charged spheres that exist as monodisperse suspensions due to hydrostatic repulsions. They are further stabilized when halos of protein are non-covalently adsorbed to the particles, thus a receptor-specific gold conjugate can be formed. Since the gold particles are conductive metals, the electric field strength will be more dense in the region surrounding the gold particles. Theoretically, electro-gene transfer should be enhanced in cells labeled with sufficiently large gold particles in close proximity to the cell membrane. To investigate this hypothesis, Hela cells were labeled with gold particles conjugated to transferrin receptor (TfR)- targeting proteins. First, a mouse monoclonal anti-human TfR and a sheep anti-mouse IgG conjugated to FITC were bound to Hela cells. Fluorescence of cell surface confirmed the presence of the transferrin receptor. Then, the primary TfR-recognizing antibody was bound to a 6nm immunogold particle coated with goat anti-mouse IgG. Luciferase plasmid was introduced into the cells using a T820 ElectroSquarePorator™. The presence of gold particles on the cells was confirmed using an Aurion Silver Enhancement Reagent. Under these conditions, no significant difference was noted between the control and gold labeled cells. Assuming the gold particle was too small or too far away from the membrane to effect electro-gene transfer, we made two adjustments to the protocol. First, the experiment was conducted with 25nm immunogold particles with goat anti-mouse IgG. Second, the electroporation was conducted with 25nm gold particles conjugated directly to holo-transferrin protein, thus decreasing the distance between cell and gold particle. The indirect conjugation using the 25nm gold particle did not significantly increase transfection efficiency. However, the cells with the gold-holo-transferrin conjugate showed significantly higher luciferase activity relative to the control group after 24 hours (95%CI, $P<0.015$). These results indicate that direct gold particle attachment is a promising method for increasing the efficiency of electro-gene transfer.

147. Chemical Enhancers of Non-Viral Salivary Gland Gene Transfer

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Gene transfer to the major salivary glands is an attractive method for the systemic delivery of therapeutic proteins. To date, non-viral gene transfer to these glands has resulted in inadequate systemic protein concentrations. We believe that identification of the barriers responsible for this inefficient transfection will enable enhanced non-viral gene transfer in salivary glands and other tissues. Our efforts to identify and transcend these barriers have resulted in the discovery of a group of compounds that enhance non-viral, salivary gland gene transfer by four orders of magnitude relative to unformulated DNA. These data as well as data directed towards understanding the mechanism-of-action responsible for the observed enhancement in non-viral gene transfer will be presented.

148. The Altered Binding Properties of Sleeping Beauty Transposase Hyperactive Mutants May Explain Their Enhanced Efficacy

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The Sleeping Beauty (SB) transposon system has been shown as an effective vector to mediate stable, long-term gene transfer. We have previously identified SB hyperactive mutants through an alanine scan of the DNA binding region of the transposase. In total, 96 single-substitution mutants were generated and those individual missense mutations leading to increased activity were combined to generate four hyperactive Sleeping Beauty (HSB) mutants. In this study, we have begun to delve into the mechanism behind the increased activity of these mutants by comparing the four HSB mutants and wild-type transposase for their ability to bind the DNA-recognition sites. Truncated (N123) derivatives of wild-type and hyperactive SB proteins were generated by *in vitro* transcription/translation and tested for their ability to bind to oligos corresponding to transposase binding sites in an electrophoretic mobility shift assay. Each SB transposon inverted repeat contains two transposase binding sites, an outer binding site (OBS) adjacent to the transposon boundary and an inner binding site (IBS) internally situated about 165bp from the OBS. We found that there was no change in the binding affinities of the hyperactive mutants versus wild-type for the IBS but that binding to the OBS was increased 2-4 fold. Our results in conjunction with previous work, where it was shown that simply increasing the binding affinity of the transposase at the transposon terminal end causes the abolishment of transposition (Cui et al, J Mol Biol, 1221-1235; 2002), suggest that the mechanisms underlying hyperactivity may be due to an enhanced ability of these mutants to form an active synaptic complex and we are in the process of investigating this possibility. We are also examining all of the single-substitution mutants to determine which individual residues contribute to increased binding affinity. Through these experiments we hope to gain additional insight into the mechanisms involved in transposase function which, in turn, will lead to further enhancement of SB as a tool for gene transfer.

149. Anti-Idiotypic CDR3 Vaccination Against Chronic B-Cell Lymphoma: DNA Vaccine Strategy

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B-cell lymphomas express tumor-specific immunoglobulin. Its variable region [idiotype (Id)] can be considered a tumor-specific antigen and a target for vaccine immunotherapy. Promising results have been obtained in clinical studies of Id vaccination using Id proteins or naked DNA Id vaccines. Several reports have indicated that the immunodominant epitopes of the clone-specific Ig lie mainly in the CDR3. We have previously demonstrated the possibility of using the short peptide encompassing the CDR3 of immunoglobulin heavy chain (VH-CDR3) as a target for eliciting a tumor specific immune response via DNA-based vaccination. DNA immunization of outbred mice with different patient-derived VH-CDR3 peptides elicited antibodies able to recognize native antigens on individual patient's tumor cells. In the present study, we evaluated the humoral and cellular immune response recruited by VL-CDR3-directed DNA vaccines using the murine 38C13 B-cell lymphoma tumor as a model system. The nucleic acid sequence of the idiotypic IgM (38C-Id) light chain was analyzed and the region corresponding to the CDR3 sequence was chosen for the production of a synthetic mini-gene. A high-level expression bicistronic plasmid DNA vaccine was designed

to express both the short VL-CDR3 and the mouse IL-2 sequences. IL-2 was chosen as immunomodulating cytokine to enhance T cell-mediated immune response, to improve antigen-specific T cell proliferation, differentiation and Ig secretion of antigen-activated B cells. Vaccination of syngenic C3H/HeN mice with the described plasmid DNA vaccine was found to generate an immune response to the 38C13 tumor, inducing both specific circulating antibodies and specific cytotoxic T-cell (CTL) activity. Combined, our data indicates that a novel CDR3-based DNA vaccine can be improved and used to develop a protective vaccine against B-cell lymphoma. We have recently developed multiple complementary strategies to enhance the efficacy of a CDR3-based DNA vaccine. The first utilizes i*n vivo* electroporation to increase the uptake of vaccine given intramuscularly and to yield powerful humoral and cellular responses. The second one entails the use of plasmid containing a DNA nuclear targeting sequence. The combination of both approaches has been reported to increase plasmid DNA vaccine delivery and expression, thus improving the antigen dose achievable *in vivo*. The third imply the use of a pathogen-derived sequence, proven to be potent adjuvants for several different DNA vaccines when fused to the tumor antigen in the expression vector. Lastly, we have developed a DNA vaccine encoding artificial strings of defined epitopes. The efficacy of the fusion gene vaccine and the epitope string DNA vaccine, in term of anti-idiotypic response and protection against B-cell lymphoma, will be tested and evaluated.

150. Cooperative Strand Invasion of Super-Coiled Plasmid DNA by Mixed Linear PNA and PNA-Peptide Chimeras

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Peptide nucleic acid (PNA) is a DNA analog with broad biotechnical, and in the future possibly also medical applications. Among suggested functions are as specific anchor sequence for binding biologically active peptides to plasmids in a sequence specific manner via strand invasion. Such complexes, referred to as Bioplex, has already been used to enhance non viral gene transfer in vitro. Here we present a method to quantify the specific binding of a PNA to super coiled DNA by labeling the PNA with the fluorophore thiazole orange (TO). Using this method we have studied how the hybridization of multiple PNAs and PNA-peptide chimeras to sequences on the same DNA strand in a super coiled plasmid are effected by the distance between the binding sites (BSs). The kinetics of the strand invasion into a set of plasmids with different distances between the BSs indicate that the optimal space between two sites are two bases. At this distance the remaining cooperative effects are overriding the negative steric hindrance of a neighboring PNA-peptide. In addition we show increased binding kinetics when using two PNA binding to overlapping sites on the opposite strands without the use of chemically modified nucleobases in the PNA molecules.

151. Gene Delivery in Larger Animals

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Intramuscular injection of plasmid DNA followed by electrical stimulation (electroporation) is an efficient mean for non viral delivery of DNA *in vivo* in smaller animals like mice and rats. In animals with increased body mass however, transfection is less efficient, often caused by the difficulty of DNA distribution relative to the electric field. We have developed a new device for the combined DNA injection and electroporation in which the injection needles also serve as electrodes. DNA is injected through two needles during insertion into the muscle and is thus distributed along the entire needle path enabling a perfect match between the DNA and the electric field. Using this device we have aimed to optimize electroporation conditions such as injection volume, DNA concentration, and field strength for use in larger animals.

152. Nucleofection and Phage phiC31 Integrase Mediate Stable Introduction of a Dystrophin Fusion Gene into Muscle Derived Stem Cell and Human Myoblasts

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Ex vivo gene therapy offers a potential treatment for Duchenne muscular dystrophy by transfection of the dystrophin gene into the patient's own myogenic precursor cells, followed by transplantation. This approach requires a safe procedure to stably modify myogenic cells so that they express the large dystrophin transgene. We used nucleofection to introduce DNA plasmids coding for eGFP or eGFP-dystrophin fusion protein and the phage phiC31 integrase into myogenic cells and to integrate these genes into a limited number of sites in the genome. This combination of methods eliminates the need for viral vectors and reduces the risk of insertional mutagenesis. Following nucleofection of a plasmid expressing eGFP, 50% of MD1 cells, a mouse muscle-derived stem cell line, and 60% of normal human primary cultured myoblasts transiently expressed the fluorescent protein. But stable expression was rare. In both cell types, co-nucleofection of a plasmid expressing the phiC31 integrase and a plasmid containing the eGFP gene carrying a 285 bp *attB* sequence produced 15 times more frequent stable eGFP expression, due to site-specific integration of the transgene into the genome. Co-nucleofection of the phiC31 integrase plasmid and of a large plasmid containing the *attB* sequence and the gene for an eGFP-full-length dystrophin fusion protein produced fluorescent human myoblasts that were able to form more intensely fluorescent myotubes after one month of culture. The presence of eGFP-full-length dystrophin protein in myotubes was confirmed by Western blotting. Finally, MD1 stem cells expressing integrated eGFP were successfully transplanted into leg muscles of *mdx* mice, leading to the presence of green fluorescent fibers. A non-viral approach combining nucleofection and the phiC31 integrase may eventually permit safe auto-transplantation of genetically modified myogenic cells to muscular dystrophy patients.

153. VEGF Associated with TP To Refine Angiogenesis in Gene Therapy

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Angiogenesis (formation of new blood vessel from pre-existing vessel) is a complex process that needs the interaction of different growth factors.

Stimulation of angiogenesis would be beneficial in the treatment of diseases derived from ischemia such as coronary artery disease