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Rapid Development of a DNA Vaccine for Zika Virus

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Abstract

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Zika virus (ZIKV) was identified as a cause of congenital disease during an explosive outbreak in the Americas and Caribbean in 2015. Because of the ongoing fetal risk from endemic disease and travel-related exposures, a vaccine to prevent viremia in women of child-bearing age and their partners is imperative. Vaccination with DNA expressing the prM and E proteins of ZIKV was immunogenic in mice and nonhuman primates, and protection against viremia after ZIKV challenge correlated with serum neutralizing activity. These data not only indicate DNA vaccination could be a successful approach to protect against ZIKV infection, but also suggest a protective threshold of vaccine-induced neutralizing activity that will prevent viremia following acute infection.

The emergence of Zika virus (ZIKV) in the Americas and the Caribbean follows a series of global threats to public health from mosquito-borne viral diseases over the last three decades. Because of the profound impact on individuals and society from a disabling congenital disease, WHO declared ZIKV infection a global health emergency in February 2016. Although it is likely that the incidence of ZIKV infection will decline significantly within 1–2 years (1), it is also likely to become endemic in tropical and subtropical regions with sporadic outbreaks and potential for spread into new geographical areas, as observed with other emerging arboviruses like West Nile (WNV) and chikungunya viruses. Therefore, unless immunity is established before child-bearing age, pregnant women will continue to be at risk for an infection that could harm their fetus. Further, because men can harbor ZIKV in semen for several months following a clinically unapparent infection and can sexually transmit virus to a pregnant partner (2), even women in nonendemic regions will have some ongoing risk if exposed to men who have traveled to endemic regions. These unique features of transmission and disease suggest there will be an ongoing need for a ZIKV vaccine to maintain a high level of immunity in the general population and in travelers to endemic regions to reduce the frequency of fetal infection.

To rapidly address the critical need for a preventive vaccine to curtail the current ZIKV outbreak in the Americas, we chose a gene-based vaccine delivery approach that leverages our prior experience with a DNA-based WNV vaccine (3). Advantages of DNA vaccines include the ability to rapidly test multiple candidate antigen designs, rapidly produce GMP material, an established safety profile in humans, and a relatively straightforward regulatory pathway into clinical evaluation.

Antigen design was guided by prior knowledge about humoral immunity to flaviviruses. Vaccine-elicited neutralizing antibodies (NAb) are associated with protection from flavivirus-mediated disease (4). Because the most potent monoclonal flavivirus NAbs map to conformational epitopes in domain III (DIII) of the E protein (5), or more complex quaternary epitopes that bridge between antiparallel E dimers or between dimer rafts arrayed on the virus surface (6, 7), our goal was to identify constructs that produced particles that faithfully capture the antigenic complexity of infectious virions. Expression of the structural proteins premembrane (prM) and envelope (E) are sufficient for the production and release of virus-like subviral particles (SVPs) with antigenic and functional properties similar to those of infectious virions (8, 9).

To identify promising vaccine candidates, prM-E constructs were synthesized and screened for expression and efficiency of particle release from transfected cells. prM-E sequences were inserted into a CMV-immediate early promoter-containing vector (VRC8400) evaluated clinically in several prior studies (3, 10, 11). These constructs are distinct from one reported in recent studies from Larocca et al. (12) and Abbink et al. (13) that was based on a Brazilian isolate (strain BeH815744) and did not express the first 93 amino acids of prM, encoding only the short M peptide that is the product of furin cleavage of prM during natural infection. Because prM plays a critical role in folding of the E protein and release of particles from cells (14, 15), it is not known how the antigenicity of that product compares to the prM-E product described here. The prM-E sequence in the current constructs was selected from a French Polynesian isolate (strain H/PF/2013) identical or highly related to strains circulating in the Americas. Neutralization studies using contemporary sera against temporally and geographically diverse strains indicate ZIKV exists as a single serotype, suggesting a single vaccine antigen will provide protection against all ZIKV strains (16). To improve expression, the ZIKV prM signal sequence was exchanged with the analogous region of Japanese encephalitis virus (JEV), as previously reported (17), to create vector VRC5283 (Fig. 1A). A second chimeric ZIKV/JEV prM-E construct, VRC5288, also encoding the JEV signal sequence, was designed with the final 98 amino acids of E, comprising the stem and transmembrane regions (ST/TM), exchanged with corresponding JEV sequences and previously shown to improve SVP secretion (18). Both vectors exhibited expression by mammalian cells (Fig 1B, right panel), with more efficient SVP release into supernatants by VRC5288 (Fig. 1B, right panel, and Fig. 1C) (19). Electron microscopic analysis of negative-stained purified VRC5288 SVP preparations revealed roughly spherical particles consistent with the appearance of other flavivirus SVPs (Fig. 1D) (8, 20).

Next we assessed the immunogenicity of each DNA candidate in BALB/c and C57BL/6 mice. Mice were immunized intramuscularly once with 50 μg of DNA in the quadriceps using electroporation as previously described (21). Serum was evaluated for binding to ZIKV SVPs (fig. S1A) and neutralizing activity using ZIKV reporter virus particles (RVPs) (fig. S1B–D) (16). Vaccination with either VRC5283 or VRC5288 elicited ZIKV-specific NAbs after a single immunization with titers up to 10^5 reciprocal EC_{50} serum dilution in C57BL/6 mice (fig. S1D). NAb titers were similar in mice vaccinated with 2, 10, or 50 μg DNA (fig. S2), and were of similar magnitude to titers induced by a previously described WNV DNA vaccine (fig. S3) (3).

Immunogenicity in rhesus macaques was evaluated after delivering vaccine intramuscularly by a needle-free injection device (PharmaJet) (Fig. 2, fig. S4 and S5). Six animals per group received two 1 mg (VRC5283) or 4 mg (VRC5283 and VRC5288) doses of vaccine at 0 and 4 weeks, while one group received a single 1 mg dose of VRC5288 at week 0. After a single dose of DNA, binding and neutralizing antibody were detectable by week two and peaked at week three. All ZIKV vaccine groups had significantly higher NAb responses than macaques that received VRC8400 control vector when comparing area-under-the-curve (AUC) using a Kruskal-Wallis test (p=0.022, Fig. 2D). Macaques that received a single 1 mg dose of VRC5288 had significantly lower NAb titers than macaques that received two doses of either vaccine at either dose level (p=0.022). There were no significant differences in NAb titer between animals that received two doses of VRC5283 or animals that received two

doses of VRC5288 comparing AUC. Sera collected at week 6 were also evaluated for NAb activity by the conventional focus-reduction neutralization test (FRNT) (22, 23) and a microneutralization (MN) assay (12, 13, 24). The results of both assays strongly correlated with EC_{50} RVP values (fig. S6, table S1), although the RVP assay was more sensitive as demonstrated by detection of neutralizing activity in macaques that received only a single 1 mg dose of VRC5288 compared to MN results (average week 6 EC_{50} reciprocal serum NAb titers of 322 versus <10 for RVP and MN assays, respectively). Further comparison of these values suggested MN values above the limit of detection corresponded more closely to EC_{90} RVP values (1.3-fold versus 9.6-fold average difference in RVP EC_{90}/MN EC_{50} and RVP EC_{50}/MN EC_{50} NAb titers, respectively, for all animals at week 6). These data indicate that both VRC5283 and VRC5288 elicit substantial ZIKV-specific NAb in macaques.

Eight weeks after the first immunization, all animals were challenged subcutaneously with 10^3 focus-forming units (FFU) of the Puerto Rican ZIKV strain PRVABC59 (GenBank KU501215.1) and blood was collected daily for quantitative PCR analysis of ZIKV genome copies in plasma (13, 19). Control animals showed peak virus load (VL) on day 3 or 4 between 10^4 and 10^6 genome copies/ml. Animals that received two doses of 4 mg or 1 mg of VRC5283 or 4 mg of VRC5288 were largely protected from viremia with 17 of 18 animals having no detectable viremia (Fig. 3A). One animal that received two 4 mg doses of VRC5288 had a low-level positive PCR in one of two assays performed on day 3 and another positive blip at day 7. All six animals that received a single dose of 1 mg of VRC5288 were viremic with peak VL on day 3 between 10^2 and 10^5 genome copies/ml. This VL was significantly reduced compared to animals that received two doses of VRC8400 control vector comparing AUC by a Wilcoxon Exact Test (two-sided p=0.041). The cutoff for low values has been established at <100 genome copies/ml, so it cannot be ruled out that low level viremia may have occurred in other animals.

Seventeen of eighteen (94%) of animals that received two doses of vaccine had no detectable viremia post-challenge. The animal with blips above background at day 3 and 7 in the VRC5288 two-dose 4 mg group had a prechallenge reciprocal EC_{50} NAb titer of 1218, which was among the lowest titers of all two-dose vaccine groups (Fig. 4A). A probability analysis indicated that one could anticipate a 70% protection from viremia if a reciprocal EC_{50} serum NAb titer of 1000 is achieved in the RVP assay (Fig. 4B). This corresponds roughly to a reciprocal EC_{50} MN titer of ~100 (fig. S6) which is similar to the titer of NAb shown to prevent viremia in nonhuman primates passively treated with immune serum (13).

Animals receiving a single dose of 1 mg VRC5288 had prechallenge reciprocal EC_{50} NAb titers measured by the RVP assay between 203 and 417. The two animals with the highest NAb activity were the ones with delayed onset of viremia at day 3. MN titers at the 6-week timepoint, as noted above, were <10 in the 1 mg single dose group animals that uniformly had breakthrough infection (table S1). Therefore, the larger dynamic range of the RVP assay will allow a more precise definition of the protective threshold needed to prevent viremia in a particular model or against a particular challenge inoculum (fig. S5 and S7).

One concern routinely raised about vaccination against flaviviruses is the possibility of enhanced disease if there is incomplete or waning immunity, as observed in a subset of

secondary dengue virus infections (25). In this study, the 1 mg single-dose group that received VRC5288 had low, sub-protective levels of NAb that resulted in breakthrough infections. In those animals, there were reduced levels of viremia compared to unvaccinated controls and no visible signs of illness or enhancement of replication. Retrospectively, we also determined that one animal in the mock-immunized control group and one in the single-dose 1 mg VRC5288 group with detectable levels of ZIKV antibody binding, but no neutralizing activity, had pre-existing WNV-specific NAbs (fig. S8). The levels of virus replication in these animals were near the group average and there was no evidence of disease enhancement in the setting of prior flavivirus exposure.

Vaccine development for ZIKV must be specific and guided by an expanded understanding of ZIKV virology, pathogenesis, immunity, and transmission. It must also be strategic, matching technical and manufacturing feasibility with the target populations that will benefit most from vaccination. In addition, to achieve both rapid deployment and long-term protection, it should be staged. This means that a rapid response to the global health emergency may require a different vaccine approach than the longer term goal of achieving durable immunity in the general population as ZIKV becomes a sporadic, endemic infection. Both VRC5288 and VRC5283 will be evaluated in humans. A Phase 1 clinical trial (NCT02840487) of VRC5288 has launched to test a variety of regimens and doses for safety and immunogenicity. These trials represent the initial efforts to define the level of vaccine-induced NAbs required for prevention of ZIKV viremia. Establishing a functional serological correlate of sterilizing immunity is key for leveraging the information gained from efficacy trials from one candidate vaccine to the next. The Phase 1 clinical trials with VRC5288 and VRC5283 are being designed in parallel with other groups who will evaluate a purified, protein-based, whole-inactivated ZIKV vaccine (ZPIV) or live-attenuated vaccine approaches. These studies and others to evaluate alternative antigen designs, delivery approaches, and combination vaccine regimens will provide safety and immunogenicity data in humans that will inform the next steps of vaccine development and provide options for achieving both the short-term goal of identifying an intervention to protect women of child-bearing age in the current ZIKV outbreak, and the long-term goal of vaccinating the general population of endemic regions and travelers to those regions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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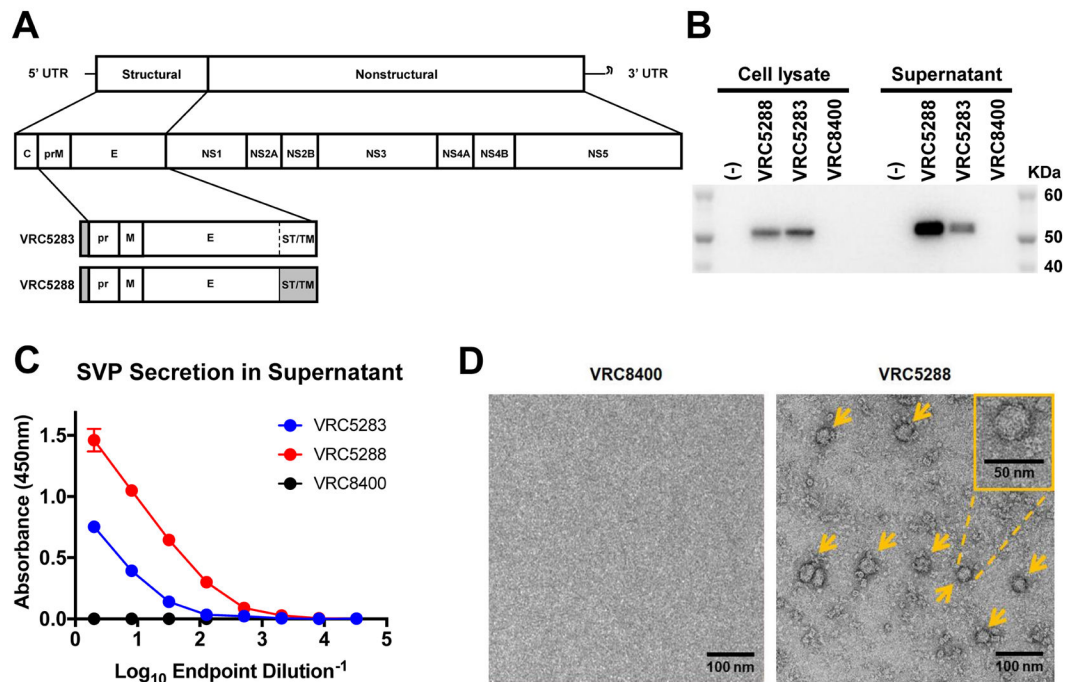
Figure 1. ZIKV DNA vaccine design and characterization

(**A**) Schematic representation of ZIKV genome and ZIKV DNA vaccine constructs VRC5283 and VRC5288. (**B**) Expression and secretion of ZIKV E was analyzed by Western blot of transfected 293T cell lysates and SVP precipitate pelleted from culture supernatants through a 20% sucrose cushion demonstrating that the VRC5288 construct secretes more particles than VRC5283. (**C**) Particle-capture ELISA quantifying the secretion of ZIKV SVP from transfected cells. (**D**) ZIKV subviral particles (SVP) were purified from the culture supernatant of VRC5288-transfected 293-F cells and subjected to negative staining and electron microscopy. SVP are labeled with arrowheads. The VRC8400 empty backbone plasmid vector was used as a control.
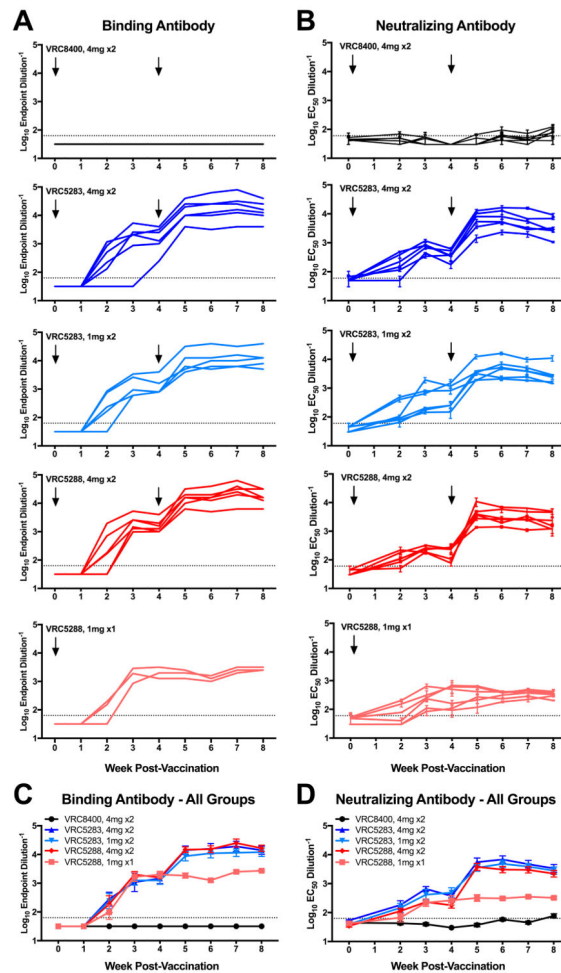
Figure 2. ZIKV DNA vaccines elicit robust binding and neutralizing antibodies in nonhuman primates

Rhesus macaques (n=6/group) were either mock immunized with VRC8400 empty backbone expression plasmid or with VRC5283 or VRC5288 vaccine plasmids intramuscularly with the indicated doses and number of vaccinations. **(A)** Macaque sera were assayed weekly for ZIKV binding antibodies by ELISA. Each line represents the average titer of an individual animal from 1–2 technical duplicates and the dashed line indicates the limit of detection (reciprocal titer of 64). Any measurement below the limit of detection was assigned a value of half the limit of detection for graphing and statistical purposes. **(B)** The NAb response elicited by vaccination was analyzed using ZIKV reporter virus particles (RVPs). The dilution of sera required for half-maximal inhibition of virus infection (EC_{50}) was estimated by non-linear regression analysis. Lines connect the average EC_{50} values of 2–5 independent experiments, each performed with duplicate technical replicates, for the individual monkeys in each group at each timepoint. Error bars denote the standard error of mean. The dotted line denotes the limit of confidence for the RVP assay (reciprocal titer of 60). Measurements below the limit of detection were assigned a value of 30. The average binding antibody **(C)** and NAb **(D)** responses for each vaccine group are shown. Error bars denote the standard error of the mean.
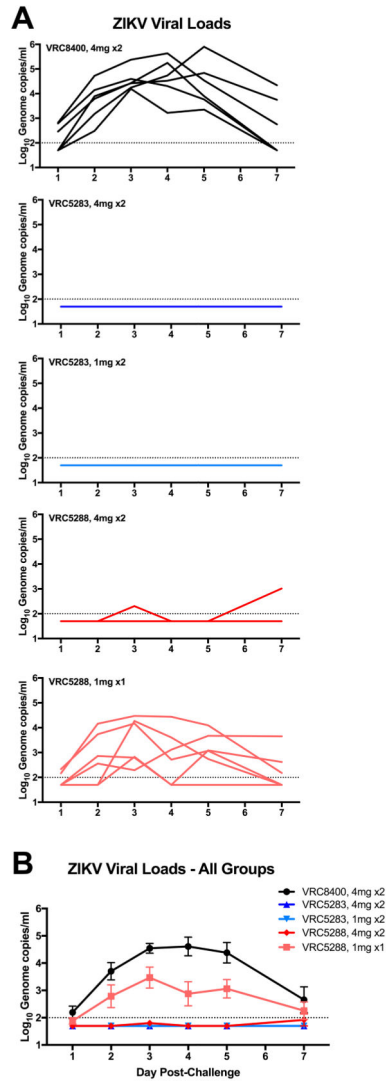
Figure 3. ZIKV DNA vaccines reduce viremia in ZIKV-challenged rhesus macaques
Eight weeks after the first vaccination, macaques were challenged with 10^3 FFU of ZIKV PRVABC59. **(A)** qPCR of the capsid gene was used to determine the genome copies/ml on days 1–5 and 7 post-challenge. Each line represents an individual animal. **(B)** Mean viral load after challenge in each group. Error bars represent the standard error of the mean. Dashed line indicates the limit of detection (100 copies/ml). Any value below the limit of detection was assigned a value half the limit of detection for graphing and AUC calculation.
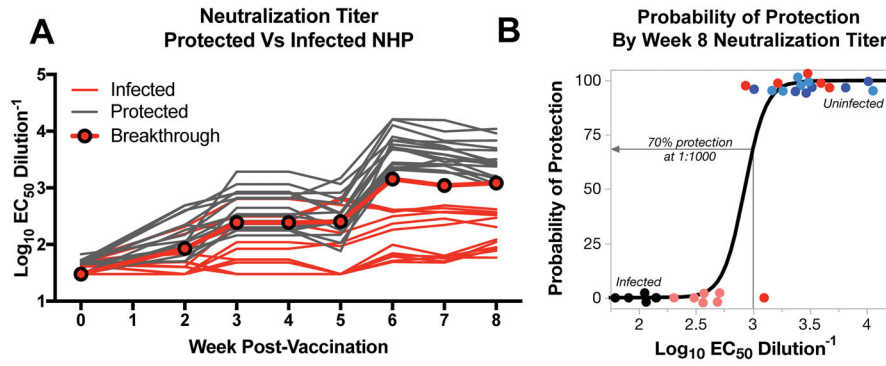
Figure 4. Protection from ZIKV challenge correlates with NAb titers present at challenge
Animals that had detectable viremia post-challenge were analyzed with respect to pre-challenge NAb activity. **(A)** The reciprocal EC_{50} NAb titer of each animal is individually plotted to reflect whether infection occurred or not. Lines indicate individual animals. Protected (no detectable viremia) and infected (viremia detectable on two successive days) animals are represented by gray and red lines, respectively. The sole animal that received two 4 mg doses of VRC5288 and was found to have a low level of viremia on days 3 and 7 after challenge is denoted as "breakthrough" (black outlined dots). **(B)** The probability of infection (Logit) based on the reciprocal EC_{50} NAb titer is demonstrated and indicates that prevention of viremia would be expected in approximately 70% of animals with NAb titers >1000.