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

Grudzinska, Frances S.; Sapey, Elizabeth

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# Antagonist then Protagonist? – the dual role of neutrophils in lung injury and repair

Dr Frances Grudzinska and Dr Elizabeth Sapey

Our current understanding of the pathogenesis of acute lung injury (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[1] and are predictive of mortality[2]. Furthermore, there is evidence of increased neutrophil proteinase (especially neutrophil elastase)[3] and oxidant[4] activity in ALI which correlates 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 injury is associated with microvascular permeability, increased tissue oedema[5] and an early accumulation of activated neutrophils to the lung[6].

Second, in animal models of ALI, reducing neutrophil accumulation to the lung (for example, by targeting CXCR2[7]) or/and inhibiting the neutrophil respiratory burst or proteinase activity are consistently associated with improved outcomes[8-10]. Sivelestat, a small molecular weight inhibitor of neutrophil elastase, has been associated with a reduction in the clinical features of ALI and improved survival in a number of animal models of ALI (for example, [11]). This protective effect was enhanced when Sivelestat was given with Edaravone (a free radical scavenger)[12], targeting neutrophilic products by combining an anti-proteinase with an anti-oxidant. Together, these data suggest that not only are neutrophils injurious in ALI, they may form a therapeutic target to improve outcomes.

However, third and unfailingly, ALI outcomes are worse in neutropenic patients[13,14] 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 STRIVE trial 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[15]. Similarly, N acetylcysteine given as an anti-oxidant either pre-emptively [16] or following the establishment of ALI [17] has not been associated with a reduction in

inflammation, oxidant burden or mortality. 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 an antagonist or protagonist in the development of this condition. The current paper by Blázquez-Prieto et al[18] , in a series of elegant experiments, provides mechanistic insight into this dilemma and suggests neutrophils might be both.

In previous work in a murine model, the authors noted that acute ventilator induced lung injury (VILI) was associated with neutrophil accumulation to lung tissue. Survivors experienced a sustained rise in matrix metalloproteinases (MMP)-2 and -9, with MMP-9 associated with a continuing presence of inflammatory cells 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[19]. Together, these results suggested that neutrophil accumulation and MMP-release were important components of the reparative process.

The current paper[18] builds on this finding, by studying the effects of neutropenia when induced twenty four hours following a VILI. Histological lung injury scores were significantly higher in the VILI and the neutrophil depleted mice at 48 hours recovery, as were the pro-inflammatory mediators TNFα, IFNγ and MIP-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 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 and without neutropenia with ARDS. The 4 neutropenic patients 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 (although time to death was unclear). The four subjects without neutropenia were more diverse; three had sepsis and one had poly-trauma, they were older (median age 79.5 years) and again had a 50% survival rate. Concordant with the murine models, BALF from neutropenic patients showed higher

levels of TNFα, IFNγ and CXCL8, with no significant differences in MMP-8 and MMP-2, but lower concentrations of pro- and active MMP-9.

To assess the effects of MMP-9 in tissue repair, the immortalised bronchial epithelial cell line, BEAS-2B, were used in wound closure studies in the presence of BALF from the previously described patients with and without neutropenia, ventilated for ARDS. BALF from neutropenic patients 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 associated with tissue repair, as MMP activity is associated with subsequent release of proangiogenic factors such as vascular endothelial growth factors and fibroblast growth factors[20]. The same mechanisms have also been associated with tumour angiogenesis and intravasation, so MMP-9 activity is not universally beneficial[20]. MMP-9 is secreted as a latent pro-enzyme that requires activation in the extracellular space, by cleavage of a cysteine and zinc interaction which exposes its catalytic site[21]. Activators of MMP-9 include all neutrophil derived proteinases[22] including neutrophil elastase[23], cathepsin G, proteinase 3, but also a number of other MMPs (for example, MMP-2[24] MMP-3[25]). MMP-9 is not expressed in healthy lungs, but is released under inflammatory conditions by macrophages, mast cells, fibroblasts and lymphocytes, however, in a major inflammatory event such as ALI, the predominant source is the neutrophil. There is thought to be a positive feedback loop between MMP-9 and neutrophil recruitment, as MMP-9 also enhances neutrophil migration into the respiratory tract in response to TLR-induced chemotactic factors[26] and by cleaving interleukin-8 to its more potent truncated form[27]. But what sort of neutrophils might MMP-9 activity attract?

There is growing recognition of the complexity of neutrophils. These cells have an adaptable life expectancy[28], can release a large array of products[29] and are more transcriptionally active than initially thought[30]. Furthermore, a number of neutrophil phenotypes have been identified in different experimental models, and these phenotypes

seem to display different functional characteristics. For example, neutrophils have been described as senescent[31], immunosuppressive[32] (thought to potentially contribute to the immunosuppression seen after sepsis[33]), reverse transmigrated[34], to name but a few. A neutrophil phenotype of potential relevance to the current study[18] is the so called "angiogenic neutrophil"; a subset which makes up 3% of neutrophils, identified by being $CD49d^{+}VEGFR1^{high}CXCR4^{high}$ and characterised by increased MMP-9 release.
The angiogenic neutrophil is found in hypoxic tissues (as seen during ALI) where it is hypothesized to help restore oxygenation through new vessel formation[35,36]. Placed in tertiary granules, proMMP-9 is released more readily, and at a lower activation status than contents of secondary or primary granules[37], and this might favour tissue repair after the cytokine storm of injury or infection has subsided.

It is possible that neutrophil recruitment to the lungs in ALI comes in two waves, the first being a more pro-inflammatory sub-population to clear infection or necrotic tissue and the second being a less inflammatory, MMP-9 producing sub-population, involved in tissue repair; and this second sub-population was depleted in the current study[18] (see figure 1).

Neutrophil phenotypes aside, there are many other reasons for the divergence of animal and cell-based experimental results and clinical observations which this paper cannot address. It is clear from neutropenic adults that ALI can occur without a functional neutrophil response, and most adults with acquired neutropenia who go on to develop ALI also have deficits in other immune cells, which might alter clinical outcomes.
ALI is most commonly seen in older adults with multi-morbidites[38]. Also, ALI in humans is often associated with an infective origin and sepsis[38] and both age and infections have been associated with reduced neutrophilic responses which might impede bacterial clearance and amplify tissue injury[33,39]. In contrast, most murine models do not include bacterial infection and most studies are performed in young adult mice, where immunosenescence (impairments in the immune response associated with age) cannot be studied.
Additionally, there are well documented differences in the biology of mice and men which might account for the disparities in murine and human studies of ALI (reviewed in [40]).
Furthermore, the doses and timings of investigative medicines have been different in mice and men. For example, in STRIVE, patients received a continuous effusion of

0.16mg/kg/hour of Sivelestat after the onset of established ALI[15], in murine studies the doses utilised tend to be much higher (for example, 3mg/kg/hour [11]) and dosing regimens started much earlier after the initial insult[41].

In summary, this paper highlights the potential of neutrophils to be involved in tissue repair (via MMP-9 activity) as well as tissue damage in ALI. The next challenge is to understand why and how neutrophils are able to develop these separate functions during ALI and then develop treatments that maintain the bacteriocidal functions of neutrophils while reducing host tissue damage or harnessing their potential for repair.

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