

Closing in on a Zika virus vaccine

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Over the past 2 years, Zika virus (ZIKV) has emerged as a pathogen capable of causing devastating congenital malformations in the developing fetus and significant neurological disease in adults. In 2017, substantial progress has been made towards the development, immunological analysis and preclinical evaluation of vaccine platforms to prevent the pathologies associated with ZIKV infection.

The potential worldwide impact of Zika virus (ZIKV) infections on human health is staggering: 48 countries and territories have confirmed cases associated with vector-borne transmission of ZIKV, with 5 countries reporting cases of sexual transmission. Since 2015, thousands of infants have been born with ZIKV-induced neurological sequelae that will considerably impair their ability to attain normal cognitive function. Even the least debilitating outcomes of ZIKV infections *in utero* may adversely affect quality of life and require life-long intervention and care. In addition to ZIKV-induced fetal disease, infection in adults is associated with rare neurological disorders such as Guillain–Barre syndrome (GBS), which can cause muscle weakness and even paralysis.

The teratogenic potential of ZIKV was unexpected, as the virus has been present in central Africa since at least the 1940s and before 2013 was associated with only a mild disease. Not since the 1962–1965 rubella virus (RuV) epidemic that occurred in the United States has the devastating impacts of a viral

infection during pregnancy been so widely feared. The subsequent development of a successful RuV vaccine saved the lives of millions of infants and has inspired a similar global effort to eradicate congenital ZIKV syndrome. In this Year in Review article, we discuss the most significant advances of the past year with a focus on the development of vaccines to mitigate the effects of ZIKV infections.

Several formalin-inactivated and live-attenuated vaccines against related flaviviruses (for example, Japanese encephalitis, Dengue and yellow fever viruses) are approved for use in humans. Analyses of these vaccines have shown that antibody responses against the flavivirus envelope (E) protein correlate with protection in animals and humans. An effective vaccine that targets all strains of ZIKV should be feasible given the limited (~3% to 5%) amino acid variability between E proteins of contemporary and historical strains.

In 2016, DNA plasmid and formalin-inactivated whole virus vaccines against ZIKV were described. DNA plasmid-based vaccines incorporating the precursor membrane (prM)

and E genes of contemporary ZIKV isolates produced secreted E proteins or virus-like particles, elicited neutralizing antibody responses and protected against infection in non-pregnant mice and non-human primates (NHPs)^{1–3}. Similarly, alum-adsorbed inactivated ZIKV induced neutralizing antibody responses in non-pregnant animals and conferred protection against ZIKV challenge. DNA plasmid-based vaccines are useful owing to their ease of production, physical and genetic stability and low reactogenicity. Plasmid-based and inactivated viral vaccines are desirable, especially for populations that are relatively immunocompromised, as other live-attenuated vaccines may be contraindicated. Both DNA plasmid and inactivated virus vaccine candidates against ZIKV advanced to clinical trials in humans in 2017.

Several papers were published in 2017 that described new vaccine platforms against ZIKV (FIG. 1), with some addressing protection against fetal injury in mice. Pardi and colleagues⁴ developed a novel lipid-encapsulated, modified mRNA vaccine encoding the prM and E genes of a French Polynesian strain of ZIKV. Modified mRNA vaccines contain untranslated regions (UTRs) that optimize translation efficiency and intracellular stability, as well as nucleoside modifications to minimize unintended activation of innate immunity. A single intradermal dose of this vaccine induced strong neutralizing antibody and CD4⁺ T cell responses in non-pregnant mice that persisted for months. Challenge with an American ZIKV strain at 2 and 20 weeks after vaccination completely protected against viraemia. Furthermore, a single inoculation of this mRNA vaccine also induced high levels of neutralizing antibody and protected against viraemia in NHPs⁴.

A second study using a different lipid-encapsulated mRNA vaccine encoding the prM and E genes from an Asian ZIKV strain and a prime–boost regimen also induced durable neutralizing antibody responses in mice. Challenge with a heterologous African ZIKV strain in immunized immunodeficient or immunocompetent mice showed protection against viral infection and lethality⁵. A modified version of this mRNA vaccine encoding mutations that destroy the conserved fusion–loop epitope in the E protein protected against ZIKV, but immunized mice

Key advances

- New anti-Zika virus (ZIKV) vaccine platforms developed in 2017 show promise to effectively limit congenital ZIKV syndrome.
- Lipid-encapsulated mRNA vaccine-based immunity established before pregnancy in mice is sufficient to protect against congenital infection and disease.
- Live-attenuated vaccines induce high levels of neutralizing antibodies, prevent infection and damage to the testis in males and abrogate *in utero* transmission and fetal infection during pregnancy.
- Immunological correlates of vaccine protection are established in preclinical models of ZIKV infection and pathogenesis.

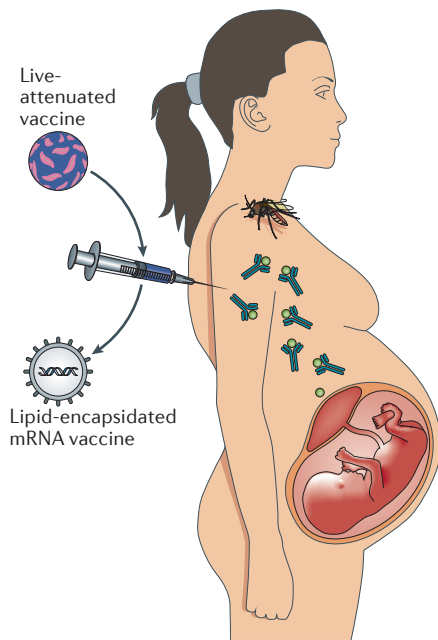


Figure 1 | Vaccine-based approaches to protect against congenital ZIKV syndrome. Zika virus (ZIKV) infection of pregnant women can have devastating impacts on the developing fetus. Two vaccine platforms developed in 2017 show promise to reduce fetal infection and ZIKV-induced fetal disease in pregnant animal models. These include live-attenuated and lipid-encapsulated modified mRNA-based approaches that induce the production of neutralizing antibodies that prevent vertical transmission of ZIKV across the placental barrier, thus limiting fetal infection.

produced decreased amounts of antibodies that enhance Dengue virus (DENV) infection⁵. This is important as the related flavivirus DENV circulates in the same areas of the world as ZIKV. As one recent study suggests that prior exposure and immune response to ZIKV can enhance DENV infection in rhesus macaques⁶, a potential vaccine candidate that minimizes antibody-dependent enhancement of DENV infection may be desirable.

Richner and colleagues⁷ tested a modified mRNA lipid nanoparticle vaccine encoding the prM and E genes of an Asian ZIKV strain in pregnant mice. This vaccine generated robust neutralizing antibody responses in immunocompetent non-pregnant female mice. Subsequently, vaccinated females were mated and, after they became pregnant, were challenged approximately one-third through the gestation period with a heterologous African strain of ZIKV. Relative to placebo controls, dams immunized with the prM–E mRNA vaccine showed markedly

diminished levels of viral RNA in maternal, placental and fetal tissues, and the majority of fetuses showed no disease. This study demonstrated for the first time that, at least in mice, vaccines administered before pregnancy can prevent congenital ZIKV syndrome.

This year, Pei-Yong Shi's group described novel, live-attenuated ZIKV vaccines that are protective in mice and NHPs. Live-attenuated vaccines can be advantageous as they may be more cost-effective than other vaccine platforms and promote durable immunity even after a single dose. Thus, a live-attenuated vaccine may be practically important when immunizing people living in and travelling to ZIKV-endemic areas, especially in developing countries. Three live-attenuated ZIKV vaccines were developed: one with mutations in the *N*-linked glycosylation sites of the viral *NS1* gene⁷ and two with small attenuating deletions in the 3'-UTR of the genomic RNA^{8,9}. In mice, these live-attenuated vaccines induced high levels of neutralizing antibody, conferred sterilizing immunity, prevented infection and damage to the testis in males and abrogated *in utero* transmission and fetal infection during pregnancy. In NHPs, a single immunization with the 3'-UTR deletion vaccines elicited a robust antibody response, which prevented viraemia upon challenge with an epidemic strain of ZIKV⁹.

Although the subunit and live-attenuated vaccines have established the activity of antibodies in controlling ZIKV pathogenesis, progress was also made this year in defining the functions of effector CD8⁺ T cells. An initial study showed that polyfunctional, cytotoxic CD8⁺ T cells reduced ZIKV burden in mice, whereas their depletion or genetic absence led to higher tissue burdens and higher mortality after ZIKV infection¹⁰. A second study, using HLA-transgenic mice, identified human-relevant ZIKV CD8⁺ T cell epitopes in naive and DENV-experienced immune settings and demonstrated that both ZIKV-specific and ZIKV/DENV cross-reactive CD8⁺ T cells can protect against ZIKV infection¹¹. Collectively, these results demonstrated that CD8⁺ T cells protect against ZIKV infection and imply that ZIKV and possibly DENV vaccine efforts should be tailored to optimize CD8⁺ T cell responses.

Notwithstanding the rapid success in pre-clinical studies, there are unique issues that may delay implementation of a ZIKV vaccine. These include our lack of understanding of how ZIKV induces GBS, whether ZIKV vaccine-induced antibodies could sensitize humans to more severe DENV infection or

promote mother-to-fetus transmission, and whether pre-conception maternal immunity is sufficient to protect against fetal transmission. A major hurdle to the development of any approach to limit fetal disease during pregnancy is the need to design ethical human trials. Although the studies published in 2017 are promising, considerable work is still needed to better define the biology of ZIKV in humans and the design and analysis of clinical trials. The collective efforts of the global scientific community have generated exciting insights into ZIKV pathogenesis and substantive progress towards a ZIKV vaccine. In the years ahead, the careful evaluation of these approaches in women of child-bearing age will prove challenging, but will be essential for the eventual eradication of ZIKV-induced congenital disease.

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Competing interests statement

The authors declare competing interests. See Web version for details.

Author contributions

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