

LENTIVIRUS GM-CSF GENE THERAPY AMELIORATES AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS

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Autoimmune pulmonary alveolar proteinosis (aPAP) is a lung disease characterised by the progressive accumulation of surfactant, leading to respiratory failure, due to anti-granulocytemacrophage colony-stimulating factor (GM-CSF) auto-antibodies. The standard of care is whole lung lavage (WLL) resulting in temporary remission of symptoms. This procedure is not only highly invasive but also needs to be performed repeatedly, carries risks and can solely be performed in highly-specialised centres. Administration of recombinant GM-CSF outcompetes the auto-GM-CSF antibodies and stimulates clearance of pulmonary surfactant by alveolar macrophages. However, this treatment is expensive, needs to be nebulised frequently and is not available to all patients. Therefore, we hypothesised that rSIV.F/HN, a lentiviral vector specifically pseudotyped for efficient gene transfer to the respiratory epithelium, carrying (m)GM-CSF cDNA, may be of therapeutic benefit. We were able to produce high-titer rSIV.F/HN carrying murine (m)GM-CSF ($1e9$ TU/ml) using scalable, serumfree suspension cultures. As previously shown, vector transduction of A549 cells (MOI 0.1–1000) led to a dose-related expression of mGM-CSF which, compared to recombinant mGM-CSF protein, showed similar activity in a mouse myeloid FDC-P1 cell proliferation assay. Transduction of air-liquid interface cultures (MOI 185), an ex vivo human model, resulted in stable, long-term (7 months) expression of mGMCSF ($n=6$) (Mean 230.3 pg/ml). More recently, characterisation of a GM-CSF-knockout mouse showed that the model recapitulates the human disease phenotype, with significantly ($p<0.05$) increased BALF turbidity and phosphatidylcholine and surfactant protein D (SP-D) levels in BALF and lung. Transduction of mice ($1e7$ TU/mouse, $n=3$ /group) generated significant ($p<0.05$) high levels of mGM-CSF in both lung (treated: median 825 (range 460–3790) pg/ml; control: 0.1 pg/ml) and bronchoalveolar lavage fluid (BALF) (treated: 3330 (range 2307–7958) pg/ml; control: 0.1 pg/ml) samples. The increase in GM-CSF levels translated into significant changes in disease biomarkers two months after a single administration of the mGM-CSF lentivirus ($1e7$ TU/mouse, $n=8$ /group) with decreased BALF turbidity ($p<0.0001$) and SP-D levels ($p<0.0001$) compared to age-matched mice transduced with a control lentivirus. These results are a proof-of-concept for gene therapy as a novel treatment for aPAP. Future experiments will further evaluate the efficacy and safety of lentiviral gene transfer in this murine model for aPAP