S103 A DECREASE IN ELAFIN DUE TO PROTEOLYTIC CLEAVAGE MAY CONTRIBUTE TO ALVEOLAR INFLAMMATION IN PATIENTS WITH ALI

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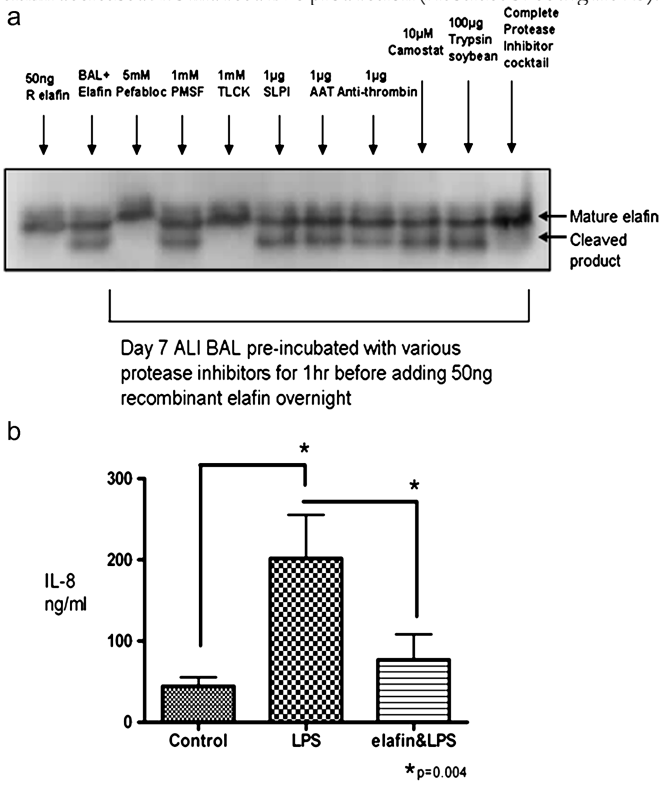
Introduction Unregulated protease activity is implicated in the pathogenesis of acute lung injury (ALI). Elafin is a potent serine protease inhibitor produced by epithelial and inflammatory cells with anti-inflammatory actions.

Aim To assess the temporal changes in elafin concentration in patients with ALI and test the hypothesis that a decrease in elafin levels due to proteolytic degradation may drive pulmonary inflammation.

Methods Patients with ALI within 48 h of onset of ALI (n=37), day 3 (n=19) and day 7 (n=9) as well as five healthy volunteers underwent bronchoalveolar lavage (BAL). Elafin was measured by ELISA. To determine whether elafin was susceptible to proteolytic cleavage, western blot analysis of recombinant elafin was incubated with BAL±protease inhibitors. Alveolar macrophages from healthy volunteers were isolated and pre-treated with 10 μg/ml elafin for 1 h, followed by 24 h stimulation with 100 ng/ml LPS. IL-8 was measured in supernatants by ELISA.

Results Elafin was significantly increased at the onset of ALI compared to healthy volunteers (39±5 ng/ml vs 0.5±0.1 ng/ml; p<0.0004). Elafin levels fell significantly by day seven compared to baseline (16±4 ng/ml vs 39±5 ng/ml; p=0.02). Incubation of exogenous elafin with day 7 ALI BAL revealed that elafin underwent proteolytic cleavage (Abstract S103 Figure 1A). In contrast, proteolytic cleavage was not observed following incubation of exogenous elafin with day 0 ALI or healthy volunteer BAL. Pre-incubation of Day 7 ALI BAL with TLCK and Pefabloc abrogated this degradation suggesting that a trypsin-like protease may be responsible for the cleavage of elafin (Abstract S103 Figure 1A). Pretreatment of alveolar macrophages with elafin decreased LPS induced IL-8 production (Abstract S103 Figure 1B).



Abstract S103 Figure 1

Conclusion Elafin concentrations fall in the pulmonary compartment over the course of ALI. This decrease was found to be due to proteolytic degradation. Furthermore elafin decreases LPS stimulated IL-8 release from alveolar macrophages. Together these data suggest loss of elafin in the alveolar airspace may result in dysregulated inflammation in ALI. Elafin augmentation may be a potential therapeutic strategy in ALI.

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S104 MONOCYTE INFLUX ACCOMPANIES THE EARLY NEUTROPHILIC INFLAMMATION SEEN IN BRONCHOALVEOLAR LAVAGE FLUID FOLLOWING LIPOPOLYSACCHARIDE INHALATION

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Introduction Acute lung injury (ALI) has a mortality rate of over 30%, with no proven pharmacological treatment. Inhalation of lipopolysaccharide (LPS) in healthy volunteers induces transient inflammation resembling that found in patients with ALI. Inhaled LPS causes neutrophilia that is detectable in bronchoalveolar lavage fluid (BALF) and blood, but its effect on BALF and blood monocyte populations is not well established.

Methods 12 healthy volunteers were recruited and randomly allocated to receive either 60 μg of inhaled LPS or saline (n=6 each arm). Clinical parameters, including temperature, and any reported symptoms were recorded. Full blood counts were taken at baseline and 2, 4, 6, 8 and 24 h post-inhalation. BAL was performed at 8 h. BALF cell populations were analysed morphologically using cytospins and cytometrically by flow cytometry after staining for cell surface markers (alveolar macrophages: CD163, CD206, CCR5; neutrophils/monocytes: HLA-DR, CD14, CD16).

Results 4 LPS volunteers developed pyrexia, two reported cough and one myalgia. The mean maximal increment in temperature was significantly greater in the LPS arm (p=0.047). Compared to saline inhalation, LPS caused a peripheral blood neutrophilia (p=0.006) that was evident from 4 h and greatest at 8 h. There was no significant difference in peripheral blood monocyte counts between treatment arms at any point measured (p=0.87). Although mean total alveolar macrophage numbers were similar between the two groups, their relative proportion in the LPS volunteers was significantly reduced due to the expansion in neutrophil and monocyte populations. Flow cytometry revealed a 24-fold expansion of the neutrophil population following LPS (in parallel with morphological data). These neutrophils were distinguishable by HLA-DR-/CD14-/CD16+ staining. There was a concomitant similar rise in the population of HLA-DR+/CD14+/CD16- 'classical' monocytes. Further analysis of these monocytes revealed that macrophage cell surface marker expression was absent.

Conclusion Morphological analysis of BAL fluid in previous LPS inhalation studies has consistently suggested that there is no change in the monocyte population. Using flow cytometry enables a more detailed analysis. This study is the first to clearly demonstrate that an early expansion in the monocyte population accompanies the neutrophil influx seen in BALF 8 h following inhalation of LPS.