

**P13** Re-Vaccinating children previously vaccinated with Edmonston-Zagreb (EZ) measles vaccine.  
M. Khalil<sup>1</sup>, A.A. Poltera<sup>2</sup>, M. AL-Howasi<sup>3</sup>, C. Herzog<sup>2</sup>, E. Gerike<sup>4</sup>, B. Wegmüller<sup>2</sup>, R. Glueck<sup>2</sup>. <sup>1</sup>STAT Cairo, <sup>2</sup>Swiss serum and Vaccine Institute Berne, <sup>3</sup>MOH Riyadh, <sup>4</sup>Robert Koch Institute Berlin.

In earlier studies, EZ measles vaccine had shown significantly higher seroconversion rates compared to Schwartz at the age of 6 months in Saudi children. The aim of this study was to evaluate the effect of two measles vaccine strains as a second dose in children previously vaccinated with E-Z. Children were randomized at 12 months of age to receive one of two MMR vaccines (Triviraten Berna containing E-Z measles strain or MMR II MSD containing Edmonston-Enders B). In both groups, the seroprotection rate increased virtually to 100%. No significant difference was found regarding GMT or fold increase between the two groups. Therefore, EZ or Edmonston-Enders B measles vaccine can be used to boost an EZ induced primary response.

**P14** Expression of the Recombinant Dengue 4 Virus Truncated E Glycoprotein Made in *Pichia pastoris* and Evaluation of the Immune Response Induced in Mice.

R. Rodríguez,<sup>1\*</sup> M. Muné,<sup>1</sup> G. Márquez,<sup>2</sup> B. Sierra,<sup>1</sup> A.B. Pérez,<sup>1</sup> M. Alvarez,<sup>1</sup> G. Guillén,<sup>2</sup> M.G. Guzmán.<sup>1</sup> <sup>1</sup> Institute for Tropical Medicine Pedro Kourí, Havana, Cuba; <sup>2</sup> Center for Genetic Engineering and Biotechnology, Havana, Cuba

The recombinant dengue 4 virus truncated E glycoprotein made in *P.pastoris* could have similar antigenic characteristics to the native E glycoprotein and may elicit an adequate immune response in mice. The E protein was expressed using the vector pFAO containing MFα prepropeptide secretion leader from *S.cerevisiae*. Yeast fractions were tested by SDS-PAGE and Western blotting (WB) using anti dengue 4 murine polyclonal antibodies. The insoluble protein detected associated to the cellular fraction was extracted with 8M urea and 25 % clarified using ammonium sulfate. The soluble preparation was tested by ELISA and WB using anti flavivirus murine polyclonal and monoclonal antibodies and anti dengue human sera. Humoral immune response was studied in mice immunized with this preparation by ELISA, WB, inhibition of haemagglutination (IH) and plaque reduction neutralization test (PRNT). The cellular immune response was also explored using lymphoproliferation assays. Finally, the capacity of induce protection against lethal dengue infection in mice was assessed. A 64 kDa glycoprotein recovered in urea extract was recognized by all murine polyclonal antibodies against flaviviruses and some of the monoclonal antibodies employed. Most of the human sera reacted with the protein. Mice immunized with the protein responded with anti dengue 4 antibodies detected by ELISA, WB, IH and PRNT. Spleen cells from the immunized mice proliferated in response to dengue virus antigens. Mice were protected against lethal challenge with dengue 4 virus. Despite the crude nature of the immunogen, our results suggest the utility of *P.pastoris* system for getting a future candidate recombinant subunit vaccine against dengue virus.

**P15** Expression of Truncated Dengue-2 E Protein by a DNA vaccine candidate is Dependent of its Signal Peptide but Independent of the Whole prM Protein. R. Occazionez J. and B.A.L. Fonseca\*. Department of Internal Medicine, School of Medicine of Ribeirão Preto, University of São Paulo.

Dengue viruses are the most important arthropod-borne viruses of medical importance. They are RNA viruses whose genome is constituted by an open-reading frame encoding 3 structural (C, prM, E) and 7 non-structural proteins. There are four serotypes, named dengue-1 -2, -3 and -4, that cause a broad spectrum of disease ranging from asymptomatic infections to severe disease characterized by shock and hemorrhage. There is no treatment or vaccine available for these infections and recombinant expression of the structural E protein has been used to develop a dengue vaccine. The correct expression of E protein in vaccinia virus needs the prM protein but this finding has not been extended to DNA vectors. We investigated the correct expression of a truncated protein by a plasmid DNA. Three different portions of the gene coding for dengue-2 E, but no prM, were inserted into an expression vector (pCI) and the E protein expression was analyzed. C6/36 cells were infected with dengue-2 virus, New Guinea C strain, and when they were 100% infected, the RNA was extracted, and the cDNA first-strand was synthesized. The segments of the E gene were amplified by PCR, cloned into the plasmid and transfected into eukaryotic cells. All of these fragments encoded a truncated E protein at the carboxyl side and included the signal peptide in only one of them. Biochemical analysis showed that E protein was correctly synthesized only when the signal peptide was present. It became evident that the expression of E by this DNA vaccine candidate was not dependent on the prM protein but depended on the presence of E signal peptide.

**P16** Dengue-2 DNA Vaccine Candidate Expressing a Truncated E Protein Prolongs Mice Survival After Challenge with a Virulent Strain. R. Occazionez J. and B.A.L. Fonseca\*. Department of Internal Medicine, School of Medicine of Ribeirão Preto, University of São Paulo.

There are four serotypes of dengue viruses, named dengue-1, -2, -3 and -4, and they are the most important arthropod-borne viruses of medical importance. These viruses cause a broad spectrum of disease ranging from asymptomatic infections to severe disease characterized by shock and hemorrhage. The severe form of the disease are usually associated with sequential infections by different dengue serotypes and due to this fact the dengue vaccine needs to be tetravalent. Recombinant DNA technology has shown that the envelope (E) and membrane (M) protein are the most immunogenic antigens of flaviviruses and this concept has been used to develop a dengue vaccine. We engineered a DNA vaccine candidate (DEN-2 DNAvac) that expresses a truncated E protein without concomitant expression of prM. The rationale for this approach is that decreasing the size of the insert it will be possible to manufacture a tetravalent vaccine using only one vector. Balb/c mice were inoculated twice, intramuscularly, with 100 µg of the DEN-2 DNAvac and bled at days 8 and 18 after first and second inoculation, respectively. Negative and positive control groups were constituted by mice inoculated with the plasmid vector and with dengue-2 live virus, the latter, inoculated intraperitoneally. On day 20, they were challenged by the intracerebral route with 100 LD<sub>50</sub> of dengue-2 wild strain. We were not able to detect neutralizing antibodies on the pooled sera of inoculated mice but mice inoculated with DNAvac started dying later than mice inoculated with the plasmid and dengue-2 virus (10-14 weeks vs 8-12 weeks). The number of mice surviving the experiment was not different between the dengue-2 and DNAvac groups while 100% of mice in the plasmid group died. Based on our data, it is possible to manufacture a tetravalent DNA vaccine candidate using only one vector but further studies are necessary.