Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Generating Powerful Immune Responses Against Lentiviral Antigens: Using rSV40 Vector-Delivered Proinflammatory Cytokines to Potentiate Immune Responses vs. rSV40-Delivered Lentiviral Antigens

Hayley J. McKee,1 David S. Strayer.1

1Pathology, Jefferson Medical College, Philadelphia, PA.

Background: The importance of strong anti-lentiviral immune responses for prevention and therapy of AIDS, combined with the failure of classical immunization techniques to provide either effectively, has led to consideration of the potential utility of immunostimulatory cytokines as adjuvants in improving the magnitude and rapidity of such responses. Delivery of such cytokines, in concert with the antigen in question, might be facilitated by using recombinant Tag-deleted SV40 vectors (rSV40s). These vectors do not elicit detectable neutralizing antibody responses, and therefore can be used multiply, both to prime and boost immune responses.

Methods: IL-12 and IL-15 were used as immunopotentiating cytokines; among other things, both stimulate type I T cell responses. IL-15 also stimulates T cell memory cytolysic responses. rSV40s carrying the cDNAs for mouse IL-12, mouse IL-15, and SIV and HIV Env and Gag antigens were injected into BALB/cJ mice monthly x2. HIV Env (gp120) and Gag were used with or without IL-12 or IL-15. SIV Env (gp130) was used with or without IL-15. Antigen-specific cytotoxic lymphocyte activity was measured by direct lysis of 51Cr-labeled P815 cells stably-transfected to express lentivirus antigens. Unselected effector cells were used. Specificity of lysis was ascertained by subtracting both lysis of wild type P815 cells by antigen-exposed lymphocytes, as well as specific lysis of antigen-expressing P815 cells by lymphocytes from mice that had received a control rSV40 vector. Cytolytic responses in recipients of IL-15-carrying vectors were “memory responses”, and were tested 1 month after the last injection; those in recipients of SV(mIL-12) were tested 4d after the last injection.

Results: Mice co-immunized with cytokines and antigens gave powerful antigen-specific CTL responses at very low E:T ratios, 10:1 and 20:1. Cells from mice given IL-12 and gp120 or Gag showed >50% specific lysis. The effectiveness of the cytokines in augmenting the cytolytic response depended upon the antigen in question and the order in which cytokine- and antigen-carrying vectors were injected. Thus, SV(mIL-12) given simultaneously with SV(Gag) provided maximal stimulation, but SV(mIL-12) was most effective when injected 3d before or after SV(gp120). IL-15 augmented cytolytic responses against all antigens: specific “memory” cytolysis of cells carrying HIV and SIV Env and HIV Gag was very strong: >50% specific lysis vs. gp120 was seen when SV(mIL-15) was given first. However, for SIV Env, the most powerful cytolysis was seen when IL-15 was given after SVgp130. When SIV Gag was the target, all 3 groups of mice receiving SV(mIL-15) + SV(SIV Gag) showed strong Gag-specific responses (>50% lysis).

Conclusions rSV40 vectors can be used to deliver HIV and SIV antigens multiple times, or to deliver multiple transgenes in sequence. Combining rSV40s carrying antigens with those bearing immunostimulatory cytokines gave stronger, faster cytolytic responses than did vectors with antigen alone. Cytolytic memory is elicited in IL-15-containing regimen. Our data argue in favor of further study rSV40 as a vaccine delivery vehicle against agents such as HIV.

Induction of Prion Protein-Specific Antibody Responses Using PrP-Retroparticles

Daphne Nikles,1 Patricia Bach,2 Klaus Boller,2 Christoph A. Merten,1 Fabio Montrasio,1 Ulrich Kalinke,2 Klaus Cichutek,1 Christian J. Buchholz,1

1Medical Biotechnology, Paul-Ehrlich-Institute, Langen, Germany; 2Immunology, Paul-Ehrlich-Institute, Langen, Germany; 3Prion Research, Paul-Ehrlich-Institut, Langen, Germany.

Passive immunization with antibodies directed against the cellular form of the prion protein can protect against prion disease. However, so far active immunization with recombinant prion protein failed to induce in vivo protective prion protein (PrP)-specific antibody responses.

To enhance the B cell immune response, a retroviral display system presenting the murine PrP on the surface of retroviral particles was established. Either the full length PrP or the C-terminal 110 aa of PrP (PrP110) were fused to the transmembrane domain of the plateau derived growth factor receptor (PDGFR) or to the N-terminus of the murine leukaemia virus (MLV)envelope (Env) protein. The constructs were transfected into cells expressing the MLV or the HIV gag/pol genes. Western blot analysis and immunogold electron microscopy of particles sedimented from the supernatant of the transfected cells using an anti-PrP antibody revealed that the PrP-PDGFR protein as well as the PrP110-PDGFR and the PrP110-Env proteins were successfully incorporated. Particle numbers and incorporation efficiencies were highest with the PrP110-PDGFR construct, reaching above 10 exp10/ml in concentrated stocks.

Upon intravenous injection, both, HIV or MLV based PrP-retroparticle preparations devoid of adjuvant were able to induce high antibody titers in PrP-deficient mice. Already 7 days after priming, high PrP-specific titers were induced. More importantly, in heterozygous PrP+/- mice and also in wild type mice, IgM and IgG antibodies were induced recognising the native cell surface exposed PrP molecule. Intravenous injection of the particles in the absence of adjuvants led to the strongest response.

The data show that the PrP molecule can be functionally displayed on retroviral particles and that PrP-Retroparticles hold promise for a novel vaccination strategy against transmissible spongiform encephalitis.

Recombinant Adenovirus Carrying Spike Gene Induces Effective Humoral and Cellular Immunity Against SARS-CoV in Rats

Ranyi Liu,1 Lizhe Wu, Bijun Huang, Jialin Huang, Wenlin Huang.

1Cancer Center.

Severe acute respiratory syndrome (SARS) is life threatening contagious disease caused by a novel coronavirus, named SARS-associated coronavirus (SARS-CoV). Now there still aren’t specific effective medicine and remedy to prevent or cure SARS. The patients suffering SARS are always administered heteropathy or conservative therapeutics. In order to prevent SARS, it may be necessary to develop vaccines against SARS-CoV. Inactivated SARS-CoV by irradiating is the most facile and convenient vaccine, but inactivated vaccine presumably possess a potential risk which results in the infection of the vaccinated, e.g. inactivated measles virus vaccine.

Infectious Diseases and Vaccines
Therefore, it could be a promising strategy to develop DNA vaccine. S protein is responsible for recognizing and binding its receptor of host cells, and directing the fusion between viral and cell membranes. We constructed recombinant adenoviruses carrying S1 fragment (Ad-S1) or S1 and S2 fragment(Ad-S12) of spike gene of SARS-CoV strain BJ01. We have investigated the ability of these recombinant adenoviruses to induce SARS-CoV virus-specific immunity in Wistar rats. Rats were immunized subcutaneously or through airway with the two recombinant adenoviruses respectively at day 0, 7 and 21. Preliminary results showed that all of vaccinated animals generated the antibodies and T-cell responses against SARS-CoV spike protein, and showed strong neutralizing antibody responses to SARS-CoV infection in vitro. These results indicate that adenoviral-based vaccine carrying spike gene fragment is able to induce strong SARS-CoV-specific immune responses in rats, and promising for development of a protective vaccine against the infection of SARS-CoV.

562. Human T-Cells Modified as Antigen Presenting Cells Are Superior to Professional APC in Stimulating T-Cell Responses
Eleni C. Adamopoulou, Christian Sinzger, Gabriele Kuntz, Christina Neff, Hermann Einsele, Hans G. Rammensee, Max S. Topp.1

1Department of Hematology, Oncology, Rheumatology and Immunology, Universitätsklinikum Tübingen, Tübingen, Germany; 2Institute for Medical Virology, Universitätsklinikum Tübingen, Tübingen, Germany; 3Department of Immunology, Institute for Cell Biology, Tübingen, Germany.

Adoptive transfer of T-cells expressing herpes simplex thymidine kinase (HSV-TK) induces an immune response against the transgene in HIV patients and in allogeneic stem cell transplant recipients’ resulting in eradication of transfected cells. The elimination of transferred cells is mediated by HSV-TK-specific CD8+ T-cells. As a vigorous T-cell response to the modified T-cells has been detected in clinical trials we reason that genetically modified human T-cells may represent a novel class of antigen presenting cells for eliciting CD8+ T-cell responses. In the current study human T-cells are oncoretrovirally transduced with a model antigen, CMV tegument pp65 protein. The ability of these genetically modified cells to function as antigen presenting cells (T-cellAPC) was investigated. Both CD4+ and CD8+ T-cells genetically modified with CMV pp65 gene can stimulate CMV-specific T cells as determined by IFN-γ production of activated T-cells. Furthermore, the potential of T-cellAPC to induce and expand CMV pp65-specific CTLs was addressed and compared to CD40-B–mediated CTL expansion. Both CD40-B and T-cellAPC induced and expanded simultaneously antigen-specific CTLs in vitro to more than one epitope with different HLA restriction alleles from healthy CMV seropositive donors. After only one round of seven days in vitro stimulation with T-cellAPC, CMV pp65-restricted T-cells were expanded up to 97-fold as determined with tetramer staining. In contrast, CTL cultures stimulated with autologous CD40-BApC expanded only up to 41-fold. The observed difference in expansion potential mediated either with T-cellAPC or CD40-BApC could result in phenotypic functional differences in the Ag-specific CTL populations. To address this issue the expression of co stimulation and chemokine receptors on tetramer positive cells was determined. Despite differential expression profile of costimulatory ligands on T-cellAPC and CD40-BApC, both expanded CTL populations are of effector/ memory phenotype and efficiently kill CMV-infected fibroblasts. Thus, T-cells transduced with oncoretroviral vectors to express full-length antigens are potent APC and can potentially be utilized as a new vaccine strategy in vivo against viral pathogens and cancer.

563. Determinants of a Humoral Immune Response Against the Transgene Product after Adenoviral Gene Transfer
Bart De Geest,1 Sophie Van Linthout,1 Jan Snoeys,1 Joke Lievens,1 Désiré Collen.1

1Center for Molecular and Vascular Biology, Katholieke Universiteit Leuven, Leuven, Belgium.

Background: Long term expression after gene transfer is dependent on the absence of an adaptive immune response against the transgene product. In contrast, genetic vaccination requires the induction of a vigorous immune response. The effect of promoter, transgene product, vector dose, murine strain and animal species on humoral immune responses against the transgene product after adenoviral transfer was evaluated.

Methods: Gene transfer was performed with E1E3E4-deleted vectors containing the 1.5 kb human α1-antitrypsin promoter in front of the genomic human apo A-I sequence and 4 copies of the human apo E enhancer (AdAT4.Apo A-I). Alternatively, human plasminogen (92 kDa) (AdAT4.plasm) or human microplasminogen (28.6 kDa) (AdAT4.plplasm) was expressed under control of the same promoter and enhancers. To determine the effect of promoter, human apo A-I was expressed under control of the cytomegalovirus promoter (AdCMV.ApoA-I) or the murine MHC II Eβ promoter (AdEβ.ApoA-I).

Results: Gene transfer with 5 x 1010 particles of AdAT4.Apo A-I was not associated with an immune response against human apo A-I in C57BL/6, Balb/c, C3H/HeJ or Swiss Webster mice. Classical immunization with human apo A-I protein formulated in complete Freund adjuvant followed by rechallenge with human apo A-I protein formulated in incomplete Freund adjuvant induced a vigorous immune response in naive Balb/c mice, but not so in Balb/c mice injected with 5 x 1010 particles of AdAT4.Apo A-I, indicating that transfer with this dose of AdAT4.Apo A-I induced immunological tolerance for human apo A-I. In contrast, gene transfer with 1 x 1010 particles of AdAT4.Apo A-I in Balb/c mice did not induce antibodies but classical immunization with human apo A-I protein induced a vigorous immune response in these mice, indicating that the absence of an immune response after transfer with this dose is solely due to immunological ignorance. Gene transfer with AdCMV.ApoA-I induced high titers of antibodies against human apo A-I in Balb/c mice, but not in the other three strains. In contrast, transfer with AdEβ.ApoA-I induced a strong immune response in Balb/c, C3H/HeJ and Swiss Webster mice and moderate antibody titers in C57BL/6 mice. Antibodies against human plasminogen were observed in C57BL/6 mice after transfer with AdAT4.plasm, but there were no antibodies against microplasminogen after transfer with AdAT4.plplasm, indicating that tolerance and/or ignorance for the transgene product in mice is not only determined by the expression cassette, but also by the transgene product. In contrast to mice, neutralising antibodies against human apo A-I were present after transfer with 4 x 1012 particles/kg of AdAT4.Apo A-I in three rabbit strains. In Sprague Dawley rats, a large interindividual variability in immune response after transfer with AdAT4.Apo A-I was observed.

Conclusion: The effect of expression cassette on the immune response against the transgene product in mice should be extended with caution to other species. The induction of immunological tolerance against the transgene product in mice is dependent on the antigen levels. The murine MHC II Eβ promoter appears to be more efficient than the CMV promoter for inducing antibodies against the transgene product in mice.