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 immuno-histochemistry, 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. Neutrophil depletion was 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 Src family kinase inhibitor dasatinib modifies multiple pro-inflammatory neutrophil functions in vitro and this approach warrants further study as a therapeutic strategy in ARDS.

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

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 A4, 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 A4 may promote both apoptosis in neutrophils, and stimulate macrophage efferocytosis, acting as an antagonist to HMGB1.

Methods Neutrophils were obtained from healthy volunteers and cultured for 24 h with or without lipoxin A4. Apoptosis of neutrophils was determined with Annexin V/SyTox staining by flow cytometry. HMGB1 levels in Acute Respiratory Distress Syndrome (ARDS) bronchoalveolar lavage fluid (BALF) was measured by ELISA. The effects of HMGB1-1 and lipoxin A4 upon alveolar macrophage efferocytosis was assessed by measuring the ingestion of CFDA labelled apoptotic neutrophils by flow cytometry. The PI3K (P83) protein expression was measured by western blotting.