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Cell pathways in lung inflammation and injury

S73 MACROPHAGES AS VEHICLES FOR DELIVERING CELL THERAPY TO INJURED LUNG

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Introduction Injury to the alveolar epithelium underlies a number of important lung diseases, exemplified by the syndromes of acute lung injury and acute respiratory distress syndrome, which currently have a poor prognosis. Keratinocyte growth factor (KGF) is a mitogen for, and exerts beneficial effects on, type II alveolar epithelial cells. Pre-treatment with KGF is associated with improvements in outcomes in animal models of lung injury, but the use of recombinant KGF as a clinical therapy is limited by its short bioavailability and lack of specificity. We sought to determine whether macrophages could be used as vehicles to deliver KGF therapy to the injured lung.

Methods Macrophages from a murine macrophage cell line (IC-21) were transduced with a lentiviral vector expressing KGF and the reporter gene GFP. Mice were given oropharyngeal (OP) bleomycin to induce lung injury. On days one and three after induction of lung injury, mice were given 3×10^6 KGF-transduced IC-21 cells (or controls) by OP instillation. Mice were sacrificed on day 5, and bronchoalveolar lavage fluid (BALF) was harvested and lungs were processed for histology. For in vivo tracking experiments, IC-21 macrophages were transduced with a lentiviral vector expressing luciferase and mice were imaged longitudinally using real-time bioluminescence imaging.

Results KGF-expression was confirmed in KGF-lentivirus-transduced macrophages, however delivery of these cells was not associated with improvements in measures of alveolar-capillary membrane permeability (BALF albumin) or inflammation (total and differential cell counts) after lung injury. Cells expressing GFP were recovered in BALF, and immunohistochemistry showed groups of cells close to conducting airways. Longitudinal imaging of mice after OP delivery of luciferase-transduced IC-21 cells suggested that cells initially localised to the lungs, but did not persist at 48 h after delivery.

Conclusions KGF-expressing macrophages can be generated using lentiviral vectors, but therapeutic delivery of these cells to the lungs did not improve measured outcomes in the mouse bleomycin lung injury model. Longitudinal imaging suggested that the lack of therapeutic efficacy of KGF-transduced macrophages may be due to their limited survival, and future work should focus on optimising macrophage delivery and survival in vivo.

S74 INCREASED PLASMA LEVELS OF SYNDECAN-1 AND sFLT-1 DURING CARDIOPULMONARY BYPASS SURGERY: ASSOCIATIONS WITH sRAGE

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Introduction and Objectives Endothelial barrier dysfunction contributes to the systemic inflammatory response syndrome (SIRS) for which cardiac surgery necessitating cardiopulmonary bypass (snCPB) is a human model. A growing body of evidence suggests that the receptor for advanced glycation end products (RAGE) is also implicated in the pathogenesis of SIRS. While RAGE activation, in vitro, is known to decrease barrier function of cultured endothelial cells, the role of RAGE in regulating vascular permeability in patients undergoing snCPB is unknown. We hypothesised that plasma levels of markers of endothelial barrier dysfunction, syndecan-1, an endothelial glycocalyx component; and sFlt-1, a soluble form of the vascular endothelial cell growth factor (VEGF) receptor-1, will increase during snCPB and levels relate to patient outcome.

Methods ELISA measurements/western blotting was used to determine syndecan-1 and sFlt-1 levels in pre-, intra- and post-snCPB plasma samples; for comparison, glypican-1, another glycocalyx component and Robo4, an endothelial barrier stabilising protein, were also measured. Clinical indices: age, nature of operation, CPB time, ischaemic time, intensive care unit (ICU)/ hospital length of stay (LOS) were collected from electronic databases.

Results Syndecan-1 was significantly higher during CPB (77.17±39.72 ng/ml) compared with pre-snCPB levels (35.26±25.81 ng/ml, n=14, $p<0.01$). Plasma levels of sFLt-1 were significantly ($p<0.001$) higher during (10 000 pg/ml±3601, n=10) and post-snCPB (4282±3271 pg/ml) compared with preoperative levels (69.68±35.22 pg/ml). Preoperative plasma syndecan-1 correlated positively with ICU LOS ($r^2=0.486$, $p=0.006$); whereas, intraoperative sFlt-1 correlated negatively with ICU LOS ($r^2=0.406$, $p=0.048$). Intraoperative syndecan-1 levels positively associated with ischaemic time ($r^2=0.383$, $p=0.018$) and plasma sRAGE levels ($r^2=389$, $p=0.040$); postoperative syndecan-1 levels correlated with sRAGE ($r^2=0.790$, $p=0.0003$). Glypican-1 and Robo4 were also detected in snCPB plasma samples.

Conclusion Plasma levels of syndecan-1 and sFlt-1 were highest during snCPB. A positive association between preoperative syndecan-1 and ICU LOS is consistent with a relationship between endothelial barrier dysfunction and outcome. By contrast, higher intraoperative sFlt-1 correlating with shorter ICU LOS implied a protective role of sFlt-1. Associations between syndecan-1 and sRAGE suggest a link between RAGE and endothelial barrier dysfunction that merits further investigation; as do the novel findings that glypican-1 and Robo4 were detected in plasma of patients undergoing snCPB.

S75 DO "CLINICALLY RELEVANT" TIDAL VOLUMES REALLY CAUSE VENTILATOR-INDUCED LUNG INJURY IN MICE?

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Background Ventilator-induced lung injury (VILI) caused by excessive lung stretch during mechanical ventilation, is an important determinant of intensive care mortality. In recent years the mouse has increasingly become the pre-clinical model of choice, and studies using mice have identified numerous pathways and mediators all apparently vital during VILI. However, findings have not translated into clinical benefit, and it is conceptually extremely difficult to reconcile this plethora of mediators into a single paradigm. We propose that this confused situation has arisen from a somewhat naïve belief that the wide variety of tidal volumes (V_T) used within such studies all induce over-stretching of the lungs.

Methods Anaesthetised mice were ventilated (3 cm H_2O positive end-expiratory pressure, using air ±CO_2 to regulate pH) with a variety of V_T ranging from "clinically relevant" (10 ml/kg) to "very high" (40 ml/kg) for up to 3 h.

Results Both 10 ml/kg and 40 ml/kg V_T evoked deterioration in arterial pO_2 and mean arterial blood pressure (BP), although intermediate V_T (20–30 ml/kg) did not (see Abstract S75 table 1). Lung