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A recombinant virus vaccine that protects against both Chikungunya and Zika virus infections

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ABSTRACT

Chikungunya virus (CHIKV) and Zika virus (ZIKV) have recently expanded their range in the world and caused serious and widespread outbreaks of near pandemic proportions. There are no licensed vaccines that protect against these co-circulating viruses that are transmitted by invasive mosquito vectors. We report here on the development of a single-dose, bivalent experimental vaccine for CHIKV and ZIKV. This vaccine is based on a chimeric vesicular stomatitis virus (VSV) that expresses the CHIKV envelope polyprotein (E3-E2-6K-E1) in place of the VSV glycoprotein (G) and also expresses the membrane-envelope (ME) glycoproteins of ZIKV. This vaccine induced neutralizing antibody responses to both CHIKV and ZIKV in wild-type mice and in interferon receptor-deficient A129 mice, animal models for CHIKV and ZIKV infection. A single vaccination of A129 mice with the vector protected these mice against infection with both CHIKV and ZIKV. Our single-dose vaccine could provide durable, low-cost protection against both CHIKV and ZIKV for people traveling to or living in areas where both viruses are circulating, which include most tropical regions in the world.

1. Introduction

Chikungunya virus (CHIKV) and Zika virus (ZIKV) are two arboviruses (arthropod-borne viruses) transmitted to humans by mosquito vectors. In recent decades both viruses have caused large and geographically widespread epidemics. CHIKV, previously endemic to the tropical areas of Africa and Southeast Asia, recently spread into tropical and subtropical areas of the New World [1]. CHIKV infection is rarely fatal, but infection typically results in severe, debilitating arthralgia that can last for weeks, months, or even years [2]. Perinatal CHIKV infection has also been reported [3–5] where neonatal infection can lead to cases of encephalitis (50%) and of acute respiratory failure (8%). Only sporadic and mild human infections by ZIKV were reported until 2007, but more recent large outbreaks in several Pacific islands [6,7] and the Americas [8–10] were associated with Guillain–Barré syndrome [11] and also linked to a dramatic increase in the incidence of infants born with microcephaly and other congenital defects [12,13]. Additionally, ZIKV was also found to be transmitted by sexual contact [14,15] and blood transfusion [16].

In addition to tropical and subtropical regions inhabited by the principal urban vector *Aedes aegypti*, CHIKV and ZIKV also pose a serious transmission risk in temperate regions of the world where the invasive mosquito vector *A. albopictus* is now widely distributed [17]. Recent reports also indicate occurrences of concurrent or sequential CHIKV and ZIKV human infections [18–20].

Currently, there are no licensed vaccines available for either CHIKV or ZIKV [21,22]. Especially in the context of co-circulation of CHIKV and ZIKV in much of the tropics, an effective bivalent CHIKV/ZIKV vaccine could have a significant impact on global health. An ideal vaccine would confer rapid protection against CHIKV and ZIKV after a single dose, and also confer long-term protection.

CHIKV (an alphavirus) and ZIKV (a flavivirus) are both enveloped, single-strand, positive-sense RNA viruses [23,24]. Neutralizing antibodies to their glycoproteins play dominant roles in protecting against infection by both viruses [21,25].

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We previously developed a potent, single-dose CHIKV vaccine vector (VSVΔG-CHIKV) consisting of a recombinant vesicular stomatitis virus (VSV) vector expressing the CHIKV E3-E2-6K-E1 envelope polyprotein in place of the VSV G protein [26]. In contrast to wild-type VSV, this recombinant virus lacks neurotropism and is non-pathogenic in adult and neonatal mice [27]. In the current study we used this recombinant as a vector platform to express ZIKV envelope glycoproteins and generated a bivalent vaccine vector that protects against CHIKV and ZIKV infection in a mouse model.

2. Materials and methods

2.1. Cells and viruses

Baby hamster kidney (BHK-21) cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% Fetal Bovine Serum (FBS). BHK-21 (clone 15) cells were grown in DMEM containing 10% FBS and supplemented with non-essential amino acids (NEAAs). African green monkey cells (Vero E6) were maintained in Minimum Essential Medium (MEM) supplemented with 10% FBS. ZIKV (Brazilian strain PE243), for neutralization assays, was grown in Vero E6 cells and titrated using BHK-21 (clone 15) cells. ZIKV (MR766 strain) and CHIKV [La Reunion (LR) strain] were used for challenge at UTMB, TX.

2.2. Plasmid constructions and virus recovery

To generate pVSVΔG-CHIKV-ZIKV we first designed a codon-optimized (http://www.jcat.de/) synthetic gene encoding a ZIKV ME (matrix-envelope) polyprotein (Brazilian strain BeH815744: amino acids 216-794) preceded by a tissue plasminogen activator (tPA) signal sequence (MDAMKRGLCCVLLLCGAVFVS). The synthetic gene (ss-ME) flanked with Xho I, Not I, and Nhe I sites (Genscript, Inc.) was inserted into a Xho I-Not I digested pVSVΔG-CHIKV [26] vector into which we had previously added additional flanking VSV transcription start/stop signals. The resulting plasmid was designated pVSVΔG-CHIKV-ZIKV. The ss-ME gene was also inserted into Xho I-Nhe I digested pVSV-XN2 vector [28] to generate pVSV-ZIKV. Recombinant VSVs were recovered from pVSVΔG-CHIKV-ZIKV and pVSV-ZIKV using helper plasmids as described previously [29,30].

2.3. Antisera and antibodies

The anti-VSV G monoclonal antibodies (MAbs) I1 and I14 [31] were used to detect VSV G protein expression. The pan-Flavivirus envelope MAb clone D1-4G2-4-15 (IgG2a) (EMD Millipore Corp., MA) was used to detect ZIKV envelope protein. MAb clones CHK48 (IgG2c, NR-44002) and CHK 263 (IgG2c, NR-44003) (BEI Resources, NIAID, NIH) were used to detect CHIKV E2 expression. Rabbit polyclonal anti-VSV serum was used to detect VSV proteins in Western blots.

2.4. Western blot analysis

Western blots were performed as described previously [32]. Briefly, whole cell extracts were collected from BHK-21 cells that were infected with VSVΔG-CHIKV, VSVΔG-CHIKV-ZIKV or VSV-ZIKV. Clarified lysates were analyzed on a 4–12% Bis-Tris NuPAGE gel (Invitrogen, CA) and western blot analysis was performed using MAbs specific for Flavivirus envelope (at 1:1000), CHIKV E2 (at 1:1000) or VSV (Indiana serotype) polyclonal antibodies at (1:5000). Goat anti-mouse IgG2c and goat anti-mouse IgG2a antibodies labeled with Alexa Fluor® 680 and Alexa Fluor® 647 (Invitrogen), and goat anti-rabbit antibody labeled with IR800 (LICOR) were used as secondary antibodies. The membrane was scanned and imaged using Odyssey near-infrared imaging system (LICOR). The anti-flavivirus blot was stripped and reprobed with anti-CHIKV antibodies and again stripped and reprobed with anti-VSV antibody.

2.5. Indirect immunofluorescence microscopy

BHK-21 cells on coverslips were infected with VSVΔG-CHIKV, VSVΔG-CHIKV-ZIKV or VSV-ZIKV at a multiplicity of infection (MOI) of 1 plaque forming unit (PFU)/cell. At 5.5 h post infection, cells were fixed with 3% paraformaldehyde, washed with PBS containing 10 mM glycine (PBS-Glycine) and incubated with a 1:200 dilution of VSV G MAbs or a 1: 200 dilution of anti-CHIKV E2 MAbs. One set of fixed cells was permeabilized and incubated with anti-flavivirus MAb at a 1:200 dilution. Following a PBS-Glycine wash the cells were further incubated with 1:500 diluted goat anti-mouse AlexaFluor 488 IgG (Molecular probes, Eugene, OR). Washed cells were then mounted on slides using ProLong Gold antifade reagent with DAPI (Molecular probes, Eugene, OR) and imaged with a Nikon Eclipse 80i fluorescence microscope (40× objective).

2.6. Animal experiments

Initial immunogenicity experiments were performed in 6–8 week-old Balb/c mice obtained from Charles River Laboratories (Wilmington, MA). The mice were housed under BSL-2 conditions. Groups of mice (n = 4) were vaccinated with indicated viruses at 10^7 PFU per mouse by the intramuscular (i.m.) route, in a volume of 50 μl of PBS, into the right hind leg muscle. Boosting was performed with the same vectors and same dose at 5 weeks post immunization. Mice were bled retro-orbitally. Sera from 28, 63 and 259 days post immunization (dpi) were used to determine the ZIKV and CHIKV neutralizing antibody (NAb) responses. The immunization/challenge studies employed 7-week old A129 mice that were vaccinated (n = 4/group; 10^7 PFU, i.m.) with VSVΔG-CHIKV-ZIKV, VSVΔG-CHIKV, or mock-vaccinated with PBS. Blood was collected at 33 dpi to ascertain the ZIKV and CHIKV NAb responses prior to challenge. At 60 dpi, immunized mice were divided into two groups and challenged with either ZIKV [MR766 strain; 10^5 PFU per mouse by the intraperitoneal (i.p.) route] or with CHIKV [LR strain; 10^4 PFU per mouse by the subcutaneous (s.c.) route]. Animals from each challenge group were divided into two subgroups and were bled on alternate days post challenge to measure ZIKV and CHIKV viremia by plaque assay for the first 3 days post challenge. Animals were monitored for 14 days post-challenge.

Yale University and UTMB Institutional Animal Care and Use Committees approved all animal experiments.

2.7. Virus neutralization assays

To measure ZIKV NAbs, approximately 50 PFU of ZIKV (PE243) were mixed with pooled and serially diluted serum samples from each immunization group, incubated for 1 h at 37 °C, and added to monolayers of BHK-21 (clone 15) cells in 6-well plates, overlayed with 1% methyl cellulose and incubated for 5 days at 37 °C. The plates were then washed, stained with crystal violet, dried and plaques were counted. Percent neutralization was calculated compared to samples containing the same dilutions of control serum from unimmunized animals. All assays were performed in duplicate. The VSVΔG-eGFP1/CHIKV pseudotype neutralization assay was performed as described previously [26].

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