-reactivity of T cells to conserved ZIKV epitopes since these can produce a strong humoral response or a pre-existing immunological memory induced by the natural infection by viruses or through vaccination with heterologous

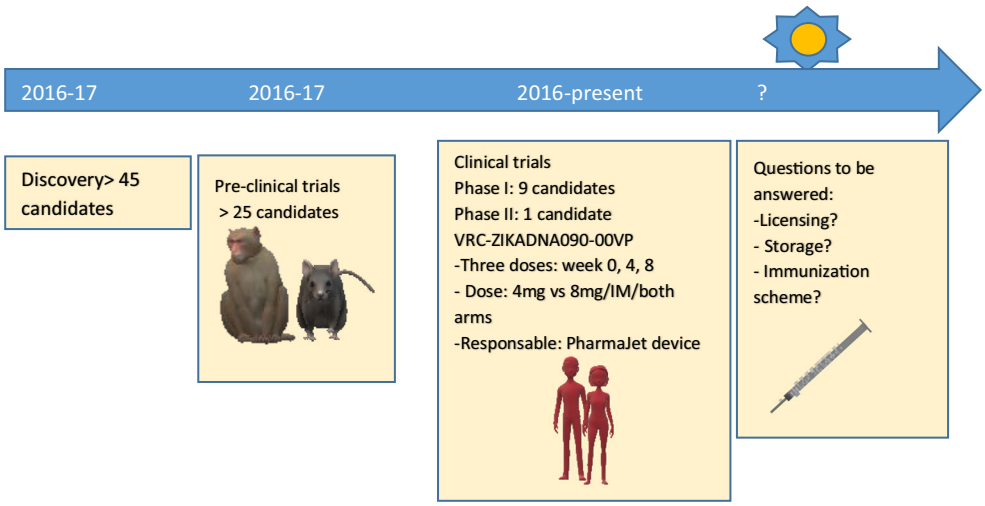

Fig. 1 Zika virus vaccine development path. The vaccine development stages include science, basic discovery, and a leader candidate vaccine, which undergoes a preclinical safety and immunogenicity assessment in animal models. High-quality information is required to justify to a regulator agency (e.g., Food and Drug Administration (FDA) or the European Medicine Agency (EMA)) that the candidate vaccine can be evaluated in a clinical trial. Once the clinical trials are successful, the vaccine will obtain a license for use. Adapted from Barrett [37]

Table 2 DENV and ZIKV vaccines designed to reduce cross-reactivity by antibody-dependent enhancement (ADE)

Vaccine	Format	Antigen designed	Location
DENV	DNA	G106R, L107D	DII/FL
		K310D,E311K,P364Q	close to DIII/FL
ZIKV	mRNA	T76R, Q77E	close to DIII/FL
		W101R, L107R	close to DIII/FL

Adapted from Lin et al [32]

flaviviruses [41]. Zhao et al. found that some specific antibodies against ZIKV generated by natural infection can facilitate the infection by DENV in vitro. A key question that remains is if neutralizing antibodies can protect pregnant women and their fetuses against infection by ZIKV and from congenital malformations, including microcephaly [42].

Different studies have shown that DENV-specific serum, as well as monoclonal antibodies, can increase ZIKV replication in vitro and in vivo in AG129 mice. Cross-reactivity between ZIKV and DENV is mainly mediated by sub-neutralizing or non-neutralizing antibodies that can promote infections by facilitating the expression of FcγR in myeloid cells; thus, enabling virion entry by ADE [16, 40, 43–47]. However, other studies that performed the passive transfer of neutralizing antibodies at sub-protective doses before challenging with ZIKV did not observe a greater replication of the virus or the presence of ZIKV disease in mice or monkeys [48, 49]. Barouch et al. recently observed that monkeys immunized with DENV or YFV and then challenged with ZIKV did not show an increase in ZIKV replication or the presence of adverse clinical results compared with controls not infected with flaviviruses [50]. Yet, there are also reports that serum collected from individuals infected with DENV and WNV favor infections by ZIKV in vitro through the ADE mechanisms previously described [44].

Future efforts in the design of a vaccine against ZIKV must focus on obtaining neutralizing antibodies with a reduced level of cross-reactivity, yet still conferring a sterilizing immunity. To minimize the effects of cross-reactivity, several novel mutations have been introduced in or close to the FL region of domain II (DII) of the E protein to attenuate the production of cross-reactive antibodies in candidate vaccines against DENV and ZIKV (see Table 2) [51].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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