

Repeated Exposure to pDNA/PEI Aerosols Results in Minimal Detectable Toxicity in the Mouse Lung

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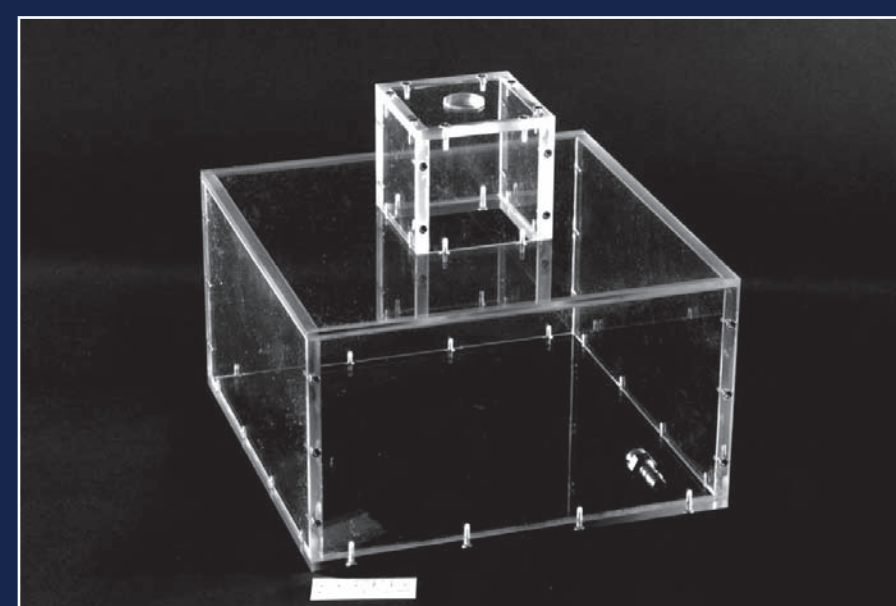
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INTRODUCTION

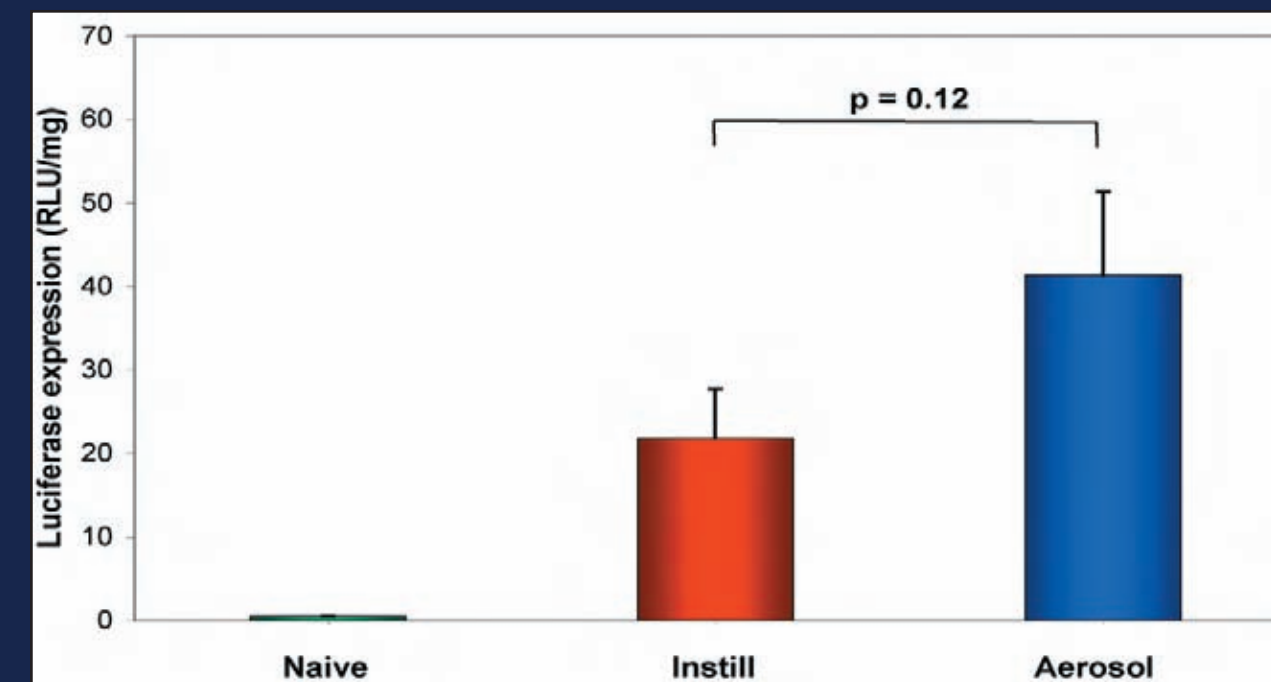
- ▶ The UK CF gene therapy consortium is committed to the testing and development of gene therapy vectors for CF clinical trials
- ▶ Successful lung gene therapy is likely to require repeated topical delivery of gene transfer agents to the airway epithelium via aerosol delivery
- ▶ The cationic polymer 25kDa polyethylenimine (PEI) has demonstrated successful gene expression following aerosol delivery to the mouse lung
- ▶ Clinical development of PEI gene therapies is currently hampered by the perceived toxicity of PEI in vivo
- ▶ We have investigated lung toxicity associated with repeated, weekly exposure to PEI/pDNA aerosols using a mouse aerosol delivery model

RESULTS



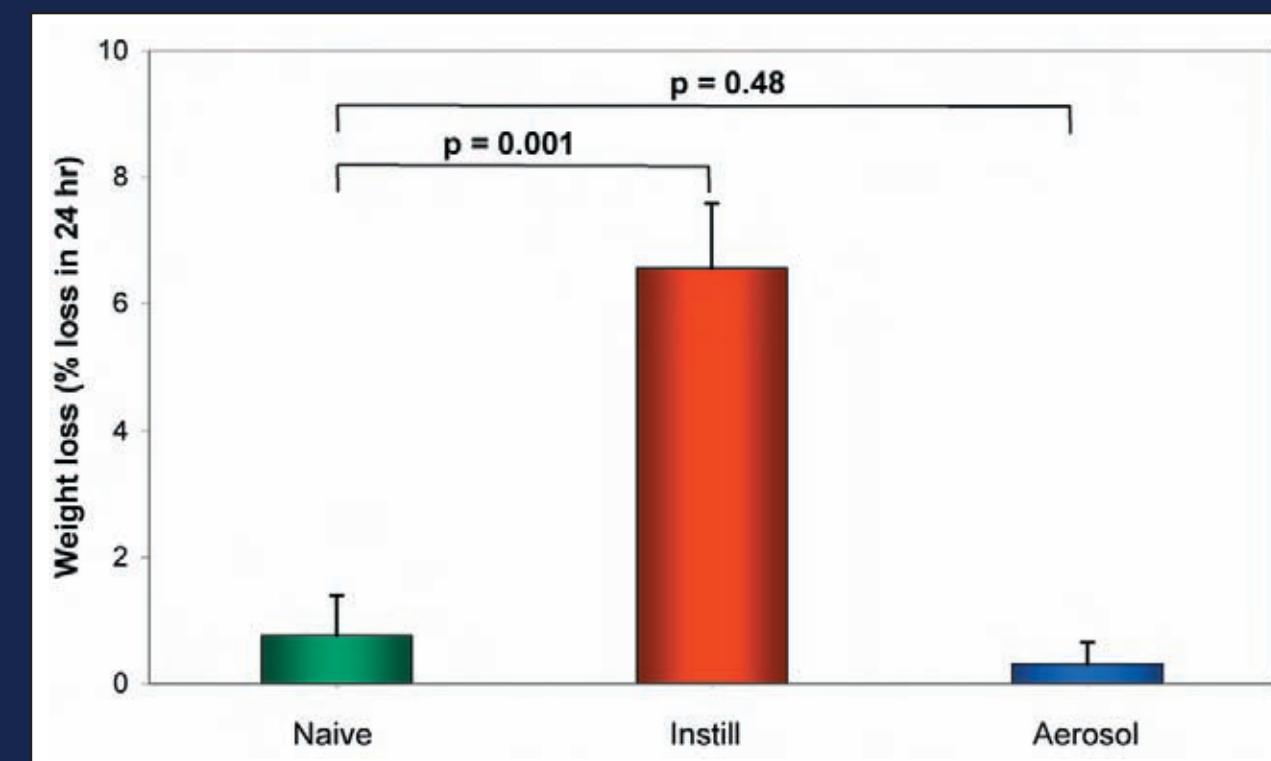
Aerosol delivery to the mouse lung

- ▶ BALB/c mice (n=6) were exposed to aerosols of pCIKLux/PEI using an 8L perspex whole-body exposure chamber (above)
- ▶ 20 ml of pCIKLux/PEI complexes (0.2mg/ml in water and N:P of 10:1) were aerosolised into the chamber using an Aerotech II nebuliser (CIS-US Inc. Bedford, MA. USA)
- ▶ Mice were exposed to a single 20ml aerosol every 7 days for up to 10 weeks



Luciferase expression in mouse lungs

- ▶ Robust luciferase expression (41.3 ± 10 RLU/mg) was detected in whole-lung homogenates 24 hr after a single aerosol exposure
- ▶ Equivalent ($p=0.12$, t-test) luciferase expression (21.8 ± 5.9 RLU/m) was observed following instillation of 20µg of pCIKLux/PEI (100µl of aerosol formulation)



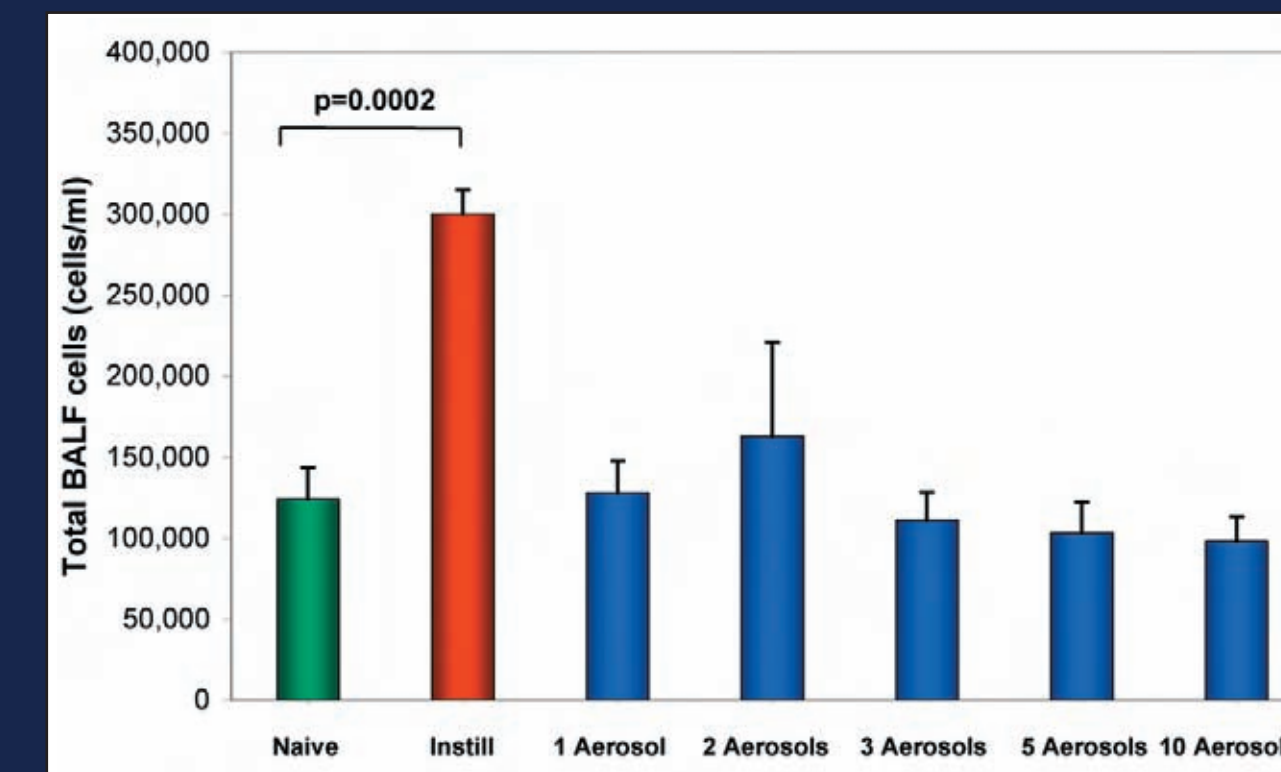
Weight change following delivery of PEI complexes

- ▶ Instillation of pCIKLux/PEI complexes was associated with a significant ($p=0.001$, TTest) toxicity related weight loss ($6.6 \pm 1.0\%$ body weight) in the 24 hr following delivery
- ▶ Aerosol delivery of pCIKLux/PEI complexes resulted in no significant weight loss in treated mice
- ▶ Mice exposed to up to 10 aerosols showed equivalent weight gain to untreated mice (data not shown) over the duration of the study



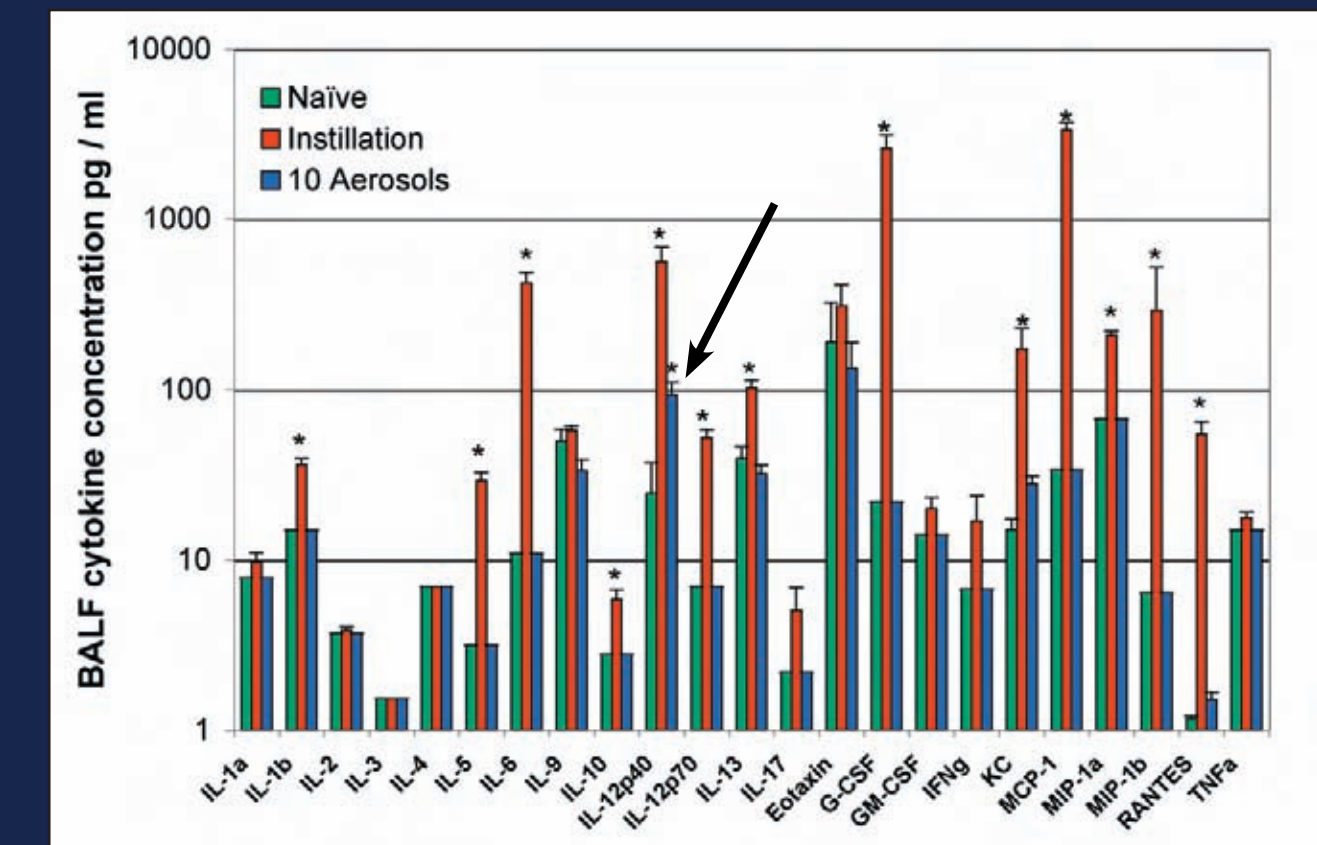
Lung histology following delivery of PEI complexes

- ▶ 7µm H&E paraffin sections were prepared from all mice 24 hr after final exposure
- ▶ Instillation of pCIKLux/PEI complexes associated with localised destruction of alveolar architecture and infiltration of inflammatory cells
- ▶ No evidence of acute or chronic histological changes observed in the lungs of mice exposed to pCIKLux/PEI aerosols



BALF inflammatory cells

- ▶ Bronchoalveolar lavage fluid was collected from all mice 24 hr after final exposure and total cell counts performed
- ▶ Instillation of pCIKLux/PEI complexes was associated with a significant ($p=0.0002$ ANOVA) increase in inflammatory cells (predominantly neutrophils)
- ▶ No increased cellular infiltrate was observed in BALF collected from mice chronically exposed to pCIKLux/PEI aerosols



BALF cytokines

- ▶ Bronchoalveolar lavage fluid was collected from all mice 24 hr after their final exposure and assayed for a panel of 23 cytokines
- ▶ Chronic exposure to pCIKLux/PEI aerosols resulted in a significant ($p = 0.02$, t-test) elevation of the pro-inflammatory cytokine IL12p40 in BALF (arrowed)
- ▶ Instillation of pCIKLux/PEI complexes resulted in a significant increase in the levels of 13 tested cytokines including the pro-inflammatory cytokines MCP 1, G-CSF, IL6 and IL12p40

CONCLUSIONS

- ▶ Aerosol delivery of PEI/pDNA complexes results in robust lung gene expression in the absence of detectable toxicity
- ▶ Chronic exposure to PEI/pDNA aerosols does not result in increased toxicity
- ▶ Delivery methodology is a critical determinant in PEI/pDNA mediated lung damage
- ▶ PEI/pDNA aerosols demonstrate potential for a variety of clinical lung gene therapy applications