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419. Cell-based tissue modulation: the good and the ugly?

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Increased vascular endothelial growth factor (VEGF) secretion from activated T cells in COPD

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Introduction: Vascular endothelial growth factor (VEGF) is important for lung development and angiogenesis but is also anti-apoptotic and chemotactic. Chronic obstructive pulmonary disease (COPD) is an inflammatory disease characterised by accumulation of T-cells and remodelling of the airways and lung parenchyma. We hypothesized that stimulated T-cells from COPD-patients secrete more VEGF than T-cells from healthy subjects.

Methods: Peripheral blood was obtained from ten COPD-patients (FEV1: 23–63 % of predicted) and from eleven healthy individuals. CD3+ cells were isolated through cell sorting and stimulated with anti-CD3/CD28 coated beads. The conditioned media was stored and analysed for VEGF and other mediators by Cytometric Bead Array flex set in a flow cytometer.

Results: T-cells from COPD-patients released significantly more VEGF compared to T-cells from healthy individuals; 65.6 (54.9–75.1) pg/mL vs. 46.7 (39.6–59.4) pg/mL (median, p25-p75), $p=0.0167$. When the COPD-patients and the control group were analysed together there was a negative correlation ($r=-0.43$, $p<0.05$) between the concentration of VEGF in conditioned media and lung function measured as FEV1 %. There were no differences in concentrations of the Th1/Th2 cytokines INF- γ , IL-4, IL-5 and IL-13 between healthy individuals and COPD patients.

Discussion: Activated T-cells from COPD-patients have a significantly increased secretion of VEGF compared to activated T-cells from healthy individuals. Since VEGF has multiple roles in the inflammatory response, we suggest that T-cell secretion of VEGF may be one mechanism by which recruited T-cells participate in tissue remodelling leading to COPD.

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Bone marrow derived mesenchymal stem cells accelerate alveolar epithelial repair *in vitro*

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Abnormal alveolar epithelial wound repair may result in pulmonary fibrosis after injury. Bone marrow derived mesenchymal stem cells (BMSC) are multipotential cells capable of differentiation to various cell types. They secrete a large number

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of growth factors and cytokines that are critical to the repair of injured tissues. We hypothesized that BMSC in co-culture with alveolar epithelial cells may accelerate alveolar epithelial wound repair *in vitro*. A co-culture system was developed using transwell inserts with a membrane of 3 μm pore size. A549 alveolar epithelial cells were grown to a monolayer on top of the membrane inside the insert and rat BMSC were either grown attached on the opposite side of the membrane or in the lower compartment. The confluent A549 alveolar epithelial cell monolayer was wounded and the areas of the wound were measured at different time points using a computerized imaging system. 6 hours after wounding, alveolar epithelial wound closure *in vitro* was increased in presence of BMSC compared to medium control. If the BMSC were grown in the lower part of the compartment, wound closure was 37% compared to 8.9% for serum free medium control. However, if the BMSC were grown on the lower side of the membrane the wounded alveolar epithelial cells, wound closure was even more accelerated (53% wound closure 6 hours after wounding). 24 hours after wounding, the epithelial wound was completely closed in presence of BMSC, whereas wound closure was 51% closed with serum free medium. In presence of BMSC, alveolar epithelial wound repair *in vitro* was increased, in particular if the BMSC were grown in close proximity to the wounded alveolar epithelial cells.

4254**Cigarette smoke induced changes in gene expression in CD117+ mononuclear cells of the bone marrow in mice**

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Cigarette smoking is a major risk factor for chronic obstructive pulmonary disease (COPD) and cardiovascular disease. We postulated that cigarette smoking-induced emphysema might occur due to a failure of the cellular and molecular maintenance program of the lung. It has recently been suggested that bone marrow-derived progenitor cells could contribute to the maintenance and repair of the lung. We hypothesize that cigarette smoke (CS) may affect the number of bone marrow-derived progenitor cells and their gene expression. C57BL/6J mice were exposed to CS for 1 day, 1, 4 and 36 weeks. The number of endothelial progenitor cells (EPCs) positive for stem cell antigen-1 (Sca-1) and vascular endothelial growth factor receptor-2 (VEGFR-2/KDR) in blood samples of CS-exposed mice were significantly increased compared with control mice at 1 day. However, CS-exposed mice had a significantly decreased number of EPCs in bone marrow at 1 and 4 weeks. Mean lung VEGF levels of CS mice were increased at 1 day and conversely decreased at 1 and 4 weeks compared with control mice. Microarray analysis detected 230 transcripts that were differentially expressed in bone marrow stem cells to a significant degree between 1-week CS mice and control mice. Genes related to cell cycles, cell proliferation, cell differentiation and anti-apoptosis were decreased in 1-week CS exposed mice. We conclude that cigarette smoke decreased the number of EPCs in bone marrow and induced significant changes in gene expression in bone marrow-derived progenitor cells.

4255**Immune modulatory strategies for prevention of autoimmune emphysema**

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While cigarette smoking is the major risk factor in the pathogenesis of Chronic Obstructive Pulmonary Disease (COPD)/emphysema, the precise mechanisms of chronic progressive alveolar septal destruction are not well understood. We hypothesize that there may be an autoimmune component to the chronic progressive emphysematous lung tissue destruction in COPD. Here we used our autoimmune model of emphysema¹, which is based on immunization of rats with xenogeneic human umbilical vein endothelial cells (HUVEC), to explore immune modulatory strategies. We tested whether induction of central T cell tolerance (thymic inoculation of antigen) or innate immune responses (pristane, poly I:C or lipid A) can be applied to protect against HUVEC-induced emphysema. Here we demonstrate that each of the immune modulatory strategies preserved alveolar airspaces, inhibited anti-endothelial cell antibody production and protected against cell death when compared with lungs from HUVEC-immunized rats. The increased levels of active caspase-3, MMP-2, and MMP-9 induced by HUVEC-immunization were reduced by each of the treatments. In conclusion, immunomodulatory treatments targeting conceptually distinct innate and adaptive immune pathways prevent the development of HUVEC-induced autoimmune emphysema. These data further support the concept that the HUVEC immunization model is an autoimmune model of emphysema.

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4256**Fibrocytes are a potential source of fibroblasts in idiopathic pulmonary fibrosis**

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Background: Fibrocytes are a unique population of circulating progenitors of fibroblasts implicated in wound healing and fibrosis. However, their contribution to the fibroblast/myofibroblast expansion in idiopathic pulmonary fibrosis (IPF), remains unresolved.

Objective: To determine the role of fibrocytes as a source of fibroblasts/myofibroblasts in IPF.

Methods: Lung fibrocytes were evaluated by microscopy using fibrocyte-specific combinations of antibodies. Stromal-derived factor-1 (CXCL12) was determined in plasma and bronchoalveolar lavage (BAL) by ELISA and its cellular source in lungs was examined by immunohistochemistry.

Results: Fibrocytes were identified in 4 out of 5 IPF lungs. CXCR4/procollagen-1 or prolyl 4-hydroxylase was the combination that stained most fibrocytes (10.3 /mm²), while with the other three combinations we found 2.8 (CD34/procollagen-1), 1.9 (CD34/ α SMA), and 1.8 (CD45RO/prolyl 4-hydroxylase) fibrocytes/mm² respectively (p < 0.05). There was a correlation between the abundance of fibroblastic foci and the amount of fibrocytes. The two patients with higher number of fibrocytes identified in the lungs displayed 13.3 and 9.4 FF/cm² while the other three IPF lungs showed less than 6.0 FF/cm² respectively. Immunoreactive CXCL12 was expressed by alveolar epithelial cells of IPF patients. Plasma levels of CXCL12 were increased in IPF patients compared to healthy controls (median: 2763.6 pg/ml versus 1810.6 pg/ml; p < 0.003). Likewise, CXCL12 was detectable in the BAL fluid of 40% of patients with IPF.

Conclusion: These findings indicate that circulating fibrocytes recruited through the CXCR4/CXCL12 axis may contribute to the expansion of the fibroblast/myofibroblast population in IPF.

4257**The influence of stem cell supernatants on alveolar epithelial cell repair**

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Stem cells have a known role in repair and regeneration of tissue but their role in disorders of aberrant wound repair such as lung fibrogenesis is unclear. We hypothesize that factors produced by stem cells during their differentiation will influence reparative responses in the lung. In this study we test the wound repair of mechanically wounded human alveolar epithelial cell (AEC) line A549 in response to human embryonic stem cell (hESC) supernatants. This study examines how fractionated stem cell supernatants influence AEC wound repair using an in-vitro wound closure model. Fluids retrieved from hESC cultures were able to manipulate wound closure responses of AEC. Specifically, supernatants taken between day 7 and day 10 of differentiation could significantly improve AEC repair (22% improvement over undifferentiated supernatants) (p < 0.05). Mechanisms involved in augmenting wound closure include enhanced cell migration and cell spreading (internuclear distances) and increased cell proliferation (BrdU incorporation) (p < 0.05). We have established that the response is stem cell specific and relates to protein/s produced by these cells; these specific factors secreted at 7–10 day of differentiation need further elucidation. Our data report for the first time the influence of factors produced by stem cells and their involvement in wound repair of lung AEC. This has potential implications for regenerative strategies in disorders of aberrant wound repair such as lung fibrosis.

4258**Embryonic stem cell engraftment in healthy and injured mouse lung**

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Embryonic stem cells (ESC) are a potential source for cell therapy strategies and, for respiratory disease, distal, gas exchange epithelium is an obvious target. We have previously demonstrated enrichment of differentiating murine ESC cultures with mature distal airway epithelial cells and their progenitors. In this proof-of-concept study we aim to establish whether these cells engraft in the lung following systemic delivery.

Differentiated, pneumocyte-enriched mESC populations were labelled using the fluorescent cell tracker CFDA SE. One million cells were implanted via the tail vein into mice with an LPS-induced model of ARDS, an elastase model

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of COPD and healthy mice. Mice were culled at 24 hrs, 48 hrs and 5 days after implantation. Implanted cells were traced using the fluorescent label and phenotyped by immunohistochemistry with antibodies to epithelium, endothelium, macrophage and lung-specific markers.

Implanted cells are seen embedded in the lungs at all time points and in the largest numbers after 24 hours in all mice. The frequency of labelled cell engraftment was noticeably greater in the lungs of healthy mice and they persisted longer in healthy lungs. The cells were commonly embedded in the distal lung epithelium and were positive for epithelial markers. As expected, cells of the mixed implanted population were also seen in diverse regions of the lungs including near small airways and blood vessels. It is possible to speculate that the cells embedded in locations according to their phenotypic niche.

Our findings support the contention that differentiated ESC, enriched for a specific phenotype, can colonize a specific organ and strongly suggest that ESC have potential in cell therapy for lung diseases.
