BIOMARKERS, 1999, VOL. 4, NO. 5, 361–372

Depressed urokinase activity in bronchoalveolar lavage fluid from patients with sarcoidosis, silicosis or idiopathic pulmonary fibrosis: relationship to disease severity

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Received 20 July 1998, revised form accepted 21 January 1999

Intraalveolar fibrinolysis, is regulated by the concerted actions of plasmin, plasminogen activators (PAs), and their specific inhibitors (PAIs). This event is considered as a critical step in the pathogenesis of pulmonary fibrosis. The aim of this study was to evaluate whether local PA activity can be held as a marker of fibrosis in chronic interstitial lung disorders (ILD). Changes in both PA activity and PA-related proteins (urokinase-type PA (uPA), tissue-type PA (tPA), PAI-1 and PAI-2) were assessed in bronchoalveolar fluid (BALF) of 60 subjects: 18 healthy controls, 18 non-fibrotic sarcoidosis patients, 16 patients with idiopathic pulmonary fibrosis (IPF) and eight silicotic patients with established fibrosis. We observed a significant decrease of BALF PA activity in the three groups of patients as compared with controls. Reduction in BALF PA activity was compatible with lower uPA protein levels associated, especially in IPF patients, with an increased occurrence of PAI-1 and PAI-2 antigens. Soluble tPA antigen was never detected either in control subjects or in patients. Most importantly, the reduction in BALF PA activity and uPA protein levels was found to be most severe in patients with advanced fibrotic disease, namely IPF, while moderate and only weak alterations were found in silicosis and non-fibrotic sarcoidosis, respectively. In addition, significant positive correlations were found between BALF PA activity and functional impairment as assessed by TLC % and DL_{CO}%. Finally, the reduction in uPA and PA activity levels observed in BALF from sarcoidosis patients was found to be proportional to the degree of BAL lymphocytosis. These findings indicate that an intense reduction in BALF PA activity is associated with severe stages of the parenchymal disease, possibly reflecting the degree of the fibrotic process.

Keywords: plasminogen activators, plasminogen activator inhibitors, inflammatory lung diseases.

Introduction

Inflammatory lung diseases, characterized by an important cellular infiltration of the lung interstitium and intra-alveolar fibrin deposition, include a large spectrum of heterogeneous disorders resulting, in many instances, in severe impairment of lung function. Lung sarcoidosis, characterized by exacerbated immunocellular activity, can be either self-limited or chronic, with episodic recrudescences and remissions which, however, rarely progress to fibrosis (Newman *et al.* 1997). Silicosis, usually a chronic fibrosing disease, develops after several years of exposure

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Biomarkers ISSN 1354-750X print/ISSN 1366-5804 online © 1999 Taylor & Francis Ltd
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to crystalline silica (Ziskind *et al.* 1976) and idiopathic pulmonary fibrosis (IPF), a severe interstitial fibrotic disease, often shows rapid clinical deterioration with an almost invariably poor prognosis (du Bois 1993). Different clinical and functional parameters as well as cellular or soluble markers in serum and bronchoalveolar lavage fluid (BALF) have been studied in an attempt to monitor inflammatory lung disease activity and progression to fibrosis. Degradation and repair of connective tissue is an important aspect in the pathogenesis of pulmonary fibrosis. Known markers associated with the extracellular matrix remodelling process and directly linked to the mechanism of fibrosis, such as type I and type III procollagen peptide (Bacchella *et al.* 1996), fibronectin (Blaschke *et al.* 1990), TGF-β (Vanhee *et al.* 1994) and more recently matrix metalloproteinase (Torii *et al.* 1997) and alkaline phosphatase (Capelli *et al.* 1997), clearly differ according to the pattern of the disease but have shown poor or uncertain clinical value.

Intraalveolar fibrin accumulation, as a result of vascular leakage associated with acute lung injury, initiates the building up of extracellular matrix scaffolds that support the healing process. Because fibrin scaffolds also promote fibroblast ingrowth, fibrin removal represents a crucial event to recovery from lung injury as illustrated by the development of fibrosis at sites of fibrin persistence in adult respiratory distress syndrome (ARDS) and IPF (Crouch, 1990). Intraalveolar fibrinolysis is mediated by the enzyme plasmin, the production of which is regulated by the concerted actions of plasminogen activators (PAs) and their specific inhibitors (PAIs) (Hart and Rehemtulla 1988, Vassalli *et al.* 1991). The normal bronchoalveolar surface is endowed with high levels of functional urokinase-type PA (uPA) mainly produced by alveolar macrophages (Vassalli *et al.* 1992) and pneumocytes (Marshall *et al.* 1991) two cell types which also express PAIs. Through the interaction with its specific receptor (uPAR), uPA catalyses plasminogen activation at the cell surface and restricts extracellular proteolysis at site of fibrinous scaffolds and extracellular matrix (ECM) contact (Behrendt *et al.* 1995). In addition, uPA/uPAR interaction also promotes cell adhesion to vitronectin (Waltz and Chapman 1994) and migration through ECM (Waltz *et al.* 1997). Recent observations demonstrating the acceleration of the lung fibrotic process in uPA-deficient mice treated with crystalline silica (Lardot *et al.* 1998) and in PAI-1-overexpressing mice treated with bleomycin (Eitzman *et al.* 1996) provide direct evidence for a causal link between reduced PA activity in the lung and the development of fibrosis. Moreover, local intratracheal instillation of uPA protein has been shown to limit the fibrotic process induced experimentally by bleomycin (Hart *et al.* 1994).

We compared changes in PA activity and PA-related proteins (uPA, tPA, PAI-1 and PAI-2) in BALF of patients with chronic inflammatory lung diseases and controls. The lung fibrinolytic system was studied in lung sarcoidosis patients characterized by an intense immunocellular reactivity without end stage fibrosis, and in patients showing established lung fibrosis either due to silicosis or of idiopathic origin. The relationship between reduced BALF PA activity and disease severity was examined.

Material and methods

Study population

The study population consisted of 60 subjects referred to the University Hospital of Mont-Godinne (Mont-Godinne, Belgium) and to the University Hospital A. Calmette (Lille, France). Four groups were considered including 18 normal volunteers without any history of pulmonary disease. The main

Table 1. Characteristics of the study population and BALF data.

	Normal subjects	Patients with sarcoidosis	Patients with silicosis	Patients with IPF
Study population				
Subjects, *n*	18	18	8	16
Age, years, mean ± SEM (range)	35 ± 2 (19–51)	47 ± 4[a] (28–76)	59 ± 3** (46–70)	57 ± 3*** (32–69)
Sex, M/F	8/10	12/6	8/0	10/6
Smoking, NS/ExS/S	10/0/8	15/2/1	3/0/5	4/0/12
BALF analysis				
Recovery, ml	124 ± 4	105 ± 10	111 ± 15	101 ± 7
Total proteins, μg^{-1} ml	23 ± 4	93 ± 18**	39 ± 9	57 ± 9**
Cell ml^{-1}, $\times 10^4$	31 ± 5.8[b]	39 ± 5.2	21 ± 5.6	29 ± 3.2
Macrophages, %	96 ± 0.6	81 ± 2.7***	82 ± 4.5**	69 ± 5.5***
Lymphocytes, %	2.9 ± 0.5	15 ± 2.6***	14 ± 4.9*	24 ± 5.5***
Lymphocytes, $CD4^+/CD8^+$	1.9 ± 0.3	10.4 ± 4.9**	nd	nd
Neutrophils, %	0.6 ± 0.1	1.4 ± 0.3	1.5 ± 0.4	4.3 ± 1.2***
Eosinophils, %	0.1 ± 0.1	0.9 ± 0.6	0.2 ± 0.1	1.5 ± 0.5*
Medication[c], *n*	0	2	0	6

All values are means ± SEM.

[a] not significantly different from silicosis or IPF patients.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from normal subjects.

NS = non-smokers, ExS = former smokers, S = smokers, IPF = idiopathic pulmonary fibrosis, nd = not determined.

[b] Total cell number from smokers and non-smokers were 51 ± 8.6 and 15 ± 1.9, respectively.

[c] medication is defined as corticosteroids and/or immunosuppressive agents.

characteristics of normal subjects and patients are summarized in table 1. A group of 18 patients had sarcoidosis with compatible clinical, functional and chest X-ray data. Final diagnosis was supported by confirmative biopsy. On the basis of chest X-ray, we identified three patients with Stage I sarcoidosis (hilar adenopathy without parenchymal involvement), five patients with Stage II sarcoidosis (hilar adenopathy with parenchymal involvement), eight patients with Stage III sarcoidosis (pulmonary infiltrates without adenopathy) and two patients with Stage IV sarcoidosis (end stage pulmonary fibrosis). Two patients with Stage III and one with Stage I received corticosteroid therapy. According to conventional clinical and radiological data, the disease was classified either as active or non active sarcoidosis as previously described (Minshall *et al.* 1997). Increased BAL lymphocytosis and $CD4^+/CD8^+$ ratio were included as additionnal indicators of active granulomatous sarcoidosis. A group of eight silicotic patients was diagnosed on the basis of a positive history of occupational exposure and chest X-ray (five patients with simple silicosis and three patients with progressive massive fibrosis). Pulmonary function tests from silicotic individuals showed both restrictive and obstructive ventilatory defects. Sixteen patients had IPF. The diagnostic of IPF was obtained on the basis of case history, physical examination, pulmonary function tests (restrictive defect and/or reduction of gas transfer) compatible with chest X-ray, exclusion of other similar diseases (collagen vascular disease, asbestosis) and confirmed by open lung biopsy or video-assisted thoracoscopy. Four IPF patients received corticosteroid therapy only and two received both corticosteroids and immunosuppressive medication.

Bronchoalveolar lavage (BAL)

Oral informed consent was obtained for BAL from both patients and normal volunteers. BAL was performed, as previously described (Delacroix *et al.* 1985), by instillation of sterile saline (4 × 50 ml) into the bronchoalveolar tree under fibreoptic bronchoscopy. BAL fluid was filtered through sterile gauze and centrifuged ($400 \times g$ for 10 min at 4 °C). Total protein content of the supernatant was assessed spectrophotometrically using a commercial kit (Systemes Technicon, Doumon, France). Part of the supernatant was concentrated 20-fold by lyophilization at 4 °C followed by extensive dialysis (membrane cut-off 10 000 MW, Medicell, London, UK) in phosphate buffered (pH 7.4) saline (Gibco, Paisley, Scotland). Differential cell counts were performed using eosin/methylene blue stained cytocentrifuge preparations of recovered cells. Total cell and lymphocyte $CD4^+$ and $CD8^+$

subpopulations counts were carried out on an automatic counter after staining with specific antibodies. BAL recovery, total protein content and cellular characteristics of BALF are summarized in table 1.

Enzymatic assay for plasminogen activators (PA)

A chromogenic assay (Lardot *et al.* 1998) was used to assess the presence of soluble PA activity in unconcentrated BALF samples. Briefly, 50 µl of BALF were incubated in 0.1 M Tris (Tris-hydroxymethyl-aminomethane)/0.1 M glycine/0.5 % BSA (bovine serum albumin) buffer (pH 8.5) in the presence of the 0.7 mM S2251 plasmin substrate (Val-Leu-Lys-*para*nitroanilide, Chromogenix, Mölndal, Sweden), 530 pg of fibrinogen fragments (Boehringer, Mannheinn, Germany) and 0.14 mM of human plasminogen (Chromogenix, Mölndal, Sweden) in a final volume of 250 µl. The measurements were performed at 37 °C in triplicate in 96-well plates. Negative controls in which plasminogen was omitted were assayed simultaneously for each sample. The formation of *para*-nitroaniline from the plasmin-mediated hydrolysis of S2251 was followed spectrophotometrically at 405 nm (Twinreader Titertek, Techgen International) each 15 min over a period of 10 h. The difference in optical density between plasminogen-containing and plasminogen-free wells was plotted against the square of the incubation time and the slope of the regression line calculated. Reference curves for PA activity were prepared by serial dilution of standard human urokinase (Calbiochem, La Jolla, CA, USA). The results are expressed in IU (µmol of substrate hydrolysed by min) per ml of BALF.

Measurement of PA-related antigens

Determinations of human tPA, uPA and PAI-1 proteins were performed on unconcentrated BALF samples with a two-site non-competitive ELISA as previously described (Declerck and Collen 1990) using a conventional peroxidase detection method with 1,2-phenylenediamine (Fluka, Buch, Switzerland) as substrate. Monoclonal antibodies directed against human tPA, uPA and PAI-1 as well as human purified tPA, uPA and PAI-1 proteins were kindly provided by P. Declerck (KUL, Leuven, Belgium). The detection limits for tPA, uPA and PAI-1 ELISA were 0.15, 0.015 and 0.02 ng ml^{-1}, respectively. PAI-2 protein measurement was performed on 20-fold concentrated BALF samples using a commercially available ELISA (Imubind PAI-2 kit, American Diagnostica, Greenwich, USA) with a detection limit of 0.5 ng ml^{-1}.

Statistical analysis

Data are expressed as means ± SEM. ANOVA was applied to compare groups under study and multiple comparisons were made using the Kruskall–Wallis non-parametric test. The correlation between enzymatic activity and functional variables, clinical data and BAL cells were computed using the Spearman non-parametric test. Statistical signification was defined as $p'' 0.05$.

Results

Characteristics of the study population

BALF cellular patterns are presented in table 1. Total cell count in BALF from normal subjects was significantly ($p'' 0.001$) higher in smokers than in non-smokers, which was the reflection of a significant ($p'' 0.001$) increase in the number of alveolar macrophages only (data not shown). Compared with normal subjects, no significant difference could be found in the total number of BAL cells in sarcoidosis, silicosis and IPF patients. However, we noted a significant reduction in the percentage of alveolar macrophages in the three groups of patients with a more substantial decrease in IPF. Lymphocytic alveolitis was found in patients with silicosis and sarcoidosis. Clinically active sarcoidosis was evidenced by significant increase of the $CD4^+/CD8^+$ BAL lymphocyte ratio compared with normal subjects. Increased $CD4^+/CD8^+$ ratio in sarcoidosis resulted from a significant ($p'' 0.01$) increase of the $CD4^+$ subpopulation representing two-thirds of the lymphocyte counts and a significant ($p'' 0.04$) reduction in the $CD8^+$ subpopulation (data not shown). A mixed-type alveolitis (lymphocytic and neutrophilic) was observed in IPF patients. Moderate but significantly increased levels of eosinophils were noted in IPF only.

Table 2. Respiratory function data.

	Patients with Sarcoidosis	Patients with Silicosis	Patients with IPF
VC, % pred.*	88 ± 7.0#a	89 ± 3.5a	83 ± 5.5a
FEV_1, % pred.	78 ± 6.3a	77 ± 7.7a	78 ± 4.0a
FEV_1/VC	75 ± 5.5a	71 ± 6.7a	79 ± 2.2a
T LC, % pred.	93 ± 4.2a	90 ± 3.0ab	73 ± 4.2b
$D L_{CO}$, % pred.	81 ± 7.2a	88 ± 7.4ab	55 ± 5.7b

* Functional data are expressed as percentage of predicted values for an age and height matched standard population.

Values (means ± SEM) with the same letter are not statistically different ($p \leq 0.05$).

IPF : idiopathic pulmonary fibrosis.

Respiratory function data are reported in table 2. Patients with sarcoidosis, silicosis and IPF showed a mild to moderate restrictive pattern of lung functions. A severe reduction in diffusing capacity for carbon monoxide was found in IPF patients.

Change in the PA/PAI system

PA activity was readily detectable in BALF from all individuals. Results are illustrated in figure 1(A). In normal subjects, no significant change in BALF PA activity was associated with cigarette smoking (12 ± 1.5 n IU ml^{-1} in smokers versus 9 ± 1 in non-smokers, $p = 0.27$). Compared with normal subjects, we found a significant reduction in BALF PA activity in the three groups of patients. BALF PA activity observed in IPF patients was however also significantly lower than in sarcoidosis and silicosis patients. No significant difference could be observed between sarcoidosis and silicotic patients. No significant difference in BALF PA activity could be evidenced between IPF patients treated or not with corticosteroid (1.25 ± 0.43 n IU ml^{-1} in corticoid-treated IPF versus 1.69 ± 0.44 in non-treated IPF, $p = 0.79$).

tPA antigen was never detected in BALF from neither normal subjects nor patients. A substantial amount of uPA protein was detectable both in normal, sarcoidosis, silicosis and IPF BALF samples (figure 1(B)). No difference in BALF uPA levels could be found between smoking and non-smoking normal subjects (33.8 ± 9.4 pg ml^{-1} in smokers versus 38.8 ± 8.4 pg ml^{-1} in non-smokers, $p = 0.61$). Compared with the normal subjects, we found no statistically significant reduction in uPA levels in sarcoidosis and silicotic patients. A marked, however not statistically significant, reduction in uPA levels was noted in IPF patients compared with normal subjects.

Determination of PAIs antigens in BALF was undertaken to further characterize the reduction of BALF PA activity observed in the patients under study. PAI-1 protein was never detected in BALF of normal subjects (< 20 pg ml^{-1}) and was found in 11 % sarcoidosis (2/18 with values of 278 and 748 pg ml^{-1}), 12.5 % silicosis (1/8 with a value of 44 pg ml^{-1}) and 31 % IPF patients (5/16 with values ranging from 42 to 5644 pg ml^{-1}). PAI-2 protein was also variably detected. We found soluble PAI-2 in BALF of 17 % normal subjects (3/18 with values of 10, 12 and 108 pg ml^{-1}), 25 % sarcoidosis (5/18 with values ranging from 39 to 85 pg ml^{-1}), 37 % silicosis (3/8 with values of 19, 99 and 104 pg ml^{-1}) and 50 % IPF patients

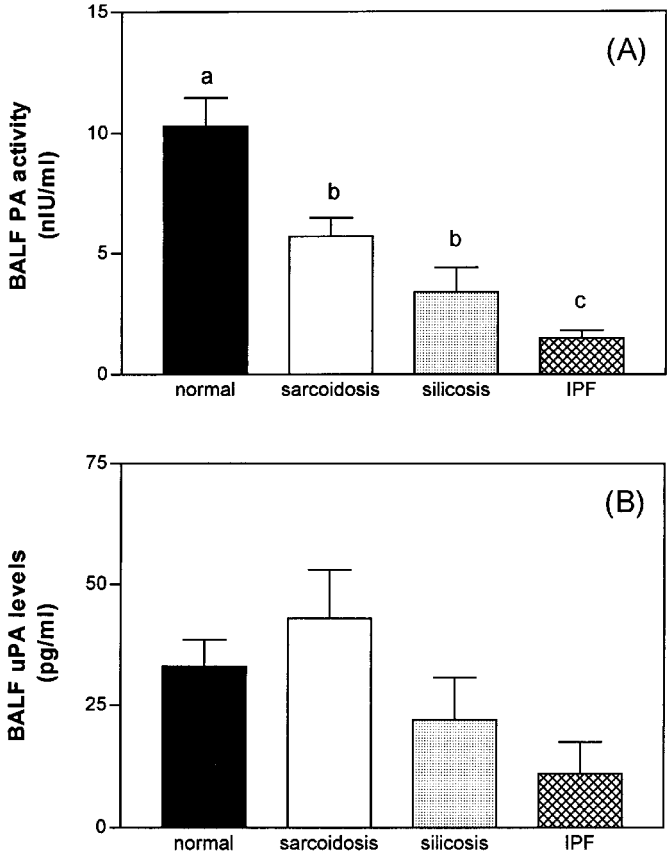

Figure 1. Plasminogen activator (PA) activity and urokinase type plasminogen activator (uPA) protein levels in unconcentrated bronchoalveolar fluid (BALF) from sarcoidosis ($n = 18$), silicosis ($n = 8$), idiopathic pulmonary fibrosis (IPF, $n = 16$) patients and normal subjects ($n = 18$). Data are expressed as means ± SEM. Significant difference were assessed by Kruskall–Wallis non-parametric test. Bars with the same letter are not statistically different from normal subjects ($p'' 0.01$). No significant difference could be found when considering BALF uPA levels ($p = 0.12$).

(8/16 with value ranging from 10 to 144 pg ml^{-1}). No association could be found between PA activity and PAI-1 or PAI-2 levels.

Relationship with BAL cell findings

Correlation studies considering all patients together, showed a weak ($r = 0.32$, $p = 0.036$) association between BALF PA activity and the percentage of alveolar macrophages. No association could be found between BALF PA activity and eosinophils, neutrophils, lymphocytes counts or total cell number ($p > 0.05$).

Relationship with disease activity

The clinical and radiological presentation of pulmonary sarcoidosis can be divided into active and non-active stages. No significant difference in BALF PA activity and uPA levels could be demonstrated between stage I/II and stage III/IV

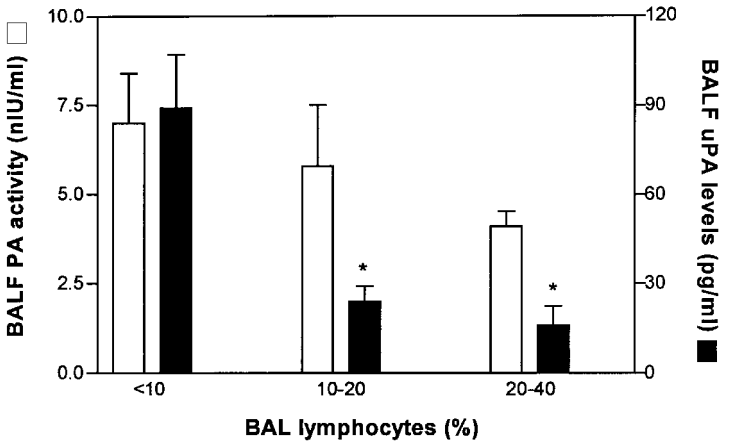

Figure 2. Relationship between plasminogen activator (PA) activity and urokinase (uPA) levels detected in unconcentrated bronchoalveolar lavage fluid (BALF) from sarcoidosis patients and the percentage of lymphocytes recovered by bronchoalveolar lavage (BAL). The results are expressed as means ± SEM, $n = 6$ for each range of lymphocytes. Multiple comparisons were assessed using ANOVA and the Kruskall–Wallis non-parametric test. Asterisk denotes significant difference (p " 0.01) compared with < 10 % lymphocyte group. No significant difference ($p = 0.3$) could be found when considering BALF PA activity parameter.

groups (5.65 ± 1.12 nIU ml^{-1} versus 5.7 ± 1.29, $p = 0.96$). Since, activated T-cells have been suggested to play a crucial role in orchestrating the inflammatory process associated with sarcoidosis, the increases of BAL lymphocytosis and of BAL $CD4^+/CD8^+$ ratio are considered as indicators of active granulomatous sarcoidosis. The reduction in uPA and PA activity levels observed in BALF from sarcoidosis patients appeared proportional to the BAL lymphocytosis (figure 2). In contrast, when considering the $CD4^+/CD8^+$ ratio (> 2.5 versus < 2.5, or > 3.5 versus < 3.5), non significant differences in BALF PA activity and uPA levels were observed.

Relationship with functional impairment

Functional parameters, namely TLC % and DL_{CO} % showed a positive association with BALF PA activity when considering sarcoidosis, silicotic and IPF patients together (figure 3). No significant association with TLC % or DL_{CO} % was found when considering each group separately. No correlation was found between TLC % ($r = -0.05$, $p = 0.71$) or DL_{CO} % ($r = -0.13$, $p = 0.43$) and total protein in BALF. No associations were found between VC %, FEV_1 % or FEV_1/VC and BALF PA activity.

Discussion

A crucial biological mechanism in fibrosis is the activation of fibroblasts and the subsequent excessive formation and deposition of collagen. In the lung, removal of alveolar fibrinous exudates, through plasminogen activation, is considered a decisive process in the pathogenesis of inflammatory lung disease because persistent fibrin deposits promote fibroblast ingrowth and lead to interstitial fibrosis.

Particular interest has been devoted to search for indicators of aberrant

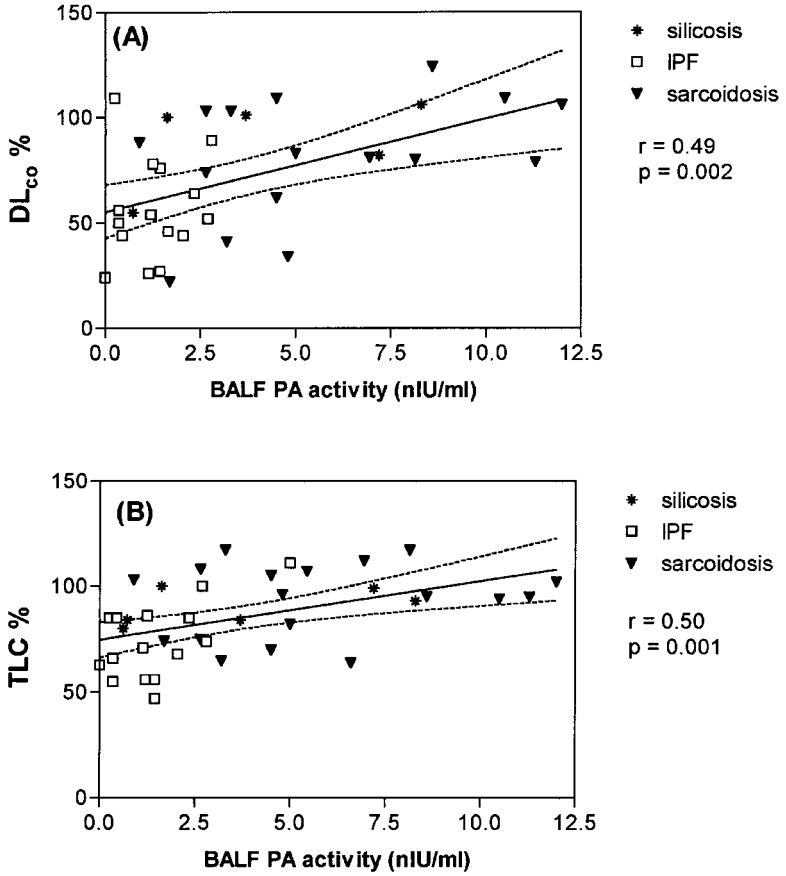

Figure 3. Relationship between plasminogen activator (PA) activity detected in bronchoalveolar fluid (BALF) and the lung respiratory function data of all sarcoidosis, silicosis and idiopathic pulmonary fibrosis patients. (A) Correlation with diffusing capacity of the lung to carbon monoxide (DL_{CO} %). (B) Correlation with total lung capacity (TLC %). DL_{CO} % values were not available for three silicosis, two sarcoidosis and one IPF patients. TLC % values were not available for two silicosis and one IPF patients.

extracellular repair process with the purpose of using them to assess or predict lung fibrosis. Reduction in soluble PA activity has been previously reported in BALF of patients with adult respiratory distress syndrome (ARDS), advanced asbestosis, pneumonia, chronic obstructive lung disease, lung cancer, advanced sarcoidosis and IPF diseases (Chapman *et al.* 1986, Cantin *et al.* 1989, Bertozzi *et al.* 1990, Nakstad *et al.* 1990, Kotani *et al.* 1995), suggesting that decreased BALF PA activity is a common manifestation in chronic lung disorders and conferring to BALF PA activity a potential merit as indicator of abnormal repair process. We confirm here these findings as sarcoidosis and IPF patients had significantly lower levels of PA activity than normal subjects. In addition, we report that silicosis with established fibrosis is also associated with a significant reduction in BALF PA activity. Lung fibrinolysis appears to mainly depend on a uPA component which is easily detected in all BALF samples whereas soluble tPA antigen was never detected either in normal subjects or in patients. The reduction of lung fibrinolysis noted in the three groups of patients appears to be related to a reduction in uPA protein and

associated with the presence of its specific inhibitors. Increased occurrence of detectable levels of PAI-1 and PAI-2 proteins in BALF from sarcoidosis, silicosis and especially IPF patients indicates that PAIs might be operative in limiting soluble PA activity in fibrotic lung diseases. Previous studies failed to evidence free PAI activity in the BALF of normal subjects and sarcoid patients (Chapman *et al.* 1986). Overexpression of PAI-1 antigen has previously been found in the BALF of IPF patients which however, contrary to our study, did not show reduced uPA levels (Kotani *et al.* 1995). Significantly increased PAI-2 antigen has previously been found in BAL cell lysate from IPF patients but without corresponding increase in BALF (Kotani *et al.* 1995) and only low levels of PAI-2 antigen were detected in ARDS BALF (Idell *et al.* 1989). Alternatively, the reduction in soluble uPA antigen could be due to alterations in uPA synthesis and/or secretion but could also be explained by the increased binding of uPA to specific cell surface receptors expressed locally. This latter possibility is supported by previous observations reporting increased expression of uPA activity in alveolar macrophages from IPF patients and in fibroblasts from IPF lung biopsy (Robinson 1988, Shetty *et al.* 1996).

Our results also suggest that the extent of reduction in PA activity reflects an advanced stage of the disease. We found good correlations between PA activity and the functional impairment as assessed by TLC % and DL_{CO} %, two advanced clinical features reflecting the development of fibrosis. In addition, reductions of uPA protein and PA activity levels were found to be more severe in the most evolutive fibrotic disease, namely IPF, compared with sarcoidosis and silicosis. Sarcoidosis subjects, mainly represented by non-fibrotic patients, showed no alterations of uPA protein levels and moderate reduction of BALF PA activity, which might reflect the much less fibrotic nature of sarcoidosis. Intermediate levels of uPA protein and PA activity were found in BALF of silicotic patients showing established fibrosis associated however with minimal impairment of lung function.

Cigarette smoking was suggested to act as a cofactor in the development of interstitial lung disease and was found to alter protease/antiprotease activities in emphysema (Janoff 1985). The smoking histories of sarcoidosis and the two other groups of ILD were indeed markedly different; however, several lines of evidence indicate that cigarette smoking could not explain the greater reduction of BALF PA activity noted in silicosis and IPF patients. Several studies demonstrated no alteration in BALF PA activity in smokers with (Reilly and Chapman 1988) and without (Bowen *et al.* 1985) lung dysfunction. In agreement with these findings, we did not observe a significant reduction in BALF PA activity in control smokers.

By contrast with sarcoidosis and silicosis patients, 37 % (6/16) of the IPF patients received corticosteroid treatment. Corticosteroids were found to inhibit many functions of alveolar macrophages including cytokines and metalloproteinases secretion (Shapiro *et al.* 1991). In human monocytes, dexamethasone was also found to lower both uPA activity (Hamilton *et al.* 1991) and basal PAI-2 levels (Hamilton *et al.* 1992) and to stimulate PAI-1 production (Hamilton *et al.* 1993), suggesting that corticosteroids given *in vivo* could reduce the fibrinolytic response of inflammatory macrophages and subsequently the BALF PA activity. However, dexamethasone was not found to suppress the PA activity associated with freshly isolated human alveolar macrophages (Bowen *et al.* 1985). In the present study, we also found no significant difference in BALF PA activity between corticosteroid-treated and non-treated IPF patients and BALF PAI-1 was detected in only one of the six treated patients.

Altogether, our findings do not support a potential downregulating effect of tobacco smoke or corticoid medication on BALF PA activity and suggest a relationship between disease severity and the intensity of lung fibrinolytic impairment. The follow-up of that simple marker could be of particular value with regard to sarcoidosis, a disease that shows spontaneous remissions in most cases, clinical stability in many, despite persistence of local inflammation and progressive deterioration in a minority of patients. Early diagnosed sarcoidosis patients showed increased level of BALF PA activity in a previous study (Sibille *et al.* 1990) while at later stages, as in the present study and others (Chapman *et al.* 1986), a decrease of PA activity was found.

Lung alveolitis and fibrosis are under the control of inflammatory cells, mainly alveolar macrophages able to release proinflammatory and profibrotic mediators but also several serine proteases including elastase, collagenase, cathepsin-L and uPA. In the present study, the BALF PA activity was significantly correlated with the percentage of alveolar macrophages indicating that the lung source of PA could largely be related to this cell type. The contribution of pneumocytes (Marshall *et al.* 1991) as additional sources of BALF PA activity can however not be excluded. The immunocompetent T lymphocytes appear to play an important role in modulating the influx and function of macrophages particularly in sarcoidosis where the accumulation of $CD4^+$ T lymphocytes in the lung is regarded as a distinctive immunologic feature (Newman *et al.* 1997). The number of lymphocytes and the ratio of $CD4^+/CD8^+$ in BALF are elevated in many patients with sarcoidosis, and have been proposed as monitors of disease activity. In the present study, active sarcoidosis patients, characterized by high BAL lymphocytes counts, showed also a greater reduction in BALF PA activity and uPA levels compared with the less active sarcoidosis group, which suggests a possible value of PA activity in terms of disease activity. The reduction in BALF PA activity in sarcoidosis might indicate a redistribution of soluble PA activity to the surface of activated lymphocytes. Indeed, uPAR has been recently identified as a pan T lymphocyte activation antigen (Nykjaer *et al.* 1994) and T-cells were found to bind extracellular uPA upon activation (Nykjaer *et al.* 1992). The physiological role of lymphocyte surface uPA activity has not yet been elucidated. Recent *in vitro* and *in vivo* observations indicated however that cell surface PA promotes lymphocyte migration (Kramer *et al.* 1994, Bianchi *et al.* 1996) which might play a role to infiltrate into sarcoid granulomatous lesions. Our results suggest therefore that the association found between lymphocytosis and PA activity most likely represents an epiphenomenom which might reflect the proportion of activated lymphocytes. The evaluation of the value of this marker in assessing sarcoidosis activity needs however further investigation.

To summarize, the impairment of soluble lung fibrinolytic activity observed in sarcoidosis, silicosis and IPF patients may contribute to the persistence of fibrin scaffolds promoting thereby fibroblast ingrowth. Reduction in BALF PA activity could be explained by a reduction of uPA protein while concomitant over-production of inhibitors cannot be excluded. Intense reduction in BALF PA activity was associated with advanced stages of the disease possibly reflecting the severity of the fibrotic process. Follow-up studies with serial determinations of BALF PA activity are however needed to better evaluate the diagnostic or prognostic merit of this parameter, particularly in sarcoidosis where reduced PA activity seems to parallel disease activity.

Acknowledgements

This study was supported by grant EV5V-CT94-0399 from the European Community (DGXII - Environment), CH and U from Lille and PHRC 1994.

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