Thorax

Editorials

The acute respiratory distress syndrome: fibrosis in the fast lane

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The acute respiratory distress syndrome (ARDS) is an acute and severe form of microvascular lung injury which is frequently seen in intensive therapy units. Reductions in mortality have been reported by some centres; however, 40–70% of patients still die from this syndrome.[1 2] Treatment at present is largely supportive and, despite our increased understanding of the pathological processes involved, there are no specific treatments of proven benefit.

Interstitial and intra-alveolar fibrosis are hallmarks of the more advanced stages of ARDS and are characterised by the abnormal and excessive deposition of extracellular matrix proteins, in particular collagen.[3 4] Histologically and biochemically this is similar to the fibrosis seen in other more chronic forms of interstitial lung disease[4]; however, more is known of the mediators and cellular events that occur in these disorders. The decrease in pulmonary compliance and progressive hypoxia resulting from fibrotic change leads to ventilator dependence. As a result, progressive fibrosis is a direct cause of respiratory death in up to 40% of patients[3 5] but is also an indirect cause of death due to nosocomial infection and progressive multi-organ failure in up to 70% of patients who die from ARDS.[6] Thus, the fibrotic process is an important determinant of outcome and a potential target for therapeutic intervention.

Fibroproliferation in ARDS

ARDS is traditionally divided into three phases: exudative, proliferative and fibrotic (fig 1). The initial exudative phase involves the leakage of proteinaceous fluid and the migration of cells, in particular neutrophils, from the circulation into the interstitium and alveolar space following diffuse damage to the endothelial and epithelial surfaces. The proliferation of fibroblasts and type II pneumocytes characterises the second phase during which activated fibroblasts secrete a number of extracellular matrix proteins within the interstitium but also migrate into the alveolar space where they form attachments to damaged basement membranes[7] and contribute to the intra-alveolar fibrosis which can predominate in some cases. Unabated, this process leads to established fibrosis and the obliteration of alveolar spaces with a dense irregular matrix.[4] The lung collagen content more than doubles in patients with ARDS who survive more than two weeks.[3] Qualitatively, the fibrillar collagens (types I and III) predominate but their relative contribution is unclear. Some reports suggest that type III collagen predominates in the early proliferative stage, whereas type I collagen—comprised of thicker more cross-linked fibrils—is more prevalent in the fibrotic stage.[4 8] Other studies report the converse[9] but differences in patient characteristics, stage of disease, lung sampling technique, and the biochemical analyses performed could account for these discrepancies. The composition and degree of cross-linking of matrix proteins deposited is an important issue as this influences its susceptibility to degradation which, in turn, could determine the degree to which established fibrosis might be reversible in ARDS.

Current hypotheses concerning both the proliferation of matrix synthesising cells and the increased deposition of alveolar and interstitial collagen propose that these events occur relatively late in the course of ARDS.[3] However, recent evidence pointing to an increase in lung collagen turnover at the very earliest stages challenges this view. The serum level of N-terminal procollagen peptide-III (N-PCP-III) is a marker of collagen turnover for which there is a transpulmonary gradient in normal adults, reflecting active type III collagen synthesis in the lung.[10] The serum N-PCP-III level is raised in patients with ARDS at an average of seven days of mechanical ventilation.[11] Although serum levels are likely to reflect collagen turnover in tissues in addition to the lung, an increase was observed even in comparison with patients with ventilation trauma, following surgery, and in those with cirrhosis. Raised levels correlated with the duration of mechanical ventilation and inspired oxygen concentration and the authors speculate that the lung is the main source of increased collagen turnover in ARDS. This view is supported by the increased levels of N-PCP-III in bronchoalveolar lavage (BAL) fluid reported in most patients within three days of the diagnosis of ARDS, although no comparison was made with other ventilated patients.[12] Recently, Chesnutt *et al* reported the earliest detection to date of a raised N-PCP-III concentration in endotracheal aspirates taken within 24 hours of mechanical ventilation.[13] A concentration above 1.75 U/ml

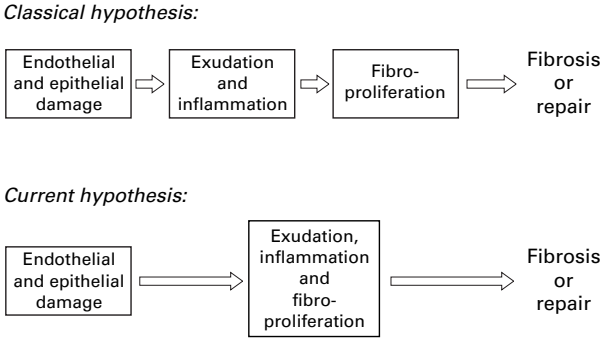

Figure 1 The classical model for the pathogenesis of ARDS suggests that damage to the endothelial and epithelial surfaces leads to exudation and inflammation. Fibroproliferation then ensues which, if excessive and unabated, results in established fibrosis. There is now mounting evidence to suggest that fibroproliferation is an early event in the pathogenesis of ARDS and we propose that this process occurs in parallel with exudative and inflammatory events. Thus, therapies preventing the progression to established fibrosis (with its devastating influence on mortality) will need to impact upon both proinflammatory and profibrotic mechanisms.

carried a relative risk of 4.5 compared with survivors in this study. The report in this issue of *Thorax* by Liebler *et al* adds further to the evidence, demonstrating both an increase in the number of α-smooth muscle actin-positive cells with a myofibroblast phenotype and procollagen immunoreactivity in lung tissue from ARDS patients ventilated for a mean of 4.7 days.[14] Qualitatively, an increase in collagen deposition was not detectable by histological analysis at this stage.

Procollagen measurements reflect collagen turnover and not deposition, and the progression from these early increases in the rate of collagen turnover to established fibrosis is likely to rely on a number of factors controlling the balance between synthesis and degradation rates. For example, matrix metalloproteinases (MMP) capable of digesting collagens such as MMP-2 and MMP-9[15] and gelatinases[16] are increased in the lungs of patients with ARDS but their relationship to the development of fibrosis or other outcomes is not known. In addition, agents governing apoptotic rates amongst inflammatory and mesenchymal cells could result in a failure to clear these cells from sites of injury and lead to a persistence of the fibrotic process.[17 18]

Profibrotic mechanisms

A number of mechanisms exist which could lead to an early activation of the fibroproliferative response in ARDS. An intense, predominantly neutrophilic infiltration into the lung parenchyma occurs almost immediately and persists throughout the course of ARDS, mediated by changes in adhesion molecule expression—for example, selectins and CD11b/18—and chemotactic stimuli—for example, IL-8 and IL-4.[19] A host of proinflammatory cytokines including TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-8 that are released by these and other inflammatory cells are increased within 24 hours of the onset of acute lung injury and persist in non-survivors.[20] In addition to their proinflammatory activity, these cytokines are also potentially fibrogenic. For example, TNF-α and IL-1β are both chemotactic and mitogenic for lung fibroblasts and stimulate collagen synthesis by these cells.[21]

COAGULATION CASCADE PROTEINS

An additional "early" source of profibrotic cytokines are products of the coagulation cascade entering from the circulation such as tissue factor/factor VII, thrombin, and fibrin. Fibrin deposition is a major component of the hyaline membrane, a typical pathological feature seen throughout the pulmonary interstitium in ARDS.[22] An increase in thrombin generation is implied by increased fibrin and thromin antithrombin III complexes measured in BAL fluid and serum[23] but is difficult to detect by direct measurement. Thrombin and fibrin are both mitogenic for fibroblasts and stimulate collagen synthesis in these cells.[24] The persistence of fibrin in ARDS is also favoured by a suppression of the fibrinolytic system suggested by increased BAL fluid levels of antiplasmin and plasmin activator inhibitor-1. Thus, fibrin incorporated into the evolving matrix may be an enduring fibroblast activator and reduction of its deposition or enhancement of its degradation could both be important treatment strategies. In this respect, observations made in endotoxin[25] and hyperinflation[26] models of acute lung injury are of great interest. In both studies hirudin, a specific thrombin inhibitor, effectively abrogated lung fibrin deposition, although effects on fibroproliferation were not assessed.

DOES MECHANICAL VENTILATION EXACERBATE LUNG INJURY?

Clinical variables such as the nature and severity of the initiating insult, patient age, genetic factors, and co-morbidity all influence the aggressiveness and progression of ARDS. Perhaps of particular concern is the potential contribution of excessive mechanical forces generated during mechanical ventilation to the perpetuation of lung injury. Evidence suggests that abnormal shear forces are generated between lung units of differing compliance and at the epithelial/endothelial interface, particularly when high pressure/volume ventilation strategies are employed.[27 28] This leads to the exposure of damaged basement membranes—with implications for inflammatory and mesenchymal cell migration—and further vascular leakage. Experimentally, maintaining high pressure or high volume ventilation in animal models results in an acute lung injury syndrome resembling ARDS.[27 29] Furthermore, mechanical forces can directly stimulate matrix synthesis by a number of cell types in vitro[30] but their importance in lung injury has not been studied. High oxygen tensions themselves, comparable to those used in the treatment of ARDS and given for short periods of time, can also induce lung injury experimentally, possibly by the generation of reactive oxidant species.[31]

Such observations make an exacerbation of lung injury by mechanical ventilation likely. In an attempt to try and avoid these problems, lung protective ventilation regimes aimed at reducing volutrauma whilst tolerating hypercapnia and lower oxygen tensions are currently in use. In the future, perfluorocarbon based liquid ventilation and high frequency oscillatory ventilation could, in theory, limit such forces in the lung, but we currently lack confirmation of clinical efficacy.[32]

PROFIBROTIC CYTOKINES

A number of cytokines implicated in the pathogenesis of other fibrotic lung disorders over the past 20 years have received little attention in the context of ARDS.[33] This is perhaps surprising, given that animal models with the pathological and temporal features of acute lung injury rather than chronic fibrosis have been used to establish a number of these factors. TGF-α levels are increased in the oedema fluid of ARDS patients[34] and a PDGF-like peptide which is chemotactic and mitogenic for fibroblasts has been detected in BAL fluid. However, clinical studies examining the role of other important profibrotic mediators such as TGF-β, endothelin, platelet derived growth factor, insulin-like growth factor, and basic fibroblast growth factor which are implicated in chronic fibrotic disorders are lacking.[33] There is likely to be an overlap between the various interstitial lung diseases and these mediators warrant further attention.

Conclusion: a hypothesis

Studies of matrix turnover in patients with ARDS and the presence of potentially profibrotic factors at the very onset of acute lung injury demand that the temporal relationship between inflammation and fibroproliferation be reconsidered. We propose an alternative to current hypotheses in which fibroproliferation represents a primary mode of response to lung injury, occurring in parallel with the inflammatory reaction rather than in series with it (fig 1). Altering the balance between matrix deposition and degradation in the first few days following acute lung injury could therefore have a significant impact on outcome. This has obvious implications for the design of future treatment regimes which will need to be pluripotent if they are to be effective. Further clinical studies are required to clarify the importance of factors that might govern the progression or resolution of fibrosis in patients with ARDS. In particular, sequential measurements of profibrotic agents and markers of fibroproliferation need to be made and placed in the context of clinical outcome. Proteins of the coagulation

cascade, proinflammatory cytokines, and the influence of ventilation induced lung injury deserve particular attention. Moreover, similarities and differences between the profibrotic factors involved in ARDS and other less acute forms of interstitial lung disease need to be explored in the search for both mechanism-specific *and* disease-specific therapies. Intriguingly, such antifibrotic agents may be of benefit even in established fibrosis where the potential for reversal appears to exist in some ARDS patients.[35 36] This capacity of the lungs to at least partially restore normal lung architecture following intense fibroproliferation can only encourage us to understand and control the processes responsible.

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