Transgene Expression In The Mouse Lung Following Administration Of Gene Transfer Vectors Expressing EGFP

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Overview:

- The UK Cystic Fibrosis Gene Therapy Consortium is commited to the testing and development of gene therapy vectors for CF clinical trials
- Successful CF gene therapy vectors will need to demonstrate not only adequate levels of transgene expression, but also appropriate distribution of expression in relevant lung cell types
- Reporter genes such as enhanced green fluorescent protein (EGFP) are valuable tools for the localisation of gene expression
- Cell type specific antibodies can be used to positively identify EGFP transfected cells *in vivo*
- We investigated the level and cellular distribution of transgene expression in the murine lung following nasal delivery of naked DNA and DNA conjugated to the cationic lipid GL67[1] (Genzyme Corporation, Framingham, USA)

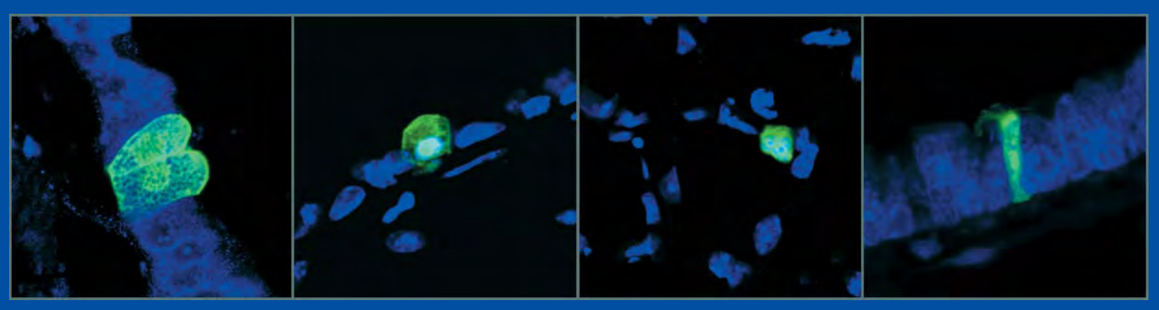

Figure 1: Visualisation of GFP positive cells in the mouse lung

Female BALB/c mice (n=6) were dosed intranasally with 300µg of the plasmid pEGFP-N1 (Clontech, Oxford, UK) or 80µg of plasmid conjugated with GL67. 24 hours later, mice were sacrificed and 7µm cryosections examined for GFP expression. GFP positive cells were found in all treated mice with expression observed in several different cell types (Figure 1). No GFP positive cells were ever observed in sections from animals dosed with control vectors (data not shown).

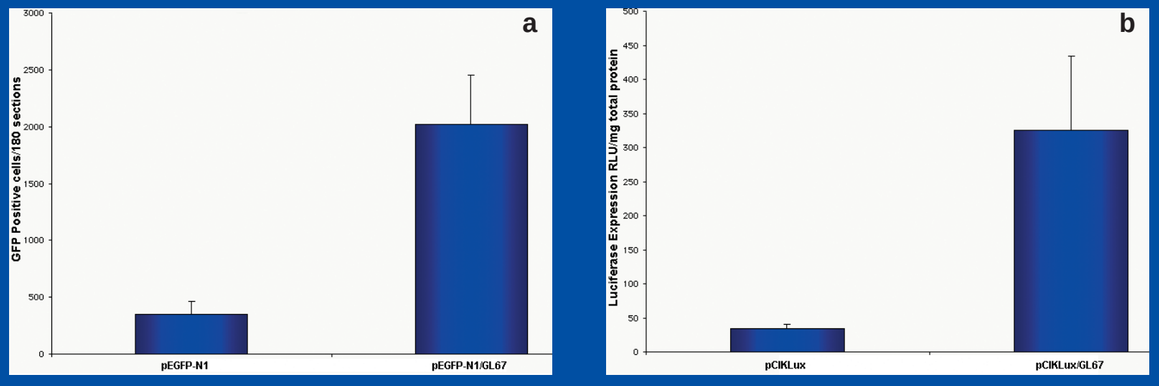

Figure 2: Comparison between GFP and luciferase reporter gene expression

For each animal treated with pEGFP-N1 or pEGFP-N1/GL67, the total number of GFP positive cells identified in 180 representative lung sections (see methods) was recorded. In a parallel study luciferase expression in lung homogenates from mice (n=6) dosed with the luciferase plasmid pCIKLux or pCIKLux/GL67 was also measured. Mice dosed with pEGFP-N1 demonstrated relatively few GFP positive cells (347 ± 114 cells/180 sections) compared to mice dosed with pEGFP-N1/GL67 (2,020 ± 435 cells/180 sections) (Figure 2a). Similarly, luciferase gene expression in whole lung homogenates was 9 fold greater for pCIKLux/GL67 compared to pCIKLux (Figure 2b). These results indicate that quantification of GFP positive cells can accurately indicate the level of overall gene expression in the mouse lung.

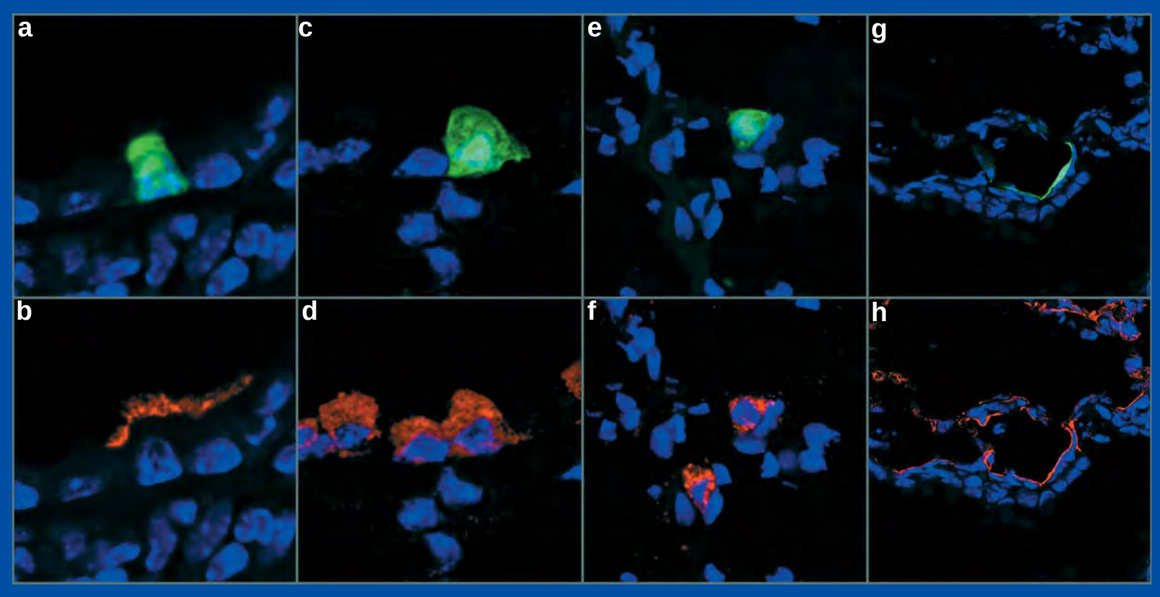

Figure 3: Identification of GFP positive cells using cell type specific antibodies

Cryosections containing GFP positive cells were stained with a range of cell specific antibodies (see methods) conjugated to the red fluorophore AlexaFluor 546. Co-localisation of GFP expression and antibody labelling was examined using confocal microscopy. Co-localisation of GFP expression with cell specific antibody labelling was observed for ciliated cells (Figures 3a and b), Clara cells (Figures 3c and d), type II pneumocytes (Figures 3e and f) and type I pneumocytes (Figures 3g and h) in animals treated with both pDNA and pDNA/GL67. However, the relative proportion of cells identified with each antibody was very different between the two vectors (Table 1).

	pEGFP-N1	pEGFP-N1/GL67
Ciliated Cells	61.6%	1.3%
Clara Cells	24.4%	1.3%
Type I Pneumocytes	1.2%	95.4%
Type II Pneumocytes	12.8%	2.0%
Macrophages	0%	0%

Table 1: Expression profiles for pDNA and pDNA/GL67

Cryosections from pEGFP-N1 and pEGFP-N1/GL67 treated animals were stained with a range of cell type specific antibodies. Following antibody staining the distribution of antibody labelled GFP positive cells was recorded for each vector. For animals treated with pDNA, the majority of positive cells (61.6%) were identified as ciliated epithelial cells with the remainder largely made up of Clara cells (24.4%) and type II pneumocytes (12.8%) (Table 1). In contrast, the vast majority of GFP positive cells in animals treated with pDNA/GL67 were identified as type I pneumocytes (95.4%) with relatively little expression in other cell types.

Conclusions:

- GFP expression from non-viral gene transfer vectors can be visualised in the murine lung
- Different gene transfer vectors can target different cell populations within the lung
- Identification of target cells should be considered an important parameter in the assessment of new gene therapy vectors

Methods:

Preparation of lung sections

Mice were killed by cervical dislocation and the lungs were inflated with 4% paraformaldehyde at a pressure of 20cm H_2O for 6 hours. Lungs and trachea were then cryopreserved in 30% sucrose in PBS overnight at 4°C. The trachea, apical, azygous, cardiac, diaphragmatic and left lung lobes were dissected away and individually embedded in OCT (Cellpath, Newtown, UK) for cryosectioning. Sections were cut at 7µm and mounted onto glass slides. For each lung, 30 non-consecutive cryosections were taken from each lobe and the trachea - a total of 180 sections per mouse.

Antibody staining

Lung sections were initially blocked with 10% foetal calf serum (FCS) in phosphate buffered saline containing 0.5% Triton X-100 for 30mins. Blocking solution was then replaced with primary antibody diluted in blocking solution and incubated at room temperature for 1 hour. Identification of Clara cells was performed using the rabbit anti-human urine protein 1 antibody (DAKO, Ely, UK). Ciliated cells were identified using the mouse anti-ß-tubulin IV antibody (BioGenex, San Ramon, CA, USA). Type I pneumocytes were identified using the hamster anti-mouse T1alpha antibody (Developmental Studies Hybridoma Bank, University of Iowa, Hybridoma #.8.1.1, courtesy of Dr Andrew Farr). Type II pneumocytes were identified using the rabbit anti-sheep surfactant protein B (SpB) (Chemicon, Temecula, CA, USA). Alveolar macrophages were identified using the rat anti-mouse F4/80 antigen antibody (Serotec, Oxford, UK). Detection of primary antibodies was performed using appropriate biotinylated secondary antibodies and streptavidin conjugated to AlexaFluor 546 (Molecular Probes, Leiden, Netherlands).

References:

[1]Alton E.W. et al (1999) Lancet 353, 947-954.