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.

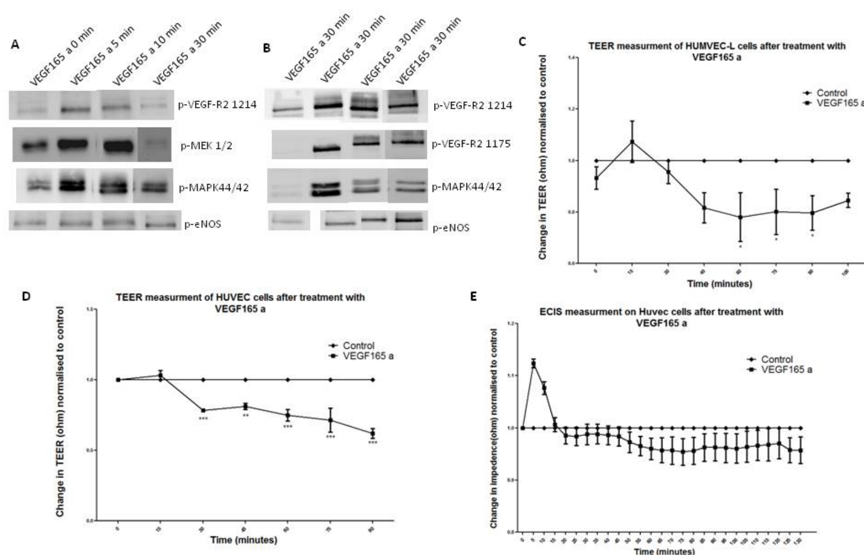

Figure1. Treatment of HUMVEC-L and HUVEC cells with VEGF solution at 20ng/ml. *A*, immunobblotting of primary HUMVEC-L treated for 0 to 30min and immunoblotted for the phosphorylation of VEGFR-2, MEK, MAPK and eNOS using phosphospecific antibodies. *B*, immunobblotting of primary HUVECs . *C*, VEGF165a 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

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

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instillation. Cell infiltrates, expression of several inflammatory markers within bronchial lavage fluid (BALF), as well as tissue permeability were examined to evaluate the immune response and resulting lung damage.

Results Dietary Vitamin D deficient mice (n=12) had elevated BALF protein concentration (p=0.0369) and red blood cell (RBC) extravasation (p=0.084) 48hrs post IT-LPS, suggestive of increased alveolar epithelial damage. BALF levels of VEGF (p=0.0023) and CXCL1/KC (p=0.0121) were significantly increased 48hrs post-LPS, indicating an increase in the inflammatory response in deficient mice. Moreover, inflammation was prolonged with both the total number of cells recruited into the BALF (p=0.0479), and the number of apoptotic neutrophils (p=0.034) significantly higher at 96hrs post LPS in Vitamin D deficient mice. Furthermore, wild type mice with normal Vitamin D levels pre-treated with cholecalciferol had reduced BALF cellular inflammation (p=0.0093), with a lower BALF protein concentration (p=0.076) and RBC accumulation in BALF (p=0.0274) 48hrs post-LPS.

Conclusion Our data indicate that Vitamin D deficiency significantly augments both the severity and duration of murine lung injury and that exogenous Vitamin D reduces lung responses to LPS even in mice with normal Vitamin D levels. These data support the use of Vitamin D to both prevent and potentially treat established ALI.

S58 SURFACTANT PHOSPHOLIPID KINETICS IN PATIENTS WITH ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

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Introduction and Aims ARDS is a significant health burden. Mortality still remains high between 30–50%. Surfactant is a mixture of phospholipids and proteins. Phosphatidylcholines (PC) account for 80% of total phospholipids. PC16:0/16:0 is the main PC with surface tension reducing characteristics. Surfactant abnormalities are well recognised in patients with ARDS However, replacement strategies remain unhelpful in improving mortality. Existing diagnostic definitions fail to identify a homogeneous population and this lack of phenotyping of patients according to surfactant biology may in part explain the absence of therapeutic benefit. The aims of this study are to assess surfactant PC kinetics by the incorporation of *methyl*-D_9-choline in patients with ARDS.

Methods ARDS patients were identified according to American European Consensus Conference (AECC) criteria. Patients were infused with 3.6mg/kg *methyl*-D_9-choline. Small volume bronchoalveolar lavages were performed via a fibre optic bronchoscope at serial time points. Healthy volunteers were used as controls. The phospholipid fraction was extracted and analysed by triple quadrupole electrospray ionisation mass spectrometry.

Results Ten patients and nine healthy controls were recruited. The endogenous PC composition consisted primarily of PC16:0/16:0, PC16:0/18:1 and PC18:0/18:2. There was significant reduction in the relative proportion of endogenous PC16:0/16:0 in patients. Compared to healthy controls, newly synthesised deuteriated PC16:0/16:0 was much lower in patients (26%) than controls (47%).

Total **surfactant PC** D_9**-**incorporation was linear until 48 hours (0.019%/h, r^2=0.9734, P<0.05) and reached its maximum at 48 hours (0.93±0.15%). Steady state of incorporation was achieved between 48–96 hours. There was ~80% increase in the fractional D_9 labelling in patients at 48 hours compared to healthy controls.

Total **plasma PC** D_9-incorporation was linear until 24 hours (0.032%/h, r^2=0.9825, P<0.05) and reached its maximum (0.755±0.056%) at 24 hours. Linear decline in enrichment was noted after 24 hours at a rate of 0.003% per hour (r^2=0.9915, P<0.05). The total surfactant PC D_9-incorporation was much higher for patients at 24 hours and 48 hours reflecting increased synthetic rate.

Conclusions By labelling surfactant PC precursors, it is possible to study surfactant kinetics in patients with ARDS. The methodology may be utilised to phenotype patients according to alveolar surfactant kinetics prior to replacement strategies.

S59 THE ROLE OF LYMPHOCYTES IN ACUTE LUNG INJURY

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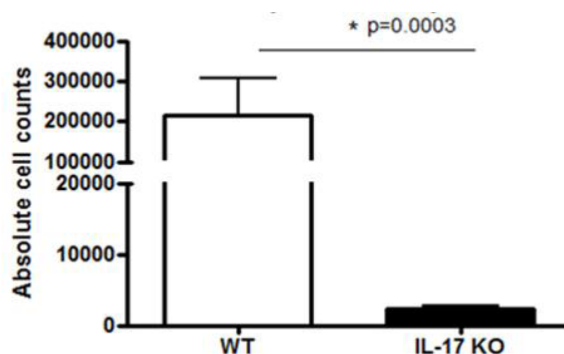
Introduction Acute lung injury (ALI) is caused by the uncontrolled activation immune cells, resulting in dysregulated inflammation which irreversibly damages the lung architecture and function. Whilst the role of myeloid cells in the pathophysiology of ALI is well established, the function of lymphoid cells is less well understood.

Hypothesis Lymphocytes may play a role inthe regulation of inflammation in acute lung injury.

Methods We have utilised both human and murine *in vivo* models of lipopolysaccharide(LPS) induced ALI, as well as patient samples, to characterise lung infiltrating cells by flow cytometry. Lymphocyte associated cytokines were quantified by luminex or via ex vivo stimulation andintra-cellular cytokine staining.

Results Rapid infiltration of T cells(both αβ and αβ T cells), including regulatory T cells (Tregs), NKT and NKcells was observed. The cytokine Interleukin (IL)-17 is a potent recruiter of neutrophilsto sites of inflammatory sites. We observed a significant elevation in IL-17 levels in bronchoalveolar lavage fluid (BALF) in the humans postLPS inhalation; high levels of IL-17 were also seen in BALF from ALI patients. AnIL-17 KO mouse was utilised to confirm the key role for this cytokine in neutro philrecruitment to the lung during ALI. The transcription factor RORγt induces transcription of the genes encoding IL-17. Flow cytometric analysis revealed $ROR\gamma t^+$ lung lymphocytes were predominantly CD^4+ T cells (18%), CD^8+ T cells (20%)and the recently described innate lymphoid cells (40%). Small percentages of $ROR\gamma t^+$natural killer (NK) cells, NK T cells, gamma delta T cells were also seen. The role of Tregs in the early regulation of ALI was examined using a Treg inducibleKO murine model (FOXP3$^{GFP\ DTR}$). When Tregs were depleted prior to LPS administration there was an increased recruitment of neutrophils into the lung.

Conclusion We have demonstrated that both lymphocyte released IL-17 and Tregs modulate the recruitment of neutrophils to the lung in ALI. These results reveal the importance of lymphocytes in the immunopathology of ALI. Increased understanding of the lymphocyte function could open new avenues of exploration for therapeutic strategies to regulate immune responses in ALI.



Abstract S59 Figure 1

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