Delivery of NF-κB Decoy Related Oligodeoxynucleotides Reduces Pro-inflammatory Cytokine Responses Associated with Plasmid DNA/Lipid Mediated Gene Transfer to Murine Lungs.

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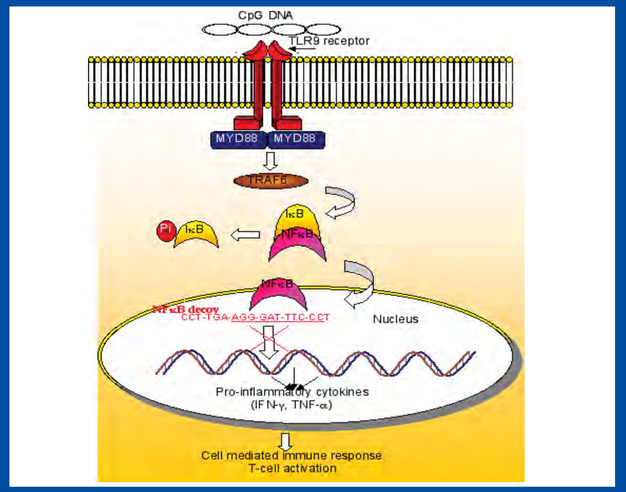
Introduction

- Cationic lipid-mediated gene transfer has been shown to be a potential approach to treat lung diseases such as Cystic Fibrosis (CF). However, pre-clinical and clinical studies have reported vector associated inflammatory responses [1]. This response is caused partly by the recognition of unmethylated CpG motifs contained within the plasmid DNA [2].
- The inflammatory response is characterised by increased production of pro-inflammatory cytokines.
- Nuclear transcription factor –κ B (NF–κB) is an important transcription factor that regulates this response.
- Recently the inhibition of NF-κB activation using synthetic double stranded and single stranded oligodeoxynucleotides (ODNs) had reduced pro-inflammatory cytokines successfully in conjunction with a lipid vector delivered systemically to the endothelium of the murine lung [3].

Figure 1. NF-κB signal transduction pathway



NF-κB is held inactive in the cytoplasm by the binding of an inhibitor molecule, IκB. TLR9 recognises unmethylated CpG motifs in DNA and initiates a signalling cascade, leading to the phosphorylation of the IκB protein and activation of NF-κB to activate a program of gene expression (Figure 1). NF-κB decoy ODN can inhibit this activation and prevent binding to the promoter region of target genes.

Aim

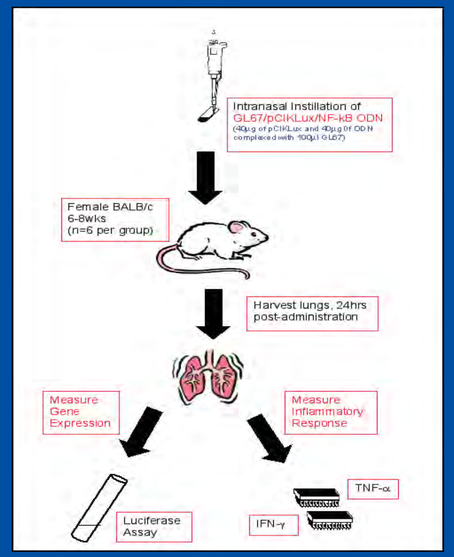
To investigate the effect of NF-κB decoy ODNs on the inflammatory response following topical delivery of Genzyme lipid 67 (GL67)/pDNA complexes to the airways of the mouse lung.

Figure 2. Sequence of NF-κB related decoy ODNs

S+	5' CCT TGA AGG GAT TTC CCT CC 3'
S11	CTG ACT GTC CTG ACC TGA CT

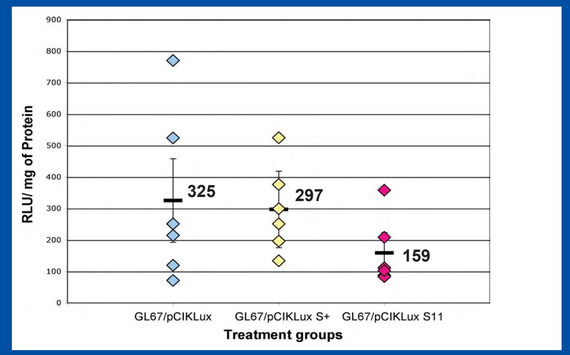
S and S11 are single-stranded 20-mer ODNs with a phosphothioate backbone. The underlined region in S+ indicates the consensus binding sites to the p50 subunit of NF-κB. ODN S11 contains a scrambled sequence with no NF-κB p50 binding potential and was used as a control.

Figure 3. Experimental procedures



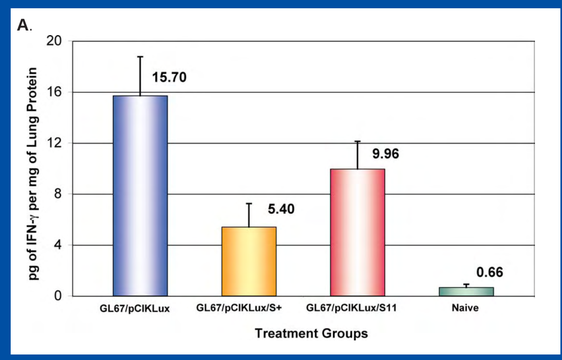
Results

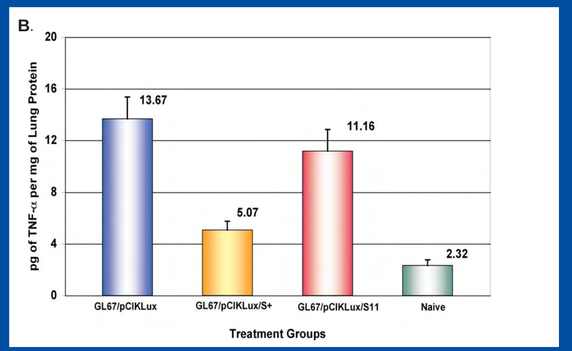
Figure 4. Co-delivery of NF-κB ODNs with GL67/pCIKLux does not affect transgene expression



Mice were dosed with pCIKLux (containg a CMV immediate early enhancer and promoter) and ODN S+ or S11 complexed with GL67 in 100μl. The addition of synthetic ODNs did not compromise the reporter gene expression with GL67/pCIKLux S+ or S11 compared to GL67/pCIKLux alone (Mann Whitney U Test p=0.75 or p=0.2, respectively).

Figure 5. NF-κB decoy ODNs reduce TNF-α and IFN-γ levels produced by GL67/pCIKlux





Mice were dosed with pCIKLux and ODN S+ or S11 as described in Fig 4. TNF-α and IFN-γ levels were determined in lung lysates 24hrs post-dosing using mouse cytokine ELISA kits. With topical delivery of the S+/pCIKLux/GL67 complex there were 80% and 70% reductions in TNF-α (Figure 5A, ANOVA with Fishers PLSD, p=0.0001) and IFN-γ (Figure 5B, p=0.004), respectively, compared to dosing with pCIKLux/GL67 alone.

Conclusions

1. The addition of the NF-κB ODNs did not compromise high levels of gene expression from GL67/pCIKLux.
2. Co-delivery of NF-κB decoy ODNs with GL67/pDNA complexes resulted in reduced pro-inflammatory cytokine levels.
3. Delivery of NF-κB ODNs with GL67/pDNA may reduce CpG related inflammation in the clinic.
4. Further studies should confirm whether this reduction in pro-inflammatory cytokine levels is specific to the NF-κB ODNS.
5. The reduction in cytokine levels may allow increased duration of transgene expression.

References

1. Alton *et al.* 1999, Lancet, 353, 947-957.
2. Yew *et al.* 2000, Molecular Therapy, 1, 255-262.
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