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SATURDAY – CIV-192

A Virus-Like Particle (VLP) Vaccine Displaying Zika Envelope (E) Protein CD Loop Antigen Elicits Protective and Specific Immune Response in a Murine Model

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Abstract

Zika virus (ZIKV) is a mosquito-borne flavivirus associated with Congenital Zika Syndrome (CZS), which comprises a wide range of congenital abnormalities in fetuses and infants infected with ZIKV before birth (1 and 2). In a small number of patients, ZIKV infection is strongly associated with the neurological autoimmune disorder Gullian Barré Syndrome (GBS) (3). To date, no vaccines or antiviral strategies are licensed for Zika Virus. Our aim is the development of a novel Zika vaccine candidate safe from antibody-dependent enhancement (ADE).

Methods

We developed novel ZIKV vaccine candidates by using a Woodchuck Hepatitis core antigen (WHcAg) virus-like particle (VLP) scaffolding system (4) for displaying specific antigens from the ZIKV Envelope (E) protein, which is conserved across ZIKV strains, as the vaccine immunogen (Fig. 1).

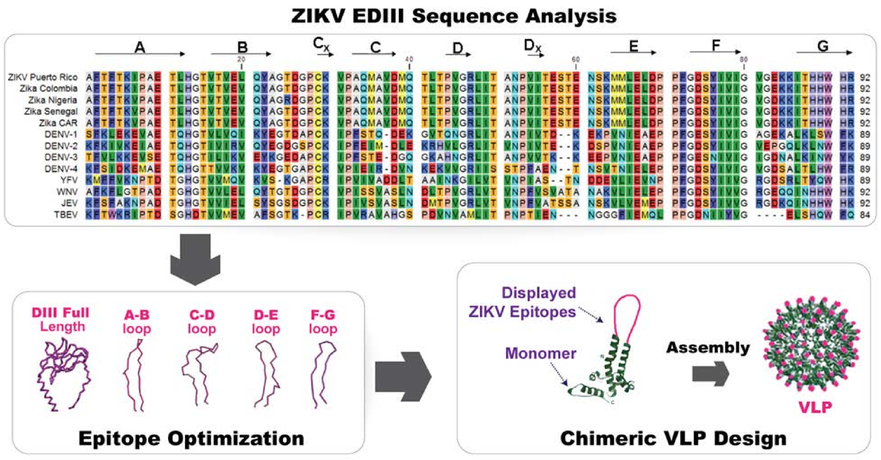

Figure 1. Structural vaccinology has been applied for developing chimeric Zika-VLPs on a Woodchuck Hepatitis core antigen backbone.

Results

Vaccine candidates were developed and tested using WHcAg chimera VLPs displaying selected ZIKV antigens from the E protein: (i) full length EDIII (WHcAg EDIII), (ii) the EDIII sub-structural domain CD Loop (WHcAg CD Loop), and (iii) the EDII sub-structural domain Fusion Loop (WHcAg Fusion Loop). While EDIII domain antigen sequences are very specific for ZIKV, the Fusion Loop antigen from EDII is highly conserved or identical among flaviviruses. VLPs were recombinantly expressed in *Pichia pastoris* (ATCC® 76273™). The purity of the control and chimeric VLPs was assessed by SDS-PAGE and Coomassie blue staining (Fig. 2A). Recombinant WHcAg control (WHcAg CTRL) and WHcAg-Zika EDIII epitope chimeras (Zika-VLPs) displayed distinct bands of predicted MW. In order to test Zika-VLPs antigenicity, dot blot was performed using mouse serum antibody from an animal challenged with 1 X 10^6 PFU of live ZIKV Puerto Rico strain (BEI Resources NR-50240 Zika virus, PRVABC59 (Human/2015/Puerto Rico)) (Fig. 2B).

Zika-VLPs demonstrated a high level of antigenicity comparable to control ZIKV prME VLPs and recombinant full-length E protein (WHcAg EDIII), (Figure 2B). We tested the assembly and morphology of the WHcAg chimera VLPs by using transmission electron microscopy (TEM); Zika-VLPs demonstrated icosahedral morphology and a size range of 25-30 nm (Fig. 2 C).

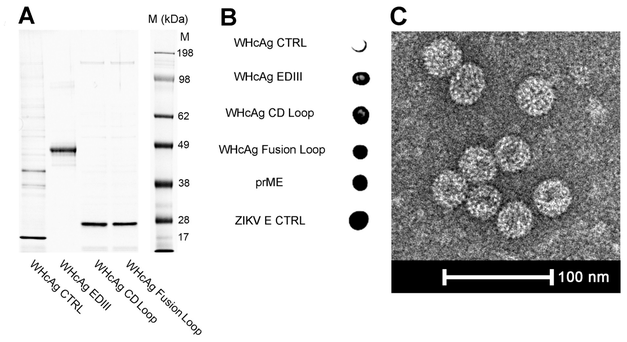

Figure 2. Expression and characterization of Zika-VLPs. **A**. Coomasie Blue staining of purified control and Zika-VLP epitope chimeras. **B**. Dot blot analysis show Zika-VLP vaccine candidate antigenicity. **C.** TEM image of WHcAg CD Loop VLPs.

ELISA analysis of serum from mice immunized with Zika-VLP vaccine candidates demonstrated that the WHcAg CD Loop induced a strong immune response and elicited a strong antibody response against ZIKV after prime immunization alone (Fig. 3A). Analysis of serum immunoglobulins demonstrated induction of both Th1- and Th2-mediated immune response (Fig. 3B).

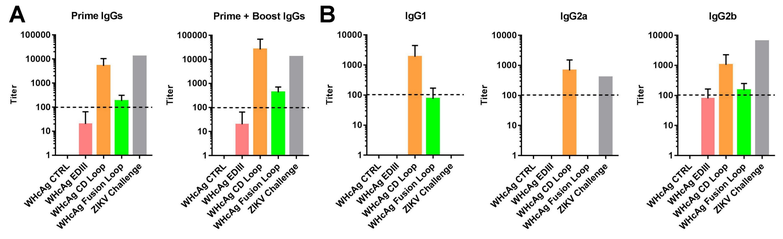

Figure 3. A. ELISA serum analysis for animals immunized with Zika-VLPs vaccine candidates. Titer represents the reciprocal of the serum dilution. The limit of detection (dotted line) is 100. A. The WHcAg CD Loop VLP induced a high level of IgG response after prime and prime + boost immunization. **B.** WHcAg CD Loop VLP induced IgG1, IgG2a and IgG2b antibody isotypes.

The WHcAg CD Loop VLP induced serum antibodies specific for ZIKV and without cross-reactivity with DENV-2 (Fig. 4). Dot blot analysis of mouse serum pooled from 5 animals immunized with WHcAg CD Loop recognized the recombinant E protein from ZIKV but did not recognize the recombinant E protein from DENV-2 (Fig. 4A). Mice challenged with live ZIKV develop antibody against ZIKV E protein; 60% of the animals showed antibody able to cross-react with DENV-2 E protein (Fig. 4B). Commercially available monoclonal antibodies against ZIKV, DENV-2, and 6-Histidine tag (6-His) are used as a control for the assay (Fig. 4C).

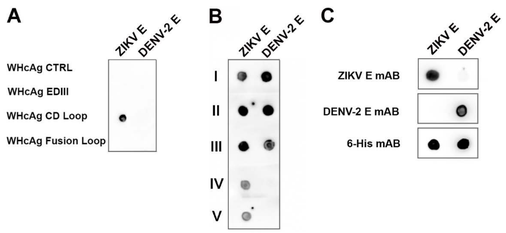

Figure 4. A. Dot blot analysis of mouse serum pooled from 5 animals immunized with WHcAg CD Loop VLP recognized recombinant E protein from ZIKV but did not recognize recombinant E protein from DENV-2. **B.** Mice challenged with live ZIKV (1 X 10^6 PFU) develop antibody against ZIKV E protein; 60% of animals developed antibody cross-reactive with DENV-2 E protein. **C.** Control monoclonal antibodies against ZIKV E protein, DENV-2 E-protein and 6-Histidine tag (6-His) demonstrate specificity for the dot blot assay.

The WHcAg CD Loop vaccine candidate demonstrated immunoprotection in a C57BL/6NHsd murine model of ZIKV infection that employs anti-IFNAR1 antibody preconditioning prior to viral challenge (Fig. 5A). The WHcAg CD Loop immunization stimulated protective, but not neutralizing (Fig. 5B), antibodies associated with antibody-dependent cell-mediated cytotoxicity (ADCC) (Fig. 5C), and complement-dependent cytotoxicity (CDC) (Fig. 5D) activities.

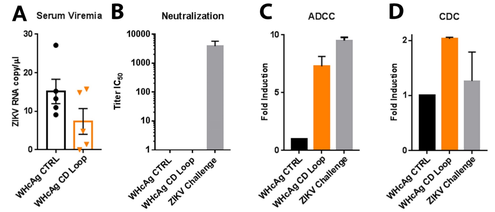

Figure 5. A. Immunization with WHcAg CD Loop reduce viremia burden in a mouse model of ZIKV infection. Viremia from animals infected with ZIKV is statistically lower after immunization (Student's T-test) with WHcAg CD Loop in respect the placebo control (WHcAg CTRL). **B.** Serum antibody elicited by WHcAg CD Loop immunization did not show detectable neutralization activity.
C and D. WHcAg CD Loop induced ADCC and CDC activities.

Conclusions

- The WHcAg CD Loop VLP vaccine candidate induces a high level of protective, ZIKV-specific antibodies in the mouse model that do not cross-react with DENV antigens.
- Antibodies elicited by the WHcAg CD Loop vaccine are safe from ADE
- Immunization with the WHcAg CD Loop reduces the ZIKV viremia burden after challenge
- The protective immunity of the WHcAg CD Loop vaccine is mediated by ADCC and CDC
- This vaccine candidate should represent a safer immunogen than whole virus-based or conserved flavivirus antigen-based vaccines for preventing ADE.

References

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