BTS/BLF/BALR Early Career Investigators Symposium

T1 FLUTICASONE PROPIONATE ALTERS THE RESIDENT AIRWAY MICROBIOTA AND IMPAIRS ANTI-VIRAL AND ANTI-BACTERIAL IMMUNE RESPONSES IN THE AIRWAYS

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Background Inhaled corticosteroids are the cornerstone of therapy in asthma and COPD but cause only modest reduction in exacerbations and are associated with increased pneumonia frequency. This has raised concern about potential detrimental effects on host-defence against respiratory pathogens. The aim of this study was to evaluate the effects of fluticasone propionate on airway anti-viral and anti-bacterial host-defence.

Methods C57BL/6 mice were intranasally treated with fluticasone propionate (1 mg/kg) or vehicle control. 16S Quantitative PCR was used to evaluate total bacterial loads and pyrosequencing was used to evaluate microbiota community composition in lung tissue. Using mouse models of infection with rhinovirus 1B and *S. pneumoniae* D39, effects of fluticasone administration on anti-viral and anti-bacterial immune responses, airway inflammation and pathogen control were evaluated.

Results Mice treated with fluticasone had increased lung bacterial loads compared to vehicle-treated controls at 8 h post administration ($p < 0.05$). Evaluation of community composition revealed that fluticasone treatment was associated with significant increases in *Stenotrophomonas* genera ($p < 0.05$). In a mouse model of *S. pneumoniae* infection, fluticasone administration suppressed anti-bacterial responses including expression of cytokines IL-6 and TNF-α (4 h post-infection; $p < 0.001$) and airway neutrophil recruitment (8 h post-infection; $p < 0.001$) and was also associated with increased lung bacterial loads measured by quantitative culture (8 h post-infection; $p < 0.001$). In a mouse model of rhinovirus infection, fluticasone suppressed innate anti-viral responses including BAL protein levels of interferon-β and -λ2/3 (day 1 post-infection; $p < 0.001$). Virus clearance was impaired by fluticasone with increased viral RNA copies observed in lung tissue (day 1&2 post-infection; $p < 0.001$). The late expression of rhinovirus-induced airway mucins MUC5AC and MUC5B BAL proteins was increased by fluticasone ($p < 0.01$ and $p < 0.05$ respectively at day 7). Administration of recombinant interferon-β in combination with fluticasone and rhinovirus led to upregulation of interferon-stimulated genes and improved virus clearance, thereby demonstrating that adverse effects of fluticasone on RV clearance are causally related to interferon suppression. Recombinant IFN-β did not alter the increased mucins observed with fluticasone treatment.

Conclusion Fluticasone alters the airway microbiota and impairs airway anti-viral and anti-bacterial host-defence in mice. Human studies are required to confirm the relevance of these effects in the context of inflammatory airway diseases.

T2 VITAMIN D SUPPLEMENTATION REDUCES PERIOPERATIVE SYSTEMIC AND ALVEOLAR INFLAMMATION IN PATIENTS UNDERGOING OESOPHAGECTOMY: RESULTS OF THE VINDALOO TRIAL

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Vitamin D deficiency is associated with increased risk of ARDS post-oesophagectomy. We recruited patients to a double-blind, randomised controlled trial of high dose Vitamin D supplementation 3–14 days pre-oesophagectomy.

79 patients were randomised to receive placebo or 300,000 IU oral Vitamin D liquid 3–14 days prior to oesophagectomy. Blood samples were collected pre-dose, post-dose (pre-op) and post-op and analysed for 25-OH and 1,25-dOH Vitamin D, inflammatory cells and cytokines. Broncho-alveolar lavage fluid was collected at the end of the operation. PICCO biomarkers of alveolar capillary damage (EVLWI and PVPI) were measured pre- and post-op.

Pre-operative supplementation with Vitamin D was well tolerated with no SUSARs and significantly increased circulating 25-OH and 1,25-OH Vitamin D ($p < 0.0001$). This was associated with reduced systemic inflammation (IL-6 ($p = 0.02$) and IL-8 ($p = 0.002$)) and an increase in circulatory Treg ($p = 0.027$).

Changes in PICCO biomarkers were lower in supplemented patients suggesting lower perioperative alveolar oedema (EVLWI $p = 0.05$, PVPI $p = 0.04$). This did not result in a significant difference in oxygenation at 24 h.

Post-op, systemic and alveolar alarmin (IL-1B, IL-6, IL-8) response was similar in treated and untreated patients but the systemic release of IL-1ra ($p = 0.046$), sTNFR-1 ($p = 0.05$) and s-TNFR-2 ($p = 0.02$) were elevated in treated patients. There was also evidence of decreased alveolar macrophage efferocytosis in patients with Vitamin D deficiency ($p = 0.003$).

Clinical diagnoses of ARDS were significantly lower in this cohort than in previous cohorts, but the study was not powered to detect that outcome. Mortality post-operative was not significantly different at 30 or 90 days but there is a significant difference after 300 days of follow-up (placebo 33% mortality, Vitamin D 8% mortality $p = 0.033$).

In conclusion, vitamin D supplementation was a safe, well tolerated pre-operative intervention that reduced systemic inflammation and biomarkers of alveolar oedema. With evidence of enhanced anti-inflammatory mechanism that may have influenced longer term post-operative survival, Vitamin D deficiency should be identified and treated in patients at risk of ARDS.

T3 MITOCHONDRIAL TRANSFER IS AN IMPORTANT MECHANISM BY WHICH MESENCHYMAL STROMAL CELLS (MSC) FACILITATE MACROPHAGE PHAGOCYTOSIS IN THE *IN VITRO* AND *IN VIVO* MODELS OF ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

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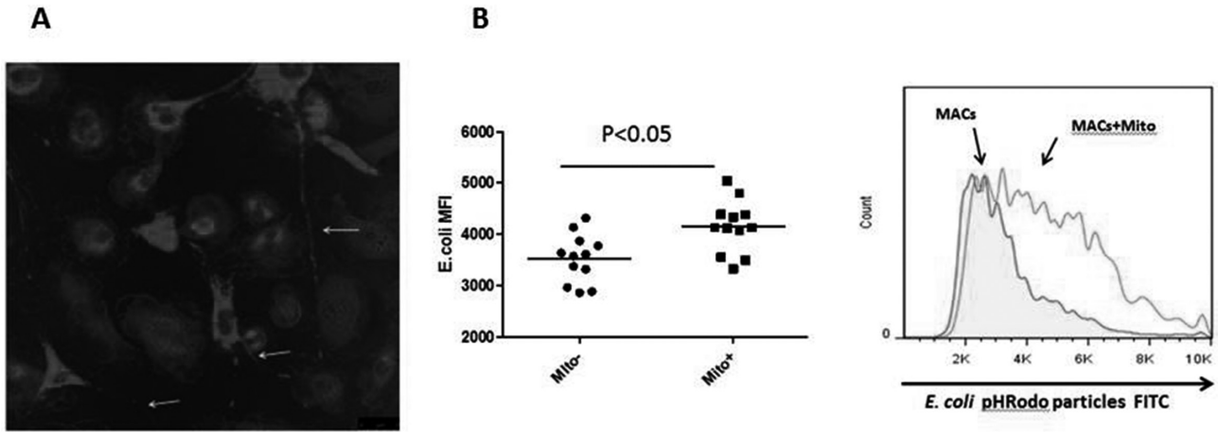

Abstract T3 Figure 1 Mitochondrial transfer from MSC to macrophages can enhance macrophage phagocytic activity *in vivo*. (A) MSC use tunnelling nano tubules (TNT) structures to transfer mitochondria (arrows). MSC were pre-stained with MitoTracker Red before co-culture with macrophages, 6 hr later sides were fixed and stained for(blue) to visualise macrophages. Almost allpositive cells demonstrate acquisition of red mitochondria from MSC. (B) In the *in vivo* model, MSC (MitoTracker)-treated mice BALF was taken and alveolar macrophages assessed for phagocytic activity using fluorescent E.coli bioparticles by flow cytometry. Macrophages that had acquired MSC mitochondria showed a higher phagocytic index in comparison to those without. This was assessed by an increase in Mean fluorescence Intensity (MFI). Some of the materials employed in this work were provided by the Texaz A&M Health Science Centre College of Medicine Institute for Regenerative Medicine at Scott and White through a grant from NCRR of the NIH, Grant #P40RR017447

Background ARDS remains a major cause of respiratory failure in critically ill patients with no specific therapy. MSC based cell therapy is a promising candidate and is being used in clinical trials for ARDS. However the mechanisms of MSC effect in lung injury are not very well understood. Islam *et al*., 2012 showed mitochondrial transfer from MSC to alveolar epithelial cells was protective in the mouse model of LPS induced pneumonia. Pathophysiology of ARDS is underpinned by dysregulated inflammation and pulmonary macrophages are key cellular mediators of the lung immune response. This study was undertaken to test if MSC could transfer their mitochondria to macrophages and to investigate the effects of MSC mitochondria transfer on macrophage function in the *in vivo* and *in vitro* models of ARDS.

Methods *In vivo* studies were performed using a mouse model of *E.coli* pneumonia induced ARDS. C56BL/6 mice were infected with *E.coli*, human bone marrow-derived MSC or PBS instilled intra-nasally 4 h after infection. For *in vitro* studies primary human monocyte-derived macrophages (MDM) were infected with *E.coli* and co-cultured with MSC in contact. MSC mitochondria were pre-stained with MitoTracker Red and MDM stained for CD45 expression. Double positive cells were visualised with confocal microscopy and quantified using flow cytometry. Phagocytosis was assessed using fluorescent *E.coli* bioparticles by flow cytometry.

Results When co-cultured with MSC >90% of MDMs acquired MitoRed fluorescence, indicating mitochondrial transfer from BM-MSC. Confocal imaging revealed presence of Mito-Red positive tunnelling nanotubules (TNTs) formed by MSC. *In vivo* >78% of $CD11c^{hi}$/F4–80+ alveolar macrophages retained MSC mitochondria at 24 hr post infection. Alveolar macrophages that had acquired MSC mitochondria had a significantly higher phagocytic index compared to those without suggesting enhancement of phagocytic capacity. Inhibition of TNT formation in MSC resulted in decreased transfer to macrophages by 60%, coupled with significant abrogation of MSC effect on macrophage phagocytosis *in vitro* and anti-microbial effect seen with MSC *in vivo*.

Conclusions Our findings suggest that anti-microbial activity of macrophages is enhanced at least partially by transfer of BM-MSC mitochondria through TNTs, representing an important mechanism of MSC effect in ARDS.

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REFERENCE

1 Islam MN, Das SR, Emin MT, *et al*. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat Med*. 2012;**18**:759–65

T4 OPTICALLY DETECTABLE ANTIMICROBIAL PEPTIDES ENABLE THE IMMEDIATE DETECTION OF BACTERIA AND FUNGI IN THE LUNG

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Introduction The immediate detection of pathogens in the lungs of patients with unexplained pulmonary opacities in the intensive care unit would represent a significant advance in their management. An optical imaging strategy, including the endobronchial administration of bacterial specific Smartprobes, would confer a number of advantages over conventional techniques such as bronchoalveolar lavage, principally real-time detection to immediately inform antimicrobial therapy. The aims of this study were to fluorescently label and iteratively develop anti-microbial peptides to image bacteria *in situ* in the lung using fibered confocal fluorescence microscopy (FCFM).

Methods Antimicrobial peptides (AMP) have been synthesised on a dendrimeric scaffold (AMP-1) and conjugated to an environmentally sensitive fluorophore called NBD, following the continuous development a linear counterpart. A further construct consists of an AMP with gram-selectivity conjugated to the NBD fluorophore (AMP-2). These are combined with FCFM to