Influence of CpG-Dinucleotide Motifs on the Duration of Gene Expression From Plasmid Vectors After *In Vivo* Lung Delivery

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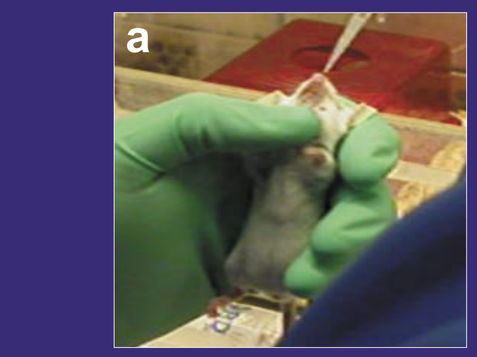
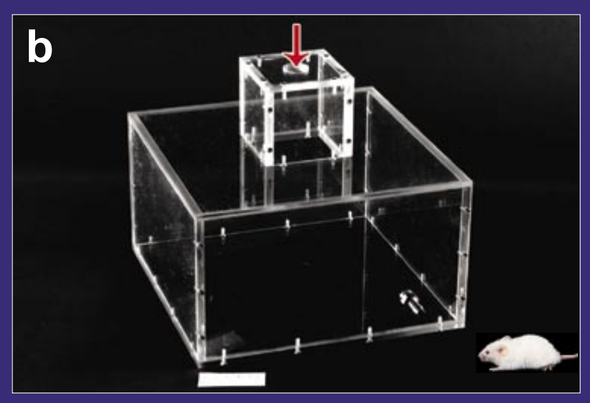
Overview

- Gene therapy is being developed as a treatment for the lung disease associated with Cystic Fibrosis (CF).
- Gene therapy will likely require long-term CFTR expression, but many gene transfer agents (GTAs) generate only transient expression.
- In non-viral GTAs the design of the plasmid (pDNA) is critical to maintain persistent transgene expression.
- Inclusion of the CpG-free hCEFI promoter allows persistent expression following aerosol delivery to the mouse lung.
- We investigated the effect of changing the CpG content of the plasmid backbone on the persistence of expression from the hCEFI promoter.

Mouse Models for Lung Gene Expression





We used BALB/c mice (8-10 weeks) as a model for lung gene transfer.

a) Intranasal instillation: Plasmid DNA (80µg) was complexed with Genzyme Lipid GL67 and delivered to the mouse lung.

b) Aerosol delivery: 2.5mg/ml pDNA was complexed with 6mM GL67 and the resulting 10ml nebulised using a Pari LC+ nebuliser and a whole body exposure chamber capable of holding 30 mice. Red arrow indicates entry route for aerosol.

Total lung extracts were assayed for luciferase reporter activity at Days 1, 2, 7, 14 and 28.

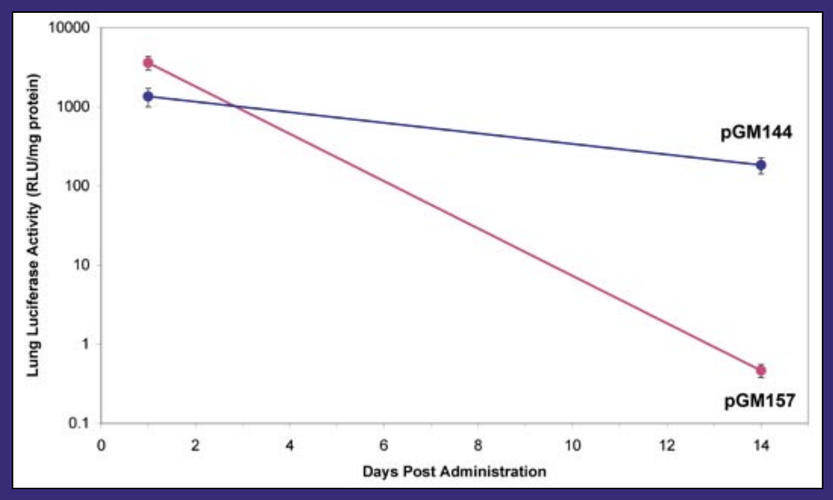
Construction of Plasmids Containing the hCEFI Promoter

We constructed two plasmids and measured their performance in the two mouse lung models:

pGM144: hCEFI (CpG-free human CMV enhancer/ EF1α) promoter expressing luciferase in CpG-free plasmid backbone.

pGM157: hCEFI promoter expressing luciferase in a backbone containing a small number of CpG motifs, in the luciferase gene (97 CpGs) and in the backbone sequence (52 CpGs).

Instillation to the Mouse Lung

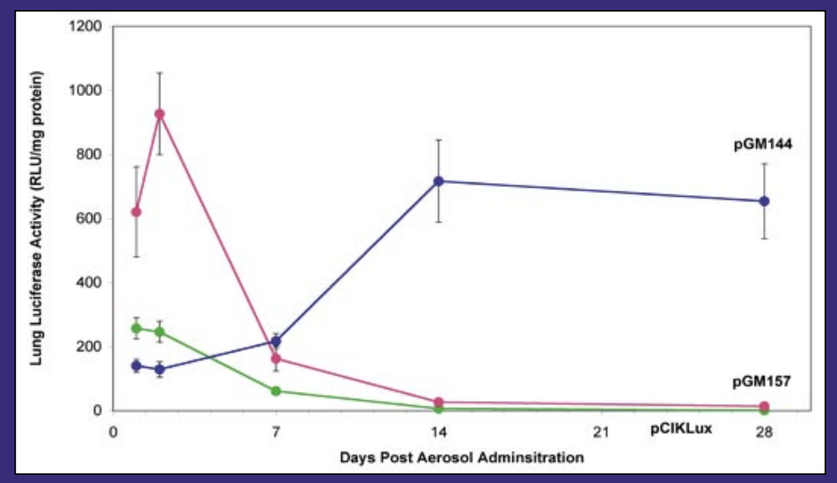


Plasmids pGM144 and pGM157 were complexed with GL67 and delivered to the mouse lung via nasal instillation (n=6).

2 days post delivery: Luciferase activity from both plasmids was initially high, with expression from pGM157 approximately 2-fold higher (3621 ± 717 RLU/mg) than pGM144 (1356 ± 360 RLU/mg).

14 days post delivery: Expression from pGM157 had fallen by 4 orders of magnitude to background levels (0.47 ± 0.1 RLU/mg), whereas expression from pGM144 had fallen less than 10-fold (184 ± 43 RLU/mg).

Aerosol Delivery to the Mouse Lung



Plasmids pGM144 and pGM157 were complexed with GL67 and delivered to the mouse lung via aerosol delivery. pCIKLux: control plasmid using CMV promoter. Total lung extracts were assayed for luciferase activity at Days 1, 2, 7, 14 and 28 after delivery (n=6).

The CpG-containing plasmid pGM157 initially directed approximately 2-fold higher levels of reporter gene expression than the CpG-free pGM144 plasmid ($p<0.05$ both Days 1 and 2 post-delivery).

Expression from the CpG-containing pGM157 fell to background levels, but expression from the CpG-free pGM144 increased and remained at peak levels for at least 28 days (CpG-free plasmid 4-fold, 16-fold and 50-fold higher expression at Days 7, 14 and 28 post-delivery respectively; $p<0.05$ for each).

CONCLUSIONS

- The hCEFI promoter directs persistent reporter gene expression in the mouse lung
- Persistence is dependent on the sequence of the plasmid backbone

Discussion

It is possible that an (as yet) unidentified sequence exists in the CpG-containing backbone that triggers transcriptional silencing of the hCEFI promoter. Alternatively, the well-described host inflammatory response to CpG-containing DNA may be involved. Thus the CpG content of the vector backbone could be responsible for these differences in expression. We are currently evaluating the potency of CpG-free plasmids expressing CFTR prior to the initiation of clinical studies of non-viral gene transfer in CF patients.

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