Mechanisms of alveolar epithelial repair in acute lung injury – a translational approach

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Summary

In patients with acute lung injury (ALI) or acute respiratory distress syndrome (ARDS), extensive damage to the alveolar epithelial and endothelial barrier is observed, resulting in the influx of protein-rich oedema fluid into the air spaces. Efficient alveolar epithelial repair is crucial to ALI/ARDS patients’ recovery. Future therapeutic strategies may therefore include acceleration of the epithelial repair process in the injured lung. However, a better understanding of the cellular and molecular mechanisms that promote alveolar epithelial repair is needed if novel therapeutic strategies are to be developed. Pulmonary oedema fluid from patients with ALI/ARDS and from patients with hydrostatic oedema as control was obtained, and the effect on alveolar epithelial repair in vitro using our alveolar epithelial wound repair bioassay was studied. In contrast to the initial hypothesis, pulmonary oedema fluid from ALI/ARDS patients increased alveolar epithelial repair by an interleukin-1β (IL-1β)-dependent mechanism, demonstrating a novel, possibly beneficial role for IL-1β in patients with ALI/ARDS. Further studies using primary alveolar epithelial cells from rats revealed that IL-1β induced alveolar epithelial repair by an epidermal growth factor (EGF)/transforming growth factor-α (TGF-α)-dependent pathway. Besides EGF and TGF-α, keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF) – both present in pulmonary oedema fluid obtained from patients with ALI/ARDS – stimulate alveolar epithelial repair in vitro. Further experimental and clinical studies will show whether acceleration of alveolar epithelial repair by modulating cytokines and growth factors in the injured lung represents a promising new therapeutic strategy in patients with ALI/ARDS.

Key words: epithelial repair; acute lung injury; ARDS

Introduction

The clinical course of patients with acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) is variable and influenced by different factors. One of the most important mechanisms that determines the severity of lung injury is the magnitude of injury to the alveolar epithelial barrier [1]. The possibility of repairing the epithelial injury at an early stage is a major determinant of recovery. Specific treatments to accelerate alveolar epithelial repair do not exist, although progress in studies with experimental models of ALI suggests that specific treatment may be possible in the future [2]. Most of the treatment modalities tested recently were based on diminution of the inflammatory response in the lung in order to minimise the initial injury. However, an alternative therapeutic approach is to accelerate the repair process in the alveolar epithelium in the early stages of ALI/ARDS, to enhance the resolution of pulmonary oedema and improve outcomes in these patients. Little is known about the cellular and molecular mechanisms of alveolar epithelial repair in ALI/ARDS. In particular, soluble mediators which play a key role in alveolar epithelial repair by modulating cytokines and growth factors in the injured lung represent a promising new therapeutic strategy in patients with ALI/ARDS.
Injury to the alveolar epithelium in acute lung injury

The normal alveolar barrier is composed of three different structures: (1) the capillary endothelium, (2) the interstitial space including the basement membrane and the extracellular matrix, and (3) the alveolar epithelium. The alveolar epithelium consists of alveolar type I and alveolar type II cells. The flat alveolar type I cells line more than 90% of the alveolar surface area. The attenuated cytoplasm provides for close approximation of the alveolar lumen and the bloodstream, optimising the exchange of respiratory gases. The cuboidal alveolar type II cells are multifunctional cells. They produce surfactant, are important for active alveolar liquid clearance, and represent the progenitor cells which regenerate the alveolar epithelium after injury [6]. Under normal conditions the epithelial barrier is much less permeable than the endothelial barrier and prevents cells and plasma from flooding the air spaces, thereby maintaining normal gas exchange [7]. Several studies have demonstrated the critical importance of the alveolar epithelium in the pathogenesis of and recovery from severe ALI/ARDS. Efficient alveolar epithelial repair is therefore crucial for ALI/ARDS patients’ recovery.

Pathology of ALI/ARDS

Diffuse alveolar damage is a hallmark of patients with ALI/ARDS, whether the latter is caused directly (e.g. pneumonia or acid aspiration) or indirectly (e.g. sepsis or severe trauma). In histological sections from patients dying of ALI/ARDS, the first lesions appear to be interstitial oedema, followed by severe alveolar epithelial damage [8]. The alveolar epithelium usually exhibits extensive necrosis of alveolar type I cells, leaving a denuded, but mainly intact, basement membrane with overlying hyaline membranes. The type I alveolar epithelial cell is highly vulnerable to injury, whereas the alveolar type II cell is more resistant and can therefore function as a progenitor cell for regeneration of the alveolar epithelium after injury. The loss of the alveolar epithelium’s integrity has several pathological and functional consequences: there is an influx of protein-rich oedema fluid into the air spaces, with deposition of hyaline membranes on the denuded basement membranes.

Hyperplastic alveolar type II cells typify the proliferative phase of ALI/ARDS. Alveolar type II cells migrate and begin to proliferate along the alveolar septa, in an attempt to cover the denuded basement membrane and re-establish the continuity of the alveolar epithelium. The arrows indicate typical alveolar type II cells with microvilli and lamellar bodies containing surfactant. Capillaries containing erythrocytes can be seen in the interstitial space (by courtesy of M. + H. Bachofen).
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The epithelial repair process includes cell-cell interactions and interactions between the alveolar type II cell and the extracellular matrix which are coordinated by a variety of soluble mediators released into the alveolar space during ALI/ARDS. Many of them have been detected in elevated concentrations in bronchoalveolar lavage fluid from ALI/ARDS patients [10]. However, although bronchoalveolar lavage procedure is standardised, the dilution factor may differ from patient to patient, rendering direct comparisons between patient groups difficult. Recently, to minimise the risks involved in bronchoalveolar lavage in critically ill patients with ALI/ARDS, undiluted pulmonary oedema fluid was obtained by direct suction through a wedged endotracheal catheter [11]. This safe procedure allows repeated drawing of several mL of undiluted pulmonary oedema fluid from the distal air space which can be used for further evaluation in the laboratory. Cytokine concentrations are usually determined by means of enzyme-linked immunosorbent assays (ELISA) or radioimmunoassays. These assays determine the total immunoreactive content of the cytokine, without providing any information regarding its biological activity. Since naturally occurring specific inhibitors may also be increased in patients with ALI/ARDS [12], the net biological activity of a cytokine may well turn out to be less relevant than was first thought. It is therefore crucial to supplement immunoreactivity assays with standardised bioassays serving to determine the biological activity of clinical samples. We therefore further developed an in vitro alveolar epithelial wound repair assay that makes it possible to quantify the epithelial repair activity of pulmonary oedema fluid and plasma from patients with ALI/ARDS [4, 14]. As a control, pulmonary oedema fluid and plasma from ventilated patients with hydrostatic pulmonary oedema was used [4].

Pulmonary oedema fluid from patients with ALI/ARDS increases alveolar epithelial repair in vitro by an IL-1β-dependent mechanism

Because of the extensive damage to the alveolar epithelium in patients with ALI/ARDS, we initially formed the hypothesis that pulmonary oedema fluid inhibits alveolar epithelial repair in vitro. To test this, pulmonary oedema fluid or plasma was added to a mechanically wounded monolayer of alveolar epithelial cells and the rate of wound closure over time was determined by means of a digital imaging system connected to the microscope and an appropriate image analysis software (Figure 2). Surprisingly, alveolar epithelial repair activity induced by pulmonary oedema fluid from patients with ALI/ARDS was markedly increased compared to plasma obtained from the same patients or pulmonary oedema fluid from patients with hydrostatic oedema [4] (Figure 3). These results indicate that biologically active mediators capable of enhancing alveolar epithelial repair in vitro are released into the alveolar space in patients with ALI/ARDS. The epithelial repair process probably begins in the early phase of ALI/ARDS, since pulmonary oedema fluid obtained during the first 12 hours after intubation enhanced epithelial repair activity to a greater extent than pulmonary oedema fluid obtained more than 12 hours after intubation.

Since IL-1β was shown to be biologically active in pulmonary oedema fluid in early ALI/ARDS [13] and to be upregulated in skin wounds, it seemed likely that this early response cytokine mediates epithelial repair activity in pulmonary oedema fluid from patients with ALI/ARDS. Specific inhibition of IL-1β by IL-1 receptor antagonist (IL-1ra) significantly reduced alveolar epithelial repair in vitro, whereas blocking of TNF-α, another inflammatory early response cytokine, had no effect, indicating that IL-1β mediated a major fraction of the enhanced in vitro...
alveolar epithelial repair. This conclusion was supported by experiments with recombinant IL-1β that showed a concentration-dependent increase in alveolar epithelial repair in our in vitro wound repair model using the human alveolar A549 epithelial cell line or freshly isolated rat alveolar type II epithelial cells. Concentrations of IL-1β showing significant alveolar epithelial repair in vitro were in a similar range to the concentrations of IL-1β found in pulmonary oedema fluid from patients with ALI/ARDS, indicating that IL-1β may contribute to repair of the alveolar epithelium in early ALI/ARDS.

The EGF/TGF-α pathway is involved in IL-1β-induced alveolar epithelial repair

There is increasing evidence that epidermal growth factor (EGF), transforming growth factor-α (TGF-α) and their common receptor, epidermal growth factor receptor (EGFR), may regulate epithelial repair in vivo and in vitro. TGF-α is elevated in pulmonary oedema fluid from patients with ALI/ARDS and has been shown to induce alveolar epithelial repair in vivo [14]. We therefore formed the hypothesis that the EGF/TGF-α pathway may be involved in IL-1β-induced alveolar epithelial repair. Neutralising antibodies to EGF and TGF-α decreased IL-1β-induced alveolar epithelial repair and blocking of the EGFR or its intracellular signalling pathway by inhibitors of the mitogen-activated protein kinase (MAPK) pathway specifically inhibit the effect of IL-1β [3]. These data indicate that IL-1β enhances alveolar epithelial repair in vitro by activating the epithelial EGF/TGF-α pathway in an autocrine-paracrine fashion. The EGF/TGF-α pathway would therefore offer an attractive new therapeutic target and is being further studied in animal models with several types of lung injury. However, the potential value of TGF-α or EGF in patients with ALI/ARDS has not yet been evaluated.

Keratinocyte growth factor and hepatocyte growth factor

Recent animal experiments suggest that keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF) may also play a significant role as new prophylactic or therapeutic agents in lung injury. KGF is chiefly produced by fibroblasts and has been shown to induce alveolar type II proliferation in vitro [15] and in vivo [16]. Studies in different lung injury models show a protective effect of KGF when given before inducing lung injury [17, 18]. Elevated levels of KGF were found to correlate with alveolar epithelial cell proliferation after bleomycin-induced lung injury in rats, suggesting that the elevated levels of KGF may induce efficient epithelial repair [19]. This hypothesis is supported by our in vitro epithelial wound healing studies showing that KGF increases alveolar epithelial repair and blocking of the EGFR or its intracellular signalling pathway by inhibitors of the mitogen-activated protein kinase (MAPK) pathway specifically inhibit the effect of IL-1β [3]. These data indicate that IL-1β enhances alveolar epithelial repair in vitro by activating the epithelial EGF/TGF-α pathway in an autocrine-paracrine fashion. The EGF/TGF-α pathway would therefore offer an attractive new therapeutic target and is being further studied in animal models with several types of lung injury. However, the potential value of TGF-α or EGF in patients with ALI/ARDS has not yet been evaluated.
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In conclusion, it must be emphasised that the alveolar epithelial repair process in the lung is very complex and modulated not only by several growth factors and cytokines, but also by other secreted products from inflammatory cells that are accumulated in the alveolar space during lung injury (e.g. proteases, reactive oxygen species) and by a variety of components of the extracellular matrix. One major goal is therefore to establish more sophisticated in vitro and in vivo models to improve our understanding of the mechanisms involved in the alveolar repair process. These experimental models will also enable us to test novel treatment modalities which seem promising in patients with ALI/ARDS.

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