Malian children who had received the AMA1 vaccine or a control rabies vaccine. Post-immunization sera from AMA1-vaccinated children had 6-fold greater mean seroreactivity to peptides spanning c1L matching the vaccine strain, compared to the seroreactivity to heterologous c1L peptides. To identify the precise amino acids responsible for this allele-specific efficacy, we tested sera against a collection of peptides containing substitutions of every possible amino acid or a deletion at each c1L position. None of the substitutions were associated with differences in seroreactivity in pre-vaccinated sera. However, in post-vaccination sera, perturbation of the vaccine-homologous glutamic acid residue at position 197, whether by substitution or deletion, significantly reduced seroreactivity. These results establish an immunologic basis for the earlier molecular epidemiological evidence that a single amino acid at position 197 in the AMA1 c1L is the primary determinant of strain-specific efficacy of the AMA1 vaccine. This result suggests that strain-transcending efficacy can potentially be achieved using multiple AMA1 antigens that vary at this single amino acid position despite polymorphisms at other positions in a multivalent next-generation malaria vaccine.

1724

IMPACT OF PROTEIN TARGETING ON IMMUNOGENICITY OF PFS25 ENCODED BY DNA VACCINE PLASMIDS

Robert J. Hart, Yi Cao, Geetha Bansal, Nirbhay Kumar

Department of Tropical Medicine, School of Public Health and Tropical Medicine, Vector-Borne Infectious Disease Research Center, Tulane University, New Orleans, LA, United States

Pfs25 is a leading candidate for the development of *Plasmodium falciparum* transmission blocking vaccines (TBV). Many different vaccine delivery platforms are currently being evaluated to develop an effective vaccine and considerable progress has been made with Pfs25 based DNA vaccines, evaluated in mice and nonhuman primates. Subsequent studies have also shown improved immunogenicity of codon optimized Pfs25 DNA plasmid delivered by intramuscular electroporation. In an effort to further optimize immunogenicity of DNA vaccines we evaluated the impact of protein targeting. We designed plasmids that would allow immunogenicity differences among encoded proteins destined for (1) secretion, (2) endosomal targeting, and (3) retention in the cytoplasm. Female Balb/c mice (5-6 weeks old) were immunized (intramuscular) with three doses, at one month apart, by *in vivo* electroporation with a final Pfs25/alum protein boost. Immune response parameters analyzed included Pfs25-specific antibodies and T cell responses. Studies revealed significant differences between targeted and non-targeted encoded Pfs25 and further emphasize evaluating similar approaches for any future DNA vaccine.

1725

IMMUNIZATION WITH MULTIPLE ALLELES OF *PLASMODIUM FALCIPARUM* FULL LENGTH VAR2CSA DNA CONSTRUCTS TO GENERATE A PLACENTAL MALARIA VACCINE SHOWING BROAD HETEROLOGOUS PROTECTION

Shaji Daniel[1], Justin Doritchamou[1], Pascal Bigey[2], Nicaise Tuikue-Ndam[3], Holly Torano[1], Charles Anderson[1], Lynn Lambert[1], Michal Fried[1], Patrick E. Duffy[1]

[1]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Unité de Technologies Chimiques et Biologiques pour la Santé, CNRS UMR8258 – Inserm U1022 – Université Paris Descartes, Chimie ParisTech, Paris, France, [3]Institut de Recherche pour le Développement, MERIT UMR216, COMUE Sorbonne Paris Cité, Université Paris Descartes, Faculté de Pharmacie, Paris, France

Placental malaria (PM) is caused by adhesion of *Plasmodium falciparum* (Pf) infected erythrocytes (IE) to the placental receptor chondroitin sulfate A (CSA), mediated by the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family member called VAR2CSA. Over successive pregnancies, women acquire antibodies against VAR2CSA as they acquire clinical resistance to malaria. VAR2CSA is a 350kDa, multi-domain protein: many different domain combinations of the protein used as immunogens have failed to generate broadly neutralizing activity against diverse CSA-binding isolates. Recent studies have shown that the full length extracellular domain of the protein has a 100,000-fold higher affinity for CSA compared to any individual domain. Moreover, the extracellular domain has also been shown to be structurally more compact compared to individual domains and antibodies raised against the full length (FL) extracellular protein could completely block homologous IE binding to CSA indicating that the full-length protein could serve as a better vaccine. In preliminary studies with FL VAR2CSA from PfNF54 and PfFCR3 we found that FL VAR2CSA DNA vaccine constructs delivered by electroporation induced neutralizing activity against diverse field isolates. To extend these findings, we have prepared codon-optimized glycosylated versions of six different Pf VAR2CSA alleles: NF54, M1010, Brazil 7G8, HB3, FCR3 and Malayan Camp, and cloned them into the mammalian expression vector VRC8400 such that the proteins will be expressed on the cell surface. A rat immunization study using these constructs either individually or as a blend of six alleles has been initiated. We will report the ability of these individual constructs to show anti adhesion activity against multiple Pf isolates as well as the ability of antibodies generated against the blend of six different Pf VAR2CSA alleles to show broad anti adhesion activity.

1726

BLOOD TRANSCRIPTOME RESPONSES TO *P. FALCIPARUM* INFECTION AND IMMUNIZATION

Ken Stuart[1], Ying Du[1], Atashi Anupama[1], Carl Murie[2], Raphael Gottardo[2], Julian Rothen[3], Stephen L. Hoffman[4], Naval Medical Research Center/ WRAIR Team[5], Ifakara Health Institute team[3]

[1]Center for Infectious Disease Research, Seattle, WA, United States, [2]Fred Hutchinson Cancer Research Center, Seattle, WA, United States, [3]Swiss TPH, Basel, Switzerland and Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, [4]Sanaria, Inc., Rockville, MD, United States, [5]U.S. Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD, United States

Immunization with radiation attenuated *P. falciparum* sporozoites provides protection from infection by non-attenuated parasites. We performed RNAseq transcriptional analysis on blood samples from subjects who were immunized with purified, cryopreserved radiation attenuated sporozoites and had protection was determined by controlled human malaria infection (CHMI) challenge. We determined responses in two vaccine trials that differed by prior malaria exposure and modes of immunization and CHMI and responses of non-immunized subjects to infection. We employed statistical methods that take into account uncontrolled variations within individual volunteers and differences between trials and performed multiple comparisons to determine host gene expression responses at various time points after vaccination and/or parasite challenge. We also compared responses between protected and non-protected people after vaccination and parasite challenge. Numerous changes in mRNA expression levels occurred following immunization and CHMI. Similar kinetics of gene expression responses to vaccination and CHMI were observed including between trials. For example: genes associated with neutrophils and erythrocyte development were significantly up-regulated 7 days after the 2nd vaccination. In addition, genes associated with myeloid lineage, coagulation cascade and metabolism had similar responses to CHMI. Furthermore, genes associated with neutrophils and erythrocyte development had similar kinetics in response to vaccination and to CHMI of non-immunized subjects. Analysis of genes for interferon responses, erythrocyte development, and hematopoietic precursors showed a trend for higher expression in protected compared to non-protected subjects after CHMI. In contrast, B-cell-related genes were more highly expressed in non-protected subjects. These changes in the blood likely reflect a combination of changes in immune cell gene expression, proliferation, apoptosis and cell trafficking and provide insights into immunological processes that are associated with infection and protection.