DNA Vaccine Initiates Replication of Live Attenuated Chikungunya Virus In Vitro and Elicits Protective Immune Response in Mice

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Background. Chikungunya virus (CHIKV) causes outbreaks of chikungunya fever worldwide and represents an emerging pandemic threat. Vaccine development against CHIKV has proved challenging. Currently there is no approved vaccine or specific therapy for the disease.

Methods. To develop novel experimental CHIKV vaccine, we used novel immunization DNA (iDNA) infectious clone technology, which combines the advantages of DNA and live attenuated vaccines. Here we describe an iDNA vaccine composed of plasmid DNA that encode the full-length infectious genome of live attenuated CHIKV clone 181/25 downstream from a eukaryotic promoter. The iDNA approach was designed to initiate replication of live vaccine virus from the plasmid in vitro and in vivo.

Results. Experimental CHIKV iDNA vaccines were prepared and evaluated in cultured cells and in mice. Transfection with 10 ng of iDNA was sufficient to initiate replication of vaccine virus in vitro. Vaccination of BALB/c mice with a single 10 μg of CHIKV iDNA plasmid resulted in seroconversion, elicitation of neutralizing antibodies, and protection from experimental challenge with a neurovirulent CHIKV.

Conclusions. Live attenuated CHIKV 181/25 vaccine can be delivered in vitro and in vivo by using DNA vaccination. The iDNA approach appears to represent a promising vaccination strategy for CHIK and other alphaviral diseases.

Keywords. Chikungunya virus; Chikungunya fever; CHIKV; DNA vaccine; live attenuated vaccine; alphavirus.

Chikungunya virus (CHIKV) causes chikungunya fever, an emerging global infectious disease. The virus belongs to the Alphavirus genus of Togaviridae family, which hosts several mosquito born pathogenic arboviruses [1, 2]. CHIKV is transmitted to humans primarily by Aedes aegypti and Aedes albopictus mosquitoes [3–5] and has a major health impact, with complications that include arthralgia, respiratory failure, cardiovascular disease, hepatitis, and central nervous system problems, especially in elderly individuals and children [6, 7]. CHIKV is found worldwide, with nearly 40 countries reporting endemic or epidemic chikungunya fever, mostly in warm climates in Asia, Africa, and, recently, Europe [8]. Recent epidemics of CHIKV infection included the 2005–2006 outbreak in La Reunion islands in the Indian Ocean that caused 284 deaths, and the epidemic in India, with an estimated 1.3 million people infected [9, 10]. With an increase in global travel, the risk for spreading CHIKV to areas where it is not endemic has increased [11]. Climate changes and urbanization also favor geographical expansion of CHIKV [12–14]. Travel-associated cases have been recorded in Europe, Australia, and the United States, and some travelers were viremic [15]. Given the current large epidemics and the worldwide distribution of A. aegypti and A. albopictus, there is a risk of an emerging CHIKV infection pandemic [16]. Currently there are no approved CHIKV vaccines, in part because of the challenging task of balancing vaccine safety and
Several promising experimental vaccines have been developed, including live attenuated vaccine 181/25 (TSI-GSD-218), which has been evaluated in a phase II clinical trial [17]. The clinical trial, which enrolled 59 healthy volunteers, has shown that vaccination resulted in successful seroconversion in 98% of the volunteers, with mild reactogenicity in 8% of volunteers experiencing transient arthralgia [17]. Recent study showed that attenuation of the strain in the 181/25 vaccine relies on 2 independently attenuating mutations within the E2 protein [18]. In at least one case, adverse effects were linked to a genetic reversion [18, 19]. Therefore, although 181/25 vaccine can be useful for an emergency response [20], improvement of the vaccine is needed. Elsewhere, we described a novel immunization DNA (iDNA*) vaccination platform that is based on a molecular clone technology that combines advantages of DNA and live attenuated vaccines [21]. Because of the genetic stability of double-stranded DNA, the iDNA vaccination approach may be advantageous over the standard live attenuated vaccine approach. In the current study, iDNA vaccines were prepared that encoded the full-length infectious RNA genome of live attenuated vaccine 181/25. The characteristics of the iDNA vaccines were evaluated in vitro and in vivo.

METHODS

Cell Lines and Viruses
Chinese hamster ovary (CHO) and African green monkey Vero cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA) and maintained in a humidified incubator at 37°C and 5% CO₂ in α minimal essential medium (αMEM) supplemented with 10% fetal bovine serum (FBS) and gentamicin sulfate (10 μg/mL) (Life Technologies, Carlsbad, CA). The CHIKV strain 181/25 live attenuated vaccine TSI-GSD-218 was obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA). The neurovirulent Ross strain of CHIKV was a standard challenge stock used for challenge of mice in the biosafety level 3+ (BSL3+) laboratory at the University of Texas Medical Branch in Galveston [22].

Plasmids and Preparation of iDNA

The CHIKV clone 181/25 vaccine virus was propagated in CHO cells in a 75-cm² flask. At 48 hours after infection, the virus was harvested, clarified, and frozen at −80°C. Viral RNA was extracted by using Trizol LS (Life Technologies), and 4 overlapping complementary DNA (cDNA) fragments were generated
by using the One-Step reverse-transcription polymerase chain reaction (PCR) system and high-fidelity Pfx polymerase in the presence of CHIKV-specific oligonucleotide primers. The cDNA fragments were cloned into the pCRII-TOPO plasmid, and the cDNA sequences were compared to the 181/25 cDNA fragments were cloned into the pCRII-TOPO plasmid (Figure 1). To ensure authentic 5′ and 3′ ends of the transcribed CHIKV genomic RNA, the distance between the CMV promoter and viral genome was optimized and the hepatitis delta virus (HDV) ribozyme was placed immediately after the viral polyA sequence. A kanamycin-resistant iDNA plasmid, p181/25-1, was prepared by transferring AscI-NolI fragment encompassing the entire cDNA sequence into the pCRII plasmid. The third iDNA plasmid, p181/25-39, was prepared from the p181/25-7 plasmid by inserting a second copy of 181/25 26S subgenomic promoter between the 181/25 virus capsid and glycoprotein genes. For this purpose, a translational stop codon was introduced into the 181/25 capsid gene, while the translational start of the ATG codon was introduced into the 181/25 glycoprotein open reading frame (Figure 1).

Transfections and Assays In Vitro
CHO or Vero cells were transfected by electroporation of plasmid iDNA at concentrations ranging from 1 ng to 5 μg. Transfection of both CHO and Vero cells was performed essentially as described previously [21, 23]. As controls, cells were incubated with \(10^2\)–\(10^5\) plaque-forming units (PFUs) of CHIKV 181/25 virus. Expression of CHIKV antigens in iDNA-transfected and virus-infected cells was detected by immunofluorescence assay (IFA) and Western blot, using CHIKV hyperimmunee mouse ascitic fluid (HMAF) VR-1241AF (ATCC). CHIKV antigens were also confirmed by Western blot, using convalescent human antiserum (UTMB; courtesy of Dr Robert Tesh). Finally, virus presence in the growth medium was confirmed by plaque assay in duplicates. Averages and SDs were determined. Each experiment was done at least 2 times to ensure reproducibility of the results. For virus growth curves, samples were harvested at indicated intervals and quantitated in duplicate by a plaque assay in Vero cell monolayers in 6-well plates.

Immunizations and Serologic Analysis
Animal experiments adhered to the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. The iDNA plasmid was isolated from *Escherichia coli* by using an endotoxin-free method (Promega, Madison, WI) and formulated in phosphate-buffered saline to a final concentration of 0.2 mg/mL. Three-week-old female BALB/c mice were anesthetized with isoflurane and vaccinated intramuscularly with a dose of 50 μL of p181/25 iDNA vaccine in the medial thighs. After injection of DNA, animals were electroporated at the site of injection using a 2-pin electrode and a square wave electroporator (ECM 830; BTX Genetronics, San Diego, CA) as described elsewhere [21]. CHIKV 181/25 virus (10^5 PFU) was injected subcutaneously as a control. After vaccinations, experimental and control animals were observed daily for clinical signs of infection. Furthermore, although weight loss is not a parameter of CHIKV infection in this model, body weights were determined on days 1–7, 14, and 21 after vaccination. Sera were collected on days 2 and 4 for viremia testing and on day 21 for antibody response determination before viral challenge. For viremia detection, serum was pooled and incubated on Vero cells for 3 days, observation for cytopathic effects (CPEs) and plaque assay of the medium were performed. A plaque reduction neutralization test (PRNT), IFA, and Western blot were performed to determine antibody responses to CHIKV. Virus-neutralizing antibodies to CHIKV were determined in Vero cells by the PRNT. For Western blot, protein lysates of CHIKV-infected Vero cells were separated using 4%–12% gradient sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and probed with antisera. For IFA, Vero cells were infected with 10^2 PFUs/well in 8-well chamber slides with 181/25 virus for 24 hours in αMEM containing 10% FBS. IFA was done with or without the propidium iodide nuclear counterstain to visualize cell nuclei.

Challenge
For experimental challenge, mice were transferred into the BSL3+ facility described above and challenged with virulent CHIKV Ross strain at a dose of 6 × 10^6 PFUs in 20 μL by the intranasal route [22]. Blood samples were collected for 3 days after challenge to detect viremia. The statistical significance of differences in virus titers between vaccinated and control animals was determined by the Student t test.

RESULTS
Preparation of CHIKV p181/25 iDNA
The CHIKV live attenuated vaccine TSI-GSD-218, clone 181/25, was passed once in CHO cells. Viral RNA was isolated from passage 1 virus and used for preparation of CHIKV cDNA. Four cDNA fragments spanning the complete genome of CHIKV 181/25 virus were generated by reverse-transcription and high-fidelity PCR. The sequences of cDNA clones were determined by using CHIKV sequence-specific oligonucleotide primers to confirm cDNA sequences to the published CHIKV 181/25 sequence (TSI-GSD-218; GenBank accession L37661). Sequencing revealed the presence of genetic variants within the amino-terminal region of the nonstructural polyprotein.
For example, only 1 of 7 sequenced cDNA clones, clone 3.5–40, contained Ile301 residue in the nsP1 that was identical to the published sequence of 181/25 (Figure 1A). The remaining 6 clones contained Thr301 characteristic for the wild-type CHIKV, as well as for the VR1 isolate from the 181/25-vaccinated viremic patient who developed mild arthralgia [18, 19]. Heterogeneity was also detected at residue 314 (Figure 1A). Although neither amino acid residues 301 nor 314 are responsible for attenuation [18], the presence of genetic variants within the virus population may contribute to a phenotypic heterogeneity of the 181/25 vaccine.

Sequence-confirmed cDNA fragments were combined within the pcDNA3.1-derived plasmid to generate the p181/25-7 iDNA plasmid containing the full-length cDNA of clone 181/25 genomic RNA downstream from the CMV major immediate-early promoter (Figure 1). Because the authentic 5′- and 3′-termini of RNA are critically important for alphavirus replication [2], the CMV promoter and the HDV ribozyme regions were optimized to ensure transcription of the functional 181/25 genomic RNA. Two additional CHIKV iDNA variants were also prepared (Figure 1). To show the applicability of the iDNA approach for engineering of new CHIKV vaccines, the p181/25-39 iDNA was prepared by inserting a duplicate 26S subgenomic promoter between the 181/25 capsid and glycoprotein genes (Figure 1B). Previous studies have shown that 2 genes can be expressed from an alphavirus in a tandem fashion [24]. Finally, the p181/25-1 iDNA variant was made by replacing the pcDNA3.1 vector backbone in the p181/25-7 with the pCRII backbone to confer kanamycin resistance to the iDNA plasmid. Thus, both p181/25-7 and p181/25-1 encoded the CHIKV 181/25 sequences and differed only in the vector backbone and antibiotic resistance gene.

**Launch of Live Attenuated Vaccine Virus Replication From iDNA In Vitro**

The p181/25 iDNA plasmids were isolated from *E. coli* by a process that resulted in a sterile DNA molecule with a 95% supercoiled fraction and an A260/A280 ratio of approximately 1.9. To initiate replication of live 181/25 virus in vitro, 5 µg of iDNA plasmids were transfected into CHO cells by using electroporation. The transfected cells were analyzed for expression of CHIKV antigens, and the growth medium was examined for the presence of replicating virus. Aliquots of transfected cells were seeded into chamber slides for expression of CHIKV antigens after transfection of iDNA plasmid is shown on the right, detected by immunofluorescence assay (IFA) 48 and 96 hours after transfection. Aliquots of transfected cells were seeded in 8-well chamber slides, fixed at indicated times in cold acetone, and processed by IFA, using mouse CHIKV-specific antibody, followed by fluorescein isothiocyanate–conjugated secondary antibody. B, Detection of CHIKV antigens in transfected CHO cells by Western blot (left) and in the growth medium by plaque assay 48 hours after transfection (middle). For comparison, plaque assay for the 181/25 virus vaccine (passage 1 in CHO cells) is shown. Right panel shows growth curve of p181/25-7 iDNA-derived virus (average of 3 experiments). Western blot was done using human convalescent-phase CHIKV-specific serum (lane 1) and CHIKV HMAF (lane 2). The PE2, E2, E1, and C antigens are indicated.
antigens. Intracellular expression of CHIKV antigens was observed 48 hours after transfection by IFA, using specific antiserum (Figure 2A). At 96 hours (day 4) after transfection, all cells were positive, suggesting replication of live 181/25 virus. As expected for an alphavirus, expression of CHIKV antigen was found in the cytoplasm of cells (Figure 2A). SDS-PAGE and Western blot confirmed the presence of CHIKV structural proteins, including the glycoproteins (PE2, E1, and E2) and capsid (C) protein (Figure 2B). In Western blot, human convalescent serum reacted with CHIKV glycoproteins, whereas mouse HMAF reacted only with capsid. This can be explained by the fact that mouse HMAF has been prepared against the S27 isolate of CHIKV, which has 39 amino acid substitutions in the capsid and glycoprotein region, compared with the 181/25 virus. The presence of the virus in transfected cell culture supernatants was assessed by direct plaque assay. Both small and larger plaques were detected in the iDNA-derived virus, potentially reflecting assay variation or genotypic differences within the virus. However, some of the largest plaques were detected in the parental 181/25 virus by plaque assay (Figure 2). Comparative sequencing analysis of the viruses is underway.

We next compared replication of vaccine viruses in the iDNA-transfected and virus-infected Vero cells and determined the minimal amount of iDNA required to launch the virus (Figure 3). Briefly, $10^7$ Vero cells in 75-cm$^2$ flasks were either (1) transfected by electroporation with iDNA plasmid vaccines at the doses ranging from 1 ng to 1 μg or (2) infected with $10^4$–$10^5$ PFUs of CHIKV 181/25 virus. To confirm transfection with iDNA or infection with the virus, IFA was performed using mouse anti-CHIKV antiserum 12 hours after transfection or infection. Samples of the growth medium were collected from transfected and infected cells every 12 hours, and the viruses were quantitated by plaque assay (Figure 3). The rates of virus replication depended on the initial titer of the virus in infection or on the amount of iDNA in transfection. Virus-infected cells rapidly reached maximum titers, whereas in iDNA-transfected cells an approximately 24-hour delay in the maximum virus titer was observed (Figure 3). In either virus-infected or iDNA-transfected Vero cells, the virus titers reached $10^8$–$10^9$ PFUs/mL. All 3 iDNA clones, p181/25-7, p181/25-1, and p181/25-39, successfully initiated replication of vaccine viruses when cells were transfected with 1 μg of the respective plasmids. The virus was successfully launched in transfections containing 1 μg, 100 ng, or 10 ng of p181/25-39 DNA but not in transfection containing 1 ng of p181/25-39 DNA (Figure 3). Virus replication in the cells transfected with 1 μg or 100 ng of p181/25-39 showed comparable titers, whereas a delay in the peak titer was observed when cells were transfected with only 10 ng of p181/25-39 iDNA.

**Immunogenicity and Efficacy of CHIKV iDNA Vaccine in Mice**

To determine whether p181/25-7 “naked” iDNA plasmid can be used for vaccination against CHIKV in vivo, 2 experiments in BALB/c mice were performed. Figure 4. Detection of serum antibody in sera from vaccinated BALB/c mice, by immunofluorescence assay. A. Mice 1–8 were vaccinated intramuscularly by electroporation in vivo with 10 μg of immunization DNA (iDNA) p181/25-7. B. Mice 9–16 were injected subcutaneously with $10^5$ plaque-forming units of live 181/25 virus. Sera were obtained at day 21 after vaccination and probed at a dilution of 1:10, followed by fluorescein isothiocyanate–conjugated antimouse antibody. Chikungunya virus (CHIKV)–positive reaction is indicated by green fluorescent foci. In this experiment, we used the propidium iodide nuclear counterstain to visualize cell nuclei [28]. Red fluorescence indicates nuclear staining.
young-adult immunocompetent inbred BALB/c mice were conducted. BALB/c mice have been previously used for CHIKV vaccine evaluation [25, 26]. In the first study (immunogenicity), 2 groups of 8 mice were vaccinated with either a single intramuscular injection of 10 μg of iDNA followed by electroporation, or injected subcutaneously with 10⁵ PFUs of 181/25 virus. After immunizations, all mice remained healthy, with no detectable pathology at the site of injection or adverse effects due to vaccinations. Consistent with the clinical trial of 181/25 vaccine [27], no viremia was detected on days 2 and 4 in both iDNA- and virus-vaccinated groups either by direct plaque assay or by incubating pooled sera with Vero cells followed by CPE and plaque assays. On day 21, all experimental and virus-control mice successfully seroconverted, as shown by Western blot, IFA, and PRNT, suggesting replication of vaccine virus in vivo (Figure 4 and Table 1). To detect antibodies by IFA, Vero cells were initially infected with CHIKV, fixed, and then probed with the mouse sera. In one experiment, IFA was done in the presence of the propidium iodide nuclear counterstain, and all antisera showed green CHIKV-specific fluorescent foci on the red fluorescent nuclear background (Figure 4). In the PRNT, the titers of neutralizing antibodies were approximately 6-fold higher in the iDNA-vaccinated animals, compared with the virus-vaccinated controls (Table 1).

In the second study (immunogenicity and efficacy), a group of 10 BALB/c mice were similarly injected/electroporated intramuscularly with 10 μg of iDNA. Again, on day 21, all mice except unvaccinated controls have successfully seroconverted, as determined by IFA and PRNT findings (Figure 5 and Table 2). In other studies, similar antibody titers have proven protective in the lethal immunocompromised A129 mouse model [29, 30]. For vaccine efficacy testing, mice were transferred into a BSL3+ facility and challenged intranasally at day 28 after vaccination with 6 × 10⁶ PFUs in 20 μL of neuro-adapted Ross CHIKV strain [31]. Since Ross CHIKV BALB/c model does not result in fatal disease when mice exceed six weeks of age, viremia was monitored in all challenged animals. On day 1, viremia was not detectable in any vaccinated mice, whereas all unvaccinated controls had high levels of viremia (Table 2). Similarly, viremia was not detectable in vaccinated animals on day 2 after challenge, whereas virus was still detectable in 2 of 5 animals in the unvaccinated group at levels of 100 PFUs/mL (Table 2), thus demonstrating a significant (P < .05) protective efficacy of experimental p181/25 iDNA vaccine in immunocompetent BALB/c mice.
DISCUSSION

CHIKV is an emerging pathogen causing outbreaks of chikungunya fever. There is no approved CHIKV vaccine or specific antiviral therapy. Current treatments are supportive and involve antinflammatory drugs, steroids, and fluids. Experimental treatments include ribavirin, chloroquine, interferon-α, and furin inhibitor dec-RVKR-cmk [32–35]. Candidate vaccines include standard DNA-based vaccines expressing CHIKV genes [26, 36], a vaccine composed of formalin-inactivated virus in combination with aluminum hydroxide (Alhydrogel) adjuvant [37], as well as rationally designed live attenuated virus [22, 29], virus-like particle [38], viral vector [39], and subunit [40] vaccines. The most-advanced experimental human vaccine in the clinic contains live attenuated CHIKV strain 181/25 [17, 20]. In a phase II clinical study, the 181/25 vaccine showed favorable safety and efficacy, with minor adverse effects [17]. In the absence of licensed vaccines and a global threat of CHIKV pandemic, the 181/25 vaccine can be an option for emergency vaccinations and a logical starting point for further improvement [20]. In this study, we configured recently developed iDNA methods [21] for the 181/25 vaccine to prepare a novel CHIKV vaccine. The 181/25 iDNA plasmid allowed production of live vaccine virus in vitro, as well as successful vaccination against CHIKV in vivo. Generally, the iDNA approach resembles the traditional so-called infectious clone technology but does not involve in vitro RNA transcription. Essentially, iDNA technology combines advantages of DNA and live attenuated vaccines [21]. Advantages of DNA vaccines include ease of production, as well as chemical and genetic stability. However, the clinical success of traditional DNA vaccines has been limited because of low immunogenicity and the need for multiple booster vaccinations with large quantities of DNA. Here we demonstrated that a single small dose of CHIKV iDNA is needed to start production of live vaccine virus. In our experiments, only 10 ng of iDNA was sufficient to start vaccine virus replication in cell culture (Figure 3). In a previous study, we found that 8 ng of pTC83 iDNA successfully initiated replication of VEEV TC-83 vaccine virus [21]. The CHIKV iDNA can also be used for successful vaccination via launching live attenuated vaccine in vivo.

In addition to extending the use of DNA immunization, the CHIKV iDNA technology can also provide improvement to the live attenuated 181/25 vaccine. Some adverse effects in 181/25 vaccine have been linked to reversion mutations [19]. It is not clear whether reversion occurred during vaccine replication in vivo or whether reversion mutation was already present in the formulated vaccine. The 181/25 vaccine was made by 18 plaque-to-plaque passages in MRC-5 cell culture [41]. Our sequencing analysis of the 181/25 cDNA fragments demonstrated vaccine polymorphism (Figure 1). Additional examination of sequence polymorphism of the parental 181/25 virus is underway. In addition to the possibility of reversion mutations, limitations for classic live vaccine such as 181/25 include the need for a cold chain, which may account for up to 80% of the vaccine cost in warm climates [42]. These limitations restricted the use of many live attenuated vaccines, including the 181/25 vaccine. In contrast, the iDNA vaccine is easy to prepare and formulate to be stable at ambient temperatures. Unlike live vaccine virus, iDNA represents a genetically defined molecular clone, which may have a safety and regulatory advantage for vaccine applications. Potentially, the iDNA clone can serve as a reverse genetic system for preparation of improved CHIKV vaccines, as well as a reference source for the production of live or killed CHIKV vaccines with improved homogeneity and reduced probability of reversion mutations. Vaccination with iDNA directly in vivo further reduces the number of vaccine replication cycles, thus also reducing the probability of reversion mutations.

In summary, CHIKV iDNA vaccine may offer certain advantages over both the traditional DNA vaccines and the live attenuated vaccines and could represent a feasible option for emergency vaccination to prevent epidemics. The requirement for emergency vaccine in resource-deficient environments is the necessity to elicit protective immunity with a single-dose vaccination [42]. We have shown that CHIKV iDNA vaccine induces a protective immune response in mice after a single vaccination. However, because this is a novel approach, more research is still

Table 2. Vaccination of BALB/c Mice With p181/25-7 Immunization DNA (iDNA) Vaccine and Challenge With Chikungunya Virus (CHIKV)

<table>
<thead>
<tr>
<th>CHIKV Vaccinea</th>
<th>Animals, No.</th>
<th>Seroconverted/Total (%)b</th>
<th>Virus Neutralization Titer, PRNT80 Range (Mean)</th>
<th>Challenge Dose, PFUc</th>
<th>Viremic/Total (%); Titer, Log10 PFU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>p181/25-7CHIKV iDNA</td>
<td>10</td>
<td>10/10 (100)</td>
<td>160–1280 (367.58)</td>
<td>6 × 10⁶</td>
<td>0/10 (0); &lt;0.9</td>
</tr>
<tr>
<td>Mock PBS</td>
<td>5</td>
<td>0/5 (0)</td>
<td>Not detected</td>
<td>6 × 10⁶</td>
<td>5/5 (100); 3.04 + 0.53</td>
</tr>
</tbody>
</table>

Abbreviations: PBS, phosphate-buffered saline; PFU, plaque-forming units.

a BALB/c mice were vaccinated by intramuscular injection-electroporation of 10 μg of p181/25-7 iDNA (Figure 1).

b Detected by immunofluorescence assay.

c Challenge was performed intranasally with 6 × 10⁶ PFU of CHIKV-Ross virus in a 20-μL volume as described elsewhere [22].
needed, including evaluation of nonelectroporative techniques, optimization of the vaccination route, examination of viral replication and antigen expression at the site of injection, as well as study of the longevity of immunity. Additional evaluation may be done in other animal models, such as young ICR and CD-1 mice [43] or interferon-deficient A129 mice [44]. As a platform technology and a reverse-genetics system, iDNA can also be used to engineer other CHIKV vaccines, including those containing live chimeric alphaviruses [22, 45]. Our data suggest that CHIKV iDNA vaccine allows elicitation of protective immunity with a single immunization, which is critical for vaccines designed to contain outbreaks or epidemics [20, 42]. Therefore, the iDNA approach may represent a feasible strategy for CHIKV vaccination.

Notes

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References


