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The use of CpG-free plasmids to mediate persistent gene expression following repeated aerosol delivery of pDNA/PEI complexes

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ABSTRACT

Aerosol gene therapy offers great potential for treating acquired and inherited lung diseases. For treatment of chronic lung diseases such as cystic fibrosis, asthma and emphysema, non-viral gene therapy will likely require repeated administration to maintain transgene expression in slowly dividing, or terminally differentiated, lung epithelial cells. When complexed with plasmid DNA (pDNA), the synthetic polymer, 25 kDa branched Polyethylenimine (PEI), can be formulated for aerosol delivery to the lungs. We show that pDNA/PEI aerosol formulations can be repeatedly administered to airways of mice on at least 10 occasions with no detectable toxicity. Interestingly, peak reporter gene activity upon repeated delivery was significantly reduced by up to 75% compared with a single administration, despite similar pDNA lung deposition at each subsequent aerosol exposure. Although the precise mechanism of inhibition is unknown, it is independent of mouse strain, does not involve an immune response, and is mediated by PEI. Importantly, using a dosing interval of 56 days, delivery of a fourth-generation, CpG-free plasmid generated high-level, sustained transgene expression, which was further boosted at subsequent administrations. Together these data indicate that pDNA/PEI aerosol formulations offer a versatile platform for gene delivery to the lung resulting in sustained transgene expression suitable for treatment of chronic lung diseases.

1. Introduction

Lung gene therapy is being evaluated for a variety of acute and chronic lung diseases including cystic fibrosis (CF), cancer and emphysema. The relative accessibility of the pulmonary epithelium makes aerosol delivery of gene therapy formulations attractive for non-invasive application to target cells in the lung, whilst minimising potential risks associated with systemic delivery. Unfortunately, due to the forces required for aerosol generation, few gene transfer agents (GTAs) have so far proven suitable for aerosol administration [1]. One non-viral GTA that continues to demonstrate promise for aerosol gene therapy is the cationic polymer polyethylenimine (PEI), which efficiently condenses plasmid DNA (pDNA) molecules to form polyplexes capable of mediating high-level gene expression both *in vitro* and *in vivo*. In contrast to the majority of GTAs, pDNA/PEI complexes remain stable during nebulisation [2,3], leading to robust gene expression in rodent [2] and sheep lungs [4]. Specifically, gene expression has been observed in ciliated epithelial cells [5] and alveolar type I pneumocytes [6], both of which are important target cell populations for lung diseases.

Chronic lung conditions such as CF are likely to require long-term treatment involving repeated administration of gene therapy formulations in order to achieve sustained gene expression over a period of months or years. Lung epithelial cells tend to be slowly dividing or terminally differentiated [7], and unless gene transfer agents can be integrated into progenitor populations, they will eventually be lost from the epithelium. Unlike the repeated

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