

on Vaccine Research

ABSTRACTS OF SUBMITTED POSTER PRESENTATIONS

P5

ELISPOT assays for evaluation of antigen specific responses in HIV-1 vaccinated individuals.

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As the ELISPOT assay replaces the cytotoxic T lymphocyte assay (CTL) and lymphoproliferation assay as a potentially more efficient, sensitive and quantitative means of evaluating cellular immune responses in vaccinees, it is increasingly important that the results of these assays are standardized and correctly interpreted. In one study, PBMC were taken at study days 42, 98, and 182 after immunization with HIV-1 ALVAC vectors (Aventis-Pasteur). Standard ⁵¹Cr release (CTL) assays were done after an *In Vitro Stimulation* (IVS), and the remaining *in-vitro* expanded PBMC were cryopreserved. It was on these frozen samples that IFN- γ ELISPOT assays were done, thawing the samples and incubating the cells with pools of overlapping 20-mer HIV env or gag peptides. After we established the criteria for ELISPOT responders/non-responders (mean # of IFN- γ secreting cells/10⁶ PBMC in the absence of HIV antigen +2SD), 16/35 volunteers who received the HIV-1 ALVAC were determined to be responders and in the placebo 0/5 were responders. The range of IFN- γ secreting cells/10⁶ PBMC was much broader in the HIV-1 ALVAC immunized volunteers seronegatives (0-8107 IFN- γ secreting cells/10⁶ PBMC) and much lower in volunteers receiving placebo vector or unimmunized HIV-1 (0-175 IFN- γ secreting cells/10⁶ PBMC). Responses to PHA were uniform in the immunized and unimmunized groups. Similar ELISPOT assays were done on fresh PBMC samples prior to IVS, with far less conclusive results due to the low frequency of antigen IFN- γ secreting cells (0-70 IFN- γ secreting cells/10⁶ PBMC). We will present data showing different ways of interpreting ELISPOT data and stress the need for developing uniform criteria for categorizing vaccine responders and non-responders.

P7

AN UPDATE ON THE PHASE I HUMAN CLINICAL TRIAL OF AN EPSTEIN-BARR VIRUS VACCINE

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Epstein-Barr Virus (EBV) infects 90% of the population and is associated with infectious mononucleosis (IM) and a range of human malignancies. CD8⁺ cytotoxic T cells (CTL) are strongly implicated as primary mediators of protection against EBV-associated diseases. We report here results from a phase-I trial designed to determine the safety and immunogenicity of a cytotoxic T cell based peptide vaccine for IM and potentially applicable for EBV-associated post-transplant lymphoproliferative disease (PTLD). This is a single blind, randomized, placebo controlled, single center study. The vaccine formulation consists of an HLA B8 restricted peptide epitope FLRGRAYGL (from EBNA3) mixed with tetanus toxoid and Montanide ISA 720 in a water-in-oil emulsion, administered in two doses (primary and boost) subcutaneously (0.5ml/dose). Peptide-specific CTL responses were detected in low dose (5ug) as well as the high dose (50ug) peptide-vaccine recipients by the limiting dilution assay and ELISPOT assay. To date, the vaccine has been well tolerated and three vaccinees have seroconverted asymptotically after a natural EBV infection.

P6

Immunogenicity of five doses of inactivated *Mycobacterium vaccae* vaccine in HIV+ subjects

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Objectives: To determine if 5 doses of inactivated *M. vaccae* vaccine evokes cell-mediated immune response against *M. vaccae* and *M. tuberculosis* antigens in HIV+ subjects with prior BCG immunization.

Methods: A total of 39 HIV+ subjects with CD4 counts >200 were randomly divided into vaccine and placebo arms in this double blinded study. The vaccine group received 5 doses of inactivated *M. vaccae* vaccine (SR Pharma, London) at 0, 2, 4, 6 and 12 months. The placebo group received hepatitis B vaccine (Engerix-B, SmithKline Beecham) at 0, 2 and 12 months and placebo (SR Pharma, London) at 4 and 6 months. In addition, 10 HIV- control subjects received *M. vaccae* vaccine. Blood samples were drawn at baseline, after 3 doses and after 5 doses. Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll-Paque (Pharmacia Biotech) density gradient centrifugation. The PBMC were stimulated on 96-well plates with media alone (background control), *M. vaccae* sonicate (kindly provided by Dr. J. Sanford), *M. tuberculosis* whole cell lysate (WCL, kindly provided by Dr. J.T. Belisle), *M. tuberculosis* antigen 85 protein complex (Ag-85, kindly provided by Dr. J.T. Belisle) or phytohemagglutinin (PHA). The proliferative response was detected as the incorporation of ³H-thymidine during the last 16-18 h of a 6-day incubation.

Results: After 3 doses of *M. vaccae* HIV- subjects had an increase in lymphocyte proliferative response to the vaccine antigen from 10,596 to 18,670 (median cpm). Data on HIV+ subjects remain blinded in two groups with significant increases in proliferative responses to the vaccine antigen in Group A vs. Group B after 3 doses.

Conclusions: Multiple doses of inactivated *M. vaccae* evoke cellular immune responses to mycobacterial antigens in persons primed with BCG. Final data will be presented.

P8

Influenza Vaccination and Stroke in the Elderly - A Community-based Observational Study in Korea

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Elderly persons are generally considered to be the primary target group for influenza vaccination, because they are most prone to serious complications from influenza virus infection. We assessed the clinical efficacy of influenza vaccination in the elderly (age over 65) against hospitalization by cardiovascular diseases including stroke. We observed the Nonsan Elder Cohort (NEC) from November 1, 1999 to February 29, 2000. NEC was composed of 7,453 elderly persons (43.1% of total elderly population in Nonsan City) who had ever visited the community health center or 13 public health offices in Nonsan City from January to October 1999. Inactivated split influenza vaccines were injected to the volunteers among the NEC in October. Neither randomization nor placebo was used. We followed the NEC up by telephone at March 2000, and interviewed successfully 4,654 persons - 3,663 were the vaccinated and 991 were not. The socio-demographic factors like sex ratio, mean age, education level, marriage status, and yearly income level were not significantly different between the two groups in bivariate analysis. The current smokers were 27.0% in vaccinee and 31.6% in non-vaccinee (p<0.05). In past medical history, 35.8% of the vaccinee and 32.6% of the non-vaccinee had one or more cardiovascular diseases (hypertension, any heart diseases and stroke) before this study (p=0.09). The proportions of other chronic medical conditions like chronic respiratory diseases, diabetes, chronic liver diseases and chronic renal failure were not significantly different, too. During this influenza season, 25 persons were hospitalized by cardiovascular diseases; 13 were vaccinee (rate: 4/1,000) and 12 were non-vaccinee (12/1,000). The result of multivariate Cox regression showed the vaccine efficacy against the hospitalization by cardiovascular diseases was 69.5%(95% CI: 32.5, 86.3). The hospitalization rate by stroke was 2/1,000 in vaccinee and 7/1,000 in non-vaccinee. The preventive efficacy of the vaccine for stroke was 68.3%(95% CI : 6.5, 89.4) by multivariate Cox regression. This result suggest the possible role of influenza vaccine to prevent the elderly from stroke.