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Chronic rejection in lung allografts: immunohistological analysis of fibrogenesis

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Abstract In ongoing chronic rejection after lung transplantation, alveolar interstitial fibrosis develops. However, little is known about the mechanisms involved. In order to investigate these mechanisms, expression of extracellular matrix molecules (ECM) (undulin, decorin, tenascin, laminin, and fibronectin) and cytokines [transforming growth factor(TGF)-β1, TGF-β3, platelet-derived growth factor(PDGF), and PDGF receptor] were semiquantitatively evaluated in chronically rejected lung allografts, using standard immunohistochemical techniques. Additionally, the presence of macrophages was analysed. The present study demonstrates an increased infiltration of macrophages with a concomitant upregulation of cytokines (TGF-β1, TGF-β3, and PDGF) and an increased deposition of ECM in chronic lung rejection. These cytokines have an important role in the stimulation of fibroblasts which are a major source of ECM. Upregulated expression of ECM in the alveolar interstitial space leads to alveolar malfunction by thickening of the wall and, thus, is one of the causative factors of respiratory dysfunction in chronic lung graft rejection.

Key words Chronic rejection · Alveolar interstitial fibrosis · Extracellular matrix · TGF-β · PDGF

Introduction

Alveolar interstitial fibrosis develops in ongoing rejection of lung allografts [1, 2] and is assumed to be a causative factor of respiratory dysfunction. In idiopathic pulmonary fibrosis and animal models of pulmonary fibrosis, alveolar interstitial fibrosis is characterized by an increase in extracellular matrix and cytokines, secreted from alveolar macrophages, i.e., TGF-β and PDGF. Particularly, these cytokines are presumed to have a potent role in the production of extracellular matrix molecules [3]. According to the results of previous studies, upregulated expression of PDGF-mRNA and PDGF were found in macrophages in chronically rejected lungs [4]. It is thought that the interstitial fibrotic change in chronically rejected lungs is immunohistochemically similar to that in idiopathic pulmonary fibropsis [5]. Yet, the mechanism of fibrotic change remains unclear. In order to investigate the process of chronic graft rejection in lungs, we analysed immunohistochemically the expression of ECM, infiltrated macrophages, and cytokines in this setting.

Patients and methods

Seven lung specimens from six patients retransplanted for chronic rejection of lung allografts were obtained at the time of retransplantation and cryopreserved. Patients' clinical data are shown in Table 1. Histological evaluation demonstrated alveolar interstitial fibrosis in all specimens. As controls, five lung specimens were obtained from donors before transplantation. All specimens were immediately frozen in liquid nitrogen and then stored in a deep freezer at – 80 °C until preparation. For immunohistochemical analysis, cryostat sections, 7 μm thick, were obtained. Using standard immu-

Table 1 Clinical data for patients with chronic rejection after lung transplantation (*CF* Cryptogenic fibrosis, *LF* lung fibrosis, *PPHT* primary pulmonary hypertension)

Chronic rejection	Sex	Age (years)	Primary disease	Time since TX
1	Female	22	PPHT	2 months
2	Female	46	LF	11 days
3	Male	27	CF	2 months
4	Male	34	CF	19 months
5	Female	33	CF	48 months
6	Female	53	CF	18 days

Table 2 Expression of extracellular matrix in the alveolar area (– Negative staining, ± weakly positive staining or few positively stained cells, + moderately positive staining or some positively stained cells, ++ strongly positive staining or high number of positively stained cells, +++ very strongly positive staining or very high number of positively stained cells, *ND* not done)

Chronic rejection	Fibronectin	Tenascin	Undulin	Decorin
1a	+++	++	+++	+++
1b	+++	++	+	+~++
2	+~++	++~+++	+~++	+~++
3	+±	++	+~++	+
4	+	±~+	±	±,~+±
5	ND	+±	–~,±	+±
6	+	+±+±,~+	+~+±	
Normal control $n = 5$	±,~+	±,~+±	–~,±	+~+±

nohistochemical techniques previously described [6], expression of ECM molecules (undulin, decorin, tenascin, laminin, and fibronectin), cytokines(TGF-β1, TGF-β3, PDGF, and PDGF receptor) and the macrophage marker(27E10) were semiquantitatively evaluated.

Results

The results of ECM and cytokine expression are shown in Tables 2, 3. In comparison to normal controls, a marked increase in ECM deposition, except for laminin, was observed in expanded alveolar interstitial tissue in chronic lung rejection. The number of infiltrated macrophages in the alveolar space and the alveolar interstitial tissue increased. A concomitant upregulation of TGF-β1, TGF-β3, and PDGF was found on alveolar macrophages in the alveolar area. Expression of PDGF was also found on alveolar epithelial cells and alveolar hyaline membrane, and PDGFr was expressed on the alveolar interstitial tissue.

Table 3 Expression of cytokines on the alveolar area

Chronic rejection	TGF-β1 Macrophage	TGF-β3 Macrophage	PDGF		PDGFr Interstitium
			Macrophage	Pneumocyte	
1a	++	+++	++	++	+++
1b	+±	+++	+++	+++	++
2	++	+++	+~++	+++	±
3	+++	+++	+	+	
4	+~++	+~++	±	±~+	±
5	ND	+~++	–	–	±
6	+	++~+++			+~++
Normal control $n = 5$	–	±~++	±,~++	–~+	±,~++

Discussion

In idiopathic pulmonary fibrosis, TGF-β and PDGF are secreted from activated alveolar macrophages. It is assumed that upon this stimulatory event, lung fibroblasts are activated and produce ECM [3]. In the present study, an increased infiltration of macrophages that stained positive for these cytokines could be demonstrated. Furthermore, severe accumulation of ECM in the interstitial space could be shown. These findings indicate that fibrogenesis is enhanced in chronic lung graft rejection. There is still debate about the cells responsible for the ECM production. From these experiments, we can conclude that cells residing in the interalveolar space demonstrated expression of PDGFr. As these cells are not macrophages, they are most likely to be fibrocytes which are known to be PDGFr positive. These data are in accordance with findings in chronic inflammation in different tissues [8]. Although the biological function of PDGFr in the lung is still debated [7], there is evidence for its pivotal role in fibrogenesis [4].

In conclusion, the present study demonstrates an increased infiltration of macrophages with a concomitant upregulation of cytokines and an increased deposition of ECM in chronically rejected lungs. These cytokines probably have an important role in the stimulation of ECM in the alveolar interstitial space which might lead to alveolar malfunction and, thus, could be one of the causative factors of respiratory dysfunction in chronic lung graft rejection.

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