Anticytokine approaches in pulmonary fibrosis: bringing factors into focus

R K Coker, G J Laurent

Abstract

The prognosis of pulmonary fibrosis is poor and current therapies inadequate. Recent progress in understanding the mechanisms underlying the pathogenesis of this disease leads us to expect that inhibitors of cytokine and polypeptide growth factor will provide novel therapeutic agents. This paper outlines the role of cytokines in the pathogenesis of pulmonary fibrosis and concludes that there are compelling reasons to explore anticytokine therapeutic approaches. It also proposes criteria that will enable us to evaluate such agents in vivo and suggests approaches which might overcome the obstacles presented by control mechanisms which recruit a multiplicity of factors.

(*Thorax* 1997;52:294-296)

Keywords: pulmonary fibrosis, anticytokine.

Pulmonary fibrosis can follow diverse insults including exposure to inhaled dusts, radiation, and certain drugs. It may complicate local or systemic pathology including pulmonary infections, sarcoidosis, and connective tissue disorders. Diffuse pulmonary fibrosis arising with no known predisposing factors is termed cryptogenic fibrosing alveolitis (idiopathic pulmonary fibrosis in the USA).

The UK prevalence of cryptogenic fibrosing alveolitis (CFA) alone is approximately five people in every 100 000. Annual mortality, around 1400, has more than doubled since 1980. Current treatment comprises corticosteroids and immunosuppressants but most clinical series show only 50% five year survival despite treatment. Recognition of the poor prognosis and long term ineffectiveness of treatment with potentially serious side effects has led many respiratory physicians to adopt conservative approaches to management.

The last decade has generated significant advances in our understanding of the pathogenesis of the condition. One current hypothesis is that polypeptide mediators or cytokines, released by resident lung cells and recruited inflammatory cells, stimulate fibroblast replication and increased synthesis of extracellular matrix proteins including collagen. Collagen deposition then impedes gas

exchange. As key cytokines involved in this process are elucidated, anticytokine therapy is emerging as a promising prospect.

Scientific basis

Alveolar epithelial and/or capillary cell injury occurs early in pathogenesis (fig 1). Circulating immune cells, including monocytes, neutrophils, lymphocytes and eosinophils, are then recruited into the lung. These effector cells release cytokines which stimulate target cells – typically fibroblasts – to replicate and synthesise increased amounts of collagen. Resident lung

Figure 1 Potential targets for anticytokine therapy in pulmonary fibrosis. Therapeutic agents could be designed to inhibit any of the stages highlighted in black. Injury promotes cytokine gene transcription in the nuclei of effector cells such as alveolar macrophages. The mRNA transcript then undergoes translation in the cytoplasm to generate a polypeptide product. After its secretion outside the cell the cytokine may undergo activation prior to receptor binding on target cells such as fibroblasts. Signal transduction results in enhanced collagen synthesis and consequent fibrosis.

Centre for
Cardiopulmonary
Biochemistry and
Respiratory Medicine,
Rayne Institute,
University College of
London Medical
School, London
WC1E 6JJ, UK
R K Coker
G J Laurent

Correspondence to:
Dr R K Coker.

Received 28 August 1996
Returned to authors
15 October 1996
Revised version received
1 November 1996
Accepted for publication
11 November 1996

cells, including macrophages, alveolar epithelial and endothelial cells, can also release such mediators.

Various cytokines have been implicated in the pathogenesis of pulmonary fibrosis, including transforming growth factor β_1 (TGF- β_1), tumour necrosis factor α (TNF- α), platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1), and the interleukins IL-1 and IL-8.¹ Recently interest has also focused on endothelin 1 (ET-1)² and blood-derived proteins including thrombin.³

Of those investigated so far, the TGF- β family has the most potent stimulatory effect on extracellular matrix deposition. TGF- β_1 , secreted as a latent precursor, stimulates fibroblast procollagen gene expression and protein synthesis. It also inhibits collagen breakdown. Our data suggest that the other mammalian TGF- β isoforms, TGF- β_2 and TGF- β_3 , also stimulate human lung fibroblast collagen synthesis and reduce degradation.⁴ In experimental lung fibrosis enhanced TGF- β_1 gene expression precedes increased collagen expression. TGF- β_1 antibodies attenuate murine bleomycin-induced lung fibrosis, and human fibrotic lung tissue shows increased TGF- β_1 gene and protein expression.

There is also evidence to implicate tumour necrosis factor α (TNF- α) in the pathogenesis of pulmonary fibrosis. It can promote fibroblast replication and collagen synthesis, and lung TNF- α gene expression increases in mice following bleomycin administration. Soluble TNF- α receptors attenuate fibrosis in murine models of lung fibrosis,⁵ bronchoalveolar lavage fluid derived macrophages from patients with CFA or asbestosis release increased amounts of TNF- α , and transgenic mice overexpressing TNF- α in alveolar epithelium develop pulmonary fibrosis.⁶

Diminished production of potentially antifibrotic cytokines such as interferon (IFN) gamma may also enhance collagen deposition in pulmonary fibrosis⁷ and various physical, chemical and hormonal agents, probably interacting with cytokines, are now recognised to modulate collagen production. Consequently cytokines can interact in vivo to produce different effects from those observed in vitro and target cell phenotype may modify the response. It may therefore be useful to establish criteria for key fibrogenic cytokines, enabling us to select and develop appropriate anticytokine treatments for patients with pulmonary fibrosis. Inspired by Koch's postulates we propose that the following criteria should be satisfied before anticytokine therapy is indicated: (1) a candidate cytokine (or polypeptide growth factor) should stimulate fibroblast replication or procollagen production; (2) mediator gene expression and protein production should increase in the lungs of patients with pulmonary fibrosis; and (3) inhibitors of mediator function should attenuate fibrosis in animal models of the disease.

TNF- α and TGF- β_1 are currently the only cytokines that fulfil all three criteria. This information provides a rational basis for developing anticytokine agents and strongly

suggests that anti-TNF- α and anti-TGF- β_1 therapy should now be evaluated in patients with pulmonary fibrosis. In the future additional cytokines may emerge that satisfy these criteria.

Mechanisms of anticytokine therapy

Anticytokine therapeutic strategies should logically target one or more key events in cytokine synthesis and receptor binding (fig 1). DNA transcription generates an mRNA transcript whose translation results in polypeptide assembly. Agents can inhibit protein production in several ways. Antisense oligodeoxynucleotides may hybridise with nuclear mRNA, activating RNases. They can also bind cytoplasmic mRNA, inhibiting ribosomal activity through steric hindrance. Alternatively, ribozymes (RNA molecules with enzymatic activity) can prevent translation by binding and cleaving the mRNA transcript in the cytoplasm.

TGF- β_1 activation requires binding to the mannose-6-phosphate receptor; agents competing for binding may therefore be antifibrotic. Inhibitors of growth factor-receptor binding on target cells include antibodies, soluble receptors, receptor antagonists, and molecules which remove the cytokine from its site of action. Elucidation of signal transduction pathways within the fibroblast may promote development of compounds which uncouple individual cytokine signalling pathways and block specific effects of profibrotic mediators.

Therapeutic potential

ANTICYTOKINE STRATEGIES UNDER EVALUATION

The following illustrations demonstrate the potential of anticytokine strategies as novel treatments for pulmonary fibrosis. TGF- β_1 antisense reduces arterial wall proteoglycan synthesis⁸ and can eradicate established intracranial rat gliomas.⁹ Mannose-6-phosphate competes with TGF- β for binding to the mannose-6-phosphate receptor, preventing TGF- β activation, and is under evaluation in clinical trials to reduce cutaneous wound scarring.

Anti-TNF antibodies reduce inflammatory markers in rheumatoid arthritis¹⁰ but immunogenicity may limit use of cytokine antibodies in pulmonary fibrosis where prolonged treatment will probably be required. Soluble cytokine receptors or binding proteins may therefore provide a safer alternative. Administration of the proteoglycan decorin, a natural inhibitor of TGF- β , attenuates fibrosis in an animal model of glomerulonephritis¹¹ as does gene therapy upregulating decorin expression.¹² Receptor antagonists effective in vitro are now available for TGF- β_1 , ET-1, and IL-1 but their value in fibrosis remains to be investigated.

OVERCOMING MULTIPLE GROWTH FACTORS

Multiple cytokines and polypeptide growth factors are likely to be implicated in the pathogenesis of pulmonary fibrosis. Their functions may overlap and eliminating one may be in-

effective therapeutically as another takes over its role. Possible solutions include blocking the actions of several cytokines simultaneously or inhibiting the actions of individual mediators at different stages of the disease process. Another approach may be to identify and block intracellular signalling pathways common to several cytokines.

CLINICAL TRIALS

There is an urgent need for clinical trials of new therapies for this disease. Although different initiating events and cytokines may be implicated in different diseases, such as CFA and sarcoidosis, there is currently no evidence that the process of fibrogenesis differs significantly between diseases. Anticytokine strategies should therefore be applicable in various settings and could be used concurrently with other treatments, new or established. Short to medium term outcome measures will be required for pharmaceutical collaboration. These could realistically include bronchoalveolar lavage procollagen peptide levels, a marker of collagen synthesis, and lung function including assessment of the alveolar/arterial gradient at rest and on exertion. Other markers of fibrosis may become available in the future as research continues.

Conclusion

There is strong evidence implicating several cytokines including TGF- β_1 and TNF- α in the pathogenesis of pulmonary fibrosis. Additional mediators are becoming implicated as research progresses, including blood-borne mediators and products of the coagulation cascade. Recognition of key profibrotic cytokines has facilitated consideration of anticytokine thera-

peutic strategies, and establishing criteria for profibrotic growth factors may guide selection of appropriate anticytokine therapy. The diversity of approaches and successful application in other fields suggest there is potential for developing novel anticytokine drugs to treat pulmonary fibrosis. Continued research is critical, especially into ways of targeting multiple growth factors, but now is the time to begin evaluating these agents in clinical trials.

- 1 Coker RK, Laurent GJ. Pathogenesis of pulmonary fibrosis: implications for pharmacological intervention. In: Walter EH, du Bois RM, eds. *Immunology and management of interstitial lung disease*. London: Chapman and Hall, 1995: 19–36.
- 2 Cambrey A, Harrison NK, Dawes KE, Southcott AM, Black CM, du Bois RM, et al. Increased levels of endothelin-1 in bronchoalveolar lavage fluid from patients with systemic sclerosis contribute to fibroblast mitogenic activity in vitro. *Am J Respir Cell Mol Biol* 1994;11:439–45.
- 3 Hernandez-Rodriguez NA, Cambrey AD, Harrison NK, Chambers RC, Gray AJ, Southcott AM, et al. Role of thrombin in pulmonary fibrosis. *Lancet* 1995;346:1071–3.
- 4 Coker RK, Laurent GJ, Shahzeidi S, Lympny PA, du Bois RM, Jeffery PK, et al. Transforming growth factor β_1 , β_2 and β_3 all stimulate fibroblast procollagen production in vitro but are differentially expressed during bleomycin-induced lung fibrosis. *Am J Pathol* 1997 (in press).
- 5 Piguet PF, Vesin C. Treatment by human recombinant soluble TNF receptor of pulmonary fibrosis induced by bleomycin or silica in mice. *Eur Respir J* 1994;7:515–8.
- 6 Miyazaki Y, Araki K, Vesin C, Garcia I, Kapanci Y, Whitsett JA, et al. Expression of a tumor necrosis factor- α transgene in murine lung causes lymphocytic and fibrosing alveolitis. *J Clin Invest* 1995;96:250–9.
- 7 Prior C, Haslam P. In vivo levels and in vitro production of interferon gamma in fibrosing interstitial lung diseases. *Clin Exp Immunol* 1992;88:280–7.
- 8 Merrilees MJ, Scott L. Antisense s-oligonucleotide against transforming growth factor- β_1 inhibits proteoglycan synthesis in arterial wall. *J Vasc Res* 1994;31:322–9.
- 9 Fakhrai H, Dorigo D, Shawler DL, Lin H, Mercola D, Black KL, et al. Eradication of established intracranial rat gliomas by transforming growth factor β antisense gene therapy. *Proc Natl Acad Sci USA* 1996;93:2909–14.
- 10 Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Katsikis P, et al. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor α . *Arthritis Rheum* 1993;36:1681–90.
- 11 Border WA, Noble NA, Yamamoto T, Harper JR, Yamaguchi Y, Pierschbacher MD, et al. Natural inhibitor of transforming growth factor- β protects against scarring in experimental kidney disease. *Nature* 1992;360:361–4.
- 12 Isaka Y, Breese DK, Ikegaya K, Kaneda Y, Imai E, Noble NA, et al. Gene therapy by skeletal muscle expression of decorin prevents fibrotic disease in rat kidney. *Nature Med* 1996;2:418–23.