Understanding the impact of the lung microenvironment to enhance the therapeutic potential of mesenchymal stromal cells for acute respiratory distress syndrome

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An enhanced understanding of the impact of the lung microenvironment on exogenously administered mesenchymal stromal cells has the potential to enhance their therapeutic potential for ARDS https://bit.ly/2S2fQ1I

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Acute respiratory distress syndrome (ARDS) is a clinical syndrome of severe acute hypoxaemic respiratory failure with clinical features including reduced lung compliance and permeability-induced pulmonary oedema, which can frequently progress to multiple organ failure [1, 2]. ARDS occurs in 10% of all critically patients in ICU and nearly one quarter of all mechanically ventilated patients [3]. Common underlying causes of ARDS include bacterial or viral pneumonia, sepsis, pulmonary aspiration and trauma [4]. The burden of ARDS is substantial, with hospital mortality rates varying, depending on ARDS severity, from 30–45% of affected patients [5]. Of further concern, ARDS survivors are often left with debilitating long-term sequelae which reduces their quality of life.

A dysregulated immune response is central to the pathogenesis of ARDS. Classically, there is activation of the immune system in response to a pulmonary or extrapulmonary inciting event, which leads to generalised inflammation of the lung, with invasion of neutrophils and macrophages into the alveolar space, the production of pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1β, IL-8 and tumour necrosis factor (TNF)-α, activation of the clotting cascade and production of reactive oxygen species [6]. This inflammatory microenvironment in the lung leads to injury, with fibrin deposition and hyaline membrane formation, necrosis and loss of type 1 and 2 pneumocytes, and damage to the capillary endothelium leading to loss of the permeability barrier and lung oedema, and diffuse alveolar damage [7].

Despite decades of research, there are no direct therapies for ARDS. Management remains supportive, with a focus on protective mechanical ventilation and fluid-restrictive strategies to minimise iatrogenic harm while treating underlying causes, such as infection [8]. Consequently, there is a pressing need to discover innovative therapies for ARDS. Attention has turned to cell-based therapies for ARDS, particularly mesenchymal stromal cells (MSCs), which possess multiple potentially relevant properties. These include immune-modulating effects [9], direct and indirect anti-microbial effects [10], enhancement of alveolar fluid clearance [11], and pro-resolution effects that restore alveolar function and pulmonary capillary permeability barrier function [12]. Other properties that make them attractive, if proven therapeutically effective, include their relative ease of isolation and availability from several tissue sources [13], including bone marrow, umbilical cord and adipose tissue, and their relatively low immunogenicity, which together facilitate their use as an allogenic therapy, which could be available “off the shelf”, in contrast to many other potential cell therapies [11, 14].

MSCs demonstrate therapeutic promise in relevant preclinical ARDS models and the mechanisms of action of MSCs are increasingly well understood. Studies show that administration of MSCs to healthy volunteers

appears safe with no adverse effects reported in relation to any inflammatory response [15]. Recent results have demonstrated that infusion of a high dose of MSCs (1.3×10^8) to healthy volunteers was well-tolerated with an anti-inflammatory effect observed 6 months after infusion [16]. Paralleling this, phase I and early phase II clinical trials accumulate attesting to the safety and tolerability of these cells. While these are important advances, clear evidence of therapeutic promise is less evident and, generally, there is a lack of strong biological signals that would signal therapeutic promise. The significant challenges to translation of cell therapies to clinical testing may partly explain this, ranging from practical scale-up challenges relating to cell dose manufacture and storage, inter-study variations in MSC dosage, source and treatment regimens, to the biological heterogeneity within ARDS populations. Consequently, it is important to continue to search for strategies that might enhance MSC therapeutic potential. One approach in this regard is to better understand the interaction between the MSC and the host. While it has long been recognised that MSCs are responsive to the host environment [17], the precise inter-play between the MSC and the lung microenvironment in health and disease, and how this might impact MSC function, remains poorly understood. A better understanding of this interaction may allow us to develop enhanced MSC therapeutics for testing in ARDS.

In this issue of *the European Respiratory Journal*, Rolandsson Enes *et al.* [18] present a paper that considerably advances our knowledge in this area, by examining the effects of bronchoalveolar lavage (BAL) fluid samples from healthy and ARDS patients on important aspects of MSC function and response. They found that the exposure to BAL fluid from healthy volunteers produced an increase in MSC pro-inflammatory cytokine gene expression, activation of coagulant pathways, and apoptotic pathways mediators such as the FAS ligand. On the other hand, BAL fluid taken from patients with ARDS did not produce this pro-inflammatory increase but stimulated neutrophil trafficking-related genes, such as CXCL1 and CXCL2. They then investigated the effect of this BAL fluid on MSC expression of surface markers related to recognition by the host immune system. The potential for MSCs to evade detection by the host immune response is due, at least in part, to their low expression of HLA-1 and no expression of HLA-2, and this facilitates allogeneic therapy. MSCs exposed to BAL fluid from healthy volunteers increased their expression of genes related to HLA-1 and HLA-2 surface proteins. In contrast, exposure to BAL fluid from ARDS patients led to reduced MSC expression of the HLA-1 and HLA-2 genes. This differential response raises the possibility that an inflammatory microenvironment might extend the presence of the MSC in the lung by reducing MSC expression of immunogenic proteins and apoptotic signalling, giving the cells more time to exert any therapeutic effect. On the other hand, MSC exposed to a healthy lung environment might become more visible to the immune system, possibly enhancing the clearance of these cells. Interestingly, a recent preclinical study of retention of administered human MSCs within the rodent lung found that MSCs were cleared from healthy lungs within 24 h but were retained for longer periods in the presence of pulmonary infection [19], supporting this concept.

These findings offer novel insights into the interaction of MSCs with the lung environment. They complement a growing understanding of the impact of the lung microenvironment on MSC effects from other recent studies. Islam *et al.* [20] found that different preclinical lung injury models produced distinct proteomic profiles, and that this appeared to modulate the MSC effects seen. Specifically, MSCs exerted effects that ranged from beneficial in a model of high stretch ventilation to deleterious in models of acid-primed lung injury. The lung environment where MSCs worsened injury were characterised by high levels of IL-6 and fibronectin, along with low antioxidant capacity. Of clinical relevance, these distinct proteomic profiles were detected in clinical samples from patients with ARDS. Of therapeutic relevance, these detrimental effects of MSCs could be abrogated by restoring antioxidant capacity in the lung microenvironment, or by enhancing MSC expression of IL-10. Similar findings have also been reported for other lung conditions, such as cystic fibrosis [21], suggesting this interaction between the MSC and lung microenvironment is of broad relevance to lung diseases and requires further study as a priority.

These insights will inform the development of strategies to favourably modulate the interaction between the MSC and the lung microenvironment to enhance beneficial effects and/or reduce the potential for adverse effects, such as fibrosis. Many strategies to “precondition” MSCs involve *in vitro* exposure to elements that replicate the inflammatory microenvironment. This strategy of pre-conditioning (also known as pre-activating, or licencing) MSCs has been a topic of much focus in the past few years. Prior exposure of MSCs to pro-inflammatory cytokine mixtures (*e.g.* cytomix) has been reported to produce an anti-inflammatory MSC phenotype [22]. This strategy enhances MSC-induced resolution of ventilation-induced lung injury in a rodent model [23]. Similarly, prior incubation of MSCs with interferon (IFN)-γ [24] and poly I:C [25] and environmental “stressors”, such as starvation [26], cell stretch [27] and different growth substrates [28], have been used to enhance MSCs effects in relevant preclinical models. We have yet to see the effects of this technique in humans, but clinical trials are in progress testing use of IFN-γ-primed MSCs for the treatment of acute graft *versus* host disease (NCT04328714).

In conclusion, the findings of Rolandsson Enes *et al.* [18] support a growing understanding of the impact of the lung microenvironment on the effect profile of MSCs. While important knowledge gaps remain, such as the optimal MSC source, the most effective pre-activation strategy to use, whether to use of MSC cell products rather than whole cell administration, and the exact effects of these cells on the immune system in health and disease, these advances offer the potential that we may be able to favourably modify the risk/benefit profile of MSC therapies for ARDS. By harnessing these insights, we can ultimately develop MSC therapeutics that are more likely to be found effective in subsequent clinical trials.

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