

S9 Cellular selection of human rotavirus enterotoxin gene variants in cell culture: A potential strategy to obtain nontoxic forms of toxic genes for subunit vaccine applications

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Rotavirus (RV) NSP4 causes cytotoxicity in cell culture. In mice, the NSP4 alone was shown to cause age- and dose-dependent diarrhea by functioning as an enterotoxin. Manifestation of NSP4 toxicity in cultured cells correlates well with its function as enterotoxin in animal models. Therefore, the hypothesis is that antibodies against rotavirus NSP4 protects infants from RV associated illness, if an attenuated nontoxic form of NSP4 is available for immunization. As a first step in obtaining an attenuated nontoxic NSP4, a novel strategy is utilized. Briefly, RV permissive intestinal cells in culture were subjected to constitutively express and forced to maintain the expression of an RV NSP4 gene (Wa strain) under G418 drug selection. Nucleotide sequence analysis of the NSP4 gene recovered from several G418 resistant NSP4 expressing cell lines demonstrated that the gene had undergone point mutations within its known cytotoxic domain. These results therefore support the idea that this strategy may be useful in obtaining naturally mutated and possibly attenuated forms of genes encoding cytotoxic proteins.

S10 Both antibody-dependent and -independent protection are stimulated after intranasal immunization of mice with inactivated rotavirus particles

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Second generation rotavirus vaccines are being developed to either supplement or replace first generation live virus vaccines which, after oral delivery, have provided only partial protection of limited duration. Intranasal (i.n.) immunization of BALB/c mice with inactivated, triple-layered (tl) particles of murine rotavirus strain EDIM was found to stimulate complete protection against viral shedding following EDIM challenge 1 month after immunization with either 1 or 2 doses (10 ug/dose). In contrast, i.n. immunization with double-layered (dl) EDIM particles lacking the VP4 and VP7 neutralization proteins or with tl or dl particles of heterotypic rotaviruses provided only partial protection against EDIM shedding. Although still incomplete, protection was significantly improved when mutant *E.coli* heat labile toxin LT (R192G) was included as adjuvant during immunization. When B cell-deficient uMt mice were immunized with tl EDIM, protection was reduced to that found with dl EDIM in either BALB/c or uMt mice. These results indicate that the greater protection provided by tl EDIM is due to serotype-specific neutralizing antibody and protection stimulated by dl EDIM and heterotypic particles is antibody-independent.

S11 "A New Vaccine Strategy: Enhancement of T Cell Responses by Recruiting CD26 to the TCR/MHC Complex with a High-Affinity CD26 Ligand Covalently Attached to Cognate T Cell Peptide Epitopes"

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The recently elucidated mechanisms of antigen recognition and T cell activation have suggested that bivalent agents, able to crosslink specific receptors on the T-cells, should have great potential for manipulating immune responses. We have synthesized two different types of such bivalent agents and have shown that they have the expected biological activities. The first, a homobivalent derivative of Lys-boroPro, designed to crosslink CD26, a costimulatory molecule found on the surface of CD4+ and CD8+ cells, costimulates murine and human T cells in culture, including naive T cells. The second, a heteroconjugate, containing a Lys-boroPro moiety covalently linked to an antigenic peptide, potently enhances T cell responses specifically to the attached peptide. These results suggest that such bivalent agents may be useful in vaccine design and development. We are now proposing to apply the above technology to construct agents able to enhance immune responses to previously established HIV antigens, and to test the agents in murine and human systems.

S12 Strategy for Isolation of Measles Vaccine Virus (MVV) Processed Peptides from Class II HLA-DR4 Alleles

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Class II HLA-DR4 homozygosity has been associated with antibody nonresponse to measles vaccine in US children (Poland GA et al, FASEB J 9:A240, 1995). To study MVV-derived peptides associated with measles nonresponsiveness, an HLA-DR4 homozygous (DRB1*0401) B-cell line was used as antigen-presenting cells. The ability of these cells to be infected with MVV and to process and present MVV-derived peptides in the peptide binding groove of HLA-DR4 molecules was demonstrated by flow cytometry (FACS), Wright's-Giemsa stain, electron microscopy, enhanced chemiluminescence (ECLTM) Western blotting, immunofluorescence and a shell vial assay techniques. Cells (1×10^5) were infected with 1,000 TCID₅₀ of MVV (Attenuvax, Merck). Expression of HLA-DR4 and CD46 (cellular receptor for measles virus) molecules on infected cells using FACS was demonstrated in 93% and 86% of cells, respectively. Cells were treated with acidic glycine buffer and HLA-DR4-peptide complexes were affinity purified from lysate of 8×10^8 cells using anti-HLA-DR mAb column. Bound peptides were released by acid elution, separated by reversed phase HPLC and matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and analyzed by membrane pre-concentration-capillary electrophoresis-tandem mass spectrometry (mPC-CE-MS/MS). By employment of two-dimensional chromatography consisting of HPLC fractionation and on-line mPC-CE-MS/MS we were able to detect peptides derived from MVV bound to class II HLA-DR4. This strategy clearly demonstrates the utility and the power of these methods. This useful model allows us to study MVV peptide processing and presentation and may provide insights into vaccine nonresponsiveness.