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ABSTRACTS OF SUBMITTED PRESENTATIONS

S25 Down Modulation of Host Inflammatory Responses by RanC/d After Adenoviral Gene Therapy.

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By functional cDNA cloning we have established that Ran is important in innate immune response (Kang et al, *Infect Immunity* 64:4612; 1996). Transduction of primary macrophages with RanC/d cDNA reduces the production of pro-inflammatory cytokine such as TNF- α , regulated via a novel post-translational mechanism. Mice treated with a lethal dose of endotoxin died of septic shock, and 35% of 40 such mice survived after adenoviral transduction of the RanC/d cDNA. This contrasted with 17% and 10%, respectively, of 35 such mice treated with endotoxin alone and 40 such mice transduced with Ran T/n cDNA, which is identical to RanC/d cDNA except a single base change from T to C at position 870 in the 3'-UTR. By histochemical analysis, we observed that while inflammation was evident in livers of mice that received no endotoxin but were transduced with RanT/n, it was absent in those transduced with RanC/d alone. This result correlated with the intensity of the respective transgenes in livers but not in peritoneal macrophages of transduced mice as revealed by results of a competitive and quantitative PCR analysis. This immune down modulation effect of RanC/d was further shown by experiments where it was tested as a prophylactic. In this model, 70% of 25 mice transduced with RanC/d cDNA survived endotoxin challenge 4 days after adenoviral gene transfer compared to 25% of the control mice. Thus, our data suggest that Ran participates in multiple signal transduction pathways in innate immune responses, and that RanC/d cDNA can be incorporated into adenoviral vectors as a key genetic element capable of down-modulating adenovirus-induced host innate immune responses.

S27 Incorporation Of CD154 Into SIVgag VLP Results In Reduced *in vivo* Immunogenicity. L. Giavedoni^{1,2}, S. Frey¹, M. Velasquillo¹, L. Parodi¹, and V. Hodara¹. ¹Southwest Foundation for Biomedical Research, and ²Southwest Regional Primate Research Center, San Antonio, TX.

SIVgag VLP are non-infectious retrovirus-like particles vaccines that usually elicit short-lived immune responses in non-human primates. CD154 is a costimulatory molecule directly involved in T cell-dependent humoral and cellular immunity. We investigated whether SIVgag VLP containing CD154 are stronger immunogens by generating recombinant baculoviruses expressing either SIVgag or both SIVgag and human CD154 (CD154/SIVgag VLP). VLP were purified from the supernatant of infected insect cells by banding in sucrose gradients. Western-blots demonstrated the presence of SIVgag in both type of particles, and flow cytometry analysis showed CD154 on the surface of CD154/SIVgag VLP. CD154/SIVgag VLP retained the ability to induce IFN- γ production by CD4+ T cells from macaques infected with an attenuated SIV in an *in vitro* stimulation assay. Additionally, incubation of immature human and baboon dendritic cells with CD154/SIVgag VLP resulted in maturation of DC, as determined by upregulation of the CD25, CD40, CD83, and CD86 markers, and reduction in the level of CD8. The immunogenicity of these antigens was evaluated in baboons by intramuscular inoculations, in the absence of any adjuvant, of SIVgag or CD154/SIVgag VLP. These studies showed that CD154/SIVgag VLP were less immunogenic than SIVgag VLP. Both SIVgag-specific humoral immunity and CD4 T-cell helper activity were reduced by the presence of the costimulatory molecule CD154. Histological analysis of draining lymph nodes also showed reduced hyperplasia in baboons inoculated with CD154/SIVgag VLP. The mechanism for this reduced immunogenicity is under investigation, including the possibility that CD154-containing VLPs are providing immune signals in the inappropriate environment.

S26 A novel adjuvant formulation containing a block copolymer with reverse gelation characteristics elicits long lasting IgG antibody responses after a single injection in mice

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We have been investigating a novel adjuvant formulation for use as a single dose vaccine delivery vehicle. The adjuvant consists of the block copolymer, Pluronic®F127, in a proprietary formulation (ProGel™ [PGZ]), and the cationic biopolymer, chitosan. The poloxamer formulation exhibits reverse gelation characteristics, potentially stabilizing antigenic conformation within the matrix and providing a depot for antigen release at body temperature. Chitosan provides the immunostimulatory component of the formulation. In studies using the prototype antigen, tetanus toxoid (TT), and subcutaneous administration to mice, a single injection of antigen formulated in PGZ/chitosan induced an accelerated rise in IgG anti-TT antibodies. Antibody levels rose to a peak at 8 to 10 weeks after injection and then persisted for at least 10 months with mean titers above 100,000. This response was more rapid and potent than that elicited by TT/alum, which did not attain equivalent levels until after three doses had been administered. Furthermore, the combination of PGZ/chitosan with TT was superior to TT/chitosan alone. Long-lived plasma cells specific for TT were found in the bone marrow of mice immunized at least 6 months previously with a single dose of TT/PGZ/chitosan. This formulation has potential as a novel adjuvant formulation that could improve compliance in immunization programs. In addition, since the components of the formulation are in human use, it is anticipated that such a formulation could receive rapid human approval. These studies have been expanded to other antigens and this work will also be presented.

S28 Improved Efficacy of a Herpes simplex Virus gD Vaccine Following Incorporation of Viral Epitope and Adjuvant in Liposomes. L.H.Song^{1*}, W. Ernst², G. Fuji² and J.P.Adler-Moore¹. ¹Biology Dept., Cal Poly University, Pomona, CA. ²Molecular Express Inc., Los Angeles, CA.

Adjuvants can increase the immunostimulatory properties of a vaccine formulation by stimulating different components of the immune system. The degree of stimulation depends upon the nature of the adjuvant and antigen preparation. A comparison was made between the efficacy of a gD1-23 HSV2 epitope mixed with adjuvant or the gD1-23 epitope fused with a hydrophobic domain (gD1-23-HD) and then incorporated into the liposomes along with adjuvant. At wk 1, 4 and 8, groups of C57BL/6 mice (n=12/group) were vaccinated subcutaneously with 6 mg/kg gD1-23-HD in one of the following preparations: liposomal gD23-HD (L-gD1-23-HD) with either monophosphoryl Lipid A (MPL) or desmuramyldipeptide (DMDP); a mixture of gD1-23-HD with either alum or MF59; L-gD1-23-HD without adjuvant; liposomal DMDP without gD1-23-HD; gD1-23-HD in buffer. At wk 9, 7 mice/group were intravaginally challenged with HSV2 and monitored for clinical signs of infection up to 60d. Sera from 5 mice/group were analyzed for neutralizing antibody (NA) titer (50% reduction of pfu). Survival after 60d was 100% for L-gD1-23-HD/MPL, 80% for L-gD1-23-HD/DMPD, 60% for L-gD1-23-HD, 43% for gD1-23-HD/MF59, 14% for gD1-23-HD /alum, and 0% for liposomal DMDP and gD1-23-HD in buffer. Vaginal lesions and neurological signs were severe in all groups, except for L-gD1-23-HD with the adjuvants MPL or DMDP. NA titers were highest for the L-gD1-23-HD with MPL or DMDP (10-14X greater than for liposomal DMDP and gD1-23-HD in buffer). The results showed that the adjuvant and the epitope-HD protein when incorporated into the liposomes produced much better efficacy in the HSV2 vaginal murine model than the epitope-HD mixed with the adjuvant in a non-liposomal form.