Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys

Peter Abbink,†, Rafael A. Larocca,†, Rafael A. De La Barrera, Christine A. Bricault,†
Edward T. Moseley, Michael Boyd,† Marinela Kirilova, Zhengfen Li, David Ng'ang'a,†
Ovini Nanayakkara,† Ramya Nityanandam,† Noe B. Mercado,† Erica N. Borducchi,†
Patricia B. Giglio, David Jetton,† Jessica Jimenez,† Benjamin C. Lee,† Amanda L. Brinkman,†
Arshi Agarwal,† Crystal Cabral,† Abishek Chandrashekar,† Ovini Nanayakkara,†
Katherine Molloy,† Mayuri Shetty,† George H. Neubauer,† Kathryn E. Stephenson,¶
Jean Pierre S. Peron,† Paolo M. de A. Zanotto,† Johnathan Mismore,4
Brad Finnefeld,† Mark G. Lewis,† Galit Alter,† Kayvon Moghadam,2,6
Richard G. Jarman,† Kenneth H. Eckels,2 Nelson L. Michael,2
Stephen J. Thomas,2† Dan H. Barouch1,5†,†

Zika virus (ZIKV) is responsible for a major ongoing epidemic in the Americas and has been causally associated with fetal microcephaly. The development of a safe and effective ZIKV vaccine is therefore an urgent global health priority. Here we demonstrate that three different vaccine platforms protect against ZIKV challenge in rhesus monkeys. A purified inactivated virus vaccine induced ZIKV-specific neutralizing antibodies and completely protected monkeys against ZIKV strains from both Brazil and Puerto Rico. Purified immunoglobulin from vaccinated monkeys also conferred protective efficacy against inactivated virus, DNA-based, and vector-based vaccines against ZIKV challenge in rhesus monkeys. These data support the rapid clinical development of ZIKV vaccines for humans.

The explosive and unprecedented ZIKV outbreak in the Americas (1, 2) prompted the World Health Organization to declare this epidemic a public health emergency of international concern. ZIKV has been causally associated with fetal microcephaly, intruterine growth retardation, and other congenital malformations in both humans (3–6) and mice (7–9), and it has also been linked with neurologic disorders such as Guillain-Barre syndrome (10). Several reports have shown that ZIKV can infect placental and fetal tissues, leading to prolonged viremia in pregnant women (11) and nonhuman primates (12). ZIKV also appears to target cortical neural progenitor cells (7–9, 13, 14), which likely contributes to neuropathology.

We recently reported the protective efficacy of two vaccines against ZIKV challenges in mice: a purified inactivated virus (PIV) vaccine from ZIKV strain PRVABC99 and a DNA vaccine expressing an optimized premembrane and envelope (prM-Env) immunogen from ZIKV strain BeH15744 (15). These studies used ZIKV challenge strains from Brazil (ZIKV-BR; Brazil/ZKV2015) (9) and Puerto Rico (ZIKV-PR; PRVABC99). ZIKV replication in mice was dependent on the mouse strain (15) and may be less extensive than in nonhuman primates (12). We therefore evaluated the immunogenicity and protective efficacy of inactivated virus, DNA-based, and vector-based vaccines against ZIKV challenge in rhesus monkeys.

ZIKV PIV vaccine study

We first immunized 16 rhesus monkeys by the subcutaneous route with 5 μg of ZIKV PIV vaccine plus alum (n = 8) or sham vaccine (alum only) (n = 8) at weeks 0 and 4 (fig. S1). All PIV-vaccinated animals developed ZIKV Env–specific binding antibodies, as measured by enzyme-linked immunosorbent assays (ELISAs), and ZIKV-specific neutralizing antibodies, as measured by micro-neutralization (MN50) assays, at week 2 after initial immunization. Median log antibody titers at week 2 were 1.87 (Fig. 1A) and 2.27 (Fig. 1B) in ELISAs and MN50 assays, respectively. After the week 4 boost immunization, median log antibody titers increased substantially to 3.54 (Fig. 1A) and 3.66 (Fig. 1B), respectively, at week 6. In contrast, sham control monkeys did not develop detectable ZIKV-specific antibody responses (fig. S2).

VACCINES

Supplementary materials

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† These authors contributed equally to this work. ‡ These authors contributed equally to this work. Corresponding author. Email: dbarouch@bidmc.harvard.edu

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Using protein G affinity chromatography, we purified immunoglobulin G (IgG) from plasma from PIV-vaccinated monkeys at week 8. Vaccine-elicited, ZIKV-specific IgG was then infused into four groups of naïve Balb/c mice (n = 5 per group) as fivefold serial dilutions of the purified IgG preparation, which had a log ELISA titer of 3.30 and a log MN50 titer of 3.30. After infusion, these groups of recipient mice (designated I, II, III, and IV) had median log ELISA titers of 2.83, 2.35, 1.40, and <1.00 (Fig. 3A) and median log MN50 titers of 2.93, 1.77, 1.14, and <1.00 (Fig. 3B), respectively. Mice were then challenged by the intravenous route with 10⁵ vp (10² PFU) of ZIKV-BR or ZIKV-PR. Each group contained six female and two male animals. Viral loads are shown for (A) plasma, (B) urine, (C) CSF, (D) colorectal secretions, and (E) cervicovaginal secretions. Viral loads were determined on days 0, 1, 2, 3, 4, 5, 6, and 7 for the plasma samples (A) and on days 0, 3, and 7 for the other samples [(B) to (E)]. Data are shown for all eight animals, except in the case of cervicovaginal secretions (E), where data for the six females are shown. The P value was determined by Fisher’s exact test.

ZIKV DNA and adenovirus vaccine study

To evaluate the immunogenicity and protective efficacy of DNA- and vector-based ZIKV vaccines, we immunized 12 rhesus monkeys with a plasmid DNA vaccine (15) or a rhesus adenovirus serotype 52 (RhAd52) vector–based vaccine (16) (fig. S1). Monkeys were immunized by the intramuscular route with 5 mg of DNA vaccine expressing prM-Env at weeks 0 and 4 (n = 4), with 10¹¹ vp of RhAd52 vector expressing prM-Env as a single immunization at week 0 (n = 4), or with sham vaccine at weeks 0 and 4 (n = 4). The DNA–prM-Env vaccine induced ZIKV-specific neutralizing antibody titers in all animals after the week 4 boost immunization, although only minimal MN50 titers were detected after the initial priming immunization (Fig. 5A). In contrast, the RhAd52–prM-Env vaccine induced ZIKV-specific neutralizing antibody responses in all animals at week 2 after the initial priming immunization (Fig. 5A). Moreover, the RhAd52–prM-Env vaccine induced a substantial breadth of antibody responses against linear ZIKV Env epitopes, as measured by peptide
**Fig. 3.** Adoptive transfer studies in mice. (A) ZIKV Env–specific ELISA titers and (B) ZIKV-specific MN50 titers in serum from recipient Balb/c mice (n = 5 per group), measured 1 hour after adoptive transfer of fivefold serial dilutions of IgG purified from PIV-vaccinated rhesus monkeys (groups I, II, III, and IV) or sham controls. (C) Plasma viral loads in mice after challenge with 10^5 vp (10^2 PFU) of ZIKV-BR. (D and E) Immune correlates of protection and infection. Red bars reflect medians. *P* values were determined by *t* tests.

**Fig. 4.** Adoptive transfer studies in rhesus monkeys. (A) ZIKV–specific MN50 titers in serum from recipient rhesus monkeys (n = 2 per group), measured 1 hour after adoptive transfer of fivefold dilutions of IgG purified from PIV-vaccinated rhesus monkeys (groups I and II) or sham controls. (B) Plasma viral loads in rhesus monkeys after challenge with 10^6 vp (10^3 PFU) of ZIKV-BR. Red bars reflect medians.

**Fig. 5.** Immunogenicity of the ZIKV DNA–prM-Env and RhAd52–prM-Env vaccines. (A) ZIKV–specific MN50 titers measured after immunization of rhesus monkeys by the intramuscular route with 5 mg of DNA–prM-Env vaccine at weeks 0 and 4 (red arrows) or with 10^11 vp RhAd52–prM-Env as a single immunization at week 0. (B) Cellular immune responses measured by IFN-γ ELISPOT assays for prM, Env, Cap, and NS1 at week 6 for the DNA–prM-Env vaccine or at week 4 for the RhAd52–prM-Env vaccine. Red bars reflect medians.
Protection against subcutaneous challenge with the RhAd52 after the initial week 0 priming immunization induced Env-specific cellular immune responses using the vaccination doses, routes, and schedules described (Fig. S7). The DNA prM-Env vaccine, or sham vaccine (n = 4 per group). Together, these findings for ZIKV vaccine development represent the official views of the Department of the Army or the Department of Defense.

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S7

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