

HEALING AND REPAIR OF THE LUNG INJURY

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Acute and chronic lung disorders such as the Acute Respiratory Distress Syndrome (ARDS), asthma, chronic obstructive pulmonary diseases, and the interstitial lung diseases are a major cause of morbidity and mortality and an enormous burden on world health systems. A feature of these diseases is the destruction and remodelling of the lung's support structures including its extracellular matrix. When this occurs in the fine structures of the lung it has deleterious effects on lung function. This is seen in many disease setting including diseases of the airways such as asthma and COPD, where excessive matrix deposition may occur in large or small airways, and parenchymal diseases, such as ARDS and Idiopathic Pulmonary Fibrosis, where there is excessive deposition in alveolar structures and severely compromised gas exchange.

In recent years we have characterized the key processes in remodelling and identified the diverse structural components of lung airway and parenchymal structures (see Dunsmore et al 2003 for review). Collagens types I and III are the most abundant proteins found in airways and blood vessels as well as alveolar septa and it is these collagens that predominate in parenchymal fibrosis (reviewed in Chambers and Laurent 1996) and asthma (Roche et al 1989). The collagens are synthesised by many cells but predominantly by mesenchymal cells- fibroblasts, smooth muscle cells, myofibroblasts, as well as epithelial cells (McAnulty and Laurent 2003). Recent data suggest plasticity between these cells in vivo and that cytokines can promote trans-differentiation processes.

Microarray studies and the fibrotic phenotype

Fibroblasts are widely distributed in all lung structures and have long been recognized to be extremely dynamic, continuously synthesising and degrading collagens and expressing the diverse matrix metalloproteases that can degrade all collagens. When activated, they express a large number of genes. We recently profiled human foetal lung fibroblast global gene expression in response to TGF- β_1 using oligonucleotide microarrays. Almost 150 genes were up-regulated at least twofold representing several major functional categories, including genes involved in cytoskeletal reorganization, matrix formation, metabolism and protein biosynthesis, cell signalling, proliferation and survival, gene transcription. There were a further 80 genes up-regulated that were not previously reported to be TGF- β_1 -responsive (Chambers et al 2003). This diversity is reflected in vivo with studies of pulmonary fibrosis in man and animal models showing almost 500 genes are expressed more than two- fold, including many of the above as well as large cluster of diverse matrix genes (Kaminski et al 2000 and 2002).

Pro- fibrogenic cytokines and pulmonary fibrosis.

A large number of molecules, produced by many different cell types, are known to promote fibroblast proliferation, collagen synthesis, migration or trans-differentiation (Coker and Laurent 1997,1998). These changes when they occur in the lung lead to excessive collagen deposition, the hallmark of fibrosis. Studies in man and experimental models have implicated many of these molecules in the pathogenesis of acute and chronic lung diseases. The understanding of this network of mediators and the redundancy in inflammatory and tissue repair cascade has brought with it a number of challenges as we seek to develop new approaches to treat patients suffering with these diseases.

Transforming growth factor β (TGF β) is one of the most potent profibrotic molecules in vitro and a strong candidate as a central player in remodelling diseases including asthma (Minshall et al fibrosis and pulmonary fibrosis (Coker et al 1997). Blocking this molecule by a number of strategies blocks pulmonary fibrosis and several groups in academia and industry are exploring inhibitors of the TGF β as a strategy to prevent fibrosis. A serious reservation, however, is the role TGF β plays as an

inhibitor of immune responses since mice deficient in this cytokine exhibit a severe wasting syndrome with evidence of mononuclear cell infiltration in the heart and lungs (Shull et al Nature 1992)

Proteases in the regulation of fibroblast function and remodelling

The serine and matrix metalloproteases have long been thought to play roles in emphysema where degradation of matrix and destruction of parenchymal lung structures is a feature. However, there is also compelling evidence that these molecules may also play roles in acute lung injury and pulmonary fibrosis (Moraes, et al 2003). Thus inhibitors of neutrophil elastase inhibit lung injury and fibrosis and recently we have demonstrated that mice deficient in this protease are protected from lung fibrosis (Dunmore et al 2001).

It is also clear that proteases of the coagulation cascade, including tissue factor, factor Xa and thrombin, have pro-inflammatory and pro-fibrotic properties and likely play key roles in acute lung injury and remodelling disorders of the lung (Dabbagh et al 1998, Chambers et al 1998, Ruf and Riewald 2003). These molecules activate cells via a family of at least four proteolytically activated receptors (PARs). These receptors have emerged as interesting targets to prevent fibrosis as thrombin inhibitors can partially block fibrosis (Howell et al 2001) and animals deficient in the main thrombin and factor Xa receptor (PAR1) are protected from lung fibrosis.

Evidence that epithelial cells are central to remodelling

Recent evidence has focussed on the epithelial- fibroblast interactions as central to remodelling both in the airways (Holgate et al 2002) and in the fibrotic foci found in the alveoli of patients with idiopathic pulmonary fibrosis (Selman et al 2001, Gauldie et al 2002). Epithelial cells release many pro-fibrotic cytokines including transforming growth factor β , insulin-like growth factor-1 and endothelin-1; all of which stimulate fibroblast proliferation and procollagen production by fibroblasts. Furthermore these cells may play key roles in the activation of growth factors via cell surface integrins. One of these molecules, expressed only by epithelial cells is $\alpha_v\beta_6$. Mice deficient in this integrin are protected from pulmonary fibrosis and lack the ability to activate TGF β . These data, taken together with the sparsity of inflammatory cells in some fibrotic conditions, and the ineffectiveness of current anti-inflammatory drugs, have led to the suggestion that remodelling and fibrosis may proceed independently of inflammation.

Endothelin and angiotensin: vasoconstrictors that regulate remodelling processes

There are parallels between the actions of agents regulating tone in the vasculature and their effects on fibroblasts- vasoconstrictors often induce remodelling whereas vasodilators are inhibitors. Two examples are endothelins and angiotensin II, both agents exhibiting pro-fibrotic features in vitro (Peacock et al 1992, Marshall et al 2000) and receptor antagonists for these agents have shown some success in blocking fibrosis in animal models. Relevance to man remains uncertain and will await the results of trials with drugs currently used in man in other settings but it is of interest that a polymorphism in angiotensin converting enzyme has been shown to influence outcome in patients with ARDS (Marshall et al 2002).

Targeting inflammation and immune processes

There is strong evidence that inflammatory processes are important in remodelling. Inflammation is a feature of all the diseases where remodelling occurs, although the precise temporal relationship is uncertain and a causal link remains unproven in man. In animal models agents that block inflammation via a variety of mechanisms have been shown to reduce the extent of the subsequent fibrosis. For example, agents targeting inflammatory cell migration, activation or gene expression can reduce fibrosis. Until the facts come in on the importance of inflammatory events we should explore with vigour agent that can influence inflammation and where possible test these agents in man. New generation agents such as the TNF α antagonists need to be assessed in the hope that they will prove to be better than corticosteroids.

Immune modifying drugs are also being explored as agents that might interfere with remodelling processes. Many diseases in which remodelling is a feature are characterized by a predominant Th2 profile (e.g. IL4, 9 and 13) and a diminished Th1 response (IFN γ)- molecules which are broadly fibrotic and anti-fibrotic, respectively, in vitro. Thus agents that might alter this balance are being explored. Immune suppressants, such as azathioprine have also been used to treat patients with IPF. The reports on its effectiveness have not been encouraging, however, the new macrolide immunosuppressants, such as the rapamycin analogue SDZ- RAD, has more recently shown promise by inhibiting bleomycin induced lung fibrosis (Simler et al 2002) and this drug is currently being explored in patients with IPF.

Cytokines and lipid mediators as inhibitors of fibroblast function and fibrosis

There is considerable interest in the use of anti- fibrotic molecules to inhibit lung fibrosis. This was given impetus by the reports in a small group of patients that interferon gamma was effective treatment for idiopathic pulmonary fibrosis (Ziesche et al 1999). Unfortunately this early promise was not born out in more recent multi-centred trials with large numbers of patients. Another molecule of interest is prostaglandin E₂, the cyclo-oxygenase product of arachidonic acid metabolism. PGE₂ is a paracrine and autocrine inhibitor of collagen deposition and its production is reduced in fibroblasts from patients with fibrosis following stimulation with mediators such as IL1 (Wilborn et al 1995) or TGF β (Keerthisingam et al 2001). Furthermore, COX-2 “knockout” mice are more susceptible to bleomycin-induced pulmonary fibrosis (Keerthisingam et al 2001). Taken together, these observations support the hypothesis that there is a defect in PGE₂ production in patients developing fibrosis. These data also suggest that strategies to target specific pro-fibrotic genes or over-express anti-fibrotic molecules might be fruitful. To begin to explore this we have developed an integrin-targeting gene delivery system which shows high delivery efficiency whilst avoiding the immune and inflammatory side effects associated with the use of adenoviral vectors (Jenkins et al 2000).

Apoptosis and pulmonary fibrosis

Apoptotic pathways are also key to the resolution of inflammation and fibrosis following lung injury (Hensen 2003, Kuwano et al 2004)). For example, for clearance of inflammatory cells or fibroblasts may be vital elements of remodelling and there is evidence for diminution of pro- apoptotic pathways in fibroblasts taken from patients with pulmonary fibrosis compared with controls (Moodley et al 2003). Furthermore, fibroblasts derived from injured lungs can induce apoptosis of epithelial cells. Furthermore, it has also been shown that excessive apoptosis of epithelial cells is a feature of experimental fibrosis and inhibitors of the pro-apoptotic molecule Fas or the caspases, which signal from the death receptors, inhibited fibrosis (Kuwano et al 1999, Wang et al 2000).

Stem cell and cell plasticity in relation to remodelling

Recent studies have challenged the concept that resident fibroblasts are the only cells that produce the matrix proteins characteristic of the remodelling response. For example, epithelial cells have been identified as possible precursors of fibroblasts in chronic renal disease and TGF β has been implicated in this trans differentiation process (Iwano et al 2002, Yang and Liu 2001). The source of differentiated fibroblasts is uncertain in remodelling disorders of the lung but one recent study showed that bone marrow derived cells expressing type I collagen populated the lung in bleomycin- induced fibrosis (Hashimoto et al 2004).

Final common pathways leading to remodelling

To date over 30 molecules have been identified as potential players in fibrosis challenging us to find final common final pathways leading to (or inhibiting) remodelling and fibrosis. There is growing evidence that transforming growth factor beta, connective tissue growth factor (Blom et al 2002) and prostaglandin E₂ may be such key molecules. As such they are primary targets for new therapeutic approaches. Finally, new approaches to target cell proliferation, apoptosis and cell trans-differentiation are also being explored. For example, agents to activate epithelial cell proliferation, and aid repopulation of the damaged epithelium are being explored; as are agents to

block transformations of mesenchymal cells, including fibroblasts and epithelial cells, to myofibroblasts. Research is therefore providing us with promising new ways treat fibrosis and halt the inexorable progression that is a feature of so many fibrotic and remodelling disorders.

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