

## Serum Clara cell protein (CC16), a marker of the integrity of the air-blood barrier in sarcoidosis

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*Serum Clara cell protein (CC16), a marker of the integrity of the air-blood barrier in sarcoidosis. C. Hermans, M. Petrek, V. Kolek, B. Weynand, T. Pieters, M. Lambert, A. Bernard. ©ERS Journals Ltd 2001.*

**ABSTRACT:** To test the hypothesis that sarcoidosis is associated with an intravascular leakage of lung epithelium secretory proteins, the occurrence and determinants in serum of sarcoid patients of CC16, a small size and readily diffusible lung-specific protein of 16 kDa secreted by bronchiolar Clara cells, was investigated.

CC16 was measured by a sensitive latex immunoassay in the serum of 117 patients with established sarcoidosis and of 117 healthy subjects matched for age, sex and smoking status. Stepwise regression analysis was used to identify extrapulmonary variables of CC16 changes in serum. These changes were then compared with biochemical and cellular parameters in bronchoalveolar lavage fluid (BALF) as well as with the number of CC16 immunostaining cells on bronchial or pulmonary biopsy samples.

CC16 concentration in serum of sarcoid patients was significantly increased, compared to their matched controls ( $25.9 \pm 16.2$  versus  $13.9 \pm 5.2 \mu\text{g}\cdot\text{L}^{-1}$ ). In nonsmoking patients without significant renal impairment, CC16 in serum increased with the severity of the chest radiograph and computed tomography changes, and was on average 50–100% higher when parenchymal involvement was present. Sarcoid patients had, however, normal levels of CC16 in BALF and an unchanged number of CC16-immunopositive cells in lung biopsy samples, suggesting that an increased secretion of CC16 in the sarcoid lung is very unlikely, and that the elevation of CC16 in sarcoidosis results from an increased intravascular leakage of the protein across the air-blood barrier.

The present study suggests that CC16 in serum might provide a useful tool to noninvasively evaluate the damage and increased permeability to proteins of the air-blood barrier associated with sarcoidosis.

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Pulmonary sarcoidosis is associated with the accumulation and activation of immune and inflammatory cells within the lung [1, 2]. These cells may damage the pulmonary tissue, in particular the alveolar-capillary barrier, leading to a decrease of the lung function and/or a loss of the gas-exchange capacity. Another consequence of the alteration of the air-blood barrier integrity is its increased permeability to solutes and proteins [3, 4] which is commonly estimated by the elevation of plasma proteins such as albumin in bronchoalveolar lavage fluid (BALF) [4–10].

According to recent human and experimental studies, the integrity of the air-blood barrier and its permeability to macromolecules might be assessed less invasively by the quantification, in serum, of

lung-specific secretory proteins, also referred to as "pneumoproteins" [11, 12]. One of these proteins is the Clara cell secretory protein, a low-molecular-weight protein of 16 kDa (CC16), secreted in large amounts into the lumen of the respiratory tract by nonciliated bronchiolar Clara cells [13, 14]. Following its passage into the bloodstream across the air-blood barrier, CC16 is rapidly eliminated by glomerular filtration [15]. Serum CC16 has been shown to increase in several situations known to be associated with a disruption of the air-blood barrier, such as pulmonary fibrosis [16] or lung injury caused by lung irritants such as firesmoke [17] and ozone [18]. By contrast, when the barrier is intact, or only slightly compromised, the concentration of CC16 in serum has been shown to be a reflection of the number of intact

Clara cells, as suggested by the diminution of CC16 in serum with tobacco smoking, a condition where the number of Clara cells and the level of CC16 in BALF are markedly decreased [14, 19–21].

The aim of this study was to determine whether pulmonary sarcoidosis is associated with an increased intravascular leakage of CC16. Whether the serum level of CC16 may be related to the degree of lung involvement in sarcoidosis as assessed by radiological staging, and biochemical markers in BALF and serum was also investigated.

### Patients and methods

One-hundred and seventeen patients with sarcoidosis were recruited from the Dept of Medicine of the Cliniques Universitaires Saint-Luc, Brussels, Belgium and from the Respiratory and Immunology Units of the Palacky University Hospital, Olomouc, Czech Republic. All patients had chest radiographs and/or clinical presentation compatible with sarcoidosis, pathological findings of noncaseating epithelioid cell granulomas on bronchial or pulmonary biopsy, absence of mycobacterial infection and of significant environmental exposure to agents known to induce granulomatous lung disease. Most patients had recent onset sarcoidosis. Ten patients were on anti-inflammatory therapy at time of inclusion (non-steroidal anti-inflammatory drugs or corticosteroids). The control group consisted of 117 healthy blood donors individually matched with sarcoid patients according to age ( $\pm 2$  yrs), sex and tobacco smoking status.

The patients provided a sample of venous peripheral blood, taken within 24 h of bronchoalveolar lavage (BAL), for determination of serum CC16, angiotensin converting enzyme (ACE), lysozyme and creatinine. For a subgroup of 71 nonsmoking sarcoid patients without renal impairment and seven non-smoking control subjects, BALF was available to measure CC16 and two plasma-derived proteins, albumin and retinol-binding protein (RBP; a low-molecular-weight protein of 21 kDa secreted by the liver [22]), as well as to assess the severity of the alveolitis on the basis of the percentage of lymphocytes and the value of the CD4+/CD8+ ratio. BAL was classically performed by infusing three successive 50-mL aliquots of 0.9% saline which were immediately aspirated by gentle suction. The first aliquot was considered representative of a bronchial wash and was not used. The two subsequent aliquots were pooled and used for cell population determinations, CC16 measurement and biochemical analyses. All patients had a chest radiograph which was interpreted by a radiologist not aware of the results of the other investigations and categorized according to the standard classification: normal chest radiograph appearance (Stage 0), hilar lymphadenopathy without parenchymal involvement (Stage I), hilar lymphadenopathy with parenchymal lesions (Stage II) and parenchymal involvement without hilar lymphadenopathy (Stage III). Chest computed tomography (CT) scan results, available for a subgroup of 59 patients,

were classified as follows: absence of changes, mediastinal lymph nodes either isolated or associated with parenchymal abnormalities, parenchymal changes only. Sarcoid patients and healthy volunteers were enrolled following ethical approval. Informed consents were obtained before BAL and blood sampling.

### CC16 assay

The concentration of CC16 in serum and BALF supernatant was determined by a sensitive immunoassay relying on the agglutination of latex particles. A detailed description of this immunoassay has been previously published in its application to urinary CC16 [23]. The assay uses the rabbit anti-protein 1 antibody from Dakopatts (Glostrup, Denmark) and as standard the protein purified in the laboratory. To avoid possible interferences by complement, rheumatoid factor or chylomicrons, sera were pretreated by heating at 56°C for 30 min and by the addition of polyethylene glycol (16%, vol/vol, 1/1) and trichloroacetic acid (10%, vol/vol, 1/40). After overnight precipitation at 4°C, the serum samples were centrifuged (3,000×g for 10 min) and CC16 was determined in the supernatants. All samples were analysed in duplicate at two different dilutions. The validity and the analytical performances of the CC16 latex immunoassay (LIA) in different biological media have been reported previously [14, 23]. Briefly, applied to serum, this assay has a detection limit of 0.5  $\mu\text{g}\cdot\text{L}^{-1}$  and an average analytical recovery of 95%. The within- and between-run coefficients of variation range from 5–10%. CC16 concentrations in serum and BALF are in good agreement with those obtained with a monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) kit recently developed by Pharmacia [20].

### Other assays

Serum ACE activity was measured by a colorimetric method using hippuryl-L-histidyl-L-leucine as substrate [24, 25]. Lysozyme was measured by a *micrococcus lysodeikticus* lytic assay [26]. Creatinine was measured by Jaffe's method. Lavage cell differentials were determined by counting a minimum of 400 cells in a cytocentrifuge preparation. The surface phenotype of bronchoalveolar lymphocytes was determined by indirect immunofluorescence using anti-CD4 and anti-CD8 monoclonal antibodies. Albumin and RBP were determined on the BALF supernatant by latex immunoassay, as previously reported, and were expressed per L of lavage fluid [27].

### CC16 immunohistochemistry

Bronchial biopsy specimens were obtained from 13 subjects without pulmonary sarcoidosis (seven non-smokers and six smokers) and 10 nonsmoking sarcoid patients. Pulmonary biopsy specimens were obtained from six autopsied nonsmoking subjects who died

Table 1. – Characteristics of the study population

Characteristics	Controls		Sarcoid patients		p-Value	
	Nonsmokers	Smokers	Nonsmokers	Smokers	S vs NS*	C vs D <sup>#</sup>
Subjects n	96	21	96	21		
Age yrs	45.2±11.8 (21–70)	36.3±9.1 (22–58)	45.5±12.0 (20–80)	34.6±7.3 (25–51)	0.0005	0.99
Sex M:F	41:55	14:7	41:55	14:7		
Serum creatinine mg·L <sup>-1</sup>	10.0±1.5 (7.3–15.1)	9.6±1.3 (7.4–11.8)	10.8±3.0 (6.5–26.0)	11.2±2.1 (6.5–15.7)	0.83	0.002
Serum CC16 µg·L <sup>-1</sup>	14.6±5.0 (3.0–34.5)	11.3±5.3 (5.0–24.1)	27.6±17.3 (4.6–132.9)	18.3±6.0 (10.3–30.3)	0.0001	0.0001

Values are given as arithmetic means±SD (range). M: male; F: female. \*: differences between smokers and nonsmokers; #: difference between controls and diseased subjects. Both differences assessed by unpaired t-tests.

from nonpulmonary diseases and four nonsmoking sarcoid patients. Biopsy samples were fixed by immersion in Bouin's fluid, paraffin embedded and cut to 6-µm thick sections. CC16-immunoreactive cells were detected by using the rabbit polyclonal antibody against human CC16 and the immunoperoxidase technique, as previously reported [16]. The number of CC16 positive and negative bronchiolar cells was determined for each bronchiolar profile examined in lung biopsies and by counting all surface epithelial cells on bronchial biopsy specimens. For each patient, the proportion of CC16-positive cells was expressed as a percentage of the whole bronchiolar epithelium cell population examined.

#### Statistical analysis

All parameters were log-transformed before the application of parametric tests. For the whole population, the determinants significantly affecting the concentrations of CC16 in serum were identified by stepwise regression analysis with the following parameters tested as independent variables: smoking status (categorized as smoker or nonsmoker), sex, serum creatinine as estimator of the glomerular filtration rate (GFR), pulmonary sarcoidosis (categorized as present or absent disease) and anti-inflammatory therapy (categorized as present or absent treatment). Regression analysis was also performed separately on the

data obtained from control subjects and sarcoid patients, testing the smoking status, sex and serum creatinine, and for the sarcoid group also the chest radiograph stage (0–I–II–III) as independent variables. Differences between controls and patients, as well as smokers and nonsmokers, were evaluated by the unpaired t-test, whereas differences according to the chest radiograph stages and chest CT categories were assessed by one-way analysis of variance followed by the Fisher's least significant difference multiple comparison test. Correlations between serum and BALF parameters were evaluated by the Pearson's correlation coefficient. Unless otherwise indicated, values are reported as the mean±SD with range when appropriate. The level of significance was set at  $p < 0.05$ .

#### Results

The characteristics of the study population are given in table 1. When factors likely to influence serum CC16 were analysed by stepwise regression combining data from controls and sarcoid patients, sarcoidosis emerged as the major determinant followed by serum creatinine and tobacco smoking (table 2). When the two groups were analysed separately, the influence of tobacco smoking was found for both sarcoid patients and controls. By contrast, the influence of the renal function appears only in the

Table 2. – Determinants of CC16 levels in serum

Populations	Regression models	Independent variables	Partial r <sup>2</sup>	Slope	p-Value
Whole population	Model 1	Sarcoidosis	0.264	0.217	0.0001
		Serum creatinine	0.055	0.658	0.0001
		Smoking status	0.05	-0.136	0.0001
Controls	Model 2	Smoking status	0.087	-0.13	0.01
Sarcoid patients	Model 3	Serum creatinine	0.15	0.996	0.0001
		Smoking status	0.071	-0.147	0.002
		Chest radiograph stage	0.05	0.066	0.006

The relative influence of the different independent variables on the CC16 levels in serum was tested by stepwise regression analysis for the whole population, the control group and the sarcoid population using different regression models (model 1: presence or absence of sarcoidosis, serum creatinine, smoking status (categorized as smoker or nonsmoker), sex and anti-inflammatory treatment (categorized as present or absent); model 2: smoking status, serum creatinine, sex and anti-inflammatory treatment; model 3: serum creatinine, smoking status, sex, radiography stage of sarcoidosis (categorized as 0–I–II–III), and anti-inflammatory treatment).

Table 3. – Levels of CC16 and other parameters in serum of control subjects and sarcoid patients classified according to the chest radiograph findings

	Controls	Sarcoid patients Chest radiograph stages		p-Value
		0-I	II-III	
Subjects n	87	53	34	
Age yrs	44.5±11.4	44.6±11.4	44.8±11.8	0.98
Sex M:F	36:51	22:31	14:20	
Serum creatinine mg·L <sup>-1</sup>	9.9±1.5	10.3±1.5	10.1±2.0	0.36
CC16 µg·L <sup>-1</sup>	14.4±4.6	22.8±11.1*	30.0±16.1*(*)	0.0001
Lysozyme mg·L <sup>-1</sup>	9.8±3.2	18.4±31.0	11.2±4.4	0.24
ACE U·L <sup>-1</sup>	29.5±15.9	31.7±13.5	30.5±18.3	0.98

Values are given as arithmetic means±SD unless stated otherwise. Differences of serum levels between controls and sarcoid patients classified according to the chest radiograph stages were assessed by analysis of variance (ANOVA) followed by the Fisher's least significant multiple comparison test. \*: p<0.05 versus control or (radiographic stages 0-I). M: male; F: female; ACE: angiotensin converting enzyme.

group of sarcoid patients, some of whom had renal impairment (table 2). Interestingly, in the latter group, a positive association was found between serum CC16 and the severity of the chest radiograph abnormalities. In the different models tested, age, sex and anti-inflammatory treatment did not emerge as significant determinants of CC16 changes.

As shown in table 1, smoking and nonsmoking sarcoid patients showed a comparable elevation of the level of CC16 in serum, which was on average 80% higher than controls. Because of the presence of renal impairment among some sarcoid patients and the influence of tobacco smoking on serum CC16 level, only the 87 nonsmoking sarcoid patients without moderate or severe renal dysfunction (serum creatinine <14 mg·L<sup>-1</sup>) were subjected to further analysis. In this subgroup, the presence of sarcoidosis appeared as the major determinant of the elevation of CC16 in serum (r<sup>2</sup>=0.249, p=0.0001) compared to the renal function which had only a minor influence (r<sup>2</sup>=0.021, p=0.03). When this subgroup of patients was categorized according to the severity of the chest radiographic involvement, CC16 concentration in serum (µg·L<sup>-1</sup>) was found to rise with increasing extent of lung involvement (controls, n=87: 14.4±4.6; stage 0, n=4:

18.9±13.7; stage I, n=49: 23.1±11.0; stage II, n=24: 28.6±13.6; stage III, n=10: 33.3±21.4; p=0.001). As shown in table 3, serum CC16 was significantly higher in patients showing parenchymal lung involvement (stages II-III) compared to those without parenchymal lung involvement (stages 0-I). A clear elevation of CC16 (µg·L<sup>-1</sup>) also emerged when patients were divided according to the degree of chest CT abnormalities (controls, n=87: 14.4±4.6, normal chest CT or isolated mediastinal lymph nodes, n=31: 21.2±9.8; mediastinal lymph nodes with parenchymal changes, n=17: 28.1±13.2; parenchymal abnormalities, n=11: 37.8±22.5, p=0.0001). Such difference was, however, not found when serum lysozyme or ACE levels were compared between the groups of patients with and without parenchymal lung involvement.

In BALF of sarcoid patients, by contrast with serum, the level of CC16 was not significantly different from that of a small group of nonsmoking healthy subjects (0.45±0.4 versus 0.46±0.18 mg·L<sup>-1</sup>, p=0.44) and showed no elevation with increasing severity of the chest radiograph abnormalities (table 4). The average CC16 level in BALF was not different between the subjects from the two participating hospitals, ruling out any confounding effect by the

Table 4. – Levels of CC16 and other parameters in BALF of control subjects and sarcoid patients classified according to the chest radiograph findings

	Controls	Sarcoid patients Chest radiograph stages		p-Value <sup>#</sup>
		0-I	II-III	
Subjects n	7	43	28	
CC16 mg·L <sup>-1</sup>	0.46±0.18	0.40±0.43	0.52±0.34	0.06
Albumin mg·L <sup>-1</sup>	25.3±10.6	53.6±37.0	73.9±78.5	0.2
RBP µg·L <sup>-1</sup>	23.2±12.1	52.5±67.2	100.6±126.1	0.053
Lymphocytosis %	8.3±5.3	25.4±11.7*	26.5±12.4*	0.0001
CD4/CD8 ratio	0.8±0.2	5.6±4.9*	4.4±6.7*	0.004
Macrophages %	91.5±5.1	70.8±12.5*	71.5±12.4*	0.008
Neutrophils %	0.6±0.5	2.9±8.2	2.3±2.8	0.36

Values are given as arithmetic mean±SD. #: differences in bronchoalveolar lavage fluid levels between controls and sarcoid patients classified according to the chest radiograph stages were assessed by analysis of variance (ANOVA) followed by the Fisher's least significant multiple comparison test. \*: p<0.05 compared to controls. RBP: retinol binding protein.

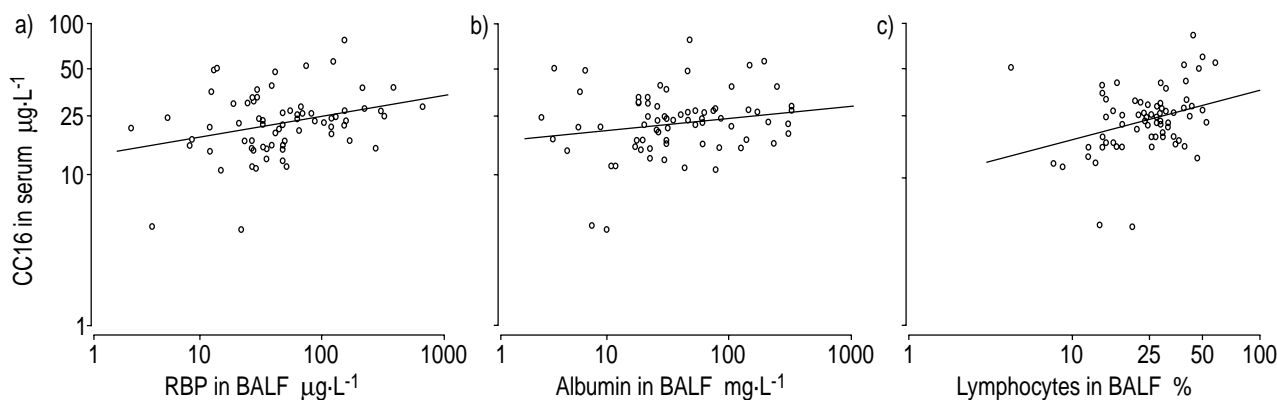


Fig. 1.—Correlations between the concentration of CC16 in serum and the bronchoalveolar lavage fluid (BALF) levels of a) retinol-binding protein (RBP) ( $n=71$ ,  $r=0.29$  and  $p=0.01$ ), b) albumin ( $n=71$ ,  $r=0.18$  and  $p=0.01$ ), and c) the percentage of BALF lymphocytes ( $n=71$ ,  $r=0.31$  and  $p=0.008$ ) among sarcoid patients. Log scales are used.

lavage procedure on the concentrations of the parameters measured in BALF. Levels of albumin and RBP tended to increase in BALF of sarcoid patients (table 4). The percentage of BALF lymphocytes, as well as the BALF CD4+/CD8+ ratio, were elevated among sarcoid patients. As illustrated in figure 1, CC16 concentration in serum of sarcoid patients was positively correlated with the level of RBP and the percentage of lymphocytes in BALF. However, no correlation was found between serum CC16 and the BALF levels of albumin, CC16 and the CD4+/CD8+ ratio. Like CC16 in serum, albumin in BALF was positively correlated with the BALF percentage of lymphocytes ( $n=71$ ,  $r=0.29$ ,  $p=0.01$ ).

On average,  $3.9 \pm 2.5$  bronchial biopsy samples and  $17.1 \pm 7.3$  bronchiolar sections on pulmonary biopsy specimens were examined for each subject. The mean number of granuloma examined was 1.63 (1–5) and 36.8 (1–100) for the bronchial and pulmonary biopsy specimens, respectively. The CC16 immunopositive cells were identified along the airways of both control subjects and sarcoid patients. In agreement with the predominant bronchiolar localization of Clara cells, the density of CC16-positive cells was higher in pulmonary than in bronchial biopsy specimens ( $38.3 \pm 3.6\%$  versus  $21.2 \pm 11.5\%$ ,  $p=0.05$ ). The analysis revealed a significant influence of tobacco smoking, the percentage of CC16 positive cells being lower in bronchial biopsy specimens of smoking, compared to nonsmoking controls ( $12.4 \pm 8.3\%$  versus  $28.8 \pm 7.8\%$ ,  $p=0.005$ ). By contrast, after matching for tobacco smoking, no difference was found between sarcoid patients and controls in the density of CC16 positive cells whether evaluated on bronchial ( $22.2 \pm 7.0\%$  versus  $28.8 \pm 7.8\%$ ,  $p=0.08$ ) or pulmonary biopsy ( $45.4 \pm 6.7\%$  versus  $38.3 \pm 3.7\%$ ,  $p=0.09$ ). No CC16 immunoreactivity was detected in sarcoid granulomas (fig. 2).

## Discussion

The increased intravascular leakage of lung secretory proteins has been investigated by measuring CC16, a major lung secretory protein, in serum of a large population of sarcoid patients. Sarcoidosis

increased the serum level of the protein on average by 80%. This elevation was independent of the effects of tobacco smoking and renal dysfunction, two factors known to decrease and increase the serum levels of the protein, respectively [14, 15, 19–21, 28]. The present findings allow the exclusion of the possibility that the elevation of serum CC16 was due to an increased synthesis and/or release by lung epithelial or inflammatory cells. The CC16 levels in BALF and the number of CC16-immunopositive cells of sarcoid patients were indeed not different from controls and no immunostaining for CC16 was detected in sarcoid granulomas. These data also suggest that, despite its immunomodulatory, anti-inflammatory and antifibrotic properties [16, 29, 30], CC16 is not functionally implicated in the pathogenesis of pulmonary sarcoidosis. Although some sarcoid patients had decreased GFR, only a small part of the elevation of CC16 in serum can be accounted for by a reduced renal clearance of the protein. The increase occurred indeed independently of the GFR as confirmed by the fact that the elevation persisted after exclusion of the sarcoid patients with significant renal dysfunction. Since the respiratory tract has been shown to be the

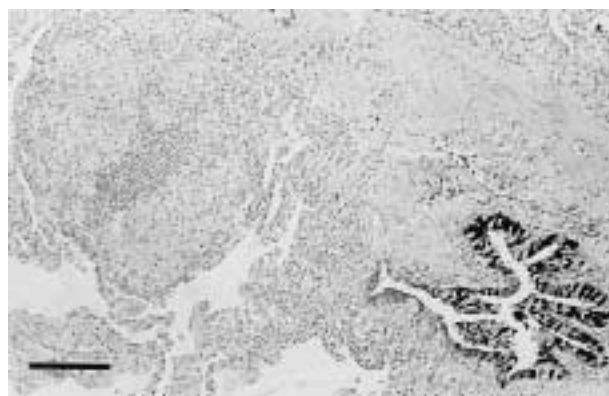


Fig. 2.—Representative micrograph showing the CC16-positive cells in a pulmonary biopsy specimen of a nonsmoking sarcoid patient. Immunoreactivity for CC16 is intense in bronchioles but absent in sarcoid noncaseating granuloma. (Internal scale bar=120 µm).

main source of CC16 in the circulation in both humans [12] and rodents [31], the only conceivable explanation is that of an increased transfer of this lung protein from the respiratory tract into the bloodstream across the air-blood barrier.

The mechanism of this elevation is probably related to the increased permeability to solutes of the air-blood barrier associated with sarcoidosis. The lack of correlation between CC16 in serum and albumin in BALF is not inconsistent with that hypothesis, since these proteins show substantial differences with respect not only to their sizes, but also their directions, routes and mechanisms of passage across the air-blood barrier. Although little is known about their exact routes and sites of passage, proteins moving across the air-blood barrier have to be transferred across the epithelial and the endothelial layers, their respective basal membranes, as well as the interstitium [11, 12, 32]. Several lines of evidence suggest that proteins cross the air-blood barrier paracellularly, through water-filled pores located at the junctional complexes linking epithelial and endothelial cells [33]. Because of the small size of most of these pores, mainly those linking the alveolar cells, the air-blood barrier should provide much more restriction to the movement of large proteins such as albumin, compared to smaller proteins such as CC16 [34–36]. This size-selective permeability of the air-blood barrier provides an attractive explanation for the correlation between CC16 in serum and RBP in BALF, two proteins of comparable size, but moving in opposite directions. Evidence has been accumulated that the blood-lung movement of albumin and the opposite passage of CC16 are both driven by a diffusional process down the concentration gradients existing for these two proteins across the air-blood barrier [14, 37]. Despite this similarity, one may logically hypothesize that the lymphatic drainage of the interstitium limits the intra-alveolar leakage of proteins originating from plasma, but, on the contrary, increases the intravascular transfer of lung secretory proteins such as CC16 following their passage across the respiratory epithelium.

Differences in fate and metabolism of proteins moving in opposite directions across the air-blood barrier is most probably another factor to take into account. Plasma proteins leaking in the epithelial lining fluid are slowly cleared from the lumen of the respiratory tract, whereas CC16 entering the circulation distributes in the vascular and extravascular compartments and is rapidly eliminated by the kidney [15]. Finally, it is very likely that the serum level of CC16 reflects the permeability of the entire air-blood barrier. By contrast, albumin in BALF only reflects the integrity in the region of the lavage. Marked differences between these two parameters may appear if the lung involvement is not homogeneous, as previously reported in interstitial lung diseases [38]. With respect to BAL, unknown and variable dilution of the ELF components during the procedure is probably another factor which accounts for the lack of correlation between the levels of plasma proteins in BALF and those of lung secretory proteins in serum [39].

Morphologically, lesions of the alveolar epithelium, the endothelium and their respective basal membranes have been evidenced suggesting that the different components of the air-blood barrier are altered in sarcoidosis [40]. Functionally, as for CC16, the intravascular clearance of inhaled radioaerosol such as  $^{99m}\text{Tc}$ -labelled diethylenetriamine-penta-acetate (DTPA) has been found to be increased in sarcoidosis and not correlated with the intra-alveolar leakage of albumin [38, 41, 42]. Although the exact mechanism of this increased permeability remains to be determined, it is plausible that the junctions between alveolar cells are abnormally permeable or increased in number in sarcoidosis, resulting in an enhanced movement of exogenous or endogenous solutes and macromolecules. However, the presence of nonselective leaks or a transcellular transport, even if very unlikely, cannot be completely ruled out [43]. As previously reported, it was found that the modifications of albumin in BALF were well correlated with the intensity of the alveolitis [4, 5, 8]. An even better correlation with the percentage of lymphocytes in BALF was found for CC16 in serum. These findings suggest that the bidirectional movement of proteins across the air-blood barrier might be associated with the degree of lung inflammation estimated by the percentage of lymphocytes in BALF.

Interestingly, serum CC16 was found to be elevated in all patients, even in the absence of marked radiological abnormalities. It has however, been shown that lung involvement and increased permeability of the air-blood barrier to proteins are present in most patients with sarcoidosis [44], even in the absence of radiologically detectable infiltrates. Moreover, CC16 tended to increase with the severity of the parenchymal lung involvement assessed by chest radiograph and chest CT. This finding supports the hypothesis that the amount of CC16 leaking into the bloodstream is influenced by the pulmonary extent of the disease. In agreement with the literature, no similar pattern of changes was, however, found for albumin in BALF [10]. As for CC16 in serum, the acceleration of the intravascular clearance of inhaled radioaerosols has been shown to be positively associated with the degree of radiological lung involvement [38, 41, 45]. By contrast, no correlation was found between serum CC16 and the serum levels of ACE or lysozyme, two markers commonly determined in serum of sarcoid patients. These findings however, are not surprising, since both ACE and lysozyme are produced within granulomas so that their levels in serum are dependent upon both pulmonary and extrapulmonary granulomatous burden [46].

The elevation of CC16 in serum is not specific for sarcoidosis since a similar change has been reported in various clinical or experimental conditions including pulmonary fibrosis [16], acute respiratory distress syndrome [15], exposure to ozone [18] and in firefighters following smoke inhalation [17]. This similarity in such different diseases supports the concept of a common mechanism, most likely an increased intravascular leakage of the protein induced by the disruption of the air-blood barrier. Very interestingly,

another lung secretory protein, the mucin-antigen KL-6 released by alveolar type II cells, has recently been found to increase in serum of patients with sarcoidosis and beryllium lung disease and to be significantly influenced by the severity of the lung involvement [47, 48]. As in the present study, KL-6 has been suggested to be a useful marker of the degree of permeability of the air-blood barrier in beryllium lung disease [48]. These observations support the concept that, in the absence of renal impairment, the elevation of CC16 in serum might be a very specific indicator of damage of the air-blood barrier and its increased permeability to proteins.

In conclusion, the results of the presented study show that sarcoidosis is associated with an increased intravascular leakage of CC16 across the air-blood barrier. This elevation was correlated with the severity of the parenchymal lung involvement assessed radiologically, as well as with the degree of alveolitis. The levels of CC16 in serum of sarcoid patients were also significantly influenced by tobacco smoking and the renal function, two factors which should be carefully considered when using this lung marker.

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