Selective tumour necrosis factor receptor-1 inhibition in acute lung injury: a new hope or a false dawn?

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A complex myriad of aetiologies and pathologies coalesce within the clinical syndrome of acute respiratory distress syndrome (ARDS) often obfuscating our understanding of the disorder. Amidst the complexity, acute lung inflammation stands out as the fundamental unifying pathophysiology underlying ARDS. A better understanding of the mechanisms driving inflammation in the lung and the ensuing response from the innate immune system, endothelial and epithelial barriers are key to developing disease-modifying drugs in ARDS. Targeted inhibition of proinflammatory molecules such as tumour necrosis factor (TNF)-□ would seem a logical and fruitful line of investigation for disease-modifying intervention in ARDS.

As suggested by its name, TNF-α was first described as a mediator in abrogating malignant proliferation of cancerous cells.[1] Since then, TNF-α has also been identified as a key cytokine in modulating inflammation. TNF-α is mainly produced by macrophages and monocytes, but it is released by most cells, including endothelial cells, epithelial cells and most other leucocytes. A pleiotropic cytokine, TNF-α has a wide range of biological activities. TNF-α has been implicated as a chemoattractant for neutrophils and eosinophils, as a mediator of apoptosis and a major stimulant of other proinflammatory cytokine release.[2]

In ARDS, elevated levels of TNF-α are found in both plasma and bronchioalveolar lavage (BAL).[3 4] In the lungs, TNF-α expression directly leads to increased endothelial permeability[5 6] and downregulates epithelial sodium channel expression leading to impaired alveolar fluid clearance.[7] Aside from ARDS, TNF-α is also a critical mediator in other inflammatory conditions such as rheumatoid arthritis, psoriasis and Crohn's disease. Moreover, in these diseases, inhibitors of TNF-α are now in widespread therapeutic use, setting a precedent for their use in ARDS.

As a ligand, TNF-α binds to two members of TNF-receptor superfamily: TNF-receptor 1 (TNFR1) and TNF-receptor 2 (TNFR2). TNFR1 is a transmembrane receptor with a cytoplasmic death domain. On activation, TNFR1 can use intracellular TNRF-associated factors to activate proinflammatory downstream signalling via ubiquitin-mediated activation of nuclear factor □-B.[8]

Alternatively, its activation can also induce apoptosis via caspase activation.[9] TNFR1 is universally expressed and, in most cells, is the primary receptor that binds to TNF-α.

In contrast, TNFR2 expression is more tightly regulated and is expressed predominantly in immune cells but may occasionally be expressed on endothelial cells, among others. In comparison with TNFR1, the role and downstream signalling of TNFR2 are less well understood. There is considerable crosstalk between the two receptors, with TNFR2 playing a vital role in switching TNFR1 activity from inflammatory to apoptotic.[10]

There are also numerous TNFR2-activated pathways that are independent of TNFR1, and activation of the two receptors can have opposing activity. For example, in a murine model of sepsis, Ebach and colleagues[11] found that in comparison with wild-type mice, TNFR2-knockout mice had more severe sepsis. Conversely, TNFR1-knockout mice were protected from the same septic insult. Similarly, in another murine knockout model of TNFR1 and TNFR2, investigators found conflicting roles of the receptors in the pathogenesis of ventilator-induced lung injury, with the absence of TNFR1, but not TNFR2, conferring protection.[12] Thus, theoretically, selective inhibition of TNFR1 in acute inflammation could result in ongoing advantageous effects of TNFR2 activation while simultaneously inhibiting the deleterious effects of TNFR1 activation.

The cleaved ectodomain of TNFR1 and TNFR2, known as soluble TNFR1 (sTNFR1) and soluble TNFR2, respectively, were observed to be elevated in ARDS with an associated increase in mortality.[13] Shed TNF receptors are known to bind to TNF with similar affinity as the membrane bound receptors. In acute inflammation, soluble TNFRs are thought to protect against excessive circulating TNF-α.[14] In contrast, in lower concentrations, soluble TNFRs can have a stabilising effect on TNF-α, thereby preserving and prolonging its activity.[15] Trials of soluble TNFR-based therapies in sepsis have been disappointing, highlighting our incomplete understanding of the biology of soluble receptors in acute inflammation.[16]

Proudfoot and colleagues[17] present a study where they used a selective antibody (GSK1995057) against TNFR1 to demonstrate attenuated inflammation in lipopolysaccharide (LPS)-induced lung injury. GSK1995057 is a small antibody (13 kDa) that has high affinity and selectivity for TNFR1 and on binding to the receptor acts as a competitive inhibitor of TNF-α. This study is an excellent example of translational research that uses multidimensional experiments across several species for hypothesis testing.

First, in human pulmonary microvascular endothelial cells cocultured with neutrophils, pretreatment with GSK1995057 reduced neutrophil migration and proinflammatory cytokine expression and decreased endothelial permeability, following LPS injury. Next, in a primate (cytologous monkey) model, pretreatment with nebulised GSK1995057 attenuated the inflammatory response following treatment with inhaled LPS as evidenced by lower neutrophil count and lower proinflammatory biomarker levels in bronchoalveolar lavage fluid (BALF). Finally, in a double-blinded trial in LPS-induced subclinical lung injury in healthy human male volunteers, pretreatment with nebulised GSK1995057 reduced BALF levels of interleukin (IL)-1□, IL-6, IL-8, macrophage inflammatory protein-1(MIP1)-α, MIP1-□, Von Wilibrand factor and monocyte chemoattractant protein-1 (MCP-1). These data strongly suggest that pretreatment with GSK1995057 was effective in attenuating pulmonary inflammatory response following LPS injury. In addition, although in a small population of healthy subjects, the drug was well tolerated.

The study has several strengths. The in vitro experiments suggest selective blockade of TNFR1 interferes with

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neutrophil–endothelial crosstalk, reducing endothelial permeability and indicating a probable mechanism by which the antibody is protective in the human LPS-induced lung injury model. The authors must also be congratulated on successfully demonstrating the feasibility and the scope of applicability of their human model of subclinical acute lung injury. The use of such models will undoubtedly increase in prominence, particularly as we look to successfully translate the plethora of animal studies of lung injury to human subjects. The direct delivery of the drug via nebulisation is a novel approach to TNFR1 blockade and is one of the first examples of direct antibody delivery in human lung injury models. Although drug delivery to the lungs and systemic compartment is clearly demonstrated, nebulised therapy in severe ARDS may have limitations. Nebulised drug delivery to severely injured areas of the lung may be inadequate or absent as ventilation in these alveolar units approaches zero. Nonetheless, targeted organ-specific therapeutics that circumnavigate the need for systemic administration are highly desirable in the milieu of critical illness.

There are some limitations. All three experimental designs were limited to pretreatment with the antibody. In addition, the non-human primate and the human models lacked direct evidence of endothelial and epithelial damage. While as a proof of concept the presented approach is sufficient, it limits generalisability and immediate clinical applicability. The models also only included sterile inflammation, and the effect of the antibody in the setting of infection therefore cannot be inferred. Finally, in theory, a great strength of this antibody is its selectivity for TNFR1 blockade. In this study, however, blockade of TNFR2 was not measured and was realistically beyond the scope of such a study. Future studies are needed to clearly demonstrate the selectivity of TNFR1 inhibition and any benefits conferred by ongoing TNFR2 activity. Currently, the observed benefits of GSK1995057 are indistinguishable from other non-selective TNF-α inhibitors.

The findings of this study are promising. However, this is not the first example of TNF-α inhibition in critical care. TNF-α monoclonal antibodies have been used in randomised controlled trials in sepsis and septic shock without any observed benefits in outcome.[18] Other anti-inflammatory strategies such as 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors (statins)[19 20] and corticosteroids[21] in ARDS have similarly shown promise in preclinical studies but not in randomised controlled trials. Negative results in these studies serve as a cautionary note. Moreover, these studies serve as an opportunity to critically explore the reasons for the near ubiquitous failure of anti-inflammatory interventions in ARDS.

Explanations for the failure of anti-inflammatory and other pharmacological agents in ARDS clinical trials are likely multifactorial. Patient heterogeneity, however, is increasingly being recognised as a central problem. The complexity of ARDS conferred by heterogeneity both in terms of underlying biology, and longitudinal variation of disease progression at presentation, makes our current approach to biological interventions too imprecise. We know that inflammation is a highly conserved, coordinated and essential response to noxious and infectious stimuli. Dysregulated or inappropriate inflammatory responses can, however, lead to irreversible damage to the epithelial and endothelial barriers leading to morbidity. Determining the appropriateness and timing of anti-inflammatory interventions, therefore, becomes a major challenge for investigators. Any clinical trial that tests novel anti-inflammatory biotherapeutics in ARDS need to factor in these inherent variances. Failure to do so will likely consign the intervention to the profusion of promising yet clinically unrealised therapies in ARDS.

Using the study by Proudfoot and colleagues as an illustration,[17] in their model of sterile inflammation, inhibition of neutrophil chemotaxis may confer benefit. Yet, in bacterial-mediated acute lung injury, the same intervention may prove catastrophic. The undesired and devastating consequences of poor neutrophil migration are neatly demonstrated in patients with leucocyte adhesion deficiency who develop recurrent and severe bacterial infections.

As a therapeutic agent, GSK1995057 offers precision in terms of receptor selectivity, and this selectivity may enhance its chances of success in instances where non-selective anti-TNF-α agents have failed. In order to be successfully studied in ARDS, however, precision in patient selection will also be essential. To that end, recent studies have sought to identify biologically homogeneous subgroups in ARDS. In one such study, investigators identified a 'hyper-inflammatory' phenotype characterised by increased levels of plasma IL-8, IL-6, sTNFR1 and angiopoietin-2 (ANG-2).[22] Patients in the 'hyper-inflammatory' subphenotype had a higher incidence of shock with increased vasopressor use and significantly worse outcome. In theory, testing the efficacy of anti-inflammatory therapies in this phenotype may be more likely to yield positive findings, and such strategies should inform future trials.

To summarise, in this promising study, Proudfoot and colleagues present the novel concept of direct delivery of TNFR1 antibody to the lung, successfully inhibiting the inflammatory response in LPS-induced lung injury in both non-human primate and human studies. In the correct setting, this drug may prove to be a useful intervention and provides an exciting new avenue of investigation in the fight against ARDS.

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