PALADINS: PROTECTIVE APPROACHES LEVERAGING AD-APTIVE AND IN-NATE SYSTEMS

Broad Spectrum Protein-peptide Antigens Against Encephalitic Alphaviruses

Catherine Schein UTMB-Galveston **Grace Rafael** UTMB-Galveston **Wendy S. Baker** UTMB-Galveston **Elizabeth S. Anaya** Los Alamos Natl. Labs **Jurgen G. Schmidt** Bioscience Division, Los Alamos National Laboratory **Scott C. Weaver** UTMB-Galveston **Surendra S. Negi** UTMB-Galveston **Werner Braun** UTMB-Galveston

Background information: The alphaviruses (AV) are a diverse group of mosquito-borne and biothreat viruses that can cause encephalitic infections with high mortality (eastern and Venezuelan equine encephalitis (EEEV and VEEV), Madariaga virus (MADV)) and chronic arthralgia (Chikungunya virus, CHIKV). Previous human trials have shown immune interference with heterologous AV vaccines. We produced broad spectrum PhysicoChemical Property Consensus (PCPcon) protein and peptide antigens to epitope-rich regions of the E2 protein of all AV (Baker et al., Antiviral Research 2020) and showed they generated broad-spectrum, neutralizing antibodies in vaccinated rabbits as determined by plaque reduction neutralization tests (PRNT50/80) (Schein et al. BioRxiv,2022). Combined protein/peptide antigens can provide a molecularly defined, easily stored vaccine requiring no animal cell culture.

Impact to the DTRA mission and warfighter: Zoonotic AV can infect troops living exposed to the elements in many areas of the world. In addition, AV are biothreat agents. As many AV circulate together, there is a pressing need for safe, broad spectrum vaccines to prevent debilitating encephalitis and arthralgia. The PCPcon/PD approach, validated for flaviviruses, enteroviruses and epitopes of allergens, has wide applicability to other emerging biothreats.
Purpose: To demonstrate that PCPcon protein/peptide vaccines can protect against lethal challenge from encephalitic AV.
Objective: To test the ability of computationally derived PCPcon E2-B domain antigens combined with peptides to surface exposed areas of the E2-A domain to protect against lethal challenge with VEEV and EEEV.

Methods: CD1 mice were inoculated IM with 3 x 20µg doses of PCPcon species-specific proteins antigens VEEVcon, EEEVcon, CHIKVcon, the consensus of these, EVCcon and Mosaiccon (AllAV consensus with specific epitopes of VEEV and CHIKV), 3 weeks apart. A mixture of 5 µg each of 4 peptides representing surface-exposed regions of the E2-A domain was administered 2x at weeks 4 and 7 to each protein-vaccinated animal. Negative controls were adjuvant only, positive were IRES-attenuated whole AV expressing the E proteins of VEEV or EEEV (Read et al. Virology, 2021). Mice were challenged with wild-type strains VEEVZPC738 or EEEV93939 three weeks after the third inoculation.
Preliminary Results: All inoculated with EEEVcon+peptides survived EEEV challenge with no weight loss or viremia 2 days after challenge. For the other antigens plus peptides, 4 of 5 CHIKVcon, 3 of 5 VEEVcon, 3 of 5 Mosaikcon and 2/5 EVCcon vaccinated mice survived; all proteins reduced viremia 2 days after challenge. All the protein/peptide inoculated had reduced viremia 2 days after VEEV challenge, but only 2/5 VEEVcon and 1/5 EEEVcon inoculated survived.
Conclusions: All the protein inocula reduced viremia but had mixed effects on survival after challenge. Further vaccination studies will use different adjuvants and increased protein dosing to enhance broad spectrum protection.

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