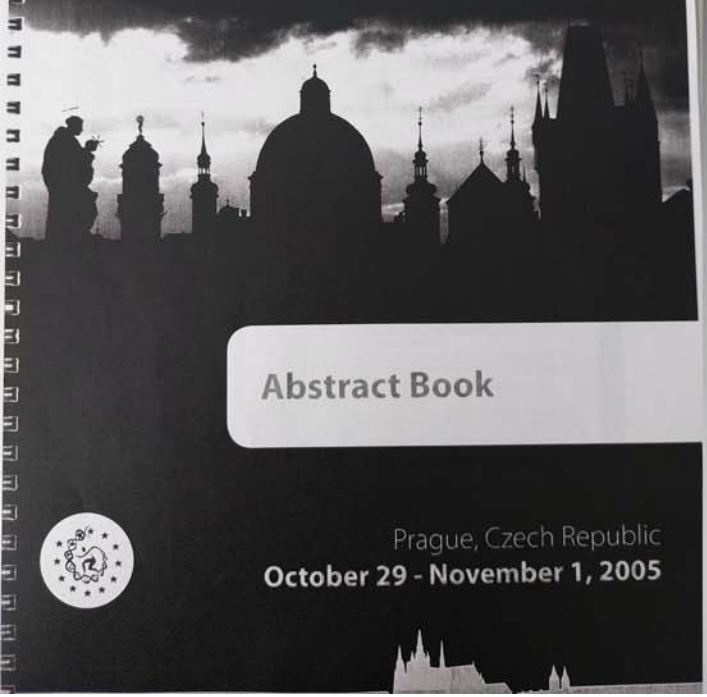
13th Annual Congress of the European Society of Gene Therapy



Abstract Book

Prague, Czech Republic

October 29 - November 1, 2005

route succeeded to induce detectable level of IgG to the E7 antigen. The data obtained gave the possibility to conclude that immune response degree achieved by DNA immunization in mice depends on the route of administration.

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A NOVEL ANTIVIRAL FACTOR IS SECRETED BY HIV-INFECTED NON-ACTIVATED T CELLS EXPRESSING A MODIFIED FORM OF CD4

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Inhibition of HIV infection by expression of genes that interfere with the replicative life cycle of HIV is a strategy that unlike current drug-based regimes may result in eradication of the virus. In this study we investigate the inhibitory effect on HIV replication of a CD4 chimera that is retained in the endoplasmic reticulum (ER) (CD4-ER). Retroviral vector mediated expression of CD4-ER interferes with HIV replication by preventing the processing of HIV env protein precursor gp160, to the mature glycoproteins gp120 and gp41. This mode of action implies that viral gene expression is permitted but infective viruses are not produced. Therefore, it could be anticipated that for gene therapy with CD4-ER to be effective most HIV target cells must be transduced. Surprisingly, HIV replication was strongly inhibited (up to 100,000-fold) even when a minority of the T cell population (10%) was transduced with CD4-ER chimera. This result indicated that CD4-ER expressing cells exerted a bystander protective effect on non-transduced cells. The bystander effect is demonstrated in turnover experiments to be mediated by a soluble factor. Furthermore, the soluble antiviral factor is released to the culture supernatant of CD4-ER-expressing cells when infected with HIV. The fact that the antiviral factor can be released by non-activated CD4+ T cells, together with its dependency on HIV infection and CD4-ER expression indicate that this factor is different to interferons, chemokines, CAF, and other previously identified antiviral factors. The identification of the antiviral factor could open a new therapy for treatment of HIV.

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RECOMBINANT SEMLIKI FOREST VIRUS PARTICLES ENCODING HEPATITIS B VIRUS S PROTEIN - EXPRESSION IN VITRO AND IMMUNIZATION

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Viral vectors as gene delivery tools for intracellular antigen expression are increasingly used for immunization purposes. We were interested to exploit Semliki Forest virus (SFV) expression system, which is characterized by high-level transient expression of heterologous genes, broad host cell range, cell replicating, and suicidal nature. It has been demonstrated in numerous studies, that SFV replicons induce specific and protective immune responses to various viral antigens, in addition the potential of SFV as an anti-tumor agent is being evaluated. Among several approaches of gene delivery offered by SFV replicon, in our study recombinant RNA packaged in SFV particles (rSFV) was chosen. Optimizational several factors affecting rSFV particle production and purification lead to rSFV titers of 109 IU/ml. Targeting of rSFV-EGFP to mice liver, spleen, and lymph nodes was shown in our preliminary experiments. We examined the potential of rSFV particles encoding Hepatitis B virus (HBV) S protein to induce humoral and cellular responses in a mouse model. HBV ayw S gene was cloned in pSFV vector (Liljestrom and Garoff, 1991) and infectious rSFV-S particles were produced. Infection of BHK and Huh-7 cells showed that S protein is translocated into ER, glycosylated and secreted. Expression of HBV S protein was also confirmed by immunocytochemistry. BalbC mice were immunized subcutaneously (s.c.) and intravenously (i.v.) with 10^6 and 10^7 IU and boosted with the same rSFV-S particle dose after two weeks. 10^7 rSFV-S particles injected i.v. were able to induce weak specific antibody response and only after a booster i.v. immunization of 10^8 IU elicited significant antibody response. Induced antibodies showed IgG2a dominance and were able to subtype specific 1st immunization route resulted in no specific antibody response at any rSFV-S dose. In future studies it is planned to use rSFV particles encoding additional HBV part, domains and core protein, as well as use various prime-boost regimens.

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IMMUNISATION WITH A LENTIVIRAL VECTOR TRANSCRIPTIONALLY TARGETED TO ANTIGEN PRESENTING CELLS

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Background: The role of dendritic cells (DC) as the principal inducers of the immune response makes them primary targets for immunotherapy. We are developing lentiviral vectors for gene delivery to DC. Our group has previously described the use of a lentiviral vector encoding the melanoma antigen NY-ESO-1, expressed from a ubiquitous promoter, to immunise mice (Palmowski et al, 2004). Strong DC-specific promoters could allow transcriptional targeting of gene expression, hence improving vaccination efficiency and safety.
Methods: Here we incorporate the promoter of the mouse dectin-2 gene into a high titre self-inactivating lentiviral vector expressing either the green fluorescent protein reporter gene (LV-Dec-GFP) or NY-ESO-1 melanoma antigen (LV-Dec-NY). Viral titre is measured by QPCR. For in vivo tracking experiments mice are immunised with LV-Dec-GFP and the phenotype of GFP transduced cells analysed. To analyse the CD8+ T cell response, HHD mice (H2-HLA-A2 chimeric) are immunised with LV-Dec-NY and the immune response to NY-ESO-1 detected by IFNγ staining using H2-HLA-A2-NY Pentamers or ELispot assay. Lentivectors incorporating the ubiquitous SFFV promoter are used as controls.
Results: We show that the dectin promoter drives GFP expression in bone marrow derived-DC in vitro experiments. Also that the dectin promoter preferentially targets antigen presenting cells (APC), displaying specificity for DC and macrophages. Immunisation of HHD mice with LV-Dec-NY results in the transduction and expression of NY-ESO-1 by murine DC in vivo, and consequently the priming of NY-ESO-1-specific T cells.
Conclusion: These data suggest that the dectin promoter can efficiently drive tumour antigen gene expression in APC, and hence the priming of T cells. The specificity of this promoter will prove useful in restricting transgene expression to APC, therefore improving the efficiency and safety of genetic vaccines.

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DNA MINIGENE VACCINATION FOR B-CELL LYMPHOMA THERAPY

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The recognition of the surface immunoglobulin of the tumour B cells as a specific tumour antigen prompted vaccination strategies aimed at the induction of antitumour responses. Preclinical models and clinical trials showed the immunogenic potential of the idiotype (Id). Several reports indicated the CDR3 regions as the immunodominant epitopes. We previously used the peptide encompassing the CDR3 of immunoglobulin heavy chain (VH-CDR3) to elicit tumor specific immune response via DNA-based vaccination. DNA immunization of outbred mice with patient-derived VH-CDR3 peptides elicited antibodies able to recognize antigens on patient's tumor cells. Thus, we investigated the antitumor response following CDR3-based DNA vaccination in a mouse model of B cell lymphoma. We have developed multiple strategies to enhance the potency of a CDR3-based DNA vaccine, in term of in vivo antigen expression, presentation and immunogenicity. Bicistronic plasmid DNA vectors were designed to express both the tumor antigen and the mouse IL-2 sequence as immunomodulating cytokine. The strategy focused on a pathogen-derived sequence as potent adjuvant when fused to the tumor antigen and on DNA vaccines encoding artificial arrays of defined peptides. Moreover the vectors were engineered by inclusion of a DNA nuclear targeting sequence and delivered by intramuscular in vivo electroporation after hyaluronidase pre-treatment. In the present study, the nucleic acid sequence of the idiotypic IgM from the murine BCL1 B cell lymphoma was analyzed. Two minigenes were constructed, one encoding for the region corresponding to the VL-CDR3 sequence and the second for one VH-CDR3 epitope with high predicted binding affinity to MHC class I. Here we show that vaccination of syngeneic C3H/HeN mice with some CDR3-based DNA vaccines improved survival rates when delivered after the initiation of tumor growth. This result suggests a potential therapeutic application of these vaccines for the treatment of B-cell lymphoma.

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MODULATION OF HEPATITIS C VIRUS MULTIPLICATION BY SPECIFIC RNA APTAMERS AGAINST NS5B RNA REPLICASE

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Characteristics of RNAs which can adopt complex structures to bind target molecules with high affinities and encode amplifiable genetic information make RNA a potentially very useful diagnostic and/or therapeutic compounds. Such short RNA ligands, termed RNA aptamers, have been elimated from a combinatorial RNA library to bind several molecules with high affinity and specificity using in vitro selection techniques, called SELEX. Hepatitis C virus (HCV) is the main causative agent of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Although the HCV infection causes worldwide health problem, efficient and specific antiviral therapies and/or vaccines have not yet been developed. Since the RNA dependent RNA polymerase (NS5B) of HCV plays a critical role in the viral replication, NS5B is a good target for anti-HCV agents. In the study, we isolated RNA aptamers to NS5B from a combinatorial RNA library using SELEX techniques. The RNA aptamers specifically and avidly bind NS5B with an apparent Kd of 1.3~4 nM. Through enzymatic probing and footprinting analysis, critical regions of the RNAs for NS5B binding have been identified. Moreover, in vitro RNA synthesis by NS5B was significantly inhibited by the RNA aptamers. Multi-bit analysis of the aptamer-bound proteins in HCV replicon-containing liver cells indicated specific interaction between the RNAs and NS5B. Importantly, the multiplications of HCV replicon in liver cells was efficiently hampered by cytoplasmic expression of the RNA aptamers, but not by nuclear expression of the RNAs. We showed that these aptamers could function as decoys to interact and hence presumably squelch NS5B proteins in the HCV replicon cells, explaining the mechanism underlying inhibition phenomena of HCV replicon replication by the specific RNA aptamers. These results suggest that the RNA aptamers could be of important utility not only for therapeutic and diagnostic agents of HCV infection but also for a powerful tool for the study of HCV replication mechanisms.

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PLGA NANOPARTICLES AS A NEW APPROCH FOR DNA VACCINATION

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Immunization with DNA encoding for antigens is emerging as a novel vaccination strategy. Advantages of DNA vaccination include: eliciting cellular response, ease of production and DNA stability. As for gene therapy, the type of vector used and obstacles associated with DNA delivery to the nucleus need to be addressed. Our work focuses on developing a non-viral nanoparticle carrier composed of biodegradable polymers. Poly lactide-co-glycolide acid (PLGA) for DNA vaccination. PLGA nanoparticles (NPs) containing DNA could serve as a sustained release system, which can be easily uptaken by antigen presenting cells. PLGA-NPs containing DNA were prepared by the double emulsion technique. The NPs were characterized in terms of: morphology, size, entrapment efficiency, surface potential and DNA release kinetics as a function of preparation parameters. The viability of different cells (macrophages, COS, BHK), the uptake and distribution of the NPs in cells, and the expression of DNA were evaluated. We achieved NPs with a narrow size distribution of 150 nm. PLGA was found to protect DNA, isolated from different preparation steps, from shearing during preparation. DNA retained its biological activity as seen after cells were successfully transfected with DNA extracted from NPs using AmPH. The viability of cells was not affected after incubation with NPs. Uptake of NPs was demonstrated by the different cell types and NPs were localized mainly in the cytoplasm. Uptake was found to be dependant on particles size and ion temperature. When transfecting COS-cells with NPs containing pLG3 (Luc) plasmid the achieved transfection was 200-fold higher than the control. Similar results were obtained when macrophages (RAW 264 cells) were treated with NPs containing pLG3 Luc. Uptake was found to be via the phagocytosis way. Transfection performed with PLGA-NPs containing DNA was also confirmed by PCR. Our results suggest that PLGA-NPs can serve as an efficient non-viral DNA delivery system for DNA vaccination.

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GENETIC IMMUNOTHERAPY TARGETED TO DENDRITIC CELLS USING A CD40-TARGETED ADENOVIRAL VECTOR

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Dendritic cells (DC) capture, process and present antigens in association with MHC class I and class II molecules to na ve CD8+ cytotoxic and CD4+ helper T cells. Through this, specific cytotoxic T cells are activated and recognize a target cell and kill it. This study was to determine the transduction efficiency of DCs using a CD40-targeted adenoviral vector expressing a tumor antigen. Recently, we characterized a new model using a mouse ovarian carcinoma cell line (MOVCAR) that expresses the SV40 large T-Ag and forms tumors in syngeneic mice. The SV40 large T-Ag is highly immunogenic, inducing both antibody and cytotoxic T lymphocyte (CTL) responses. Since this antigen is synthesized in MOVCAR cells, the SV40 large T-Ag is an attractive candidate as a model system for the development of a DC targeted cancer vaccine. We hypothesize that transduction of DCs in vitro using a CD40-targeted Ad vector expressing SV40 T-Ag (Ad5-SV40-TAg) will result in a high level of transgene expression, and be effective in inducing an antigen-specific CTL response. To target Ad5-SV40-TAg to DCs, we utilized a recombinant adapter protein consisting of extracellular portion of the native adenovirus receptor, CAR, fused to a trimerization motif from T4 fibritin protein, and linked to the extracellular domain of the mouse CD40 ligand. DCs were treated with untargeted and CD40-targeted Ad5-SV40-TAg using increasing multiplicities of infection. Western blot analysis was used to determine the level of expression of SV40 T-Ag in the transduced DCs, and flow cytometric analysis was used to determine the changes in the expression of cell surface markers. Cells transduced with CD40-targeted Ad5-SV40-TAg vector showed increased expression of transgene and expression of co-stimulatory molecules at 48 hours post-infection compared to cells transduced with untargeted Ad5-SV40-TAg vector. These results demonstrate that DCs can be successfully transduced using a CD40 targeted adenoviral vector and that transduced DCs show activation.