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Ageing is associated with increased episodes of sepsis and poorer outcomes. Statins are associated with improved outcomes during infection. We aimed to characterise the impact of age and acute severe infection on key neutrophil functions, assess whether physiologically relevant doses of simvastatin altered neutrophil functions and if benefits were seen, when during a septic episode statins could be utilised.

Methods Neutrophils from healthy volunteers and patients with lower respiratory tract infections (LRTI), pneumonia and sepsis were assessed for migratory accuracy, phagocytosis and neutrophil extracellular trap production before and after in-vitro treatment with simvastatin. Healthy elderly volunteers received 80mg simvastatin or placebo in a cross over double-blind randomised controlled trial and neutrophil functions were assessed. Data presented is for migration.

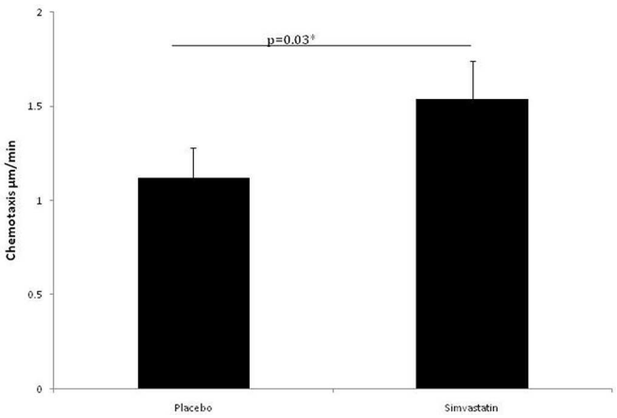
Results Neutrophils from healthy subjects (n = 70, aged 21–94) demonstrated preserved neutrophil movement) (R^2 = -0.48, p < 0.0001) towards chemoattractants (data shown for IL-8). Neutrophil chemotaxis decreased after 60yrs (comparing <35 to >65yrs: mean difference (MD)1.25μm/min, p = 0.02).

There was a progressive decrease in neutrophil chemotaxis in old patients with a LRTI, pneumonia and severe sepsis (MD compared to healthy control; LRTI (n = 10), 0.7μm/min, p = 0.04; pneumonia (n = 5), MD1.1μm/min, p = 0.02; sepsis (n = 22) MD1.6μm/min, p = 0.01) with "septic neutrophils" unable to mount targeted chemotaxis. Improvements to baseline were seen following recovery.

In-vitro treatment of neutrophils from healthy older people with simvastatin (1 M) restored "old" neutrophil chemotaxis to that of "young" cells. Simvastatin also restored neutrophil migration from old patients with LRTI and pneumonia to baseline but not in patients with sepsis.

Two weeks of oral simvastatin 80mg once daily therapy in healthy old volunteers (Age>65,n = 20) increased the accuracy of neutrophil migration (MD1.68μm/min, p = 0.02) replicating benchwork.

Conclusions "Elderly" neutrophil function is compromised in health, and deteriorates during infective episodes, in accordance with the severity of the insult. Migratory accuracy can be improved with simvastatin therapy however neutrophil function in sepsis patients cannot be modulated during short term *in-vitro* therapy. Our data suggest statin therapy might be a preventative or an early adjuvant intervention rather than a treatment in established sepsis. We are testing whether simvastatin 80mg for seven days modifies neutrophil responses in elderly patients with pneumonia and sepsis (SNOOPI Trial).



Abstract S96 Figure 1. Simavastatin 80mg once daily for 14 days improves directional migration (chemostaxis) of neutrophils from healthy elderly volunteers towards IL-8. ****Student's* **t-Test

S97 ALVEOLAR EPITHELIAL DNA DAMAGE, INFLAMMATION AND ALTERED AUTOPHAGY FOLLOWING EXPOSURE TO SILVER NANOPARTICLES IS EXACERBATED BY VIRAL LIGANDS *IN VITRO*

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Rationale Over the past decade there has been a rapid increase in the development and use of engineered nanoparticles. Silver (Ag) is the most commercialised nanomaterial in the world. Due to its antibacterial activity it is incorporated into a variety of consumer goods including silver nanoparticle sprays to treat pulmonary infection. However, little is known about silver nanoparticle (AgNPs) toxicity in the lung. We hypothesise that inhalation of AgNPs will trigger a pro-inflammatory response in the alveolar epithelium, which, in the presence of viral infection, will synergise with the innate immune response, leading to increased pulmonary epithelial inflammation, DNA damage and autophagy activation.

Methods Transformed human alveolar epithelial type-1-like cells (TT1) were exposed to AgNPs alone and in combination with Poly I:C, a synthetic analogue of double strand RNA that mimics viral infection. Oxidative stress level was measured by dihydroethidium staining. Levels of IL-6 and IL-8 were assessed by ELISA. DNA damage and autophagy marker LC3II/LC3I ratio were measured by western blot.

Results AgNPs induced oxidative stress in TT1 cells leading to enhanced inflammation, DNA double strand breakage and autophagy activation. The combination of Ag and Poly I:C potentiated IL-6 release (4-fold; p < 0.01) and DNA damage (3-fold; p < 0.01). Autophagy flux, activated by AgNPs alone, was slowed down by combined AgNPs and Poly I:C exposure.

Conclusion This study shows that Ag could induce oxidative stress in the lung, leading to a strong pro-inflammatory response and DNA damage, both potentiated by co-exposure to Poly I:C. Also, defective autophagy might result in certain human diseases such as cancer and neurodegenerative disease. This suggests that inhalation of AgNPs may have adverse health effects on the lung, that might be exacerbated by viral infection.

S98 VITAMIN D DEFICIENCY INCREASES BACTERIAL LOAD IN A MURINE MODEL OF SEPSIS-INDUCED LUNG INJURY

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Introduction We have previously shown that patients with Acute Lung Injury (ALI) are severely vitamin D deficient. Several studies have reported a high prevalence of vitamin D deficiency in critically ill patients with sepsis, associated with increased morbidity and mortality but whether this is cause or effect is unknown. Bacteraemic sepsis is more common in the winter

months when vitamin D levels are lowest. The purpose of this study was to investigate the local and systemic effects of vitamin D deficiency in a murine model of sepsis-induced lung injury where we can predictably time the initiating insult.

Methods We fed 8 wild-type C57Bl/6 mice a diet completely devoid of vitamin D for 6 weeks to induce severe vitamin D deficiency (9 nmol/l) and compared to 7 mice fed a vitamin D sufficient diet (42 nmol/l). Caecal ligation and puncture (CLP) was used to establish sepsis. Animals were culled 16h after CLP and blood, peritoneal lavage fluid (PLF) and bronchoalveloar lavage fluid (BALF) were collected. Cell infiltrates were assessed by flow cytometry. Fluid protein levels were measured and tissue protein permeability index (PPI) was calculated as the ratio between fluid and serum protein. Bacterial load was evaluated as colony-forming units (CFU) after 24h incubation on appropriate media.

Results Vitamin D deficient mice had increased bacterial load in BALF, blood and PLF compared to dietary sufficient mice. BALF protein permeability index was higher in deficient compared to sufficient mice but there was no difference in cell numbers recruited to the lung. PLF protein permeability index was also increased in the deficient group compared to sufficient mice with an associated significant increase in neutrophils recruited to the peritoneum. (See Table 1)

Conclusion Vitamin D deficiency significantly increases the bacterial load both systemically, locally and within the lung in a murine model of peritonitis. This is associated with an increase in tissue permeability locally and within the lung. These data support pre-existing vitamin D deficiency as a determinant of the severity of bacteraemic sepsis and may account for some of the seasonal variations observed in the incidence of sepsis.

Abstract S98 Table 1. Differences between dietary deficient and sufficient mice post CLP induced sepsis. Data is expressed as median values.

	Sufficient (n = 7)	Deficient (n = 8)	p-value
Bacteria (CFU x10^3)	0.51	2.51	0.038
BALF	0.28	66.1	0.019
Blood	8.22	336.6	0.005
PLF			
PPI (x1000)	1.82	3.30	0.0003
BALF	27.2	46.9	0.05
PLF			
Neutrophil Number	64.1	27.9	0.183
BALF	2.11	4.60	0.04
PLF (x10^6)			

S99 A FUNCTIONAL VARIANT OF ELAFIN WITH IMPROVED ANTI-INFLAMMATORY ACTIVITY

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Introduction and Objectives Elafin is a well-known serine protease inhibitor produced by epithelial and inflammatory cells with anti-inflammatory properties. Previous work has shown that unregulated protease activity can cause the proteolytic cleavage of elafin, therefore impairing the innate immune function of the protein. Consequently, the aim of this study was to generate a variant of elafin that would demonstrate increased protease resistance whilst retaining many of the beneficial characteristics of the parent molecule.

Methods Two elafin variants (GG-elafin and QQ-elafin) were recombinantly synthesised in a yeast-based expression system and subsequently tested for antiprotease, transglutaminase and protease susceptibility. In addition, the LPS neutralisation activity of the GG-variant was evaluated in *in vitro* based assays and an *in vivo* mouse model of LPS-induced acute lung inflammation.

Results GG- and QQ-elafin retained similar antiprotease and transglutaminase activity compared to wild-type elafin (WT-elafin). When incubated with diseased bronchoalveolar lavage fluid (BALF), the elafin variants displayed significantly enhanced resistance to degradation when compared to WT-elafin. Intriguingly, both variants, but particularly GG-elafin, demonstrated improved LPS neutralisation by inhibiting cytokine expression in monocytic cells. Moreover, the GG-elafin showed improved anti-inflammatory properties in a mouse model of LPS-induced acute lung inflammation with significantly decreased inflammatory cell counts, namely neutrophils ($p = 0.0362$). Furthermore, total BAL protein levels were significantly decreased ($p = 0.0336$) and a reduction in pro-inflammatory cytokine/chemokine levels was observed in mice treated with GG-elafin compared to those treated with WT-elafin.

Conclusions Site-specific mutants of elafin, particularly GG-elafin, showed increased functionality when compared to WT-elafin and may be of future therapeutic relevance in the treatment of lung diseases, particularly acute lung injury (ALI).

Mechanisms of cystic fibrosis

S100 NOVEL T HELPER CELL RESPONSES AGAINST PSEUDOMONAS AERUGINOSA IN HEALTHY INDIVIDUALS AND PATIENTS WITH CYSTIC FIBROSIS

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Introduction and Objectives *Pseudomonas aeruginosa* (PA) colonisation is a hallmark of cystic fibrosis (CF) resulting in damaging neutrophilic inflammation. Patients with CF produce anti-pseudomonal antibodies but the role of $CD4^+$ T cell responses to PA remains unclear. Novel T helper cell subsets, Th17 and Th22 cells, have important roles in host defense but may also enhance tissue damage. We aimed to define the antigen-specific memory T helper cell responses to *Pseudomonas aeruginosa* in healthy humans and patients with cystic fibrosis.

Methods $CD14^+$ monocytes and memory $CD4^+$ $CD45RO^+$ T cells were isolated from peripheral blood of CF patients with PA colonisation (n = 8) and healthy controls (n = 10). Monocyte-derived dendritic cells (DCs) were stimulated with live Pseudomonas strain PA103 and PA isolates derived from CF patients. Autologous T cells were co-cultured with activated DCs. The resultant T helper response was determined by measuring proliferation, immunoassay of cytokine output, and immunostaining of intracellular cytokines. Lavage samples from explanted CF lungs were assayed for IL-22 secretion.