suggest that BAL taken on day 0 of ARDS upregulated IL17 expression in normal T cells. This finding was blocked by exogenous 1,25-OH Vitamin D. By contrast, BAL taken on day 4 upregulated FoxP3 and CD25 expression, suggesting an increase in regulatory T cell activity.

Discussion These results suggest that in early ARDS an imbalance in T cells favouring expression of IL-17 may play a role in the inflammatory response to injury, and this may be attenuated by adequate vitamin D levels. Later in the course of the disease, Treg cells may predominate and play a role in resolution.

S50 EVALUATION OF SECRETORY LEUCOPROTEASE INHIBITOR (SLPI) AS AN ANTI-INFLAMMATORY THERAPY FOR DONOR LUNG INFLAMMATION

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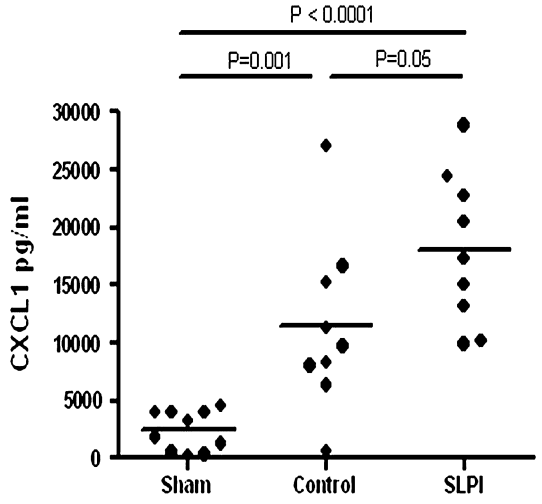
Introduction Donor lung inflammation, reflected by high concentrations of interleukin-8 in bronchoalveolar lavage (BAL) and an imbalance between pro-inflammatory IL-6 and anti inflammatory IL-10 in tissue, correlates with poor graft function and reduced survival after human lung transplantation. Secretory leucoprotease inhibitor (SLPI) is an anti-protease abundant in the lung. SLPI inhibits neutrophil elastase and down regulates inflammatory cytokine transcription via the NFκB pathway. We investigated the effect of SLPI on inflammatory mediators, in a rat model of brain death induced lung injury.

Methods Brain death (BD) was induced in anesthetised ventilated male Wistar rats (n=16) by rapid inflation of an intracranial balloon, the balloon was not inflated in non-BD sham animals. Rats received intra-tracheal human recombinant SLPI (400 ng/g) (n=8) or saline (control n=8, sham n=9) at 1 h. The experiment was terminated at 5 h. Serum samples were taken at 0, 1, 3 and 5 h, and BAL from one lung was taken for cytokine analysis. The second lung was used for wet/dry ratio and Q-PCR analysis.

Results CXCL1, TNF-α, IFN-γ and IL-6 were significantly higher in BAL and serum of control than sham rats, demonstrating that BD induced lung inflammation in this model. There was no change in lung wet-dry ratio between SLPI treated and control groups. Surprisingly, CXCL1 levels were higher in the BAL of SLPI treated rats than controls (p=0.05), however no significant difference was detected for any other cytokine. There was a non-significant trend towards a higher number of CD45+ leucocytes in BAL in SLPI treated rats compared to controls. CXCL1 mRNA was also increased 1.5-fold in the SLPI treated group compared to controls (p<0.05).

Conclusions In this study, SLPI does not appear to have an anti-inflammatory effect in the rat lung, and may exacerbate inflammation as seen by an increased concentration of the chemokine CXCL1. This surprising effect may be due to the short time course of this experiment where the initial effect of SLPI may be pro-inflammatory. Given the small window of opportunity available to treat donor lungs we believe that SLPI is an inappropriate intervention for use in lung transplantation.



Abstract S50 Figure 1

S51 THIOREDOXIN MODIFIES MIF RELEASE FROM HUMAN MONOCYTES FOLLOWING STIMULATION WITH LTA AND LPS

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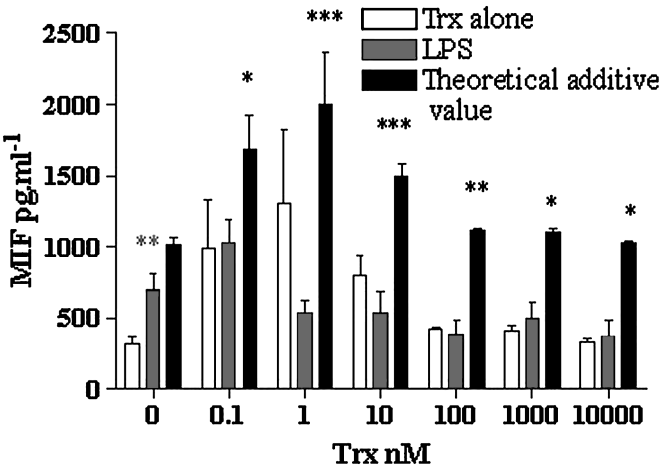
Rationale Thioredoxin (Trx) is a 12-kDa ubiquitous redox-active thiol (-SH) protein. Plasma levels of Trx are raised in numerous medical and surgical conditions associated with oxidative stress and inflammation such as sepsis (Burke-Gaffney 2005). Trx is thought to have an anti-inflammatory role, at least when released into the circulation. By contrast macrophage migration inhibitory factor (MIF) is regarded as a pivotal pro-inflammatory protein. Indeed co-injection of MIF and *E. coli* enhanced lethality, whereas, anti-MIF monoclonal antibodies conferred protection against murine caecal ligation and puncture and administration of *E. coli* (Calandra 2000). We have previously reported a positive correlation



Abstract S51 Figure 1 Effect of Trx of on release of MIF from human monocytes following stimulation with LPS. Human monocytes (1×10^6 ml^{-1}) we re pre-incubated with Trx (0.1−10 000 mM) for 24 h followed by stimulation with medium alone (white bars) or LPS 1 μg/ml (red bars) for 24 h MIF concentrations in cell supematants were determined by sandwich ELISA. The black bars represent the theoretical additive values of Trx and LPS. Data represent mean ± SEM from 5 experiments (LPS) and 3 experiments nts (no treatment). *p<0.05, **p (0.01, ***p<0.001 when conditions compared (two way ANOVA with Bonferroni's post test). Red asterisks represent significant increase following LPS alone. Black asterisks represent significant difference between LPS and Trx with the theoretical additive value. MIF, macropage migration inhubitory factor; LPS, lipopolysac charide; LTA, lipoteichoic acid; Trx, Thioredoxin.

between Trx and MIF in adults with SIRS/sepsis. (Leaver 2009) Furthermore Trx was shown to inhibit the secretion of MIF in THP-1 cells (Tamaki 2006). The aim of this study was to determine the effect of exogenous Trx on the release of MIF and for comparison IL-8 and IL-10 from primary human monocytes at baseline and following stimulation with lipoteichoic acid (LTA) or lipopolysaccharide (LPS).

Methods Monocytes were extracted from whole blood of healthy volunteers using Percoll gradients and MACS columns. Monocytes (1×10^6 cells/ml) were pre-incubated with Trx (0.1—10 000 nM) for 24 h followed by treatment (24 h) with medium alone, LPS 1 μg/ml, LTA 10μg/ml. MIF, IL-8 and IL-10 concentrations in cell supernatants were determined by ELISA.

Results Following incubation with Trx there was no significant change in MIF release from monocytes. By contrast, LPS and LTA significantly ($p<0.01$) induced MIF from base line. When monocytes were treated with LPS (Abstract S51 Figure 1) or LTA following pre-incubation with Trx, MIF release was significantly less than the theoretical additive effects of the two treatments alone. By contrast, although Trx significantly induced IL-8 and IL-10, Trx did not modulate LPS or LTA induced cytokine release.

Conclusion Trx reduced MIF release following stimulation with LPS and LTA. Extracellular Trx exerts an anti-inflammatory effect in this model. The Trx/MIF axis should be explored as a potential route for therapeutic intervention in patients with sepsis.

S52 THE ROLE OF THE RECEPTOR FOR ADVANCED END PRODUCTS (RAGE) IN ACUTE LUNG INJURY (ALI)

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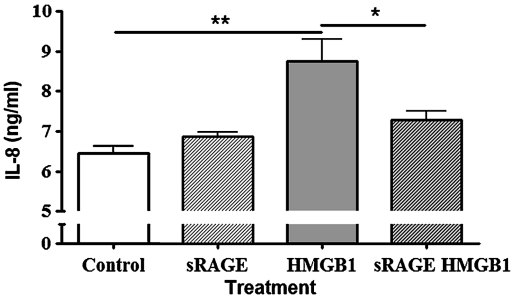
Introduction RAGE is expressed by both alveolar epithelial and endothelial cells. The receptor can bind to pro-inflammatory ligands, including HMGB1 which is elevated in ALI. RAGE negative mice show a reduced inflammatory response when challenged with LPS. We hypothesised that RAGE activation in the pulmonary circulation propagates inflammation in ALI and that soluble RAGE (sRAGE) can act as a scavenger receptor, reducing RAGE-mediated inflammation.

Aim To investigate HMGB1 RAGE-dependent activation of pulmonary microvascular endothelial cells, and resultant production of inflammatory cytokines and tissue-destructive proteases in a model of ALI.

Methods Primary HPMECs (human pulmonary microvascular endothelial cells) were stimulated with clinically relevant concentrations of HMGB1. MAPK phosphorylation was assessed by Western Blot. Supernatants collected at 72 h were analysed by ELISA for IL-8, Tissue Inhibitors of Metalloproteinases (TIMPs)-1 and -2. Experiments were repeated both with a RAGE-blocking antibody and in the presence of sRAGE.

Results HMGB1 increased phosphorylation of ERK1/2 at 15 min and p38 at 30 min after stimulation HMGB1 increased IL-8 secretion (from 6.46 ng/ml to 8.75 ng/ml, $p<0.01$) and significant decrease in TIMP-1 secretion (from 19.79 ng/ml to 16.9 ng/ml, $p<0.05$) at 72 h. MAPK activation, IL-8 increase and TIMP-1 decrease was significantly reversed in the presence of sRAGE ($p<0.05$) (Abstract S52 Figure 1). Incubating cells with a RAGE blocking antibody inhibited MAPK phosphorylation by HMGB1.

Conclusions Data suggest that RAGE ligation leads to an increase in pulmonary endothelial cell activation and IL-8 release. *In vivo* this would increase inflammatory cell influx into the pulmonary environment, propagating inflammation. This is combined with a decrease in TIMP protection potentially increasing degradation of the basement membrane by functionally unopposed proteinases.



Abstract S52 Figure 1 sRAGE and HMGB1 were used at 1 μg/ml. Statistics are one way ANOVA with Tukey post test. *$p<0.05$, **$p<0.01$.

That these changes can be partially rescued using sRAGE shows that it could potentially decrease inflammatory damage in ALI.

S53 NANOPARTICLES CAUSE PULMONARY INFLAMMATION THROUGH IL-1α AND PARTIAL ACTIVATION OF THE NLRP3 INFLAMMASOME

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Nanoparticles are increasingly used in various fields, including biomedicine and electronics. Their size and physical characteristics allow them to easily access the cytosol of tissue or immune cells. Although inorganic metal oxide nano-TiO_2 is believed to be biologically inert, an emerging literature reports increased incidence of respiratory diseases in exposed people. Here, we show that instillation of nano-TiO_2 induces lung inflammation which is strongly suppressed in IL-1R- and IL-1α-deficient mice. They have drastically reduced neutrophil recruitments in the alveolar space, together with lung inflammatory cytokine productions. Surprisingly, the NLRP3 inflammasome complex seems to be only partially involved. Nlrp3-, ASC- or Casp-1-deficient mice show only a slight reduction in pulmonary inflammatory response. IL-1β-deficient mice exhibit decreased inflammation parameters that are less pronounced than IL-1α-deficient mice. *In vitro* experiments show that primary pulmonary epithelial cells cultured in presence of nano-TiO_2 are able to produce KC and IL-1α, but not IL-1β, to initiate inflammation. In conclusion, it appears that nanoparticles-mediated inflammation is highly dependent on IL-1α and to a less extend on the NLRP3 inflammasome/IL-1β axis. Collectively, these data demonstrate that the expending use of nano-TiO_2, e.g. in cosmetics, may present a health hazard and should be taken under consideration, a situation reminiscent of inflammation provoked by asbestos exposure.

Orphan lung diseases

S54 CHARACTERISING SARCOIDOSIS USING A WEB-BASED REGISTRY: A PILOT STUDY

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Introduction and Objectives Sarcoidosis is a chronic multisystem disorder of unknown cause. Demographic and phenotypic characteristics have not been comprehensively studied in Britain.