

S21 Enhance Humoral Responses to HCV Envelope in DNA Immunization by Protein Boost
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Hepatitis C Virus (HCV) is the causative agent of most case of transfusion-associated non-A, non-B hepatitis, more than 70% of acutely infection will proceed to a chronic carrier state which may progress to cirrhosis and hepatocellular carcinoma. Currently there is no effective therapy for chronic HCV infection and no valuable vaccine has been developed. In this study, we constructed the recombinant plasmids pCI-HCV-E18 (E18) containing HCV envelope genes which encoding E1 + E2 protein and pCI-HCV-D1 (D1) containing core plus envelope genes. After the intra-muscular inoculation in BALB/c mice, we found no IgG antibody rise after second boost. To verify whether the humoral response had been primed, we boosted the DNA-immunized mice with purified recombinant HCV-envelope protein G13 (aa413-729, E2 region). The anti-envelope IgG in E18 and D1 immunized mice reached high level rapidly ($p < 0.01$), one to two weeks earlier than that inoculated with control plasmid (pCI-blank). This demonstrated the specific humoral response has been primed. In a proliferation assay, the spleen cells from E18 and D1 immunized mice gave higher stimulation index than that of control mice ($P < 0.01$). The responding cells in proliferation have been proved to be of CD4⁺ CD8⁻ phenotype by blocking using specific monoclonal antibodies, supporting that the DNA immunization has specifically primed T helper cells. A Cr-51 releasing CTL assay specific for HCV core and envelope was investigated using peptides. We demonstrated CTL activity against HCV core in D1 immunized mice, however we fail to detect CTL activity against HCV envelope in E18 and D1 immunized mice. We demonstrated that immunization of plasmid containing HCV envelope gene could induce specific antibody response and T helper cell activation. But the ability of HCV envelope gene to induce CTL remind to be further investigated.

S22 Revisiting whole inactivated HIV-1 vaccines: Retention of gp120 and enhanced antigenicity of conformation-dependent neutralization epitopes.

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Inactivation of whole viral particles is the basis for several successful vaccines currently in use. Initial attempts to use SIV to model a killed HIV-1 vaccine were unsuccessful, and limited subsequent effort has been directed towards a systematic study of the requirements for a successful whole killed HIV-1 vaccine. Recent insights into HIV-1 virion and glycoprotein structure and neutralization epitopes led us to revisit the question of whether inactivation of whole HIV-1 particles could serve as a basis an HIV-1 vaccine. Our results indicate that relatively simple processes involving thermal and chemical inactivation can inactivate HIV-1 by at least 7 logs, and maintain envelope glycoproteins on the virion surface in a strain dependent fashion. Of importance, we demonstrate retention of each of three conformation dependent neutralization epitopes. Moreover, the antigenicity of these epitopes is increased as a function of time. In contrast, treatment of free envelope under the same conditions leads only to loss of antigenicity. These inactivated virions can also be presented by human dendritic cells to drive a cell mediated recall response in HIV seropositive donors *in vitro*. These data indicate that a systematic study of HIV-1 inactivation, gp120 retention, and epitope antigenicity can provide insights that will be important in developing an effective whole killed HIV-1 vaccine.

S23 Gelatin in Vaccines As A Possible Cause of Anaphylaxis
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Background: Gelatin is used in some live viral (e.g. measles) vaccines as a heat stabilizer. A case report from the U.S. and several case-series from Japan suggest that the gelatin in such vaccines can cause anaphylaxis. The fraction of vaccine-associated anaphylaxis attributable to gelatin is unknown, however.

Methods: We conducted a case-control study to identify possible risk factors for anaphylaxis after measles vaccination used in the US. Cases were identified from the Vaccine Adverse Event Reporting System (VAERS), a national passive surveillance system. VAERS reports with symptoms consistent with anaphylaxis were reviewed by an allergist and classified as probable and possible cases. Controls matched by age, sex and the time of measles vaccination were recruited from the population served by the Mayo Clinic (Rochester, MN). Allergy histories and a blood sample for anti-gelatin IgE testing were obtained from both cases and controls. The IgE binding level of 2 standard deviations above the mean count per minute for the controls was used as a cut-off level for positive test results.

Results: Sixty one of 132 probable and possible cases were interviewed. Twenty two of them also provided a blood sample for IgE testing. To date, laboratory results are complete for 13 cases and their controls. Six cases had anti-gelatin IgE levels exceeding the positive cut-off versus none of the matched controls (McNemar's P-value=0.014). Ten of the cases had a history of allergies to food or drugs compared to 3 of the controls (P=0.001).

Conclusions: Our data suggest that gelatin may be an important cause for anaphylaxis following measles vaccination in the U.S. and elsewhere. Efforts to identify alternatives to gelatin for vaccine stabilizer may be needed.

S24 The Safety of Hepatitis B Vaccine in Adults
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Objective: Reports of permanent disability following hepatitis B (HB) vaccination in previously healthy adults raise questions about its safety. We assessed the validity of such concerns. **Methods:** We reviewed 1991-98 data for adults 18+ years old from 1) the Vaccine Adverse Event Reporting System (VAERS) and 2) Biologics Surveillance on net doses distributed in the U.S. Definitions were developed for inflammatory (IAE) and paralytic adverse events (PAE). Separately, dizziness, paresthesia, and fainting were defined as nuisance adverse events (NAE). Data for Tetanus-diphtheria (Td) vaccine was used for comparison. **Results:** Reporting rate to VAERS per million doses distributed for IAE, PAE, and NAE was higher for HB vaccine (0.8, 0.7, 5.5) compared to Td (0.2, 0.3, 3.3) ($p < 0.01$, t-test). Among vaccinees 18-65 years of age reporting disability, there is a striking female predominance (80% of 184 HB, 72% of 18 Td). **Discussion:** The higher reporting rate for NAE, as well as IAE and PAE, after HB vaccine compared to Td vaccine suggest that this may be an artifact of reporting due to the greater use of HB vaccine in health care workers - who may be more likely to detect adverse events and report to VAERS. The female predominance among disabled vaccinees irrespective of vaccine, also observed in many autoimmune disorders, bears further exploration. It suggests that there may be a generic "unmasking" of genetically susceptible individuals by vaccines.