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 proteinase (especially neutrophil elastase) and oxidant[2] 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 proteinase 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),[5] targeting neutrophilic products by combining an antiproteinase 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 studies[10 11] which suggest that neutrophil accumulation and MMP release are important components of the reparative process.

The current paper[8] 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.

Institute of Inflammation and Ageing, Queen Elizabeth Hospital, University of Birmingham, Birmingham, UK

Correspondence to Dr Elizabeth Sapey, Institute of Inflammation and Ageing, Queen Elizabeth Hospital, University of Birmingham, Birmingham B15 2TT, UK; e.sapey@bham.ac.uk

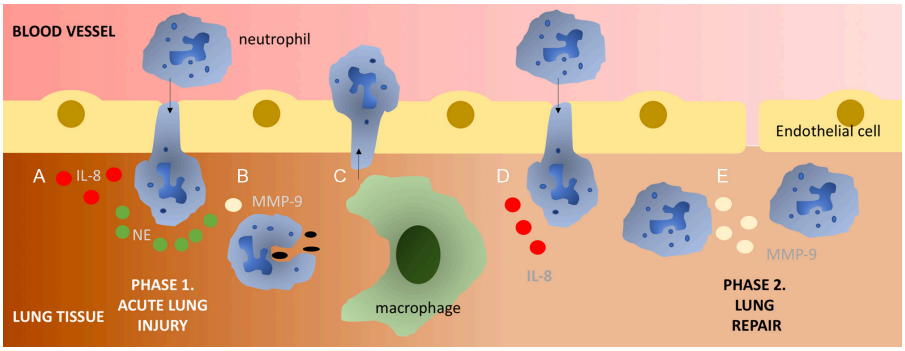

Figure 1 The proposed two phases of neutrophil recruitment to the lung in acute lung injury. (A) Activated, proinflammatory neutrophils are recruited to lung tissue during acute lung injury by the presence of chemotactic gradients formed by the release of cytokines such as IL-8 (red circles) by endothelial 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.

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 and proteinase 3,[13] and also a number of other MMPs. 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, with MMP-9 also enhancing neutrophil migration into the respiratory tract in response to toll-like receptor-induced chemotactic factors.[14] 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, can release a large array of products and are more transcriptionally active than initially thought. 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[15] and immunosuppressive[16] (thought to potentially contribute to the immunosuppression seen after sepsis[17]) although the physiological and pathological relevance of these phenotypes is as yet unclear. A neutrophil phenotype of potential relevance to the current study[8] 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.[18]

The angiogenic neutrophil is found in hypoxic tissues (as seen during ALI) where it is hypothesised to help restore oxygenation through new vessel formation.[18] Placed in tertiary granules, pro-MMP-9 is released more readily, and at a lower activation status than contents of secondary or primary granules,[19] and this might favour tissue repair after the cytokine storm of injury or infection has subsided.

A potential explanation for the divergent results in studies of neutrophils in ALI is that neutrophil recruitment to the lungs in ALI comes in two phases. First, a more proinflammatory subpopulation to clear infection or necrotic tissue. Second, a less inflammatory, MMP-9 producing subpopulation, involved in tissue repair (see figure 1). Potentially, it was the second subpopulation that was depleted in the current study, leading to delayed tissue repair and poorer outcomes.[8]

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, therefore neutrophils may not be necessary for the initiation of lung injury. The current paper[8] does not assess whether neutrophil depletion prior to VILI would still result in an inflammatory response and tissue injury consistent with ARDS using this model. Also, 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 multimorbidities with an infective origin and both age and infections have been associated with reduced neutrophilic responses which might impede bacterial clearance and amplify tissue injury.[17 20] 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. Furthermore, the doses and timings of investigative medicines have been different in murine and human studies. For example, in STRIVE, patients received a continuous effusion of 0.16 mg/kg/hour of Sivelestat after the onset of established ALI,[6] in murine studies the doses used tended to be much higher (eg, 3 mg/kg/hour) and dosing regimens started much earlier after the initial insult.[4] All these factors might contribute to the differing results seen in some human and animal models of ALI; however, it is the similarity of the human and murine results in the current study of ALI[8] which is of interest and may provide insight into the changing roles of the neutrophil following lung injury.

In summary, this paper suggests that neutrophils have a role in tissue repair in ALI, via MMP-9 activity. This supports the hypothesis that neutrophils have two functions in ALI, involved in tissue damage and then tissue repair but needs to be confirmed in further studies. The next challenge is to understand why and how neutrophils are able to develop these separate functions during ALI and then design treatments that maintain the bactericidal function of neutrophils while reducing host tissue damage and harnessing their potential for repair.

Contributors FSG cowrote the article and helped with figure preparation. ES cowrote and finalised the article and completed the figure preparation.

Competing interests ESG reports grants from Medical Research Council, grants from British Lung Foundation, grants from NIHR, grants from Alpha 1 Foundation, grants from Wellcome Trust, outside the submitted work.

Provenance and peer review Commissioned; externally peer reviewed.

To cite Grudzinska FS, Sapey E. *Thorax* 2018;**73**:305–307.

Published Online First 29 January 2018

Linked

► http://dx.doi.org/10.1136/thoraxjnl-2017-210105

Thorax 2018;**73**:305–307.
doi:10.1136/thoraxjnl-2017-211253

REFERENCES

1 Steinberg KP, Milberg JA, Martin TR, *et al*. Evolution of bronchoalveolar cell populations in the adult respiratory distress syndrome. *Am J Respir Crit Care Med* 1994;150:113–22.
2 Kooy NW, Royall JA, Ye YZ, *et al*. Evidence for in vivo peroxynitrite production in human acute lung injury. *Am J Respir Crit Care Med* 1995;151:1250–4.
3 Bai J, Tang L, Lomas-Neira J, *et al*. TAT-SNAP-23 treatment inhibits the priming of neutrophil functions contributing to shock and/or sepsis-induced extra-pulmonary acute lung injury. *Innate Immun* 2015;21:42–54.
4 Hagio T, Kishikawa K, Kawabata K, *et al*. Inhibition of neutrophil elastase reduces lung injury and bacterial count in hamsters. *Pulm Pharmacol Ther* 2008;21:884–91.
5 Yang T, Zhang J, Sun L, *et al*. Combined effects of a neutrophil elastase inhibitor (sivelestat sodium) and a free radical scavenger (edaravone) on lipopolysaccharide-induced acute lung injury in rats. *Inflamm Res* 2012;61:563–9.
6 Zeiher BG, Artigas A, Vincent JL, *et al*. Neutrophil elastase inhibition in acute lung injury: results of the STRIVE study. *Crit Care Med* 2004;32:1695–702.
7 Domenighetti G, Suter PM, Schaller MD, *et al*. Treatment with N-acetylcysteine during acute respiratory distress syndrome: a randomized, double-blind, placebo-controlled clinical study. *J Crit Care* 1997;12:177–82.
8 Blázquez-Prieto J, López-Alonso I, Amado-Rodríguez L, *et al*. Impaired lung repair during neutropenia can be reverted by matrix metalloproteinase-9. *Thorax* 2018;73:321–30.
9 González-López A, Astudillo A, García-Prieto E, *et al*. Inflammation and matrix remodeling during repair of ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol* 2011;301:L500–9.
10 Kawano T, Mori S, Cybulsky M, *et al*. Effect of granulocyte depletion in a ventilated surfactant-depleted lung. *J Appl Physiol* 1987;62:27–33.
11 Belperio JA, Keane MP, Burdick MD, *et al*. Critical role for CXCR2 and CXCR2 ligands during the pathogenesis of ventilator-induced lung injury. *J Clin Invest* 2002;110:1703–16.
12 Bekes EM, Schweighofer B, Kupriyanova TA, *et al*. Tumor-recruited neutrophils and neutrophil TIMP-free MMP-9 regulate coordinately the levels of tumor angiogenesis and efficiency of malignant cell intravasation. *Am J Pathol* 2011;179:1455–70.
13 Tschesche H, Zölzer V, Triebel S, *et al*. The human neutrophil lipocalin supports the allosteric activation of matrix metalloproteinases. *Eur J Biochem* 2001;268:1918–28.
14 Bradley LM, Douglass MF, Chatterjee D, *et al*. Matrix metalloprotease 9 mediates neutrophil migration into the airways in response to influenza virus-induced toll-like receptor signaling. *PLoS Pathog* 2012;8:e1002641.
15 Zhang D, Chen G, Manwani D, *et al*. Neutrophil ageing is regulated by the microbiome. *Nature* 2015;525:528–32.
16 Pillay J, Kamp VM, van Hoffen E, *et al*. A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. *J Clin Invest* 2012;122:327–36.
17 Sapey E, Patel JM, Greenwood HL, *et al*. Pulmonary infections in the elderly lead to impaired neutrophil targeting, which is improved by Simvastatin. *Am J Respir Crit Care Med* 2017;196:1325–36.
18 Christoffersson G, Vågesjö E, Vandooren J, *et al*. VEGF-A recruits a proangiogenic MMP-9-delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue. *Blood* 2012;120:4653–62.
19 Lacy P. Mechanisms of degranulation in neutrophils. *Allergy Asthma Clin Immunol* 2006;2:98–108.
20 Sapey E, Greenwood H, Walton G, *et al*. Phosphoinositide 3-kinase inhibition restores neutrophil accuracy in the elderly: toward targeted treatments for immunosenescence. *Blood* 2014;123:239–48.