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Research Paper

Passive Transfer of Immune Sera Induced by a Zika Virus-Like Particle Vaccine Protects AG129 Mice Against Lethal Zika Virus Challenge

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ABSTRACT

Zika virus (ZIKV) poses a serious public health threat due to its association with birth defects in developing fetuses and Guillain-Barré Syndrome in adults. We are developing a ZIKV vaccine based on virus-like particles (VLPs) generated in transiently transfected HEK293 cells. The genetic construct consists of the prM and envelope structural protein genes of ZIKV placed downstream from a heterologous signal sequence. To better understand the humoral responses and correlates of protection (CoP) induced by the VLP vaccine, we evaluated VLP immunogenicity with and without alum in immune-competent mice (C57Bl/6 x Balb/c) and observed efficient induction of neutralizing antibody as well as a dose-sparing effect of alum. To assess the efficacy of the immune sera, we performed passive transfer experiments in AG129 mice. Mice that received the immune sera prior to ZIKV infection demonstrated significantly reduced viral replication as measured by viral RNA levels in the blood and remained healthy, whereas control mice succumbed to infection. The results underscore the protective effect of the antibody responses elicited by this ZIKV VLP vaccine candidate. These studies will help define optimal vaccine formulations, contribute to translational efforts in developing a vaccine for clinical development, and assist in the definition of immunologic CoP.

1. Introduction

Zika virus (ZIKV) is a flavivirus closely related to human pathogens such as yellow fever, West Nile, Japanese encephalitis, tick-borne encephalitis, and dengue viruses (Pierson and Diamond, 2012). ZIKV was initially discovered in the Zika Forest of Uganda in 1947 in a sentinel Rhesus monkey that developed viremia (Dick et al., 1952). ZIKV is transmitted to humans by *Aedes* mosquito species, which are prevalent in African, Asian, and American tropical regions (Faye et al., 2014; Haddow et al., 2012; Hayes, 2009). ZIKV, apparently uniquely among arboviruses, is also transmitted by sexual activity. High viral loads have been detected in semen from infected patients (Atkinson et al., 2016; Foy et al., 2011; Hills et al., 2016; Musso et al., 2015), and sexual transmission from infected men and women to their partners has been reported (Hastings and Fikrig, 2017). It has been reported in mice that ZIKV infection damages the testis and leads to male infertility (Govero et al., 2016; Ma et al., 2016). Because of the usual benign course of disease and high percentage of subclinical infections, ZIKV was initially discounted as a significant human pathogen until a major outbreak occurred in 2007 on Yap Island, Micronesia (Duffy et al., 2009; Haddow et al., 2012; Lanciotti et al., 2008), followed by an outbreak in French Polynesia from 2013 to 2014 (Cao-Lormeau et al., 2016), and subsequent spread into many countries throughout the Western Hemisphere (Hennessey et al., 2016; Petersen et al., 2016). Brazil reported an estimated 500,000 to 1,500,000 human cases of ZIKV infection in 2015 (Bogoch et al., 2016) and it is likely that Zika will ultimately spread throughout most areas that have significant populations of vector mosquitos.

As the geographic range of ZIKV increased, so did appreciation that ZIKV could cause serious human disease (Chan et al., 2016; Ioos et al., 2014). Guillain-Barré syndrome linked to ZIKV infection was detected in the 2013 outbreak in French Polynesia (Ansar and Valadi, 2015, Cao-Lormeau et al., 2016, Oehler et al., 2014). Concern was also amplified with the observation of an approximate 20-fold increase in incidence of congenital microcephaly in the 2015 outbreak in Brazil (Vogel, 2016). Evidence that ZIKV infection is associated with fetal microcephaly is, in part, based on the observation that microcephaly coincided temporally with the ZIKV outbreak (offset by ~6 months) and subsequently, the detection of ZIKV in microcephalic fetal brain tissues (Besnard et al., 2014, Driggers et al., 2016, Marrs et al., 2016, Martines et al., 2016, Mlakar et al., 2016, Schuler-Faccini et al., 2016, Tang et al., 2016, Ventura et al., 2016). Association with neurologic disorders is

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also supported by an animal model in which ZIKV infects neural progenitor cells leading to microcephaly in mice (Li et al., 2016). Further studies demonstrated that ZIKV targets and infects human embryonic stem cell-derived cerebral organoids (Dang et al., 2016). From the accumulated evidence to date, it is likely that ZIKV infection during pregnancy can cause microcephaly and associated congenital defects.

Little is known about the nature and duration of protective immunity following natural ZIKV infection. To address this issue, the search for natural correlates of protection (CoP) relies on in vitro studies of post-infection immune responses and animal models of ZIKV infection. For several licensed vaccines, correlates of human protection rely on accepted levels of antibody titers, e.g., measles, influenza, pneumococcal and Hepatitis A (Plotkin et al., 2013). Specifically, for licensed flavivirus vaccines such as yellow fever and Japanese encephalitis, neutralizing antibody (nAb) immune responses are strongly correlated with protection (Belmusto-Worn et al., 2005; Hombach et al., 2005; Julander et al., 2011; Markoff, 2000; Van Gessel et al., 2011). Knowledge of CoP for ZIKV may provide a defined path to vaccine design and ultimate licensure. In contrast, clinical field trials to demonstrate vaccine efficacy are challenging to design and execute due to the fact that epidemics are difficult to predict, often sporadic, leading to a decline of ZIKV incidence in a given population before the clinical trial sites are functional.

The envelope (E) protein of flaviviruses is involved in binding cellular receptors, enabling viral entry, and is the major target for nAbs (Modis et al., 2003). Isolation of monoclonal antibodies from ZIKV-infected donors has shown that the majority of the humoral immune response is directed to the E protein, with the most potent nAbs directed against the DIII lateral ridge and complex quaternary epitopes including more than one E domain, such as the DII dimer interface (Hasan et al., 2017; Sapparapu et al., 2016; Stettler et al., 2016; Wang et al., 2016). In contrast, flavivirus-specific antibodies recognizing the DII fusion loop tend to be cross-reactive and neutralize the virus less effectively (Cherrier et al., 2009; Dai et al., 2016; Sapparapu et al., 2016; Stettler et al., 2016; Stiasny et al., 2006; Vogt et al., 2011). In addition, several animal studies have demonstrated that ZIKV-specific E antibodies confer protection against ZIKV infection and pathology (Abbink et al., 2016; Dowd et al., 2016; Larocca et al., 2016; Richner et al., 2017; Sapparapu et al., 2016; Zhao et al., 2016). For these reasons, and because of the inherent safety of VLP vaccines, we have chosen a VLP vaccine development approach that efficiently generates antibodies against the ZIKV E protein. As shown here, neutralizing antibodies induced by this VLP vaccine, upon transfer to AG129 mice, provided significantly protection against subsequent ZIKV challenge.

2. Materials & Methods

2.1. ZIKV VLP DNA Design, Production, Purification, and Characterization of VLPs

The ZIKV cassette used to generate the VLP consisted of: 1) the human IL2 signal sequence (SS) (MYRMQLLSCIALSLALVTNS); 2) the prM sequence contains 93 amino acids (aa), starting with AEI and ending with AYS from the African MR766 strain (accession #KU955594); 3) the E ectodomain from the Brazilian SPH2015 strain (accession #KU321639) contains 405 aa, starting with IRC and ending with SGS; and 4) the stem-anchor from the African MR766 strain (accession #KU955594) contains 99 aa starting with TIG and ending with VSA. This construct was designed based on an assumption that the antibody response should be directed towards the more recent Brazilian E ectodomain. The other consideration for vaccine design was VLP expression level. We evaluated African MR766, Brazilian SPH2015, and the chimeric construct and found that the chimeric DNA construct, upon transfection of HEK293 cells, generated the highest levels of VLP expression (data not shown). The cassette containing the structural genes was inserted downstream from a human CMV IE enhancer/promoter in the CMV/R plasmid described by Akahata et al. (2010). This plasmid was transiently transfected into HEK293 cells, and the ZIKV VLPs were concentrated and purified from media supernatant for C57Bl/6 x Balb/c (CB6F1) mouse immunogenicity studies.

Briefly, HEK293 cells in serum-free suspension culture were transfected with 25 mg/L of DNA in a 1:2 ratio (by weight) of plasmid to polyethylenimine (PEI) (Polysciences, PA). At 96 hour post-transfection, cell supernatant was harvested and clarified by centrifugation at 10,000 $\times g$ for 10 min at room temperature, then pooled and filtered through a Sartopore 2 XL 0.8 μm/0.2 μm filter (Sartorius, Germany) to produce a clarified harvest. The clarified harvest was concentrated and diafiltered into load buffer (25 mM Tris and 100 mM Sodium Citrate at pH 8.3) using a 500 kDa hollow fiber (Spectrum Labs, CA) prior to column chromatography.

The ZIKV VLPs were purified using a two-column chromatography process. ZIKV VLPs from the initial tangential flow filtration process were loaded onto single-step, tandem chromatography columns comprised of mix-mode chromatography resin, Capto Core 700 (GE, PA), and a Sartobind salt-tolerant interaction chromatography resin (STIC-PA). This process used both columns in a negative capture mode by using 25 mM Tris and 100 mM Sodium Citrate at pH 8.3. Chromatography experiments were performed using AKTA Start, operated with the UNICORN software (GE Healthcare, PA).

Harvest supernatant containing VLPs or column purified VLPs were resolved by NuPAGE 4–12% Bis-Tris precast protein gels (Invitrogen, CA) and stained with InstantBlue Coomassie stain reagent (Expedeon) or transferred onto a nitrocellulose membrane using iBlot dry blotting system (Invitrogen, CA). The nitrocellulose membrane with transferred protein was blocked with 5% milk (Labscientific, Inc., NJ) in phosphate buffered saline containing 0.05% Tween 20 (PBS-T) and incubated with a mouse monoclonal antibody (MyBioSource, CA) to recombinant (generated in insect cells) E protein (MyBiosource) at 1:1000 dilution. The membrane was washed three times with PBS-T and probed with goat anti-mouse HRP-conjugated antibody (Invitrogen) at 1:10,000 dilution. The western image was developed by using an enhanced chemiluminescent (ECL) substrate kit SuperSignal West Femto (ThermoFisher Scientific).

ZIKV VLP morphology was analyzed by negative stained electron microscopy at The Scripps Research Institute Microscopy Core Facility (La Jolla, CA). Briefly, purified ZIKV VLPs were absorbed on to the glow-discharged carbon-coated 200 mesh copper grid. The grid was washed and stained with 1% uranyl acetate and visualized by a Philips CM100 Transmission Electron Microscope with Soft Imaging Systems MegaView III CCD camera.

2.2. ZIKV Challenge Strain

Prototype Zika Nica 2-16 strain used for challenge was originally isolated from a ZIKV-infected Nicaraguan patient in 2016 by the National Virology Laboratory, Ministry of Health, Managua, Nicaragua (Tabata et al., 2016). The virus was propagated in C6/36 cells to generate the virus stock and titered by focus-forming assay on BHK cells (Kostyuchenko et al., 2016).

2.3. Mouse Experiments

Immune sera for passive transfer studies were generated by immunizing CB6F1 mice via the intramuscular (IM) route on days 0 and 32 using a dose of 1, 5, and 25 μg of ZIKV VLP vaccine formulated with or without 100 μg alum (Alhydrogel) adjuvant. For the IM immunization, 100 μL of vaccine was injected into the hind limbs (bilateral quadriceps) at 50 μL per hind limb using a 1 mL tuberculin syringe and 27 gauge needle. On day 68 following the booster ZIKV VLP immunization, 200 μL of blood was collected from the orbital sinus plexus using non-heparinized Natelson tubes and dispensed into serum separation tubes for serum collection. The pooled mouse immune sera were tested for nAb activity against Zika Reporter Virus Particles (RVPs) expressing luciferase

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