Methods and Results The role of neutrophilic inflammation and PAR-1 was investigated in two models of murine pneumococcal pneumonia (serotype 2 (D39) and serotype 19F (EF3030)) by using the most clinically advanced PAR-1 antagonist, SCH530348. Neutrophil depletion and chemokine neutralisation studies were also performed. Samples were analysed by immunohistochemistry, cytology, flow cytometry, ELISA and microbiological techniques. Our models were characterised by evidence of intra-alveolar coagulation, increased neutrophil recruitment to areas of bacterial infection and increased PAR-1 expression (demonstrated by quantitative image-analysis). Neutrophil depletion protected mice against barrier disruption but resulted in compromised host defence. In contrast, PAR-1 antagonist treatment significantly reduced neutrophil recruitment to the bronchoalveolar space without being detrimental to host defence. Markers of alveolar leak, coagulation activation and pro-inflammatory cytokines and chemokines (IL-1β, CXCL1, CCL2 and CCL7) were also attenuated. Neutralisation studies demonstrated that IL-1β and CCL7, but not CXCL1 and CCL2, played a key role in neutrophil recruitment to the airspaces in this model. Translational studies were performed to examine by flow cytometry the CXC and CC chemokine receptor expression on neutrophils from blood and BALF of mechanically ventilated CAP-induced ARDS patients and controls. CXCR1 and CXCR2 expression on BALF neutrophils was higher in CAP-ARDS patients compared to controls. Additionally, chemokine expression patterns on neutrophils from CAP-ARDS patients changed within different compartments, evidenced by decreased expression of CXCR1 and increased expression of CXCR2, CCR1, CCR2 and CCR3 on neutrophils from BALF compared with blood.

Conclusion These data provide preclinical proof-of-concept that recently developed PAR-1 antagonists may offer a novel therapeutic approach for controlling or preventing alveolar barrier dysfunction and excessive neutrophilic inflammation in pneumococcal pneumonia without compromising host defence. Furthermore, they highlight a role for chemokine receptor switching in CAP-ARDS with important implications for future targeting of these chemokine receptors.

S101 SRC/BCR-ABL INHIBITION WITH DASATINIB IN STERILE AND NON-STERILE ACUTE LUNG INFLAMMATION

[1]JG Macfarlane, [2]DA Dorward, [2]CD Lucas, [1]JA Scott, [1]MH Ruchaud-Sparagano, [3]CMA Khan, [2]AG Rossi, [1]AJ Simpson. *[1]Institute of Cellular Medicine, Newcastle Upon Tyne, UK; [2]MRC Centre for Inflammation Research, Edinburgh, UK; [3]Institute of Cell and Molecular Biosciences, Newcastle Upon Tyne, UK*

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Introduction and objectives Adult respiratory distress syndrome (ARDS) is a commonly fatal complication of lung infection and inflammation, with no effective treatment. It is characterised by excessive neutrophil influx and degranulation into the lungs, with alveolar leak and severe hypoxia. *Src* family tyrosine kinases are critical in integrin-dependent neutrophil degranulation. Dasatinib is a *Src*/*Bcr-abl* inhibitor used in chronic myeloid leukaemia. We investigated our hypothesis that extracellular neutrophil degranulation could be inhibited by dasatinib *in vitro* and would modulate the inflammatory response *in vivo* in models of infective and sterile lung injury.

Methods Whole blood and isolated blood neutrophils from healthy volunteers were pre-treated with dasatinib and treated with neutrophil stimuli or live bacteria. Degranulation was measured by granule receptor expression and presence of extracellular granule products. Other neutrophil functions were assessed, including adhesion, L-selectin shedding, chemotaxis, phagocytosis, oxidative burst, bacterial killing and apoptosis. Neutrophilic lung inflammation was induced in mice using intratracheal *E. coli* or hydrochloric acid.

Results *In vitro*, dasatinib inhibited neutrophil degranulation in response to lipopolysaccharide derived from *E. Coli* 026:B6, fMLF and *Staphylococcus aureus* at concentrations above 100 nM, with no effect on neutrophil viability or apoptosis. Integrin-dependent functions including adhesion, chemotaxis and phagocytosis in adherent conditions were impaired, but phagocytosis was unaffected in whole blood. Intracellular oxidative burst was maintained, with normal bacterial killing, but extracellular superoxide anion release was impaired.

In vivo, dasatinib had modest effects on the pro-inflammatory response to *E. coli*, reducing%interstitial neutrophils, alveolar myeloperoxidase and TNFα at 1 mg/kg and alveolar lactoferrin at 10 mg/kg. Bacterial killing was impaired in a dose dependent fashion, with associated alveolar leak and systemic toxicity at 10 mg/kg. In sterile acid injury, 5 mg/kg dasatinib reduced%neutrophils, degranulation (interstitial CD11b/alveolar lactoferrin) and monocyte-chemotactic protein-1 (MCP-1) in the alveolar space, but induced detrimental effects at 10 mg/kg.

Conclusions The pan-*Src* kinase inhibitor dasatinib modifies multiple pro-inflammatory neutrophil functions *in vitro* and *in vivo* with an impairment in bacterial killing observed in infective lung injury. In the context of sterile inflammation, manipulation of neutrophil degranulation also alters the inflammatory environment and this approach warrants further study as a therapeutic strategy in ARDS.

S102 LIPOXIN A4 IMPROVES EFFEROCYTOSIS VIA INHIBITION OF THE HMGB1 IN HUMAN ALVEOLAR MACROPHAGES

[1]Q Wang, [1]D Parekh, [1]VK D'Souza, [1]R Dancer, [1]JM Patel, [1]D Bartis, [1]F Gao, [2]Q Lian, [2]S Jin, [1]DR Thickett. *[1]Centre for Translational Inflammation and Respiratory Research, School of Clinical and Experimental Medicine, University of Birmingham, Birmingham, UK; [2]Department of Anesthesia and Critical Care, Second Affiliated Hospital of Wenzhou Medical University, Zhejiang, China*

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Introduction Effective clearance of apoptotic cells by macrophages, termed efferocytosis, is a pre-requisite for successful resolution of inflammation. High mobility group box protein 1 (HMGB1), is an alarmin that may promote inflammation as well as suppress phagocytosis. Lipoxin A_4, represents one of a unique class of lipid mediators that possess a wide spectrum of anti-inflammatory and pro-resolution actions. We hypothesised that lipoxin A_4 may promote both apoptosis in neutrophils, and stimulate macrophage efferocytosis, acting as an antagonist to HMGB-1.

Methods Neutrophils were obtained from healthy volunteers and cultured for 24 h with or without lipoxin A_4. Apoptosis of neutrophils was determined with Annexin V/SyTox staining by flow cytometry. HMGB-1 levels in Acute Respiratory Distress Syndrome (ARDS) bronchoalveolar lavage fluid (BALF) was measured by ELISA. The effects of HMGB-1 and lipoxin A_4 upon alveolar macrophage efferocytosis was assessed by measuring the ingestion of CMFDA labelled apoptotic neutrophils by flow cytometry. The PI3K (P85) protein expression was measured by western blotting.