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Phagocyte enzymes in bronchoalveolar lavage from patients with pulmonary sarcoidosis and collagen vascular disorders

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Phagocyte enzymes in bronchoalveolar lavage from patients with pulmonary sarcoidosis and collagen vascular disorders. Y. Sibille, J.B. Martinot, L.L. Polomski, B. Wallaert, M. Demusis, J.A. Rankin, C. Voisin, J.B.L. Gee.

ABSTRACT: The balance between proteases and antiproteases in the lower respiratory tract is believed to play a role in the outcome of interstitial lung diseases. In this cross-sectional study, we measure several phagocyte derived enzymes, namely plasminogen activator, neutrophil elastase and an ill-defined protease active on the trialanine chromophore substrate methoxysuccinyl-alanine$_3$-nitroanilide (SLAPN) in bronchoalveolar lavage (BAL) fluid from 42 patients with pulmonary sarcoidosis and from 43 patients with collagen vascular disease (CVD), 22 without lung disease (group I) and 21 associated with parenchymal lung disease (group II). The results show: a) that sarcoidosis is associated with increased plasminogen activator activity and with the presence of enzymatic activity against SLAPN corresponding at least in part to a metalloprotease; b) that CVD in the absence of radiographic lung disease is associated with an increase of plasminogen activator activity and increased levels of alpha$_1$-antiprotease-neutrophil elastase complexes; c) that the majority of untreated CVD (group I) patients have detectable levels of neutrophil elastase activity. These data show that patients with pulmonary sarcoidosis and CVD have different enzymatic profiles in their lower respiratory tract as assessed by BAL. Thus, sarcoidosis (mostly lymphocytic) is associated with enhanced macrophage-derived proteolytic activity in BAL, while CVD patients both with and without lung disease have increased neutrophil counts and neutrophil elastase complexed to alpha$_1$-protease inhibitor and presumably inactive in BAL. Finally, only BAL from untreated CVD patients with interstitial lung disease contain neutrophil elastase activity. This latter activity could contribute to the lung lesions frequently observed in these disorders.

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Impairment of the balance between proteases and antiproteases is believed to play a critical role in both acute and chronic lung injury [1, 2]. Proteolytic activity present in the lung has been related to adult respiratory distress syndrome (ARDS), emphysema and diffuse pulmonary fibrosis [3–5].

Pulmonary sarcoidosis and collagen vascular disorders (CVD) associated with interstitial lung disease represent two diseases with very different cellular mechanisms and clinical outcomes. Sarcoidosis is characterized by heightened immunocellular activity affecting both alveolar macrophages and lymphocytes (largely helper-inducer T cells) but rarely leads to lung fibrosis [6]. In contrast, CVDs involving the lung are frequently characterized by an increase in the macrophage population associated with either neutrophilia, lymphocytosis, or a combination of both [7–9]. Furthermore, CVD commonly leads to lung fibrosis [10].

Because proteolytic activity in the lower respiratory tract may contribute to architectural changes in the lungs and given the often unpredictable clinical course of interstitial lung diseases (ILD), we decided to determine whether the proteolytic burden in the lower respiratory tract could vary among different types of ILD. To address this issue, we measured several polymorphonuclear neutrophil (PMN) and macrophage-derived proteases including plasminogen activator and PMN elastase in bronchoalveolar lavage (BAL) from patients with pulmonary sarcoidosis, CVD patients with ILD and CVD patients without ILD.

Methods

Study population

Nineteen normal volunteers, 42 patients with pulmonary sarcoidosis and 43 patients with collagen vascular disease were included in the present study. All were lifelong nonsmokers. Normal values were similar in the

three referral centres. The patients were referred to the University Hospital of Mont-Godinne, University of Louvain, Belgium, or to the University Hospital A. Calmette and Hôpital Régional, Lille, France or to the Yale University School of Medicine, New Haven, USA. The group of biopsy proven pulmonary sarcoidosis was further divided empirically into two subgroups based on BAL lymphocyte counts, namely lymphocytic sarcoidosis (LS) with BAL lymphocyte counts >15%, and nonlymphocytic sarcoidosis (NLS) with lymphocyte <15% in order to separate patients between respectively high and low intensity alveolitis as previously suggested [11]. In our hands, the 15% has been reported as a reasonable cut-off [12]. None of the patients had received steroids for at least one year. Three patients had previously been treated with steroids. Sarcoidosis groups LS and NLS, respectively, comprised 14 and 4 with hilar adenopathy alone, 7 and 7 with hilar adenopathy and also parenchymal infiltrates and 7 and 3 with infiltrates alone. All but one patients of those groups had normal lung function, *i.e.* no value of static or dynamic lung volumes or diffusing capacity of <80% predicted. The one abnormal LS sarcoid showed 60% predicted values for total lung capacity (TLC), forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and pulmonary carbon monoxide diffusing capacity (DLCO).

The CVD group totalled 43 persons and their features are shown in table 1. The diagnoses are based on previously described criteria [9].

Table 1. – Collagen vascular disorders

	Group I	Group II		Total
		Untreated	Treated	
Sjögren's syndrome	8	0	0	8
Rheumatoid arthritis	5	3	2	10
Scleroderma	6	7	4	17
Dermatomyositis	2	1	2	5
Lupus erythematosis	1	2	0	3

Group I: all had normal pulmonary function tests and normal chest X-rays. None had been treated with steroid. Group II showed parenchymal radiologic infiltrates. In group II, the untreated and treated groups, respectively, showed mean and ranges of % predicted values of TLC of 82% (50–105%) and 69% (47–90%), of FEV_1 of 76% (42–102%) and 71% (52–93%) and also of DLCO of 66% (39–100%) and 66% (32–109%). There were no significant differences between these function tests in the two subgroups of collagen vascular disorders group II. Therapy comprised prednisone (30–40 mg daily) in all treated patients, one of whom also received cyclophosphamide.

Bronchoalveolar lavage

Bronchoalveolar lavage was performed in all normals and patients following the same standardized protocol using an Olympus BF-B3 Model fibreoptic bronchoscope [13, 14]. Briefly, after anaesthesia of the upper airways with lidocaine, the bronchoscope was wedged in a subsegmental bronchus and 5×50 ml aliquots of sterile 0.9% saline were instilled and then aspirated. Except for half of the normals, the first aliquot was discarded. This had no effect on protein and enzyme measurements. Further analyses were performed on the pooled 4 or 5 aliquots. Total cell and differential counts (on Cytospin preparations) were performed prior to a 900 g centrifugation to separate the cell pellet from the supernatant. In half of the normal volunteers, cell counts and cell differentials were performed after the 900 g centrifugation on the cell pellet. Part of the supernatant was concentrated using an Amicon membrane (10,000 mw cut-off). Lavage recovery volumes and volumes after concentration were recorded and their ratio used to correct the measurements of materials performed on concentrated lavage fluid to those in the initial BAL fluid.

Albumin, $alpha_1$-protease inhibitor ($Alpha_1PI$) and $alpha_2$-macroglobulin ($Alpha_2M$) were measured in unconcentrated BAL using an immunoradiometric assay (IRMA) with a sensitivity in the nanogram range and in serum by immunonephelometry [14, 15]. Data were expressed per ml of unconcentrated lavage.

Enzyme assays

Neutrophil elastase (NE) in the concentrated BAL was assayed by following the hydrolysis of a specific substrate, MeOSuc-Ala_2-Pro-Val-7-Amino-4-Methyl Coumarin (AMC) (Enzyme Systems Products, Livermore, CA) as described previously [16, 17]. Samples of 100 µl of concentrated BAL were added to 1 ml of buffer (0.05 M Tris at pH 7.5, with 0.5 M NaCl, 0.1 M $CaCl_2$ and 10% DMSO) containing 0.1 M of the AMC substrate. The fluorescence of the mixture was followed using an excitation wavelength of 370 nm and emission wavelength of 460 nm on an Aminco-Bowman spectrofluorometer.

Plasminogen activator (PA) activity was also measured on concentrated BAL according to SAKSELA [18]. Agarose plates were prepared with a mixture of 34.4 ml of 1.25% agarose in 0.1 M Tris HCl pH 8.0, 4.3 ml of 6% casein in 1% sodium azide and 4.3 ml of 0.1 M Tris HCl pH 8.0 with or without (for control) plasminogen at 0.3 $U \cdot ml^{-1}$. Sample wells (3.5 mm diameter) were punched into the gel. 10 µl of the concentrated BAL were loaded into the wells (each lavage sample being run in duplicate) and the plates were incubated for 24 h at 37°C. The diameters of the clear areas were measured and data expressed in units of plasminogen activator according to a standard curve. The lower detectable level of enzyme was 0.01 $U \cdot ml^{-1}$. The specificity of the assay for PA is conferred by the use of plasminogen.

The hydrolysis of succinyl-$alanine_3$-nitroanilide (SLAPN) was assayed at 410 nm using a spectrophotometer as initially described by BIETH and WERMUTH [19] and detailed by NIEDERMAN *et al.* [20].

The effect of various inhibitors (table 2) on the activity against SLAPN present in BAL from 7 sarcoidosis patients with high BAL lymphocytosis (mean lymphocyte percentage: 37.6) was tested by mixing the inhibitor solutions in buffer at appropriate concentrations with the BAL sample for 30 min at room temperature prior to

– Inhibition studies on SLAPN activity in BAL*

	EDTA	PMN elastase° inhibitor	Cathepsin L+ inhibitor	PMSF
	5 mM	10 μM	10 μM	5mM
	72.6* (n=7)	10.3 (n=4)	12.6 (n=5)	12.7 (n=4)

* represent the mean percentage of inhibition observed in ce of the respective inhibitors, compared to the values in the absence of the inhibitor; n: number of BAL tested; °: PMN elatase inhibitor: (Meo-Suc-Ala$_2$-Pro-CH$_2$Cl); +: Cathepsin L inhibitor: (Z-Phe-Ala-Pro-Val-CH$_2$Cl); * $p<0.05$ compared to control by paired t-test.

SLAPN assay. The same volume of buffer was added to control. All inhibitors were also tested alone in the SLAPN assay and showed no interference.

Immunoreactive neutrophil elastase-Alpha$_1$PI complexes were measured by ELISA purchased from Merck (Darmstadt, Germany) [21]. This procedure employs antibodies specific to neutrophil elastase (NE) coated to a plastic tube. After BAL samples were incubated with this antibody, the tubes were washed, leaving NE bound to the tube walls. Enzyme-labelled (alkaline phosphatase) antibody specific for the Alpha$_1$PI was then added. After these bound to the NE-Alpha$_1$PI complex, the excess labelled antibody was washed off and the remaining NE-Alpha$_1$PI complex was assayed with 4-nitrophenol phosphate.

Statistics were performed by the NIH sponsored Clinfo system using the Wilcoxon Rank Sum Test (two-tailed) and Spearman Rank coefficients since much of the data showed different group variances and some were not normally distributed. Values of $p \leq 0.05$ were considered significant. Data are expressed as mean±SD.

Results

BAL cell composition

The cellular composition in the BAL fluids of normal subjects and patients with the two disorders are shown in table 3. BAL from sarcoidosis patients as a group were characterized by increased total cell and lymphocyte counts and by normal neutrophil (PMN) and eosinophil counts. CVD patients from group I and group II had elevated lymphocytes and PMN counts in BAL but total cell counts were only increased in group II (with lung disease). Eosinophils were only elevated in BAL from untreated group II CVD patients.

BAL enzyme constituents

The data on enzyme activities are expressed per ml of recovered BAL fluid. Since the initial and recovered lavage fluid volumes were no different in these groups, the data directly represents the quantity of these constituents recovered.

Table 3 – Cellular components in BAL

	Age	Total cells*	% Mac	% Ly	% PMN	% Eos
Normals (n=19)***	33.7±7.8**	12.4±3.8	91.2±7.0	8.2±6.7	0.5±0.6	0.1±0.2
Active sarcoid (n=28)	36.6±13.5	43.1±57.3	70.5±10.6	28.0±10.2	0.6±0.8	0.4±0.7
Inactive sarcoid (n=4)	44.2±15.0	22.5±12.5	91.7±3.8	7.6±3.8	0.6±0.8	0.1±0.2
Collagen vascular disorder (n=43)	49.0±15.0	25.0±26.2	72.7±17.0	18.2±16.0	7.4±8.8	1.6±3.4
Group I	46.6±15.9	18.6±27.6	75.9±14.5	19.3±13.8	4.4±3.7	0.4±0.8
Group II	48.7±16.0	32.1±19.3	71.5±16.5	17.8±18.2	8.6±6.4	2.1±2.4
Treated (n=8)	53.9±10.7	28.9±24.3	79.1±8.7	13.9±10.4	6.3±4.9	0.8±1.4
Untreated (n=13)	49.2±18.3	34.2±16.1	66.5±18.8	20.4±22.1	10.2±7.0	2.9±2.6

* Total cells × 10·ml^{-1} recovered BAL. Instilled lavage volume and return was the same in all groups; **: mean±SD; ***: n= number of subjects, measurements that were obtained on all subjects in each group; Mac: macrophages; Ly: lymphocytes; PMN: polymorphonuclear neutrophils; Eos: eosinophils.

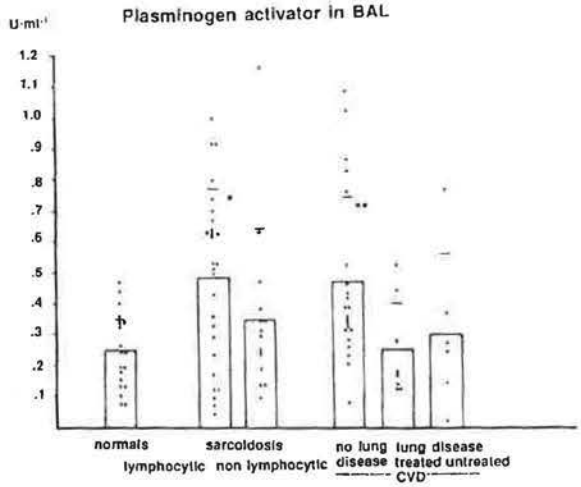

Fig. 1. – Plasminogen activator levels (U·ml⁻¹) in BAL. From left to right: normals (n=19); lymphocytic (BAL lymphocytosis >15%) (n=27) and non-lymphocytic (n=14) sarcoidosis patients and collagen vascular disease patients (CVD). The CVD group is subdivided into patients without lung disease (group I) (n=22) and patients with lung disease (group II). Group II includes treated (n=8) and untreated patients (n=6). Columns represent means, bars standard deviations. *: p<0.01; **: p<0.03 when compared to normals.

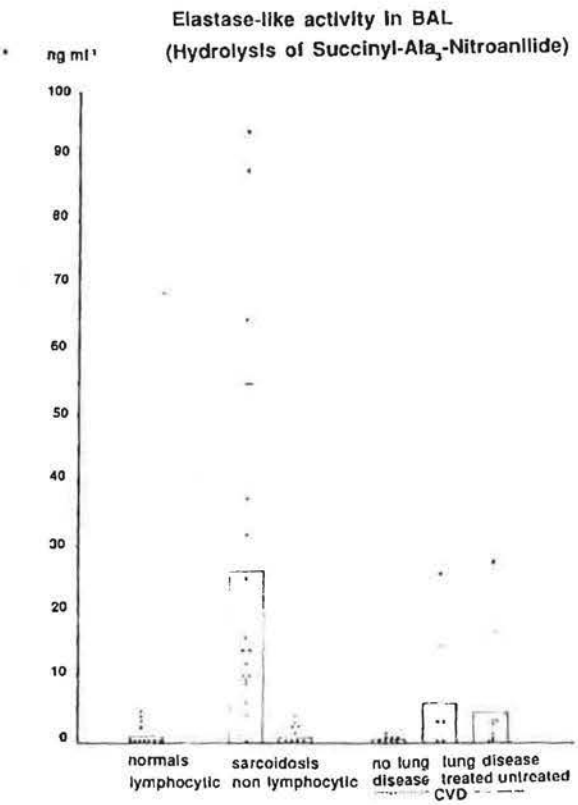

Fig. 2. – Elastase-like activity in BAL measured by hydrolysis of SLAPN substrate in the same groups as in figure 1. Normals (n=11), lymphocytic sarcoidosis (n=17), non-lymphocytic sarcoidosis (n=9), CVD group I (n=8) and treated (n=5) and untreated (n=8) CVD group II patients. Columns are means and bars standard deviations. *: p<0.001 compared to all other groups.

Plasminogen activator (PA) activity in BAL was significantly increased in the sarcoidosis patients as a group (mean±SD: 0.44±0.28 U·ml⁻¹) compared to the normal group (0.24±0.12), (p<0.01). However, no correlation was observed between BAL lymphocytosis and PA activity. When the sarcoidosis patients were subdivided into two groups according to the BAL lymphocytosis, only the group with high BAL lymphocyte counts (over 15%) had significantly higher PA values (0.49±0.28) (p<0.01) as shown in figure 1. In addition, PA activity was increased in BAL from CVD group I (0.46±0.27) (p<0.01).

The activity against SLAPN was significantly increased in BAL from sarcoidosis patients as one group (mean ±SD: 17.5±26.1) compared to normals (1.3±1.8) (p<0.05). This increase was solely due to the patients with BAL lymphocytosis >15% (26.1±29.0) as illustrated in figure 2. In CVD, none of the groups show statistically significant differences from normal subjects, and only two patients exhibited values above the normal range. Inhibition studies performed on BAL from sarcoidosis patients containing proteolytic activity against SLAPN demonstrated significant inhibition (72.6%) with the metal chelator EDTA and minimal inhibition with inhibitors of serine proteases (table 2).

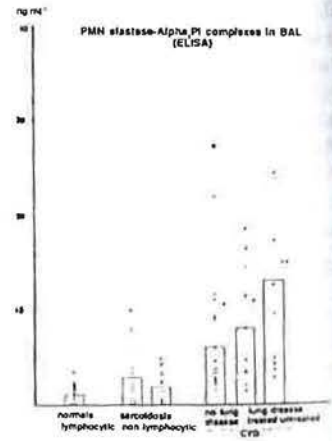

Fig. 3. – Neutrophil elastase activity against Meo-Suc-Ala$_3$-Pro-Val AMC substrate in BAL. Groups similar to figures 1 and 2: Normals (n=19), lymphocytic sarcoidosis (n=25), non-lymphocytic sarcoidosis (n=14), CVD group I (n=22) and treated (n=7) and untreated (n=12) CVD group II patients. Values are significantly elevated in the untreated CVD group II patients compared to the other groups (p<0.01).

Neutrophil elastase activities against AMC show a very different pattern to those observed with SLAPN. There was essentially no AMC activity in the normal subjects and the sarcoid groups. However, in the CVD groups, detectable levels were present in lavages from 8–12 untreated group II patients, yielding an average activity of 1.5±1.3 ng·ml⁻¹ (fig. 3). Among treated group II CVD subjects, only 1 of 8 yielded such activity. This distribution is significantly different by Chi-square, p<0.05. In group I patients, activity was only detected in 1 of 21 lavages with a value of 6.8 ng·ml⁻¹ for inapparent reasons. Thus, untreated patients in CVD group II alone are associated with NE activity.

(fig. 4). By contrast, the CVD disorders show statistically significant higher levels. The combined groups I and II of CVD showed eight-fold increases ($p<0.001$) over the normal subjects. Even those CVD without radiologic or functional lung disease (group I) showed a four-fold increase ($p<0.05$), and in group II the elevated levels are more apparent in the untreated patients, as a group, ($p<0.01$ for 3 comparisons).

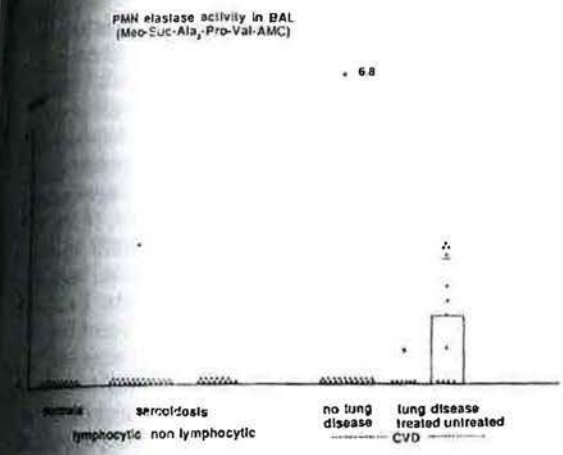

Fig. 4. – Neutrophil elastase-alpha$_1$PI complex levels in BAL (ELISA). All groups are similar to those in figures 1–3: normals (n=11), lymphocytic sarcoidosis (n=13), non-lymphocytic sarcoidosis (n=7), CVD group I (n=14) and treated (n=8) and untreated (n=8) CVD group II patients *: $p<0.05$; **: $p<0.01$ when compared to normals.

Albumin levels

The well described [14] increase in lavage albumin concentrations in LS sarcoid ($p<0.001$) but not NLS sarcoid are apparent (table 4): more interestingly, this is also apparent in all CVD disorders and in both groups I and II considered separately. The albumin concentrations in steroid treated group II CVD patients were significantly lower when directly compared with the untreated group II patients ($p<0.01$).

Antiproteases

These data are presented in table 4 as concentrations in lavage fluid rather than in coefficient of excretions relative to albumin (RCE), the form used previously [13] to attempt to correct for blood to lavage protein leak. This is appropriate since we are considering the protease antiprotease system in absolute terms. Thus, expressed in $\mu g \cdot ml^{-1}$, both Alpha$_1$PI and Alpha$_2$M are significantly increased in LS sarcoid ($p<0.001$) but not NLS sarcoid.

Table 4. – Albumin and anti-protease levels in BAL

	Albumin	Alpha$_2$M	Alpha$_1$PI
Normals (n=17)	26.0±19.9	0.04±0.03	1.55±1.25
Sarcoid LS (n=26)	90.0±94.0	1.20±1.55	6.60±5.43
Sarcoid NLS (n=14)	31.0±16.7	0.08±0.08	1.25±1.23
Collagen Vascular Disorders	46.2±35.8	0.80±1.50	3.70±4.10
Group I (n=21)	37.9±21.2	0.30±0.85	2.57±3.44
Group II (n=17)	54.9±44.8	1.44±1.84	5.02±4.54
Treated (n=5)	36.4±25.6	0.63±0.47	2.51±3.52
Untreated (n=12)	67.2±51.4	1.78±2.11	6.07±4.62

Data expressed as $\mu g \cdot ml^{-1}$ unconcentrated lavage (mean±SD)

Neutrophil Elastase-Alpha$_1$PI Complex measurement was introduced at a later stage in this study; therefore, not all the BAL materials were examined. However, normal subjects and both groups of sarcoidosis (despite two patients with increased levels) showed similar low levels of these complexes in the 1–2.5 $ng \cdot ml^{-1}$ range

In group I CVD there is a marginal and nonsignificant elevation in Alpha$_1$ PI but a significant increase occurred in Alpha$_2$M ($p<0.05$). However, group II CVD show three-fold rises in both antiprotease concentrations compared to group I ($p<0.05$). Moreover, in the group II CVD, both antiprotease BAL levels in the steroid treated

patients are at least half the levels found in the untreated patients ($p<0.05$ for both antiproteases).

Discussion

This study evaluates the presence of different phagocyte-derived enzymes in BAL from patients with interstitial lung disease and compares enzymatic measurements with the increases of the antiprotease levels reported in these disorders [12, 22].

Firstly, we measured the activity of plasminogen activator (PA) in BAL. This enzyme has been implicated in matrix degradation by human alveolar macrophages *in vitro* [23]. We have shown that PA was increased in BAL from sarcoidosis patients (mostly the ones with high BAL lymphocytosis) and group I CVD. Others [24] have reported diminished BAL PA levels in sarcoidosis and related this decrease in PA activity to an excess of PA inhibitor in sarcoidosis patient BAL fluid [25]. However, we generally studied recently diagnosed cases of pulmonary sarcoidosis, only one of which showed functional defects, while half the patients in the other study [24] had a disease duration of two years or more and 6 out of 14 had decreased FVC. Therefore, the apparent conflict in results may be related to patient selection, and reflects an increase in BAL PA activity early in disease with an association of increased levels of PA inhibitor(s) in chronic disease. This is supported by a recent study using an asbestos sheep model where acute inflammation was associated with high PA levels while chronic and fibrotic disease was associated with diminished PA levels in the lungs [26].

The assays employing SLAPN, AMC and NE-$Alpha_1$PI complexes should be considered together and also in relation to the anti-protease measurements. While NE is active against both SLAPN and AMC, there has been considerable debate concerning the significance of SLAPN activity as a measure of free elastase activity [21, 27]. Our sarcoidosis data show major SLAPN activity totally unmatched by either AMC activity or NE-$Alpha_1$PI.

This, along with the virtual absence of neutrophils in both sarcoid groups and normal nonsmoking subjects, strengthens the argument that SLAPN activity is not neutrophil-, but rather macrophage-derived, a view supported by previous studies [20, 27, 28]. The inhibition profile of SLAPN activity (inhibited by EDTA but not by $Alpha_1$PI) suggests the presence of a metalloenzyme. Furthermore, there is an indication that the activity against SLAPN is not due to either free or complex forms of NE. A recent study suggests the activity against SLAPN reflects an endopeptidase capable of elastinolytic activity in co-operation with a metalloenzyme [29]. Thus, the nature and potential role of these enzymes is obscure in sarcoidosis. However, there is no compelling evidence that they alone attack native insoluble elastin *in vivo*.

A recent report [29] described enhanced activity against SLAPN in BAL cells of non-fibrotic sarcoid patient's. Since SLAPN activity has also been reported to be increased in the lavage fluid from smokers [20], it is likely that enhanced activity against SLAPN reflects heightened macrophage activity by either immunologic mechanisms in sarcoidosis or by smoke-related mechanisms.

Activity against AMC, generally accepted as a relatively specific substrate for NE, is absent in the BAL fluid from nonsmoking controls and non-fibrotic sarcoidosis groups, confirming previous data in normal nonsmoking subjects [28]. In CVD, the NE activity is essentially present in only 8 of 12 untreated patients with lung disease as opposed to only two of the remaining 28 patients in groups I and II. This presumed neutrophil elastase activity is paradoxically associated with an increase of immunoreactive $Alpha_2$M levels. Assuming this $Alpha_2$M can still complex to NE, such complexes in the lungs may remain active against low molecular weight substrates, as previously reported in animals [30]. Whether such complexes are active *in vivo* against matrix components is, however, debatable [31].

When an immunologic assay was used to detect NE-$Alpha_1$PI complexes, most subjects, including normal nonsmokers, had detectable levels. Except for two individuals, sarcoidosis patients had complex levels similar to normals. In contrast, all groups of CVD patients exhibited an increase in NE-$Alpha_1$PI complexes with no difference among the subgroups, suggesting most of these patients, with the possible exception of the untreated group II patients, had NE complexed to $Alpha_1$PI and, therefore, present in an inactive form *in vivo*. This also suggests there is heightened activity of elastase and antielastase ($Alpha_1$PI and $Alpha_2$M) systems in the lung of CVD patients.

The balance between matrix degradation and collagen deposition is believed to be critical in the pathogenesis of fibrotic processes. Lung fibrosis is often associated with neutrophil infiltration of the interstitium and alveoli, and thus a persistent neutrophil derived proteolytic activity could contribute to tissue injury and indirect fibrosis [2, 32, 33]. On this basis, others found increased collagenase activity in BAL from idiopathic pulmonary fibrosis (IPF) and rheumatoid arthritis patients [34, 35]. Our data show that NE activity is increased in group II CVD with lung disease characteristically fibrotic in nature. In contrast, the heightened anti-protease levels (both $Alpha_1$PI and $Alpha_2$M) and the undetectable NE activity against AMC in sarcoidosis, particularly the LS group, shows the dominance of anti-protease activities in this "active sarcoid" group. This feature may be pathologically significant for the much lower risk of fibrosis in sarcoidosis.

Steroid treatment has been reported to have little effect on BAL PMN counts in IPF patients [36–38]. In a longitudinal study of CVD patients with ILD, we observed a decrease of the BAL PMN percentage without improvement in lung function tests [22]. In the present cross-sectional study of nonsmoking patients, steroid treated CVD patients with ILD only diminished their total as opposed to their percentage of PMN in BAL when compared to untreated patients with ILD. We also observed that PMN elastase activity in BAL was abolished in patients under corticosteroid treatment. This

...ation, together with a smaller albumin leak in the ...space and a decrease in BAL eosinophilia, ...reduced inflammation in the lower respiratory ... Although we did not observe significant differences ...clinical, radiological or functional data between the ...treated and untreated CVD patients, a longitudi... study is necessary to demonstrate that the observed ...are due to steroid treatment alone. However, in ...of these changes, steroid treatment generally does ...influence clinically the fibrotic processes in the lungs. ...did not detect lung functional differences in these ...subgroups of group II CVD.

We have shown that: a) sarcoidosis in the absence of ...fibrosis is characterized by the enhanced activity of ...macrophage derived enzymes, PA and an ...characterized metalloprotease active against a trialan... chromophore substrate, in addition to enhanced ...-protease levels; b) CVDs are associated with modest ...ophilia and minor eosinophilia in BAL along with ...anced levels of the elastase-antiprotease system, ...ticularly where CVD is associated with restrictive lung ...ease; c) only untreated CVD with lung involvement is ...ociated with detectable PMN elastase activity in BAL. ...This suggests an inappropriate antielastase response in ...the lower respiratory tract of these untreated patients with ...interstitial lung disease and could contribute to the lung ...damage observed in these disorders.

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Les enzymes phagocytaires dans les lavages broncho-alvéolaires de patients atteints de sarcoïdose pulmonaire et de collagénose
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RÉSUMÉ: L'équilibre entre les protéases et les anti-protéases du tractus respiratoire inférieur se voit attribuer un rôle dans le développement des maladies interstitielles du poumon. Dans cette étude transversale, nous avons mesuré divers enzymes d'origine phagocytaire, notamment l'activateur du plasminogène, l'élastase neutrophilique, et une protéase mal définie active sur un substrat de trialamine chromophore (SLAPN), dans le liquide de lavage broncho-alvéolaire chez 42 patients atteints de sarcoïdose pulmonaire, et chez 43 patients atteints de collagénose (CVD), chez 22 sujets sans atteinte pulmonaire (groupe I) et chez 21 sujets atteints de maladie parenchymateuse pulmonaire (group II). Les observations montrent: a) que la sarcoïdose est associée à une activité accrue de l'activateur du plasminogène et à la présence d'une activité enzymatique contre SLAPN, correspondant au moins en partie à une métallo-protéase; b) que CVD, en l'absence d'anomalie radiographique du poumon, est associée à une augmentation de l'activité de l'activateur du plasminogène et de niveaux accrus des complexes élastasiques alpha$_1$ antiprotéase-neutrophiles; c) que la majorité des CVD non traitées (groupe II) ont des niveaux détectables d'activité élastasique des neutrophiles. Ces observations montrent que les patients atteints de sarcoïdose pulmonaire et de CVD ont des profils enzymatiques différents au niveau du tractus respiratoire inférieur lorsqu'on les soumet au lavage broncho-alvéolaire. Donc, la sarcoïdose, en particulier sa forme lymphocytaire, est associée à une activité protéolytique d'origine macrophagique accrue dans le lavage alvéolaire, alors que les patients CVD, avec ou sans maladie pulmonaire, ont des décomptes de neutrophiles augmentés et de l'elastase neutrophilique complexée à l'inhibiteur de l'alpha$_1$ anti-protéase, et probablement inactifs dans le lavage broncho-alvéolaire. Finalement, seul le lavage des patients non traités, atteints de CVD avec maladie pulmonaire interstitielle, contient une activité élastasique neutrophilique. Cette dernière activité pourrait contribuer au développement des lésions pulmonaires fréquemment observées dans ces affections.
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