Increased Expression of CD16, CD69, and Very Late Antigen-1 on Blood Monocytes in Active Sarcoidosis*

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Background: **Different types of immune cells are involved in the formation of granulomas, a hallmark of pulmonary sarcoidosis. Proinflammatory monocytes are activated circulating monocytes thought to be related to the initial events of granuloma formation. We tested the hypothesis that peripheral blood monocytes in patients with active pulmonary sarcoidosis have an activated phenotype and, secondly, that measuring this activation status can provide a new tool for monitoring disease activity.**

Methods: **Blood was collected of 23 steroid-naive patients presenting with pulmonary sarcoidosis and 10 healthy control subjects. Expression of CD16 (Fc-γ type III receptor), CD69 (a general activation marker of cells of the hematopoietic lineage), and the integrin very late antigen (VLA)-1 (on interaction with extracellular matrix compounds mediates cell adhesion) was measured by flow cytometry.**

Results: **Percentages of monocytes expressing CD16, CD69, and VLA-1 in patients vs control subjects were 56.2 ± 4.1% vs 12.2 ± 2.4% ($p < 0.0001$), 87.3 ± 2.1% vs 8.6 ± 3.3% ($p < 0.0001$), and 66.5 ± 3.6% vs 11.2 ± 2.3% ($p < 0.0001$), respectively. Moreover, the $CD69^+VLA\text{-}1^+$ monocyte subset, abundantly present at disease presentation, was found to decrease to normal levels during follow-up with disease remission.**

Conclusions: **Peripheral blood monocytes from patients with pulmonary sarcoidosis show a highly activated phenotype. Phenotyping circulating monocytes might be a promising tool for monitoring sarcoidosis disease activity but needs further investigation.**

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Key words: flow cytometry; monocyte activation; sarcoidosis; surface molecules

Abbreviations: ACE = angiotensin-converting enzyme; ECM = extracellular matrix; MFI = median fluorescence intensity; NK = natural killer; sACE = soluble angiotensin-converting enzyme; sIL-2R = soluble interleukin-2 receptor; SSC = side-light scatter characteristics; VLA = very late antigen

Sarcoidosis is a systemic, granulomatous disease of unknown origin, primarily affecting the lungs. The disease is characterized by a mononuclear cell alveolitis dominated by activated $CD4^+$ (helper/inducer) T cells and macrophages. The coordinated interplay between these cells leads to the characteristic formation of noncaseating granulomas and, in a subgroup of patients, to fibrosis and permanently impaired lung function.[1,2] Proinflammatory monocytes are activated circulating monocytes that might be related to the initial events that lead to granuloma formation. Proinflammatory monocytes co-express CD14, CD16, and CD69. CD14 is part of the Toll-like receptor-4 membrane receptor complex and expressed on all monocytes. Peripheral blood CD16 (a low-affinity Fc-γ type III receptor)-positive monocytes have been shown to expand in different pathologic conditions, such as cancer, asthma, sepsis, HIV infection, and AIDS progression (reviewed by Ziegler-Heitbrock[3]). CD69 is a general activation marker of cells of the hematopoietic lineage and has been identified as the earliest activation marker on the surface of cytokine- or mitogen-activated lymphocytes.[4]

Studies[5,6] suggest that interactions between leukocyte-associated integrins and the interstitial matrix may promote the migration and/or activation of extravasated leukocytes (*eg*, T cells and monocytes) within the perivascular compartment. Consistent with the initial description of $\alpha_1\beta_1$ as very late antigen (VLA), in the immune system VLA-1 is expressed on T cells, natural killer (NK) cells, NK T cells, and macrophages after (long-term) activation by antigen, superantigens, or cytokines.[7] Expression of VLA-1 has been demonstrated on tissue-infiltrating T cells from a variety of chronic inflammatory settings, including the rheumatoid synovium of arthritis patients[8] and the lungs of sarcoidosis patients.[9] Moreover, increased expression of β_2-integrins on peripheral blood monocytes and alveolar macrophages has been reported in patients with active sarcoidosis compared to patients with inactive disease,[10,11] and on peripheral blood monocytes in patients with rheumatoid arthritis.[12]

Differences in phenotype of peripheral blood monocytes in patients with pulmonary sarcoidosis have been described, although not extensively.[10,13,14] We hypothesized that considering the systemic character of the disease, peripheral blood monocytes in patients with active pulmonary sarcoidosis are activated and show a proinflammatory phenotype.

The aim of our study was to study the expression of activation markers CD16, CD69, and VLA-1 on monocytes in patients presenting with sarcoidosis and compare the results with clinical phenotypes and conventional biomarkers of sarcoidosis such as angiotensin-converting enzyme (ACE) levels in serum and serum soluble interleukin-2 receptor (sIL-2R) levels. Both are well-recognized markers of disease activity,[15,16] where serum ACE levels reflect the granuloma burden and serum sIL-2R levels reflect mainly the activity of the T-cell component. In extension, we measured the activation status of blood monocytes in a subgroup of the patients showing disease remission.

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Table 1—*Characteristics of Sarcoidosis Patient Population

Characteristics	Data
Patients, No.	23
Age, yr	41.2 (24–65)
Male/female gender	70 (16)/30 (7)
Lung function parameters	
FEV_1 % predicted (n = 18)	91 (59–115)
FVC % predicted (n = 18)	95 (65–116)
DLCO % predicted (n = 18)	80 (44–105)
Chest radiographic stages I/II/III/IV, No.	14/4/4/1
Löfgren syndrome, No.	6
Organ involvement†	
Pulmonary involvement	100 (17)
Extrapulmonary involvement	35 (6)
Kidney	24 (4)
Skin	12 (2)
Extrathoracic lymph node	6 (1)
Neurologic	6 (1)
Eyes	6 (1)
Parotid/salivary	6 (1)
Muscles	6 (1)
Bone/joints	6 (1)

*Data are presented as mean (range) or No. (%) unless otherwise indicated. DLCO = diffusing capacity of the lung for carbon monoxide.
†Based on A Case Control Etiologic Study of Sarcoidosis[34] assessment instrument. Organ involvement data are from non-Löfgren sarcoidosis patients (n = 17).

Materials and Methods

Subjects

Twenty-three consecutive patients presenting to our department because of symptomatic sarcoidosis and ≥ 15% lymphocytes in BAL, a criterion for active pulmonary alveolitis, were included in this study. Table 1 summarizes the clinical characteristics of the patient group. The diagnosis of sarcoidosis was established on the basis of clinical findings and histologic evidence of noncaseating epithelioid granulomas and after exclusion of other known causes of granulomatosis in accordance with the consensus of the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and Other Granulomatous Disorders statement on sarcoidosis.[17] In six patients, the diagnosis was made without biopsy proof because these patients presented with the classic symptoms of Löfgren syndrome: fever, erythema nodosum, arthralgia, and bilateral hilar lymphadenopathy. All patients were steroid naive at time of inclusion in the study. Pulmonary disease severity at presentation was evaluated by chest radiography (in online supplemental material). For further analysis, patients were classified into a group presented with lymphadenopathy (radiographic stage I and II, n = 18) and without lymphadenopathy (radiographic stage III and IV, n = 5). In addition, 10 randomly selected healthy control subjects (employees from St. Antonius Hospital) were included in the study (mean age, 31.3 years; range, 23 to 45 years; 6 men and 4 women). The institutional review board approved the study, and all subjects gave informed written consent.

Follow-up Study

Follow-up data were available for eight patients. During routine follow-up examination of the patients, peripheral blood