

P17 Atypical Immune Responses among Dengue Vaccine Recipients Attributable to Occult Yellow Fever Exposure. N. Kanesa-htasan,^{1*} W. Sun,¹ G. Ludwig,² C. Rossi,² J.R. Putnak,¹ J.A. Mangiafico,² R. Edelman,³ B.L. Innis,¹ Walter Reed Army Institute of Research, Washington DC; ²United States Army Institute for Infectious Diseases, Ft Detrick MD; ³University of Maryland, Baltimore MD.

Three clinical studies of attenuated monovalent dengue vaccines were conducted in 69 adults with no prior flavivirus exposure. Volunteers were negative for flavivirus antibodies to dengue 1-4, yellow fever (YF), Japanese encephalitis (E), St Louis E, tick-borne E, and Powassan viruses by hemagglutination-inhibiting (HAI) assay or EIA before vaccination. Eight volunteers (12%) had atypical dengue antibody (Ab) responses after vaccination: depressed IgM/IgG Ab ratio, high-titer HAI Ab production to all 4 dengue types, and heterologous and homologous neutralizing (NT) Abs, yet Ab kinetics were identical to those observed in primary responders. Gender, age, race, dengue type vaccine, or clinical signs and symptoms were similar between the two groups. However, 6 atypical responders had detectable NT Ab titers to YF virus (range 40-640) before vaccination while 5 primary responders had YF NT Ab titer ≤ 10 , $p=0.04$. After dengue vaccination, 4 primary responders had no rise in YF NT Ab >10 (one had titer 1:40) while all 8 atypical responders demonstrated titers ≥ 160 , $p=0.02$. These findings show that occult YF exposure (perhaps unrecalled YF vaccination > 20 years previously) may confound Ab responses to dengue vaccination. Furthermore, occult exposure may be common in some populations and should be considered in screening volunteers before vaccination.

P19 Safety and Immunogenicity of a Live-Attenuated, Mutagenized Rift Valley Fever Vaccine in Humans
P.R. Pittman*, D. McClain, K. Coonan, P. Summers, R.S. Makuch, J. Mangiafico, P. Gibbs, J. Lorenz, J. Morrill, C.J. Peters. Divisions of Medicine, Diagnostics Systems, Virology and Biometrics and Informatics, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland. Rift Valley fever virus (RVFV) is endemic in sub-Saharan Africa and has been responsible for several epizootics and epidemics with significant animal and human morbidity and mortality. The U.S. Army developed a vaccine candidate against RVFV, RVFV MP-12, derived from the ZH548 strain of RVFV by 12 successive cycles of 5-FU mutagenesis and replication in MRC5 cells. It is protective and attenuated in animals. After successful phase I testing, healthy adult volunteers (N=56) were randomized into the following groups for double blind injection: A ($10^{4.7}$ pfu s.c., n = 10), B ($10^{5.4}$ pfu i.m., n = 6) or C ($10^{4.4}$ pfu i.m., n = 27), and P (placebo, n = 13). Immunogenicity was assessed by the plaque-reduction neutralization test (PRNT₅₀) and ELISA. Minor adverse events were infrequent and observed in both placebo and MP12 recipients. There were no serious adverse events. Five volunteers, all from Group C, had transient low-titer (<10 pfu/ml) viremia. There was no correlation between viremia and clinical or laboratory adverse events. All but four MP12 recipients had IgM titers of $\geq 1:200$ by day 14. IgG responses were evident by day 21. On day 28 PRNT₅₀ was $\geq 1:20$ in 10/10 subjects in Group A (95% CI 74 to 100%), 5/6 in Group B (36 to 99.6%), and 23/27 in Group C (66 to 96%). GMT PRNT₅₀ values at 1, 6 and 12 months were 174, 54, and 67 for Group A; 90, 80, and 160 for Group B; and 115, 192 and 122 for Group C, respectively. Five of nine in Group A (95% CI 21 to 86%), 3/4 in Group B (19 to 99.4%), and 20/23 (66 to 97%) in Group C maintained titers of $\geq 1:20$ for 1 year. The reverse cumulative distribution of GMT at peak is indistinguishable for the three groups. RVFV MP-12 was well tolerated and immunogenic at the doses and routes studied. Further study of safety and immunogenicity comparing i.m. and s.c. routes is warranted.

P18 High avidity virus specific-IgG antibody response after inactivated Hantaan virus vaccine immunization and its utility

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Recently, antigen-specific antibody IgG avidity assay has been suggested to be useful for evaluation of IgM antibody response caused by primary, reinfection or persistence in some infectious diseases. In this study we evaluated the avidity of the hantaan virus-specific IgG antibody following vaccination. Serum samples from 60 vaccinees and acute phase sera from 45 patients with acute phase hemorrhagic fever with renal syndrome were tested. Formalin inactivated mouse brain-derived Hantaan virus vaccine was given for primary basic vaccination(0-1month) either with a booster(0-1-13). IgG avidity index was measured by indirect immunofluorescent antibody titers with pre- and posttreatment of 8M urea. Sera from vaccinees with the completion of primary basic vaccination or with a booster have high avidity of IgG antibody (mode: 4 of avidity index), whereas sera with one dose vaccination or acute phase sera from patients have much lower avidity (mode:64 of avidity index), with statistical significance ($P=0.0001$). IgM antibody response by capture EIA were observed in 20% of vaccinees' sera. In conclusion, the increase in serum avidity observed in vaccinees is probably due to anamnestic response provided by restimulation of the second dose of vaccination. And the IgG avidity assay can be used as a surrogate marker in evaluation of vaccination status in terms of adequate immune stimulation. Its significance in relation to protectivity remains to be investigated.

P20 Development of DNA Vaccines Directing Antigens to Alternative Cellular Processing Pathways and their Effect on the Immune Response. Cohen S., Grosfeld H., Lustig S., Halevy M., Bino T., Velan B., Shaffer A., Israel Institute for Biological Research Ness-Ziona 74100, Israel.

The envelope protein E2 of Semliki Forest Virus (SFV) is a major immunoprotective antigen. We constructed DNA vaccines for three configurations of SFV-E2: (i) intact E2 (ii) the precursor polypeptide -E3E2, and (iii) a transmembrane-anchor truncated form of the latter -E3E2tr. Extensive biochemical, immunofluorescence and cellular analyses of the three E2 forms expressed in cell free system (TNT) and in transfected COS cells allowed to determine that: E2 is non glycosylated and is quickly degraded in the cytoplasm; E3E2 and E3E2tr are glycosylated and are translocated into the endoplasmic reticulum; E3E2 is anchored to the cell membrane while the E3E2tr is retained in the Golgi compartment; expression levels and cellular stability of E3E2 and E3E2tr are comparable. The E2 construct did not induce any detectable immune response - not even priming. Both E3E2 and E3E2tr induced a typical Th1 response including CTL and could protect mice from lethal challenge though full protection was achieved only with E3E2. The E3E2 construct induced in addition to Th1 a humoral response with elevated IgG2a:IgG1 ratio, resembling the response to a live SFV vaccine. Unlike the live vaccine, the E3E2-DNA vaccine induced antibodies are non-neutralizing in-vitro but still confer protection in passive transfer experiments.