

**21 Phase I Study of Two Doses of Monovalent Live-Attenuated Dengue Virus Vaccines**

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**Introduction:** WRAIR has developed live-attenuated dengue virus (LAV) of all 4 serotypes by serial passage in primary dog kidney (PDK) cells. In previous studies DEN-3 and DEN-4 had only about 50% seroconversion rates (SCR).

**Objectives:** 1) To determine clinical safety of 2 doses of dengue LAV and 2) To determine if second dose increases SCR of homologous neutralizing antibody (PRNT>1:10).

**Method:** DEN-1 (45A25) PDK20, DEN-2 (S16803) PDK50, DEN-3 (CH53489) PDK20 and DEN 4 (341750) PDK20 were each given to 8, 8, 8 and 6 flavivirus HAI-negative human volunteers, respectively. Each volunteer received 2 doses at 1 or 3 months apart. Reactogenicity is expressed as symptom severity index (SSI). PRNT<sub>50</sub> and IgM/G were done.

**Results:** Reactogenicity shown as SSI=(severity x duration in days)/# volunteers. Immunogenicity is shown as SCR and reciprocal geometric mean homologous PRNT titer at 30 days after vaccination.

Serotype	#vol*	fev	HA	rash	SCR	GMT*
1, 1st dose	7	3.14	2.86	4.71	7/7 (100%)	964
1, 2nd dose	7	0	0.43	0	—	585
2, 1st dose	7	0.29	0.57	0.29	7/7 (100%)	90
2, 2nd dose	7	0	0	0	—	302
3, 1st dose	8	0	1.63	0	2/8 (25%)	13
3, 2nd dose	8	0.25	0.38	0	2/8 (25%)	44
4, 1st dose	6	0.33	3.17	1.17	3/6 (50%)	29
4, 2nd dose	5	0	0	0	0/5 (0%)	8

**Conclusion:** 1) DEN-1 candidate is moderately reactogenic. Second dose of LAVs elicited almost no clinical symptoms. 2) Revaccination did not increase SCR compared to single dose, with the possible exception of DEN-3. Testing of the tetravalent combination is in progress.

**23 HLA-DPA1 Alleles and Measles Vaccine Antibody (Ab) Response: Preliminary Results**

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Highly polymorphic HLA class II DR, DQ and DP genes are known to encode cell surface glycoproteins that play an important role in immune response. Previous studies have shown that HLA-DRB1, HLA-DQA1 and the TAP2 alleles may influence measles vaccine-induced Ab response (Hayney MS *et al.*, J Invest Med 44:261, 1996; Hayney MS *et al.*, Vaccine 15:3, 1997). The HLA-DPA1 gene is known to be far less polymorphic than other HLA genes. To date, there are no data reported on the associations between HLA-DPA1 alleles and measles vaccine immune response. To determine whether HLA-DPA1 alleles play a role in immune response to measles vaccine and to determine the distribution of DPA1 alleles between measles vaccine non-responders (NRs) and hyper-responders (HRs), class II HLA-DPA1 typing was performed on 70 NR (IgG Ab seronegative) and 39 HR (from the upper 10th percentile of IgG levels of all subjects) school children ages 5-12 years immunized with MMR-II vaccine at age 15 months. Genomic DNA was typed for HLA-DPA1 by PCR-SSP technique. Statistical analysis of allele distribution between these two groups revealed that the HLA-DPA1\*0103/0202 genotype frequency distribution was significantly different ( $p=0.015$ ) among measles vaccine NRs (0%) and HRs (10%). However, analysis of the distribution of other allele frequencies at the DPA1 locus showed that the allele distribution at the DPA locus is not significantly different among NRs and HRs. Also, the HLA-DPA1 homozygosity rate among NRs was similar to HRs (61% vs. 56%;  $p=0.24$ ). No association was found between any one DPA1 allele and vaccine response; however, we did identify a significant association between the DPA1\*0103/0202 genotype and nonresponse to measles vaccine. Further studies with larger sample sizes are planned.

**22 Maternal Antibodies Provide Passive Immunity and Modulate Antibody Responses to Human Rotavirus (HRV) in Gnotobiotics Pigs**

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The effect of maternal antibodies (Ab) in the circulation (serum) and diet (colostrum/milk) on protection and development of mucosal antibody secreting cells (ASC) to HRV was examined in neonatal gnotobiotics pigs devoid of maternal Ab. Pigs were injected peritoneally after birth with sow serum of high (immune, group 1, 2) or low (control, group 3, 4) Ab titer and immune or control colostrum/milk was added to the diet for 13 days of groups 2 and 4, respectively. Group 5 received no maternal Ab. Pigs were inoculated (3-5 days of age) and challenged [21 post-inoculation days (PID)] with virulent HRV to assess the effects of maternal Ab on protection (diarrhea, virus shedding) and Ab responses (enumeration of ASC by ELISPOT in intestinal and systemic lymphoid tissues). Group 1 and 2 (immune maternal Ab) had significantly less diarrhea and reduced virus shedding after inoculation, but greater rates of diarrhea after challenge than the other (control) groups (3-5), which developed active immunity. Immune maternal Ab in serum (group 1) or serum and milk (group 2) led to significantly reduced numbers of IgA and IgG ASC in intestinal tissues, both after inoculation (PID 21) and challenge (PCD 7) compared to Ab negative controls (group 5). Pigs given immune serum and milk (group 2) had significantly fewer IgA ASC (PCD 7) than pigs given immune serum alone (group 1), suggesting an additive suppressive effect of milk Ab. Thus circulating or dietary maternal Ab passively protect pigs against HRV diarrhea, but have a negative impact on development of intestinal ASC responses, and could impact the efficacy of oral HRV vaccines in neonates. Current studies are in progress to examine this latter possibility in the gnotobiotic pig model.

**24 Oral Immunization with Human Papillomavirus Type 11 (HPV-11) Recombinant Virus-Like Particles (VLPs) Induces a Potentially Protective Systemic Immune Response in Mice**

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**Objective:** To evaluate the oral immunogenicity of HPV-11 VLPs in mice.

**Methods:** Purified HPV-11 VLPs were formulated in phosphate-buffered saline in three dosage levels (100µg, 50µg or 10µg), and administered orally to three groups of five female BALB/c mice (aged 8-10 weeks). Booster inoculations were also administered orally 14 and 41 days after primary immunizations. A fourth group of mice received no inoculations. Sera from immunized and control animals were evaluated by enzyme-linked immunosorbent assay (ELISA).

**Results:** Sera from immunized mice demonstrated dose-dependent antigen-specific immunoglobulin G (IgG) and IgA responses. Antibody specificities were found to be conformationally dependent, and were restricted according to virus genotype. No clear predominance of IgG subclass was observed, although the appearance of IgG2a preceded that of IgG1. Importantly, when tested in a VLP binding inhibition ELISA, orally induced antibodies efficiently inhibited VLP binding by antibodies known to neutralize infectious HPV-11 virions.

**Conclusions:** VLPs may be effective oral immunogens for the prevention of anogenital HPV infection. This observation may facilitate the development of mass immunization strategies designed to reduce the incidence of invasive uterine cervical carcinoma and other HPV-associated diseases.