Occasional review

New strategies for treatment of pulmonary fibrosis

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Fibrosis is a common response to various insults or injuries to the lung. As such, it comprises a heterogeneous group of diseases whose initiating factors may be quite different, but whose terminal stages have a common theme – namely, the progressive accumulation of connective tissue replacing normal functional parenchyma. Recent progress in the understanding of the mechanisms which mediate lung injury and fibrosis should produce more rational and specific therapeutic strategies. However, a consequence of this progress is the increasing recognition of the complexity of the targets which could be susceptible to new therapeutic agents.

In this review the pathogenetic mechanisms relevant to pulmonary fibrosis will be summarised and highlighted, with more detailed discussion reserved for the possible points of therapeutic attack. More detailed reviews of mechanisms and aetiological factors have been presented previously.¹⁻⁸

Pathogenesis

The most common features of pulmonary fibrosis are injury and inflammation, although the extent of involvement or expression may vary depending on the aetiology. The early response to injury in the lung is similar to that in other tissues. Acute inflammation occurs in response to injury of the alveolar epithelium and/or the endothelium of the lung parenchyma, with rapid recruitment and mobilisation of neutrophils to the lung interstitium. In animal models with a single non-persistent insult to the lungs this acute response rapidly subsides and, depending on the magnitude of the injury, is completely replaced by a more chronic mononuclear infiltrate by the end of the first week.²⁶ In human studies, however, neutrophils may persist,¹⁵⁸ and their role in the subsequent accumulation of connective tissue remains uncertain. Most of the animal model studies suggest a limited or absent role for the neutrophil, while some human studies imply some association with progressive disease and a worse prognosis. Development of therapeutic strategies aimed at controlling neutrophil recruitment and activation should not therefore be ignored and, as will be discussed below, currently available treatment targeted at other aspects of fibrogenesis may also have significant effects on the neutrophil response. Although

the presence of eosinophils, basophils, and mast cells has also been described in both human and animal fibrotic specimens, their role in fibrosis has not been established, despite their potential as sources of potentially fibrogenic mediators such as cytokines and eicosanoids.

During the mononuclear phase roles for macrophages/monocytes and lymphocytes have been proposed on the basis that they represent important sources of fibrogenic cytokines such as transforming growth factors α (TGF α) and β (TGF β), tumour necrosis factor α (TNF α), and eicosanoids such as leukotrienes B₄ (LTB₄) and C₄ (LTC₄).²⁻⁸ These roles are supported by recent studies demonstrating increased expression of TNF α and TGF β by fibrotic lungs, particularly at sites of active fibrosis in human lung specimens using *in situ* hybridisation and immunohistochemistry.⁹⁻¹⁴ The role of TNF α is dependent on an active T cell response, which strongly implicates the immune response in pulmonary fibrosis, even where the target antigen or the source of immunological injury is not immediately apparent.²⁵⁹ These and other more recent *in vitro* studies suggest that cells other than macrophages/monocytes may also be important sources of these mediators.^{48 15-18} Their ultimate impact is to recruit more inflammatory/immune cells, amplify this mediator network, recruit and activate fibroblasts, and to stimulate connective tissue deposition.

The fibrotic response in the lung can be subdivided into relatively distinct stages on factors which affect cellular chemotactic recruitment, activation, proliferation, and extracellular matrix gene expression. However, the events in the various stages are interrelated in terms of common cellular mechanisms and mediators which exert multiple effects on the same or different cells as the fibrosis evolves. Discussion on therapeutic strategies can therefore be subdivided to address these common mechanisms and not the stages of disease progression. As a reflection of the increased understanding at a more basic mechanistic level, the new strategies discussed below will not deal much with older and more generic approaches such as the use of immunosuppressive and anti-inflammatory agents, but will focus on specific cellular and molecular strategies which may be amenable to control by recently discovered mechanisms (figure).

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Therapeutic points of attack in pulmonary fibrosis. The major mechanistic steps in pulmonary fibrosis are in bold and are interconnected by double headed arrows to indicate that each of these processes mutually regulates each other. Hence, any treatment directed to any one of these is likely to have an impact on one or more of the other mechanistic steps. The suggested therapeutic approaches for each are listed in the respective adjacent boxes and are explained in detail in the text. The asterisk indicates that the same list of anticytokine approaches listed in the box for "cytokine imbalance" applies as well. Flap = 5-lipoxygenase activating protein; PGE₂ = prostaglandin E₂; ECM = extracellular matrix.

Cytokine imbalance

A major development in pulmonary fibrosis research is the realisation that an ever increasing number of peptide mediators or cytokines, involved in a complex regulatory interplay, are important in its pathogenesis. These cytokines, in conjunction with other mediators, are important regulators of cell functions of direct relevance to the fibrotic response. This conclusion is based on a correlation of the increased gene expression or production of a cytokine with fibrosis, and a reduction in fibrosis when endogenous cytokine activity is neutralised with specific antibody or cytokine receptor antagonists. Based on the first approach, there is support for the importance of interleukin 1 (IL-1), TNF α , platelet derived growth factor (PDGF), insulin like growth factor 1 (IGF-1), and TGF β .^{2-6 9-14 19-21} Important roles for TNF α and IL-1 are supported by the ability of anti-TNF α antibody and IL-1ra (receptor antagonist), respectively, to inhibit pulmonary fibrosis.^{9 19} The importance of TGF β is also suggested by the inhibition of renal fibrosis by specific antibody.²² These three cytokines have some known cellular regulatory properties consistent with their multifaceted and multistage roles in fibrosis. Thus, IL-1 and TNF α are active as chemotactic and growth factors in addition to their role in the promotion of inflammation and cell activation.²³ The ability of these cytokines to coordinate expression of other cytokines with fibrogenic activity may

also cause further fibrosis.²³ TGF β has similar activities and is also a potent stimulator of extracellular matrix synthesis, a key component of the chronic phase of the fibrotic response. The experimental evidence for their importance, the multiplicity of their activities, and their possible amplification role (by paracrine and autocrine means) make these three cytokines ideal first candidates for potential therapeutic attack. Neutralising these cytokines may have an ameliorating effect on initial inflammation, tissue injury, and connective tissue deposition.

MONOCLONAL ANTIBODIES

Cytokine antibodies and cytokine receptor antagonists can successfully control pulmonary fibrosis in animal models.^{9 19} Monoclonal antibodies are safe to use in human disease and are effective in certain instances. Their use in controlling specific fibrogenic cytokines should therefore be important in new methods to control fibrosis. Monoclonal antibodies to TNF α are available and have been used in clinical trials²⁴⁻²⁶; their use is feasible given the simplicity of the target antigen and its cellular receptor. On the other hand, TGF β consists of a minimum of at least five isoforms and three known cellular binding sites, although not all isoforms or binding sites are important for mediation of selected biological effects.^{27 28} The development of an anti-TGF β strategy may therefore be more difficult and must await better understanding of the mechanism of action of TGF β on cells at the level of the cellular receptor, isoform specificity, and signal transduction mechanism.

RECEPTOR ANTAGONISTS AND BINDING PROTEINS

Treatment with IL-1ra may reverse increased collagen,¹⁹ an attractive approach clinically, given the usual presentation of patients with pulmonary fibrosis.^{1 25} Recombinant material is readily available and this reagent has been used in recent trials of other inflammatory diseases, but with mixed results.²⁹ Nevertheless, this strategy remains a promising new approach with a solid experimental foundation. Endogenous receptor antagonists for TNF α or TGF β (and isoforms) have not been reported, but an alternative approach is suggested by the presence of an endogenous soluble TNF α receptor which could compete with cellular receptors for any available secreted TNF α , thus inhibiting its biological activity.³⁰ A number of endogenous substances, including certain proteoglycans and α_2 -macroglobulin,³¹⁻³³ can bind to TGF β and thus serve as potential endogenous negative regulators of TGF β activity by similar competitive binding mechanisms; however, more studies are needed to assess their potential clinical usefulness.

ANTISENSE APPROACH

New strategies should also consider even more basic molecular approaches which have been shown to be effective in vitro. They are directed

at cytokine expression and include controlling gene expression at the mRNA and perhaps even the DNA level. Control of cytokine expression at the mRNA level by antisense nucleic acid probes has been demonstrated previously.³⁴⁻³⁶ Success with this approach *in vivo* depends on the ability to introduce adequate amounts of antisense probe to suppress cytokine production for long enough to arrest fibrosis. In the long term it may be necessary to achieve stable transfection of the antisense probe using vectors. The problem *in vivo* is therefore the development of an effective delivery method which would be clinically useful. The strategy for treating pulmonary fibrosis is theoretically subject to fewer constraints than that for treatment of genetic diseases where the aim is to correct a more stable long term (if not permanent) impairment of gene expression. In pulmonary fibrosis there is a sequence of events before the end stage deposition of connective tissue which may be controllable using this antisense approach directed at the inhibition of specific fibrogenic cytokine gene expression. Recent demonstration of relatively stable delivery of nucleic acids via the airway/endotracheal route³⁷⁻³⁹ supports the feasibility of specifically targeting the lung with the antisense probes, minimising possible untoward systemic effects. This strategy could be extended to any protein whose expression may contribute to the fibrotic process, including the matrix proteins themselves; however, the likelihood for success is reduced with proteins that are highly expressed for more sustained periods of time. The peak production of fibrogenic cytokines (for example, $\text{TNF}\alpha$ and $\text{TGF}\beta$) is relatively brief during the pathogenesis of fibrosis, hence they are more susceptible to control by the antisense approach. This strategy could be an improvement on the current use of relatively non-specific immunosuppressive or anti-inflammatory agents.

SIGNAL TRANSDUCTION

An alternative approach is to target the signal transduction apparatus responsible for the regulation of cytokine production. For example, the ability to modulate $\text{TNF}\alpha$ production by eicosanoids⁴⁰ could be used to control pulmonary fibrosis when this cytokine may be playing a key role in the recruitment and activation of inflammatory cells and fibroblasts. Given the importance of intracellular Ca^{2+} in signal transduction, calmodulin inhibitors have been tried with some success in the control of experimentally induced pulmonary fibrosis.⁴¹ Other approaches will depend on future advances into more unique signal transduction mechanisms for the targeted cytokines, with the advantage of the availability of pharmacological agents which affect cellular signal transduction and the established safety of many of these agents. The disadvantages lie in the relative non-specificity of their effects due to the common sharing of signal transduction mechanisms with other cellular processes, and the lack of information on the possible mechanisms of specific relevance to the cytokine(s) of interest.

Thus, the role of these agents as primary treatments for pulmonary fibrosis is likely to be minimal, although their potential usefulness as adjuncts should not be ignored.

ANTIFIBROGENIC CYTOKINES

The enhancement of the production of antifibrogenic cytokines such as interferon- γ ($\text{IFN}\gamma$)^{34,42,43} has been shown to be beneficial in animal model studies using poly I:C as interferon inducer⁴⁴ and confirmed by the direct administration of $\text{IFN}\gamma$ itself.⁴⁵⁻⁴⁷ Other potentially useful antifibrogenic cytokines have the ability to inhibit inflammatory/immune cell activation, and these paradoxically include $\text{TGF}\beta$. Recent studies indicate the importance of $\text{TGF}\beta$ as an endogenous downregulator of inflammation.^{48,49} This approach is likely to be limited by the wide range of bioactivity of these cytokines which makes them too non-specific to be therapeutically useful.

Inflammation and tissue injury

The contribution of inflammation to tissue injury was the basis for the early use of anti-inflammatory agents (steroids, alkylating agents and non-steroidal anti-inflammatory preparations) in treating pulmonary fibrosis but with little success.^{1,2} Other data suggest that cyclooxygenase inhibitors could exacerbate fibrosis by abrogating inhibition of collagen synthesis and cytokine production by endogenous prostaglandins.⁵⁰ In contrast, the stimulatory potential of lipoxygenase pathway products, such as leukotrienes, on cytokine and collagen production^{51,53} argue for the use of lipoxygenase inhibitors and leukotriene antagonists in the treatment of pulmonary fibrosis. Many such agents have been developed recently, directed primarily at the treatment of asthma, and it is a matter now of testing their efficacy in fibrotic diseases. Recent work on the mechanism of lipoxygenase activation^{54,55} has opened up research into pharmacological agents for controlling leukotriene production. The role of steroids in the future is likely to remain limited in view of their side effects and relative lack of specificity and efficacy.

ANTIOXIDANTS

Despite early recruitment of phagocytes in many forms of lung injury and fibrosis, there is only limited support for the role of neutrophils, oxidants, and proteases in the pathogenesis of pulmonary fibrosis.^{1,2,56} However, an approach to control inflammation-associated injury should be directed primarily at the production and effects of oxidants and proteases released by activated phagocytes, and perhaps even the target cells themselves.^{56,57} In addition to direct injurious effects, oxidants may also upregulate cytokine production, possibly via activation of $\text{NF-}\kappa\text{B}$ ⁵⁸ which may indirectly promote fibrosis. Several antioxidants and inhibitors of phagocyte activation have been evaluated for possible inhibition of pulmonary fibrosis in animal models² but the results of these studies are con-

flicting. Superoxide dismutase, catalase, and desferrioxamine have been found to ameliorate asbestosis-associated pulmonary disease, while the effects are equivocal in bleomycin-induced injury.²⁵⁹⁻⁶² *N*-acetyl cysteine and combinations of taurine and niacin have been used to control the latter,⁶³⁻⁶⁵ although whether the effects are mediated by the control of oxidant-mediated tissue injury remains undetermined. More recently, with the discovery of nitrogen-centred radicals as possible mediators of tissue injury, inhibitors of nitric oxide synthetase have been investigated for control of pulmonary fibrosis; however, the evidence is still too preliminary for any therapeutic strategy. A potential drawback is that these inhibitors may inhibit the endogenous production of nitric oxide necessary for vasodilatation which may be an important beneficial physiological response to injury.

ANTIPROTEASES

Experience with antiproteases has been limited, despite evidence that proteolytic activity in bronchoalveolar lavage fluid may be associated with progressive disease and a poor prognosis.¹²⁵⁶⁶ α_1 -Antiprotease is effective in ameliorating pulmonary fibrosis in a model⁶⁷ but needs wider testing to evaluate its clinical potential. Protease inhibitors may have an indirect effect by controlling oxidant production, possibly at the level of mediation of the activation of phagocytes and/or endothelial cell xanthine oxidase by signal transduction.⁵⁷ The use of non-proteinaceous inhibitors and antioxidants such as vitamin E, *N*-acetylcysteine, and iron chelators (for example, desferrioxamine) is preferable from a pharmacological standpoint, as they are easier to administer than the proteinaceous agents. Future advances will depend on the discovery of more specific, potent but less toxic antioxidants, assuming that the culprit oxidants can be identified.

INHIBITION OF CELL ADHESION

The possibility that inflammation and its undesirable sequelae could be prevented by blocking inflammatory cell adhesion is suggested by recent studies using antibodies to selected cell adhesion molecules.⁸ Direct evidence that pulmonary fibrosis can be controlled by such an approach is provided by the ability of anti-CD11 antibodies to inhibit collagen deposition in animal models induced by bleomycin or silica.⁶⁸ The mechanism of fibrosis inhibition is not understood, but the reduction in alveolitis, lymphoid infiltrate, and platelet microthrombi suggest that generalised inhibition of intercellular adhesion may contribute to the effect. Since there is extensive and generally positive experience with monoclonal antibodies in clinical practice, the use of monoclonal anti-adhesion molecule antibodies to control pulmonary fibrosis may represent a future development.

These strategies may not be generally applicable throughout the spectrum of pulmonary

fibrosis, as tissue injury by oxidants and proteases may not be a key component of fibrosis and, at the time of clinical detection, the tissue injury process may be far advanced or irreversible. However, where investigations suggest that the disease is at an early stage with an important oxidant component, these approaches may be effective. Other treatments directed at other facets of the fibrotic process may also have an impact on oxidant and/or protease mediated injury. These are discussed in the relevant sections.

Little information is available on the role(s) (if any) of the humoral aspects of inflammation and platelets in pulmonary fibrosis. Evidence suggests an insignificant role for platelets,² and few studies have addressed the issue of kinins or the coagulation pathway. Serum complement appears to be important in animal models of fibrosis, but the mechanistic basis for its role has not been defined.⁶⁹

Immune response

The immune response is important in the pathogenesis of many forms of pulmonary fibrosis.¹⁻⁷ Although the expected role of the immune system in autoimmune and other immunologically-mediated diseases in sustaining fibrosis-associated inflammation is well known, the cell-mediated immune mechanisms responsible for lung fibrosis, and the offending antigen(s) inciting the immune response, are unknown in many forms of the disease. Both clinically and in animal models there is good evidence of a role for the immune system, despite the inability to identify a specific antigen. Cell-mediated immunity to type I collagen has been reported in some cases of idiopathic pulmonary fibrosis and in an animal model² but the importance of this response to the pathogenesis of fibrosis is unclear. Nevertheless, early treatments have primarily relied on suppressing the immune system with steroids and other immunosuppressive agents, and they are more effective where the immune response is an important aetiological factor.²⁷⁰⁻⁷²

The drawbacks, in addition to lack of efficacy in many forms of the disease, include the possibility that some of the immunosuppressive agents themselves may be fibrogenic.²

Given the important role of the immune system, however, immunosuppression with fewer side effects should be an important part of any future strategy. Recent studies using cyclosporin A in both man and animal models have yielded promising results.⁷³⁻⁷⁷ Even more selective immunosuppression can be envisaged by suppression of T cell subpopulations, with cytotoxic cell type specific antibodies or desensitisation to an offending antigen. These approaches have been used successfully in other diseases, and the increasing ability to manufacture monoclonal antibodies makes this an attractive approach for controlling pulmonary fibrosis. The advantage of using these agents is their greater specificity compared with steroids and other general immunosuppressive agents.

As with the anti-inflammatory agents, it is unlikely that immunosuppression alone would

adequately treat the whole spectrum of pulmonary fibrosis. Additional and possibly synergistic effects may be obtained by targeting the immune response at the regulatory cytokine, eicosanoid, or signal transduction levels, using the approaches described above for cytokine imbalance. Even where a specific antigen has not been identified, the approaches described in this section may be partially effective.

Extracellular matrix deposition

The ultimate effect of pulmonary fibrosis is the replacement of normal lung parenchyma by connective tissue. Although this matrix is complex, more data are available on the accumulation of collagen than other matrix components. Ideally, a selective method could be developed in which abnormal deposition of lung collagen is controlled to allow for normal repair to take place. It offers the possibility that patients could be treated during the later stages of the disease, but success will depend on the ability to inhibit matrix production selectively to allow normal repair to continue. Treatment will be limited to a short time only so that collagen synthesis in other organs is not impaired. Animal model studies suggest that an early event characterised by inflammation and increased cytokine production precedes the period of increased collagen gene expression,^{2,3,5-7} suggesting a cause and effect relationship between these two sequential stages. The control of collagen synthesis by itself may not therefore be sufficient to control progressive fibrosis if the increase in cytokine production driving the upregulation in collagen production persists beyond the period of therapeutic efficacy of the collagen synthesis inhibitors. It is likely that successful treatment will require that the cause for the increase in collagen deposition (for example, cytokine production) should also be controlled.

PROLINE ANALOGUES

Despite these theoretical limitations, there has been some success with using inhibitors of collagen deposition in animal models. A number of proline analogues have been tried in animal models – including fibrosis in organs other than lung – with variable success and toxicity.² However, toxicity associated with generalised inhibition of collagen production in non-targeted tissues makes this approach much less attractive. This problem can be partially prevented by using tissue-specific inhibitors, such as the one developed for selective inhibition of liver collagen synthesis based on synthesising inactive esters of the inhibitor (pyridine 2,4-dicarboxylate) which are activated only in the liver.⁷⁸ In the lung this would depend on the identification of lung-specific enzymes with analogous properties.

PROCOLLAGEN TERMINAL PROPEPTIDES

Another approach to controlling collagen synthesis is to exploit the negative feedback properties of procollagen terminal propeptides.⁷⁹⁻⁸¹

Synthetic analogues may be developed with similar properties for therapeutic purposes.^{79,81} However, levels of these peptide fragments are already raised in the plasma of individuals with active fibrotic processes,^{82,83} and fibroblasts isolated from fibrotic tissue may be more resistant to negative feedback control by such peptides.⁸⁴ Not all propeptides behave similarly in regulating collagen synthesis, however, some being inhibitory and others stimulatory.⁸¹ If a select group of peptides can be identified with potent inhibitory properties, this approach may therefore be feasible where collagen synthesis needs to be controlled for a brief period, where the fibrosis-inducing stimulus is temporary, or can be controlled by removing an offending agent.

POST-TRANSLATIONAL CONTROL

Another method is to control collagen cross-linking using agents such as penicillamine and lathyrogens, such as β -aminopropionitrile.² An additional aim is to disrupt the secretory process in extracellular collagen deposition which relies on disruption of the cytoskeletal machinery. Although these studies show some success, toxic effects are common. Thus, approaches to inhibit collagen synthesis directly at the post-translational level are unlikely to produce results.

ANTISENSE APPROACH AND OTHER INHIBITORS

With increasing understanding of the regulation of collagen gene expression, new approaches using antisense probes directed at the specific collagen mRNA of interest may be feasible, as discussed above for cytokines. Known inhibitors of collagen production whose efficacy has already been tested in animal models include IFN γ and prostaglandin E₂ (PGE₂) which inhibit collagen synthesis and stimulate its degradation,² and IFN γ also inhibits bleomycin-induced pulmonary fibrosis.⁴³⁻⁴⁷ There is also evidence that stable synthetic PGE₂ analogues may be effective in reducing fibrosis.⁸⁵ The mechanism for these two agents, however, is unlikely to be mediated solely via the inhibition of collagen production, and may also affect other aspects of the fibrotic process. Some of these reagents are available and have been approved for clinical use in other disorders.

Miscellaneous

Other agents have been found to be effective in controlling pulmonary fibrosis in animal studies but their mechanisms remain uncertain, making them difficult to classify. The efficacy of niacin and taurine has been mentioned above as a possible antioxidant mechanism. More curious is the efficacy of the angiotensin converting enzyme (ACE) inhibitor^{86,87} and, given the extensive clinical experience with this class of drugs, more should be done to pursue an understanding of the mechanism underlying its potential therapeutic value. Significant toxicity of penicillamine and colchicine has limited their usefulness, but this is combined with equivocal therapeutic efficacy. Another agent, malotilate,

is effective in limiting liver and pulmonary fibrosis and has the advantage of having been studied in man and found to be effective in treating cirrhosis.⁸⁸ Whether it has applicability to other forms of fibrosis remains to be seen.

Finally, lung transplantation has received attention as a strategy for treatment of end stage pulmonary fibrosis.^{89,90} Progress has been helped by the development of more selective immunosuppressive agents. This approach seems quite successful in selected patients, but will remain limited by the lack of suitable donors and side effects of the immunosuppressive agent.

Conclusions

Recent discoveries in the pathogenesis of pulmonary fibrosis and related mechanisms governing lung inflammation, injury, immune response, and extracellular matrix synthesis have provided new impetus toward the development of therapeutic approaches. New strategies should be aimed at more selective and effective methods of controlling the key elements of pulmonary fibrosis. Animal and human studies have highlighted the importance of sequential lung cytokine gene expression and networking which drives fibrosis, in conjunction with other mediator systems such as eicosanoids. As early stage fibrosis appears to be reversible before matrix deposition occurs, future strategies should focus on controlling these elements. More non-specific approaches – for example, immunosuppressive agents, anti-inflammatory agents, etc – are less likely to improve on current methods. In contrast, previous success with monoclonal antibodies in neutralising the biological activity of undesirable molecules and selectively eliminating harmful cells suggests that they may have some feasibility in pulmonary fibrosis. Development of new synthetic and recombinant cytokine (and leukotriene, etc) receptor antagonists or soluble receptors should also be possible in the near future. Molecular biological approaches such as the antisense strategy are promising but still require much work. The high selectivity and effectiveness of these methods gives optimism for their application in clinical trials, and should provide the necessary tools for the future treatment of pulmonary fibrosis.

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