Friend or foe? The dual role of neutrophils in lung injury and repair

Frances S Grudzinska, Elizabeth Sapey

Studies describing the role of neutrophils in acute lung injury (ALI) show divergent results. Some support a pivotal role for neutrophils in the development of lung injury, with improvements in outcomes following inhibition of neutrophil recruitment or their granular contents. Others describe poorer outcomes in patients with neutropenia and no improvements in clinical outcomes or inflammatory biomarkers following interventions which target neutrophils. This has led to uncertainty about whether neutrophils are a therapeutic target in this condition. The recent publication by Blázquez-Prieto et al provides some insight into why different studies may vary in outcome. It describes increased inflammation and a poorer recovery in a murine model where neutrophils are depleted 24 hours after a ventilator-induced lung injury (VILI), and links tissue repair to the sustained activity of neutrophil-derived matrix metalloproteinase (MMP)-9 in cellular, animal and human studies. These data suggest the timing of targeting neutrophils may be critical for improving outcomes in ALI. This editorial explores the evidence for this finding and discusses heterogeneous neutrophil populations in disease and the role of MMP-9 in tissue repair.

Our current understanding of the pathogenesis of ALI includes a paradox which incorporates the following four pieces of evidence.

First, in human and animal studies, neutrophil numbers in bronchoalveolar lavage fluid (BALF) correlate with ALI severity and are predictive of mortality.1 Furthermore, there is evidence of increased neutrophil protease (especially neutrophil elastase) and oxidant activity in ALI which correlate with the severity of the clinical syndrome. This suggests that neutrophils are centrally implicated in the onset and progression of ALI, where endothelial and epithelial injuries are associated with microvascular permeability, increased tissue oedema and an early accumulation of activated neutrophils to the lung.

Second, in animal models of ALI, reducing neutrophil accumulation to the lung (eg, by targeting CXCR2 or/and inhibiting the neutrophil respiratory burst or protease activity) are consistently associated with improved outcomes.3 Sivelestat, a small molecular weight inhibitor of neutrophil elastase, has been associated with a reduction in clinical features and improved survival in a number of animal models of ALI (eg, ref 4). This protective effect was enhanced when Sivelestat was given with edaravone (a free radical scavenger),3 targeting neutrophilic products by combining an antiprotease with an antioxidant. Together, these data suggest that neutrophils are injurious in ALI, and may form a therapeutic target to improve outcomes.

However, third, ALI outcomes are worse in patients with neutropenia, suggesting neutrophils are not needed for ALI onset and may be protective.

Fourth, interventional trials aimed at reducing neutrophil recruitment to the lungs or inhibiting neutrophilic products (which have been shown to be beneficial in animal models of ALI) have not, in the main, been efficacious in humans. For example, the neutrophil elastase inhibition in acute lung injury: the STRIVE study of Sivelestat in 492 mechanically ventilated adults with ALI showed no reduction in inflammation and no clinical benefit acutely and was associated with an increased 180-day mortality.6 Similarly, N-acetylcysteine given as an antioxidant has not been associated with a reduction in inflammation, oxidant burden or mortality.7 Together, these studies do not support the neutrophil being a therapeutic target in ALI.

This has led to a confusion of therapeutic strategies in ALI. There is considerable uncertainty as to whether the neutrophil is a friend or foe in the development of this condition. The current paper by Blázquez-Prieto et al,8 in a series of elegant experiments, provides mechanistic insight into this dilemma and suggests neutrophils might be both.

In previous work, using a murine model, the authors noted that acute VILI was associated with neutrophil accumulation to lung tissue. Survivors experienced a sustained rise in MMP-2 and MMP-9, with MMP-9 associated with a continuing inflammatory cell presence during lung repair but a reduction in other measured inflammatory mediators. Furthermore, a pan-MMP inhibitor or selective MMP-2 inhibitor delayed epithelial repair in a cellular wound model.9 These results support those from other studies10 11 which suggest that neutrophil accumulation and MMP release are important components of the reparative process.

The current paper builds on this finding by studying the effects of neutropenia when induced 24 hours following a VILI. Histological lung injury scores were significantly higher in the VILI and then neutrophil depleted mice at 48 hours’ recovery, as were the proinflammatory mediators tumour necrosis factor (TNF)α, interferon (IFN)γ and macrophage inflammatory protein-2, compared with the VILI and neutrophil replete group. Levels of MMP-2 and MMP-8 were equal in both groups, but there was a decrease in both pro MMP-9 and active MMP-9 in the neutropenic animals.

To assess the validity of this finding in humans, the authors then studied a small group of patients with acute respiratory distress syndrome (ARDS), with or without neutropenia. The four patients with neutropenia had received chemotherapy for haematological malignancies, had a median age of 54.4 years, were admitted with neutropenic sepsis and had a 50% survival rate. The four subjects without neutropenia were more diverse; three had sepsis and one had polytrauma, they were older (median age 79.5 years) and again had a 50% survival rate. Concordant with the murine models, BALF from patients with neutropenia showed higher levels of TNFα, IFNγ and interleukin-8, with no significant differences in MMP-8 and MMP-2, but lower concentrations of proactive and active MMP-9.

To assess the effects of MMP-9 in tissue repair, the immortalised bronchial epithelial cell line, BEAS-2B, was used in wound closure studies in the presence of BALF from the previously described patients with and without neutropenia, ventilated for ARDS. BALF from patients with neutropenia slowed wound recovery times, but this could be restored by the addition of MMP-9. The authors finally back-translated this into mice, demonstrating that inhaled exogenous (active) MMP-9 could improve tissue repair in their murine model of VILI and subsequent neutrophil depletion.
MMP-9 is a proteolytic enzyme which cleaves denatured collagens (gelatins) and type IV collagen present in basement membranes. It has long been linked with tissue repair, as MMP activity is associated with subsequent release of proangiogenic factors such as vascular endothelial growth factors and fibroblast growth factors.12 MMP-9 is secreted as a latent proenzyme that requires activation in the extracellular space, by cleavage of a cysteine and zinc interaction which exposes its catalytic site.

Activators of MMP-9 include all neutrophil-derived proteinases including neutrophil elastase (NE), leading to host damage. These proteinases activate MMP-9 (yellow circle). (C) In the initial phases of repair, neutrophils are cleared by airway macrophages (efferocytosis), expelled in sputum or leave lung tissue by reverse migration back into the circulation. (D) Lung repair is associated with further neutrophil recruitment to the airways, but these neutrophils include a higher proportion of the angiogenic neutrophil phenotype. (E) These neutrophils contribute to tissue repair by releasing high levels of MMP-9. IL, interleukin; MMP, matrix metalloproteinase.

Figure 1 The proposed two phases of neutrophil recruitment to the lung in acute lung injury. (A) Activated, proinflammatory neutrophils are recruited to lung tissue during acute lung injury by the presence of chemotactic gradients formed by the release of cytokines such as IL-8 (red circles) by endothelial and epithelial cells and resident immune cells. (B) These neutrophils phagocytose bacteria to clear infection, and also degranulate, releasing proteinases such as neutrophil elastase (NE), leading to host damage. These proteinases activate MMP-9 (yellow circle). (C) In the initial phases of repair, neutrophils are cleared by airway macrophages (efferocytosis), expelled in sputum or leave lung tissue by reverse migration back into the circulation. (D) Lung repair is associated with further neutrophil recruitment to the airways, but these neutrophils include a higher proportion of the angiogenic neutrophil phenotype. (E) These neutrophils contribute to tissue repair by releasing high levels of MMP-9. IL, interleukin; MMP, matrix metalloproteinase.
To cite Grudzinska FS, Sapey E. Thorax April 2018 Vol 73 No 4
Published Online First 29 January 2018

http://dx.doi.org/10.1136/thoraxjnl-2017-211253
doi:10.1136/thoraxjnl-2017-211253

REFERENCES


