P21 Nucleic Acid Vaccination Against Hepatitis B Surface Antigen in Mice.

A. Karami[1-3*], B. Yakhchali[1], T. Lohrasbi[1], M.N. Sarbolouki[2], National Research Center for Genetic Engineering and Biotechnology[1], Basic Science Research Institute-Imam Hossein University[3], Institute for Biophysics and Biochemistry, Tehran University[2].

We have studied expression of HBs antigen and anti-HBs response in mice by intramuscular and intradermal injection of recombinant eukaryotic vector encoding Hepatitis B surface antigen sequence adjacent to CMV promoter. Analysis of serum sample from mice injected with 50 or 100 ug of endotoxin free DNA with and without special polymer (liposome) by ELISA revealed that: small amount of HBs-Ag expressed in mice tissue strongly stimulate immune response and high level of anti-HBs were detected in both endotoxin free DNA alone and with liposome but liposome mediated DNA vaccination caused to elevate antibody production when compared with non adjuvant DNA. Detection of enhanced immune response was as 10 folds higher when compared to four times administration of human recombinant HBs Vaccine. PCR Analysis of blood and muscle tissue after injection of DNA Vaccine for presence of HBs sequence in different time intervals revealed that after 72hr we were not able to detect HBs encoding sequence.

P22 **The Attenuation Phenotype of Live, Temperature-sensitive (Ts) Vaccine Strains of Human Respiratory Syncytial Virus (RSV) at Restrictive Temperatures Is Not Associated with Enhanced Interferon Synthesis or Increased-sensitivity to Type I Interferons (IFNs).**

D. A. Loveys, S. Kulkarni and P. L. Aureva*, Laboratory of Pediatric and Respiratory Viral Diseases, Division of Viral Products, CBER, FDA, Bethesda, MD 20892.

RSV infection causes bronchiolitis and pneumonia in infants and children less than 2 years of age. At present no RSV vaccine is licensed and many vaccine candidates are under study. We have tested four, live Ts mutant strains of RSV along with their non-Ts parent strains, in human lung A549 cell line, for (1) their ability to induce IFNs and (2) their sensitivity to IFNs, at both permissive (32 °C) and restrictive temperatures (≥37 °C) to investigate if IFNs have a role in their the attenuation at the restrictive temperature. All the viruses produced very low amounts of IFN at permissive temperature except TS 1C mutant which produced the most, about 6-8 times antiviral activity compared to its parent. RT-PCR of the cellular mRNA with IFN specific primers correlated this antiviral activity to IFN-β. UV-inactivated viruses failed to produce this activity suggesting that viral replication is necessary for IFN induction even at permissive temperatures. At restrictive temperatures, no IFN specific mRNA was induced when cells were infected by either infectious or UV-inactivated viruses and viral mRNA analysis indicated lack of mutant RSV replication. Hence lack of IFN induction is due to lack of viral replication at restrictive temperatures. In addition, none of the Ts mutants were more sensitive to the antiviral activity of exogenously added type I IFNs, compared to their parents. These results suggest that the attenuation of these Ts mutants of RSV at restrictive temperatures is mainly due to the temperature-sensitivity of the viral polymerase itself and is not due to other attenuating factors such as enhanced IFN synthesis in the host or their increased-sensitivity to IFNs.

P23 RESTRICTED REPLICATION OF A CHIMERIC VIRUS FOR INDUCING PROTECT IMMUNITY AGAINST SIV INFECTION.

T. Matano*, T. Odawara, M. Kano, K. Mori, and Y. Ami. AIDS Research Center, National Institute of Infectious Diseases, Japan.

Although it has been reported that immunization with live-attenuated viruses (LAV) induced good protection against simian immunodeficiency virus (SIV) in macaques, their potential use for HIV-1 vaccines has been controversial because of safety concerns. In this study, to develop safe LAV, we tried to induce restricted replication of a chimeric virus in macaques.

A chimeric SIV, FSIV, in which SIV *env* was replaced with Friend murine leukemia virus (FMLV) *env* was obtained. Because no FMLV receptor, mCAT1, is expressed in primates, it is expected that treatment of macaques both with the mCAT1-expression vector DNA and the FSIV infectious clone DNA would result in FSIV replication restricted in the mCAT1-transduced cells.

In vitro experiments showed that the FSIV DNA-transfected cells produced the chimeric virus carrying FMLV Env. FSIV could not infect COS-1 cells but the mCAT1-expressing COS-1 cells as expected. In animal experiments, two rhesus macaques were inoculated with both the mCAT1-expression vector DNA and the FSIV infectious clone DNA, and one rhesus with the FSIV DNA only as a control. The former macaques treated with both the mCAT1 and the FSIV DNAs showed higher FSIV RNA copy number in plasma as compared with that in the control.

P24 **Altered Patterns of SIVmac251 Infection after DNA Vaccination in Macaques.**

Philippe Lena*[1], Paul Luciw[1], Murray Gardner[1], Jim Smith[1], Mike McChesney[1], Francois Villinger[2], Gary Rhodes[1]. [1]University of California at Davis, CA 95616. [2]Emory University Atlanta, GA 30322. Supported by Contract DAMD17-94-J4436 and Grant AI42608

We vaccinated juvenile rhesus macaques with different forms of SIV envelope antigens expressed in plasmid DNA. One group of animals was later boosted with recombinant envelope protein, and another group was boosted with a noninfections proviral DNA (expressing Gag). After oral challenge of all animals with pathogenic SIVmac251, we observed different patterns of infection. Two animals did not have detectable virus at any time after challenge. Another macaque had only transient low viremia. Two other animals showed initial high viremia, then cleared the virus exponentially. None of these monkeys showed any sign of clinical disease during a 9 month observation period. The group of animals boosted with recombinant env protein showed increased viral loads and early progression to disease. Increased levels of antibodies correlated with aggravated infection. Additional studies are in progress to measure other immunological effector mechanisms in protected animals. These results have implications for development of anti-HIV vaccines.