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ORIGINAL PRE-CLINICAL SCIENCE

Intratracheal instillation of alveolar type II cells enhances recovery from acute lung injury in rats

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KEYWORDS:
acute lung injury;
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macrophages

BACKGROUND: Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are characterized by excess production of inflammatory factors. Alveolar type II (ATII) cells help repair damaged lung tissue, rapidly proliferating and differentiating into alveolar type I cells after epithelial cell injury. In ALI, the lack of viable ATII favors progression to more severe lung injury. ATII cells regulate the immune response by synthesizing surfactant and other anti-inflammatory proteins and lipids. Cross-talk between ATII and other cells such as macrophages may also be part of the ATII function. The aim of this study was to test the anti-inflammatory and reparative effects of ATII cells in an experimental model of ALI.

METHODS: In this study ATII cells (2.5×10^6 cells/animal) were intratracheally instilled in rats with HCl and lipopolysaccharide (LPS)-induced ALI and in healthy animals to check for side effects. The specific effect of ATII cells was compared with fibroblast transplantation.

RESULTS: ATII cell transplantation promoted recovery of lung function, decrease mortality and lung inflammation of the animals with ALI. The primary mechanisms for benefit were paracrine effects of prostaglandin E_2 (PGE_2) and surfactant protein A (SPA) released from ATII cells that modulate alveolar macrophages to an anti-inflammatory phenotype. To our knowledge, these data are the first to provide evidence that ATII cells secrete PGE_2 and SPA, reducing pro-inflammatory macrophage activation and ALI.

CONCLUSION: ATII cells and their secreted molecules have shown an ability to resolve ALI, thereby highlighting a potential novel therapeutic target.

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Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are characterized by excess production of inflammatory factors in lung tissue, followed by non-cardiogenic dyspnea, severe hypoxemia and pulmonary

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edema. Despite significant advances in our understanding and management of patients with ARDS, morbidity and mortality associated with ARDS remain high, with around 40% of patients developing critical illness.[1–4] The low tidal volume ventilation, neuromuscular blockade and prone positioning are the only strategies that have reduced mortality.[3–5]

Decades of research have failed to find effective therapies that reduce mortality in established ARDS, and pre-clinical studies suggest that therapies effective in preventing lung injury when administered before an insult are less effective or ineffective after lung injury is well established.[6,7] New pharmacologic agents have shown promising results in pre-clinical studies,[8–10] yielding valuable insights into the mechanisms responsible for the pathogenesis and resolution of lung injury.[11] However, clinical studies have failed to extend these results to humans,[12–14] and no pharmacologic treatment for ARDS has been effective to date.

More recently, cell-based therapies have started to emerge, with a focus on improving the function of the alveolar epithelial barrier. In pre-clinical studies, mesenchymal stem (stromal) cells (MSCs) have reduced ALI, raising hope that MSCs may enhance repair of damaged lung tissue.[15]

In the alveoli, alveolar type II (ATII) cells are classically considered to be progenitor cells of the alveolar epithelium because they are also reparative in nature, rapidly proliferating and differentiating into alveolar type I cells after epithelial cell injury.[16–18] In ALI/ARDS, both type II and type I cells are damaged, and the lack of viable ATII cells compromises the reparative mechanism, favoring the development and progression of ALI.[19] When the massive inflammation cannot be controlled (in about 50% of cases), the lung damage is repaired in part by fibroblasts in a late phase. Transplanting ATII cells has proven effective in treating pulmonary fibrosis in pre-clinical studies and in 1 clinical study.[20–22]

ATII have the ability to synthesize surfactant and other proteins and lipids that have anti-inflammatory properties and anti-microbial properties.[23,24] We hypothesize that ATII cells may have beneficial properties in ALI that have not been well identified. To test this possibility, we have administered ATII cells into the distal airspaces of the acute injured rat lung to determine whether the ATII cells would decrease lung injury and, if so, by which mechanisms. We focused our studies on the possible modulating effect that ATII cells have on alveolar macrophages (AMs).

Macrophages exhibit remarkable plasticity and can change their activation in response to environmental signals. Macrophages are termed M1 when secreting pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), inducible nitric oxide synthase (iNOS), interleukin-1beta (IL-1β) and IL-6, or M2 when presenting anti-inflammatory properties, and promote cell survival, proliferation, and tissue repair. During the initial stages of the inflammatory response in ALI/ARDS, damaged and apoptotic alveolar cells release vesicles that activate the M1 phenotype; when the reparative process starts, the macrophages switch their activation to the M2 phenotype, characterized by up-regulation expression of the mannose receptor, arginase I, transforming growth factor-beta (TGF-β) and IL-10.[25–28] Thus, the switching capacity of macrophages confers a pivotal role to these cells in the evolution and resolution of ALI/ARDS.

To test the anti-inflammatory and reparative effects of ATII cells after ALI, we transplanted isolated ATII cells into the tracheas of rats with ALI.[29] We hypothesized that treatment with ATII cells would reduce the early inflammatory and physiologic changes in ALI. We aimed to determine whether the proteins and lipids released by ATII cells could activate the anti-inflammatory phenotype of macrophages.

Methods

Experimental design

A power analysis using the G*Power computer program (Düsseldorf, Germany) previously to start the experiment indicated that a total sample of 48 animals (8 animals per group) would be needed to detect large effects (0.5) with 80% power using an analysis of variance (ANOVA) between factors with $\alpha = 0.05$ by the end of the experiment. Taking into account the mortality rate in some groups, we decided to include additional animals (24 per group) before the end of the experiment (72 hours). We used 12 animals for histology studies and 12 animals for bronchoalveolar lavage (BAL), macrophage activation and cytokine lung measures. Each figure legend indicates the the number of animals included in each analysis.

Animals

Male Sprague-Dawley rats (Charles River, France), weighing 200 to 225 g at the beginning of the experiment, were used in accordance with European Community Directive 86/609/EEC and Spanish guidelines for experimental animals. This study was approved by the animal experimentation ethics committee of the University of Barcelona.

ALI model

ALI was induced in rats as previously described.[29] Detailed methods are presented in the Supplementary Material available online at www.jhltonline.org/.

Isolation, purification and characterization of ATII cells and fibroblasts

Fresh ATII cells were isolated from healthy donor animals. The protocol for purification has been described elsewhere.[20] Details are presented in the Supplementary Material online.

Experimental groups

The animals were randomly distributed into 6 experimental groups as follows:

- Control: saline intratracheal (IT) instillation at 0 hour and at 2 hours.