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should also be applicable to siRNA's, since recent research has found that delivery methods developed for ODN are also efficient for siRNA. The ultrasound-sensitive liposomes provides the distinct advantage of allowing control and localization of the release of nucleotide-based and other hydrophilic therapeutic agents. Furthermore, the destruction of microbubbles may facilitate nucleotide transport across biological membranes. Ultrasound image enhancement by these liposomes may also facilitate identification of regions of disease, allowing further improvements in site-specific delivery.

484. Longevity of Expression Following Liposome-Mediated Gene Transfer

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Although gene expression and even functional correction have been demonstrated following administration of non-viral gene therapy vectors in several clinical trials, this response is generally transient. In this report, we attempt to characterise the dynamics of expression decline in order to more fully understand the causes behind this transience, and thereby rationally design vectors for extended expression.

Several factors might contribute to loss of expression in non-viral gene therapy including: (1) plasmid shedding, (2) gene silencing, due to CpG methylation and associated heterochromatin formation, and (3) dilution of plasmid DNA/transfected cells during mitosis. We use permanently immortalised cell lines to study the contribution of these various factors to loss of expression.

In order to study the dynamics of expression decline, we transfect the COS-7 and CHO-K1 cell lines with the pUMVC-nt-beta plasmid using the Trojene liposomal transfection reagent, and measure βgalactosidase activity at various time points. In both cell lines, expression peaks two days post-transfection (for example: in COS-7 the Trojene Liposome-Mediated Gene Transfer

485. Marked Transfection Enhancement by the DPL (DNA/Peptide/Liposomes) Trimeric Complex

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A short peptide of the nuclear localization signal (NLS) of HIV-1 Tat protein, Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg, was modified by adding a specified amino acid at the NH2 and COOH termini. These peptides were simply added to DNA before being added with cationic liposomes to improve intracellular trafficking of the plasmid DNA. The Tat peptides were first mixed with reporter plasmid that express either luciferase, LacZ, or GFP, and then mixed with liposomes, to form the trimeric complex of DNA/peptide/liposome (DPL). The DPL complex was treated to various cell lines, and was compared for transfection efficiency with that obtained by the conventional DNA/liposomes complex. When tested for transfection efficiency employing peptides with end modifications, the peptide with COOH-terminal modification showed highest transfection efficiency. When the DPL complex was formed with various cationic liposomes, DOSPA/DOPE (Lipofectamine®) exhibited better transfection efficiency than other liposomes tested. Meanwhile plasmid DNA mixed only with the Tat NLS peptide showed significantly lower transfection when compared to that obtained with the DPL complex. When an optimal ratio of each DPL component was used with the DOSPA/DOPE liposomes, transfection efficiency was shown to be 100 to 600 fold better in reporter gene expression than that obtained with the DNA/liposomes (without peptide addition) complex. This study shows that the trimeric DPL complex can be simply formed and employed for enhanced expression of a transgene in many different cells with a strong potential for practical applications.

Authors are associated with the company.

486. Plasmid DNA Constructs Expressing SARS-CoV Antigens Are Immunogenic in Mice and Rabbits

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We are testing a number of expression plasmids (pDNA) encoding SARS-CoV antigens to support potential development of a pDNA vaccine against this emerging pathogen. Our constructs are all derived from SARS-CoV Urbani and include the complete coding sequence for the nucleocapsid antigen, the soluble (extracellular) portion of the spike antigen, and two subdomains of the spike antigen termed S1 and S2. The codons used in these constructs were optimized for expression in human cells. These pDNAs were assayed for expression both in vitro and in vivo. Western blots of in vitro transfected cell lysates and culture supernatants were probed with rabbit polyclonal antibody specific to either spike or nucleocapsid peptides. These assays indicate that the spike antigen is efficiently released from cells in culture whereas the nucleocapsid antigen remains predominately intracellular. All four pDNAs were formulated in either cationic lipid or poloxamer and injected into mouse (50 mg) or rabbit (1 mg) muscle tissue (i.e. 3X @ two week intervals). Sera from pDNA-vaccinated animals will be tested for virus neutralization in vitro. The use of one or more of these pDNA vaccines will be further evaluated for their immunogenicity and safety in animal models.