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

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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 is therefore an urgent global health priority. Here we demonstrate that three different vaccine platforms protect against ZIKV challenge in rhesus monkeys.

VACCINES

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, intrauterine 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 PRVABC59 and a DNA vaccine expressing an optimized premembrane and envelope (pPM-Env) immunogen from ZIKV strain BeH1815744 (15). These studies used ZIKV challenge strains from Brazil (ZIKV-Br; Brazil/ZIKV2015) (9) and Puerto Rico (ZIKV-PR; PRVABC59). 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-Env-specific neutralizing antibodies, as measured by micro-neutralization (MN50) assays, at week 2 after initial immunization. Median log antibody titer 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 titer 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).

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Supplementary Text

Figs. S1 to S5

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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 10^5 vp (10^2 PFU) of ZIKV-BR, as we have previously described (15). The higher two doses of purified IgG provided complete protection after ZIKV challenge, whereas the lower two doses of purified IgG resulted in reduced viremia compared with sham-infused control mice (Fig. 3, C to E).

Vaccine-elicited, ZIKV-specific IgG was also infused into two groups of naïve rhesus monkeys (n = 2 per group). After infusion, these groups of recipient monkeys (designated I and II) had respective median log MN50 titers of 2.11 and 1.22 (Fig. 4A). Monkeys were then challenged with 10^6 vp (10^3 PFU) of ZIKV-BR. In the animals that received the higher IgG dose, one animal was completely protected and the other showed a blip of viremia on days 3 to 5 (Fig. 4B). No enhancement of viral replication was observed at subtherapeutic IgG concentrations. Taken together, these data demonstrate that purified IgG from PIV-vaccinated rhesus monkeys provided passive protection against ZIKV challenge after adoptive transfer to both rodents and primates.

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 10^11 vp of RhAd52 vector expressing prM-Env as a single immunization at week 0 (n = 4), or with sham vaccinated 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 serial 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.
microarray assays, compared with the other vaccines tested (17) (Fig. S7). The DNA–prM-Env vaccine also induced detectable Env-specific IFN-γ ELISPOT responses after the week 4 boost immunization, and the RhAd52–prM-Env vaccine induced Env-specific cellular immune responses after the initial week 0 priming immunization (Fig. 5B). Monkeys were challenged 4 weeks after the final vaccination. Both the DNA– and the RhAd52–prM-Env vaccines provided complete protection against subcutaneous challenge with 10^6 vp (10^3 PFU) of ZIKV-BR. Plasma viral loads are shown.

**Discussion**

We demonstrate that three different vaccine platforms provide complete protection against ZIKV challenge in rhesus monkeys. No specific clinical safety adverse effects related to the vaccines were observed. We recently reported the protective efficacy of the PIV vaccine and the DNA–prM-Env vaccine in mice (15). The present data confirm and extend these prior studies by demonstrating robust protection against ZIKV challenge in nonhuman primates and specifically using the vaccination doses, routes, and schedules that are typically evaluated in clinical trials. Although the PIV vaccine and the DNA–prM-Env vaccine appeared comparably immunogenic in mice (15), the former proved more potent in rhesus monkeys under the conditions tested (Figs. 1 and 5). To generalize these observations to a vector-based vaccine, we also evaluated the RhAd52–prM-Env vaccine, which proved highly immunogenic and afforded complete protection after a single immunization in monkeys (Fig. 5). Rhesus adenovirus vectors have the potential advantage of minimal baseline vector-specific neutralizing antibodies in human populations (16).

The adaptive transfer studies demonstrate that vaccine-elicted antibodies are sufficient for protection against ZIKV challenge. Moreover, passive protection in mice and rhesus monkeys was observed at relatively low antibody titers (Figs. 3 and 4). Such antibody titers are likely achievable in humans with these vaccine platforms, thus raising optimism for the development of a ZIKV vaccine for humans. Future preclinical and clinical studies will need to address the potential impact of cross-reactive antibodies against dengue virus and other flaviviruses. Secondary infection with a heterologous dengue serotype can be clinically more severe than initial infection, which may or may not reflect antibody-dependent enhancement (18, 19). Cross-reactive antibodies for ZIKV and dengue virus have also been described (20, 21), and dengue-specific antibodies have been reported to increase ZIKV replication in vitro (22). The relevance and implications of these findings for ZIKV vaccine development remain to be determined.

The consistent and robust antibody-based correlates of vaccine protection against ZIKV challenge in both rodents and primates suggest the generalizability of these findings. Similar correlates of protection, and specifically neutralizing antibody titers >10, have been reported for other flavivirus vaccines in humans (23–25). Together, these data suggest a path forward for clinical development of ZIKV vaccines in humans. PIV vaccines have been evaluated previously in clinical trials for other flaviviruses, including dengue virus, tick-borne encephalitis virus, and Japanese encephalitis virus (26–30). Phase 1 clinical trials with the ZIKV PIV vaccine, as well as other candidate ZIKV vaccines, are expected to begin later this year.

**REFERENCES AND NOTES**

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**SUPPLEMENTARY MATERIALS**

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Materials and Methods

Figs. S1 to S7

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