Relationship Between Presentation of Sarcoidosis and T Lymphocyte Profile*

A Study in Bronchoalveolar Lavage Fluid

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One hundred patients with histologically verified sarcoidosis were studied. They were divided into three groups, based on their clinical presentation and smoking status. Group A consisted of patients whose disease was detected by routine chest x-ray film, without symptoms; group B included those with respiratory and general constitutional symptoms; and group C included patients with erythema nodosum and/or arthralgia and hilar lymphadenopathy. Group A showed an increased CD4/CD8 ratio of 4.7±1.1; group B, 8.0±1.2; and group C counted for the highest ratio of 10.7±1.5. Cigarette smoking modifies the immunologic bronchoalveolar lavage (BAL) fluid sample profile, since alveolitis was less pronounced in smokers. In addition, BAL fluid samples obtained from sarcoidosis patients with hilar lymphadenopathy showed the most characteristic features of alveolitis, suggesting a disseminated instead of a local immune response. Therefore, the clinical presentation of sarcoidosis and the smoking status of a sarcoidosis patient are crucial for interpreting individual lavage analysis results.

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BAL = bronchoalveolar lavage; NSm = nonsmoker; PB = peripheral blood; Sm = smoker

Sarcoidosis is a disorder of unknown origin, characterized by the formation of noncaseating epithelioid cell granulomas, probably antigen driven, most frequently occurring in the lungs.[1-4] In addition to granuloma formation, there is often an extensive vascular disease as seen by the appearance of microangiopathies.[4] Granuloma formation in the lungs is preceded by a mononuclear cell alveolitis with increased numbers of activated T lymphocytes and alveolar macrophages.[5-7] Besides changes in T lymphocyte and alveolar macrophage populations, changes in the humoral immunity have been reported.[8]

Clinical manifestations of sarcoidosis depend on the intensity of the inflammation and organ systems affected.[9-11] In some sarcoidosis patients, the alveolitis remains subclinical, whereas in others both alveolitis and granuloma formation are present, resulting in specific pulmonary symptoms.[12-14] Although the lung is the most frequently affected organ, extrapulmonary manifestations such as erythema nodosum commonly occur.[1,3,10]

Bronchoalveolar lavage (BAL) is regarded as an important diagnostic method in sarcoidosis.[15-17] However, conflicting results have been reported in studies evaluating the utility of BAL in assessing the prognosis of the disease. The cellular profile in BAL fluid samples reflects the presence of alveolitis as a local expression of a disseminated immunologic disorder.[8,15,18] Lymphocytes recovered in BAL fluid are predominantly T lymphocytes, and there is no more than a 5 percent proportion of B lymphocytes.[8,9] Activation of alveolar T lymphocytes is a characteristic feature of sarcoidosis[19,20] and is demonstrated not only by an increased expression of typical activation markers on the cell surface (immunophenotypic markers, such as HLA-DR antigen expression, T lymphocyte antigen receptor decrease and interleukin-2 receptors), but also by the release of specific mediators (functional markers, such as IL-2, interferon gamma, and other T lymphocyte mediators).[8,9,21-23] Moreover, activation of T lymphocytes in sarcoidosis is subset-specific. Also, inhibition of responsiveness of memory T lymphocytes to recall antigens is part of the immune response in active sarcoidosis, which has been suggested possibly to contribute to the anergy observed in these patients.[24] According to current concepts, the process of cell-mediated immunity is thought to mediate the pathogenesis of sarcoidosis.[8]

Studies on BAL fluid samples profile characteristics in sarcoidosis patients hitherto reported in literature give rise to conflicting data. These controversial results and disparity between conclusions may be explained by differences in the sarcoidosis subpopulations studied and methodologic variations, as well as the fact that sarcoidosis does not present as an entity. Only a few reports regarding the clinical presentation of the disease associated with alveolitis are available. Furthermore, many studies do not differentiate between

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Table 1—*Characteristics of the Groups Studied*

Group	No. of Cases	Age, yr*	Sex	
			Female	Male
Nsms				
Control subjects	11	39.4 (27-66)	7	4
Patients†				
A	11	38.2 (27-69)	5	6
B	50	36.6 (22-79)	28	22
C	16	37.0 (23-57)	9	7
Sms				
Control subjects	3	41.0 (30-60)	2	1
Patients†				
A	7	39.0 (24-67)	3	4
B	10	32.3 (19-48)	5	5
C	6	29.7 (21-36)	3	3

*Mean with range in parentheses.

†A = sarcoidosis patients, no symptoms; B = sarcoidosis patients, respiratory and general constitutional symptoms; C = sarcoidosis patients, erythema nodosum and/or arthralgia and hilar lymphadenopathy (*ie*, Löfgren's syndrome).

smoker (Sm) and nonsmoker (NSm) patients.

The aim of this study was to investigate whether the way in which sarcoidosis presents is associated with differences in cellular profile in BAL fluid samples, especially with regard to the number of T lymphocytes and T lymphocyte subpopulations and the smoking status in sarcoidosis patients.

Materials and Methods

Patients and Control Subjects

Bronchoalveolar lavage was performed in 100 patients with histologically proven sarcoidosis and 14 control subjects. The characteristics of the patients and control subjects are described in Table 1. The patients were divided into three groups based on their clinical presentation.

Group A consisted of patients whose disease was detected on routine chest x-ray film, without symptoms or knowledge of the exact duration and the time of onset of the disease (11 NSm and 7 Sm); group B included those with respiratory and general constitutional symptoms (50 NSm and 10 Sm); and group C included patients with erythema nodosum and/or arthralgia and hilar lymphadenopathy (*ie*, M. Löfgren; 16 NSm and 6 Sm). All patients had stage I or II disease and none had stage III disease, as evidenced on x-ray films. The majority of the patients were NSms, 77 of 100 (Table 1). The initial BAL fluid samples of consecutive sarcoidosis patients obtained at the time of the diagnosis, within 2 weeks after admission to our hospital, were used for this study. No patient was receiving corticosteroid or other treatment either at the time of or before the lavage. The control group consisted of individuals without chest abnormalities or a history of pulmonary abnormalities or disease (11 NSm; 3 Sm). The studied groups were subdivided according to their smoking status (Table 1).

Bronchoalveolar Lavage

Bronchoalveolar lavage was performed as previously reported during fiberoptic bronchoscopy.[22] At the same time, blood samples were taken. In short, the procedure was as follows. After premedication with atropine and sometimes diazepam or codeine, and local anesthesia of the larynx and bronchial tree with 0.5 percent tetracaine, BAL was performed by standardized washing of the right middle lobe with four 50-ml aliquots of sterile saline solution (0.9 percent NaCl) at room temperature. Lavage fluid samples, kept on ice in a siliconized specimen trap, were centrifuged (10 min, 350 g) and separated into cells and supernatant. The cell pellet was washed twice, counted, and suspended in minimal essential medium (Gibco, Grand Island, NY), supplemented with 1 percent bovine serum albumin (Organon, Teknika, Boxtel, the Netherlands). Preparations of the cell suspension were made in a cytocentrifuge (Shandon). Cytospin slides of BAL cells were stained with May-Grünwald-Giemsa (Merck, Darmstadt, Germany) for cell differentiation. At least 1,000 cells were counted.

If more than 15 percent lymphocytes were present in the BAL fluid samples, T lymphocyte subpopulations were determined. Identification of T lymphocytes reacting with monoclonal antibodies was performed by means of a conventional indirect immunofluorescence technique. Total T lymphocytes and subpopulations were recognized by staining with monoclonal antibodies (CD3, CD4, and CD8). Monoclonal antibodies, CD3 (OKT3), CD4 (OKT4), and CD8 (OKT8), were obtained from Ortho-Pharmaceuticals (Beerse, Belgium) and subsequently labelled with FITC-conjugated goat-antimouse-immunoglobulin (Nordic, Immunological Laboratories, Tilburg, the Netherlands and from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service [CLB], Amsterdam, the Netherlands). Results were expressed as a percentage of lymphocytes.

Statistical Analysis

To investigate whether there were statistically significant differences between the three categories of sarcoidosis patients, the Kruskal-Wallis one-way analysis of variance test was used. Each category, group A (no symptoms), group B (respiratory and general constitutional symptoms), and group C (erythema nodosum and hilar lymphadenopathy [M. Löfgren]), respectively, denoted the clinical presentation of the patient.

The Mann-Whitney U test, a pairwise comparison, was used to evaluate differences between Sms and NSms in each group, as well as the differences in each category with the control subjects. The probability values less than 0.05 were considered to be significant.

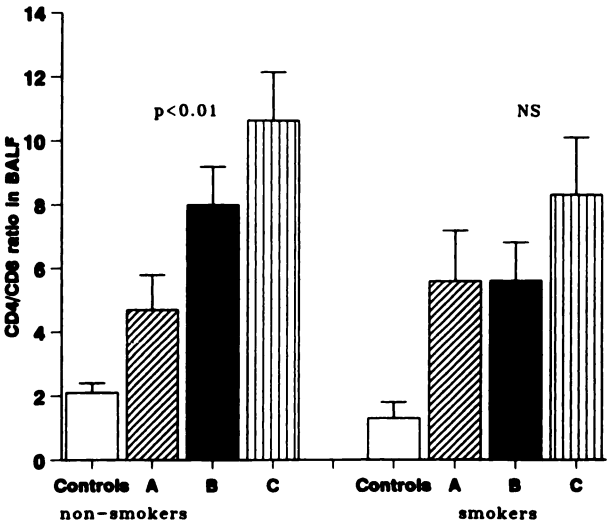

FIGURE 1. CD4/CD8 T lymphocyte ratio in BAL fluid samples in three different clinical presentations of sarcoidosis patients (A = no symptoms; B = respiratory and general constitutional symptoms; C = erythema nodosum and/or arthralgia and hilar lymphadenopathy [*ie*, Löfgren's syndrome]; mean ± SEM) and control subjects. The general effect of smoking is a reduction of the CD4/CD8 ratio.

Results

The mean values ± SEM of the immunologic marker analysis of the cells in BAL fluid and peripheral blood (PB) samples of the groups studied are summarized in Table 2. In general, the cellular components of BAL fluid samples obtained from all patient groups differed significantly from those obtained from control subjects. The mean percentages of $CD4^+$ and $CD8^+$ T lymphocytes and the CD4/CD8 ratio in BAL fluid samples showed statistically significant differences in the NSm sarcoidosis patients (Fig 1, Table 2). No significant difference could be demonstrated between the three categories of Sm patients, except the mean percentage of $CD3^+$ T lymphocytes in the BAL fluid samples, which appeared to be the highest in patients with erythema nodosum and/or arthralgia, *ie*, Löfgren's syndrome (Table 2). No B lymphocytes or plasma cells were demonstrated in the BAL fluid samples from the sarcoidosis patient groups or from the control subjects.

In BAL fluid samples of all categories of sarcoidosis patients, the total cell count was significantly increased compared with that of the control subjects ($p<0.04$). The absolute and relative numbers of lymphocytes were increased, as well as the percentages of $CD3^+$ and $CD4^+$ T lymphocytes; in contrast, the percentage of $CD8^+$ T lymphocytes was decreased most of all in patients with M. Löfgren (category C). The CD4/CD8 ratios in the BAL fluid samples were increased in all sarcoidosis patient categories, most prominent in both NSm and Sm patients with Löfgren's syndrome. The CD4/CD8 ratios in the PB samples showed no statistical differences, except in category A of the NSm patients, who demonstrated a decreased ratio compared with that of the control subjects.

Significant differences were demonstrated between Sm and NSm subjects of the studied categories. In general, total cell count and the number of alveolar macrophages were increased, while the percentages of lymphocytes were decreased in the Sm groups. In the sarcoidosis Sm groups studied, also a significant increase in the number of polymorphonuclear neutrophils and a decrease in the number of mast cells were found in BAL fluid samples (data not shown).

Smoker patients with respiratory or constitutional symptoms (*ie*, group B) showed a decreased percentage of $CD3^+$ T lymphocytes in the PB samples (data not shown) and decreased CD4/CD8 ratios in BAL fluid and PB samples compared with NSm patients of category B. Smoker patients, presenting with Löfgren's syndrome (*ie*, group C) showed an increased percentage of $CD3^+$ lymphocytes and less increased CD4/CD8 ratio in BAL fluid and lower CD4/CD8 ratio in PB samples in comparison with NSm patients. In general, most significant changes were demonstrated in the BAL fluid samples of patients with Löfgren's syndrome both in NSm and Sm patients compared with the other categories and control subjects (Fig 1, Table 2).

Table 2—*Total Cell Count, Lymphocytes, T Lymphocyte Subpopulations and the CD4/CD8 Ratios in Bronchoalveolar Fluid Samples and Peripheral Blood Samples in Three Different Clinical Presentations of Sarcoidosis Patients and Control Subjects**

Groups	Total Cell Count $\times 10^6$	Lymphocytes $\times 10^4$/ml	Lymphocytes %	CD3, %	CD4, %	CD8, %	CD4/CD8 Ratio BAL	CD4/CD8 Ratio PB
NSms								
Control subjects	11.7 (2.7)	1.0 (2.8)	10.8 (1.7)	75.8 (2.3)	49.5 (3.5)	29.6 (4.8)	2.1 (0.3)	2.3 (0.5)
Patients†								
A	19.2 (3.5)	4.7 (1.1)‖	31.3 (5.1)‖	83.5 (2.4)‖	60.9 (4.7)§	19.9 (3.5)§	4.7 (1.1)§	1.3 (0.2)§
B	20.8 (1.8)§	7.7 (0.9)‖	38.1 (2.6)‖	88.3 (1.0)‖	69.5 (2.2)‖	17.9 (2.0)‖	8.0 (1.2)‖	2.4 (0.4)
C	23.5 (3.2)§	8.7 (1.4)‖	40.2 (4.5)‖	88.8 (2.1)‖	80.3 (2.7)‖¶	8.9 (0.8)‖¶	10.7 (1.5)‖¶	2.6 (0.4)
p-value‡	NS†	NS	NS	0.05	<0.001	0.01	0.008	0.06
Sms								
Control subjects	33.3 (12.7)	8.9 (3.5)	4.2 (2.1)	82.3 (5.4)	45.7 (7.2)	40.7 (7.3)	1.3 (0.5)	1.4 (0.6)
Patients†								
A	31.0 (5.5)	6.1 (1.4)§	21.3 (3.6)‖	89.1 (2.6)§	69.4 (6.8)‖	19.4 (5.6)‖	5.6 (1.6)§	2.1 (0.5)
B	43.9 (14.9)§	10.8 (4.1)	27.1 (6.4)‖	86.1 (3.4)	66.2 (6.4)§	22.0 (6.8)‖	5.6 (1.2)‖	1.7 (0.3)
C	31.2 (7.0)	4.7 (1.8)§	18.7 (5.9)‖	95.3 (1.2)‖¶	81.0 (4.0)‖¶	12.5 (2.8)‖¶	8.3 (1.8)‖¶	1.8 (0.5)
p-value‡	NS	NS	NS	0.03	NS	NS	NS	NS

*Data are expressed as mean with SEM in parentheses.

†A = sarcoidosis patients, no symptoms; B = sarcoidosis patients, respiratory and general constitutional symptoms; C = sarcoidosis patients, erythema nodosum and/or arthralgia and hilar lymphadenopathy (Löfgren's syndrome). NS = not significant.

‡Kruskal-Wallis test for differences between groups A, B, and C.

§$p<0.04$ Mann-Whitney versus control group.

‖$p<0.02$ Mann-Whitney versus control group.

¶$p<0.05$ Mann-Whitney C versus A and B.

Discussion

The results presented in this study confirm previous observations of signs of a T lymphocyte alveolitis and the influence of smoking on T lymphocyte subsets in patients with sarcoidosis. Most remarkably, we demonstrated that the BAL fluid T lymphocyte profiles are related to the differential clinical presentation of sarcoidosis patients. This relationship was found to be less pronounced in Sms. However, comparison between the different studied categories of sarcoidosis patients is rather difficult, whereas estimating the exact time of onset of the asymptomatic patients and those presenting with respiratory symptoms is much more difficult than assessing the time of onset of erythema nodosum.[14] Although the influence of smoking on BAL fluid cell profile has been studied extensively, only a few reports concerning the influence of clinical presentation of sarcoidosis on the cell profile in BAL fluid samples have hitherto been published.

Costabel et al[25] suggested earlier that cellular immunoregulation may be disturbed in the lungs of cigarette smokers and as such, may influence pulmonary host defense. In the present study, we confirm the data previously reported by others, by showing that smoking results in increased total cell counts, less increased percentages of T lymphocytes, and less increased CD4/CD8 ratios in the BAL fluid samples in sarcoidosis patients.[26-32] Thus, alveolitis, as determined by immunologic marker analysis, is less significant in smokers.[27]

Although all sarcoidosis patient groups, both Sms and NSms, in our study appeared to have an increased CD4/CD8 ratio in BAL fluid samples, NSm asymptomatic patients (group A) had the lowest, less increased ratio (4.7) in comparison with that of NSm symptomatic patients with respiratory and general symptoms (group B). These latter patients had a mean CD4/CD8 ratio of 8.0, while NSm patients presenting with acute onset sarcoidosis (*ie*, Löfgren syndrome), had the highest ratio (10.7) in the BAL fluid samples. These significant changes in the CD4/CD8 ratio seem to be primarily due to an increased influx of $CD4^+$ T lymphocytes to the alveoli, indicating an active immune response.[2-4] The reported decreased influx of $CD8^+$ T lymphocytes also determines the CD4/CD8 ratio in sarcoidosis, most prominently demonstrated in patients with Löfgren's syndrome, who showed significant lower percentages $CD8^+$ T lymphocytes both in Sms and NSms. Interestingly, patients with pronounced alveolitis, as reflected in a high CD4/CD8 ratio in BAL fluid samples, who clinically present with acute onset such as Löfgren's syndrome, do not need corticosteroid or any other treatment and have a short recovery time period.[14] In contrast, sarcoidosis patients with a relatively low CD4/CD8 ratio in BAL fluid samples more frequently develop permanent lesions, such as pulmonary fibrosis.[33-36] These data confirm the previously suggested poor prognostic significance of "isolated" BAL fluid analysis results.[10,14] The aforementioned best prognosis and spontaneous resolution of the lesions in almost all cases suffering from Löfgren's syndrome, compared with other subgroups, suggests the beneficial role of especially $CD4^+$ T lymphocytes in the immune response in sarcoidosis and their essential function in defense.[14,20,36]

Lymphocytic alveolitis is an early event in the evolution of pulmonary involvement in sarcoidosis.[14,15] This inflammatory response precedes granuloma formation in the lung and may be latently present without clinical or physiologic impairment, while remaining undetected by radiologic investigation.[37,38] It has been demonstrated by Valeyre et al[10] that the alveolitis in sarcoidosis patients with erythema nodosum precedes the increase in serum angiotensin-converting enzyme. Furthermore, they showed that the serum IgG levels were correlated to the lymphocyte counts in BAL fluid samples. Increased levels of serum IgG are probably under the influence of T lymphocytes through the activation of B lymphocytes present in sarcoidosis lesions, as have been demonstrated by Rankin et al[39] (1983) and recently by Fazel et al[8] (1992). Also, the existence of alveolitis in patients with extrapulmonary sarcoidosis with a normal chest x-ray film findings has been reported by others.[10,12,37] Our findings that patients with Löfgren's syndrome show the most prominent alveolitis and the highest CD4/CD8 ratio in BAL fluid samples suggest the involvement of T lymphocytes activated by an unknown stimulus in the initiation of granulomatous inflammation in sarcoidosis.[20,38] In addition, the characteristic findings of M. Löfgren, *ie*, erythema nodosum and arthralgia, early in the course of sarcoidosis, histologically resembling a nonspecific vasculitis, suggest a disseminated rather than a local immune response, probably antigen-driven.[8,20,37,40] This speculated involvement of an antigen as a stimulus underlying the granulomatous response has been demonstrated by the so-called Kveim-Siltzbach test.[18,41] Recently, du Bois et al[20] provided evidence of recent stimulation of the T lymphocyte antigen receptor of T lymphocytes accumulating in the lung in sarcoidosis. Fazel et al[8] found large numbers of B lymphocytes in sarcoidosis pulmonary lesions. The B lymphocytes at these sites were suggested to be the possible origin of some of the humoral changes in serum and lesions of sarcoidosis patients. They might also influence the pathogenesis of the disorder by presenting antigens and forming immune complexes at sites of disease activity.[8] Therefore, analysis of the antigen specificity of these expanded populations is likely to provide insight into the pathogenesis of the disease.

In conclusion, patients with different clinical pres-

entations of sarcoidosis have various T lymphocyte profiles in BAL fluid samples. Patients with erythema nodosum and/or arthralgia and hilar lymphadenopathy (*ie*, Löfgren's syndrome) show the most marked characteristics of alveolitis, including the highest CD4/CD8 ratios in BAL fluid samples, suggesting a disseminated instead of a local immune response. Furthermore, cigarette smoking modifies the immunologic BAL fluid sample profile and in addition, alveolitis is found to be less pronounced in Sms. Therefore, disease presentation or activity at the time of onset and smoking status are crucial for interpretation of individual BAL fluid sample analysis results.

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