promoted neutrophil aggregation as assessed by light microscopy. Phase-contrast video-microscopy demonstrated that in WM-15 treated neutrophils, where HA was evident, the percentage of cells entering collagen 1 gels in response to IL-8 was significantly reduced (26.9% vs 71.8% in non-HA cells). WM-15 does not prime neutrophils, as assessed by superoxide production and shape change, and the cell surface expression of CD11b, CD18 and CD66b were not altered. These data suggest a novel role for CD13 in the homotypic aggregation of neutrophils, which reduces chemoattractant-induced migration through collagen 1 matrix and may predispose to neutrophil micro-aggregation within the circulation.

S56 UNRAVELLING VEGF165 SIGNALLING IN THE LUNG

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Introduction Vascular endothelial growth factor (VEGF) is a potent mitogenic, angiogenic and permeability factor that has been implicated in the development of lung injury and repair in a number of respiratory diseases such as ARDS and IPF $VEGF_{165}$a functions via VEGF receptors in particular VEGFR-2, leading to a diverse and complex network of signalling pathways including activation of both the MAPK pathway and eNOS This results in changes to cell permeability, migration and proliferation. We have investigated the downstream signalling mechanisms regulated by $VEGF_{165}$a in pulmonary and systemic endothelial cells. Understanding the signalling pathway used by $VEGF_{165}$a to regulate lung biology is critical to preferentially induce specific beneficial effects.

Methods Human Umbilical Vein Endothelial Cells (HUVEC) and Human Lung Microvascular Endothelial Cells (HUMVEC-L) were treated with 20ng/ml of $VEGF_{165}$a lysed and studied using phospho-specific antibodies which measure the phosphorylation/activation of key signalling molecules. Phosphorylation of VEGFR-2 was measured using phosphotyrosine-specific antibody to tyr^{1175} and tyr^{1214}. Phosphorylation and hence activation of MEK, MAPK and eNOS were also measured. The effects of VEGF isoforms on cell permeability in a time and dose dependent manner were measured by using a transwell system and "Electrical Cell-Substrate Impedance Sensor" (ECIS). Changes in the cellular distribution of VE-cadherin a protein known to be involved in the regulation of cell permeability was assessed by immunofluorescent labelling and confocal microscopy.

Results Phosphorylation of VEGFR-2 at tyr^{1175} and $tyr^{1214}$was induced between 5 and 10min (n=4; >5 fold increase). Activation of MEK and p44/42 MAPK (members of the MAPK pathway which regulates cell proliferation) were seen over a similar time course to that of VEGFR-2 (n=4; >5 fold increase) (Figs 1A, B). Phosphorylation of eNOS which regulates cell permeability was also observed (n=3; >2 fold) and indeed $VEGF_{165}$a increased permeability in both HUVEC and HUMVEC-L (Huvec $p<0.001$); (Humvec-l $p<0.01$) (Fig 1). Finally we showed that in both cell types VEGF induced changes in the cellular distribution of VE-cadherin.

Conclusion These results demonstrate that signalling pathways, previously suggested to induce mitogenesis or permeability are activated by VEGF 165a in HUVEC and HMVEC-l cells, identifying potential future therapeutic targets.

S57 THE ROLE OF VITAMIN D DEFICIENCY IN REGULATING THE SEVERITY AND DURATION OF MURINE LUNG INJURY

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Introduction Vitamin D has been shown to modulate both the innate and adaptive immune responses. Patients deficient have increased susceptibility to both infection and autoimmunity. Our research suggests patients with, or at risk of developing acute lung injury (ALI), are severely Vitamin D deficient/insufficient. As there are no licenced treatments for ALI, novel therapies need to be developed, therefore we investigated the effect of Vitamin D deficiency in a murine model of ALI to understand the mechanistic drivers of its action.

Methods Using a diet completely devoid of Vitamin D, we established near complete Vitamin D deficiency in otherwise wild type C57Bl/6 mice. We combined this with intra-tracheal instillations of LPS (50μg), and analysed the inflammatory response within the lungs of these mice compared to those fed on a Vitamin D sufficient diet. In addition, systemic Vitamin D supplementation was assessed by intra-peritoneal injection of cholecalciferol 48hrs prior to LPS

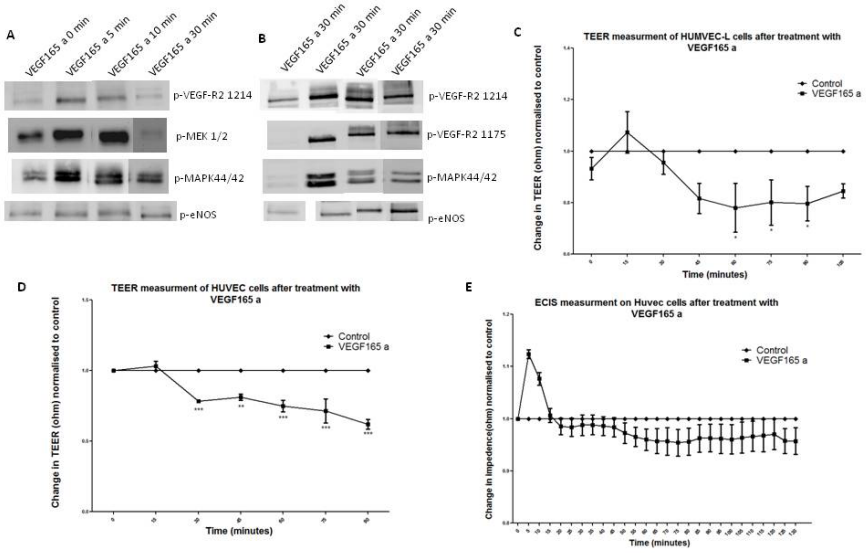

Figure1. Treatment of HUMVEC-L and HUVEC cells with VEGF solution at 20ng/ml. *A*, immunobblotting of primary HUMVEC-L treated for 0 to 30min and immunoblotted for the phosphorylation of VEGFR-2, MEK, MAPK and eNOS using phosphospecific antibodies. *B*, immunobblotting of primary HUVECs . *C*, VEGF165a reduces HUMVEC-L transendothelial electrical resistance (TEER) (increased permeability) in insert cultures monolyers.*p<0.1 (between 60 and 90min), compared with control (untreated cells). *D*, VEGF165a reduces HUVEC TEER .***p<0.001, compared with control (between 30 and 90min) . *E*, Electrical Cell-Substrate Impedance Sensor (ECIS) measurement in HUVECs using 8 well assay 8W10E+; VEGF165a reduces the resistance (increased permeability) compares to the control. Data were analysed using one-way ANOVA and Bonferroni post test analysis.

Abstract S56 Figure 1