Abstract P19 Figure 1 A549 cells plated on an iCelligence 8-well gold electrode coated plate were incubated with TNFR1 dAb™, a dummy dAb or Adalimumab™ for 1 h then exposed to exogenous TNF or vehicle control. Electrical impedance was measured continuously over 50 h. Trough normalised impedance was measured over 50 h post treatment (n = 3–5). Data are presented as mean ±SEM analysed by Kruskal-Wallis (Dunns). *p < 0.05, **p < 0.01.

Poster sessions

P20

DELINEATING THE CONTRIBUTION OF FORMYLATED PEPTIDES AND FORMYL PEPTIDE RECEPTOR 1 TO THE PATHOGENESIS OF ACUTE LUNG INJURY

1DA Dorward, 1CD Lucas, 2MK Docherty, 1GB Chapman, 1E Scholefield, 1A Conway-Morris, 1T Kipari, 1CT Robb, 1JM Felton, 1PD Whitefield, 1C Haslet, 1K Dhalwal, 1AG Ross. 2MRC Centre for Inflammation Research, University of Edinburgh, Edinburgh, UK; 3University of Highlands and Islands, Inverness, UK

Background Acute respiratory distress syndrome (ARDS) remains an often fatal condition without effective pharmacological therapies. Characteristically, a neutrophil-dominant disorder, it is associated with a dysregulated inflammatory response and tissue injury. Neutrophil migration into inflammatory sites is controlled by a variety of factors; in sterile tissue injury mitochondrial formylated peptides are released following necrotic cell death and bind to formyl peptide receptor 1 (FPR1) on neutrophils to induce migration and activation.

Hypothesis That mitochondrial formylated peptides are elevated in ARDS and drive FPR1-mediated neutrophil recruitment. Inhibition of FPR1 in sterile lung injury would therefore attenuate the inflammatory response through multiple FPR1-mediated effects.

Methods Mitochondrial DNA and formylated peptides were quantified in plasma of ARDS patients and healthy controls by qPCR, western blot and LC-MS/MS. Healthy volunteer neutrophils were stimulated with mitochondrial formylated peptides and chemotaxis assays and flow cytometry used to assess neutrophil function. Intracellular signalling was assessed by western blotting. Mouse models of infective (E. coli) and sterile (hydrochloric acid) acute lung injury were used.

Results Free mitochondrial DNA and formylated peptides were elevated in ARDS patients. Mitochondrial formylated peptides induced FPR1-dependent neutrophil chemotaxis through PI3K- and MAPK-mediated control of the β3-integrin heterodimer Mac1. In sterile acid-induced injury FPR1 inhibition resulted in reduced neutrophil migration, pulmonary haemorrhage, protein leak and pro-inflammatory cytokine expression. Furthermore, acid-induced reduction in alveolar macrophage number was inhibited while interstitial macrophages displayed an alternatively activated phenotype. FPR1 was also found to be expressed on mouse type 1 alveolar epithelial cells suggesting further possible mechanisms through which FPR1-mediated alveolar leak occurs. Importantly, delivery of FPR1 antagonists 12 h after injury also reduced acute lung inflammation demonstrating potential therapeutic relevance. In non-sterile E. coli-mediated lung injury partial antagonism of FPR1 resulted in reduced alveolar neutrophil numbers and attenuated vascular leak without altering bacterial clearance.

Conclusions Mitochondrial formylated peptides and FPR1 play an important role in the pathogenesis of sterile acute lung injury. This appears to be predominantly through neutrophil-dependent means but their role in macrophage and epithelial cell function could also be important. FPR1 antagonism may therefore represent a multi-cellular therapeutic target in the treatment of ARDS.

P21

HYPOXIA-INDUCED NEUTROPHIL SURVIVAL IS DEPENDENT ON PHOSPHOINOSITIDE 3-KINASE (PI3-K)-MEDIATED SIGNALLING

1SP pallet, 1L Porter, 1JKuss, 2EHessel, 1A Amour, 1D House, 1M Beg, 1ER Chilvers. 2University of Cambridge/School of Clinical Medicine, Cambridge, UK; 3GlaxoSmithKline, Stevenage, UK

Background Neutrophils (PMNs) are a key component of the innate immune response to invading pathogens. They accumulate at sites of inflammation and infection, which are typically characterised by low oxygen tensions (e.g. in the acute respiratory distress syndrome (ARDS)). Human PMNs undergo constitutive apoptosis, their survival contingent upon pro-survival and pro-apoptotic signals derived from their micro-environment. Hypoxia profoundly delays PMN apoptosis, resulting in persistence of PMNs at inflammatory foci and this may perpetuate hypoxia-mediated lung injury. Given the importance of phosphoinositol 3-kinase (PI3-K) signalling in cytokine-mediated neutrophil survival, we hypothesised that hypoxia-induced PMN survival may also involve PI3-K-mediated signalling.

Methods Highly pure PMNs isolated from healthy volunteers were incubated for 20 h under normoxic (20 kPa) and physiologically relevant hypoxic (3 kPa) conditions with the same panel of inhibitors, allowing comparison with physiologically relevant hypoxic conditions. PMNs were also incubated in normoxia and hypoxia in the presence of GM-CSF (1 ng/ml) with the same panel of inhibitors, allowing comparison with GM-CSF mediated survival, which is largely PI3-K dependent. PMN apoptosis was assessed using two complementary techniques – morphology and flow cytometry following annexin V-FITC and propidium iodide staining.

Introduction and objectives Neutrophils (PMNs) are a key component of the innate immune response to invading pathogens. They accumulate at sites of inflammation and infection, which are typically characterised by low oxygen tensions (e.g. in the acute respiratory distress syndrome (ARDS)). Human PMNs undergo constitutive apoptosis, their survival contingent upon pro-survival and pro-apoptotic signals derived from their micro-environment. Hypoxia profoundly delays PMN apoptosis, resulting in persistence of PMNs at inflammatory foci and this may perpetuate hypoxia-mediated lung injury. Given the importance of phosphoinositol 3-kinase (PI3-K) signalling in cytokine-mediated neutrophil survival, we hypothesised that hypoxia-induced PMN survival may also involve PI3-K-mediated signalling.

Methods Highly pure PMNs isolated from healthy volunteers were incubated for 20 h under normoxic (20 kPa) and physiologically relevant hypoxic (3 kPa) conditions with the same panel of inhibitors, allowing comparison with physiologically relevant hypoxic conditions. PMNs were also incubated in normoxia and hypoxia in the presence of GM-CSF (1 ng/ml) with the same panel of inhibitors, allowing comparison with GM-CSF mediated survival, which is largely PI3-K dependent. PMN apoptosis was assessed using two complementary techniques – morphology and flow cytometry following annexin V-FITC and propidium iodide staining.
Corrections

P20 - withdrawn
