Expression of CD14 Correlates With Lung Function Impairment in Pulmonary Sarcoidosis

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CD14 expression on alveolar macrophages (AM) was studied in patients with sarcoidosis using immunochemistry and cytometric analysis. Compared with healthy control donors, patients had elevated percentages of CD14-positive AM (22 percent vs 34 percent), and the antigen density was threefold higher (92 vs 297 channels). Furthermore, soluble serum CD14 (ssCD14) was significantly elevated in patients with sarcoidosis with an average of 5.3 ± 1.6 mg/L vs 3.2 ± 0.7 mg/L in healthy control subjects. Follow-up of one patient, whose lung function test results improved during therapy with corticosteroids, revealed a concomitant decrease of CD14 staining on AM and of ssCD14.

Sarcoidosis is a systemic inflammatory disease with a lung involvement in more than 80 percent of patients. Histologic study of the lung demonstrates formation of granulomas and diffuse infiltrations by mononuclear cells with a preponderance of CD4-positive T cells. In bronchoalveolar lavage (BAL), similar changes were observed in that among lymphocytes, the CD4-positive cells predominate. Alveolar macrophages (AMs) are, however, also increased in number and these cells show signs of activation like increased expression of HLA-DR, transferrin receptor, and interleukin 2 (IL-2) receptor.

Alveolar macrophages do express the CD14 molecule, a 55-kd phosphatidyl-inositol anchored cell surface molecule, which is crucially involved in activation by lipopolysaccharide. When looking at different stages of differentiation in the monocyte lineage, CD14 is absent from monoblasts in bone marrow, as evidenced by negativity of monoblastic cell lines like U937. In blood, CD14 is expressed at high levels in regular monocytes and at low levels in the novel subset of CD14+/CD16+ monocytes. Tissue macrophages, which are derived from blood monocytes, may express high levels of CD14 as shown for peritoneal macrophages, while AMs show only a low level of CD14 expressions.

Statistical analysis revealed a negative correlation between CD14 expression on AM and P02 at rest (p = 0.0005), and after labor (p = 0.02). Levels of ssCD14 gave a positive correlation to reduction of Dco (p = 0.006) and VC (p = 0.05). These data suggest that CD14 expression is related to severity of disease and that it may be useful for monitoring in sarcoidosis.

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AM = alveolar macrophage; BAL = bronchoalveolar lavage; Dco = diffusing capacity for carbon monoxide; IL-2 = interleukin 2; MoAb = monoclonal antibody; OD = optical density; ssCD14 = soluble serum CD14; VC = vital capacity

MATERIALS AND METHODS

Study Population

Eleven patients with sarcoidosis (8 men, 3 women, 9 nonsmokers, 2 smokers), age between 25 and 71 years, were investigated. The diagnosis was based on histologic examination either of transbronchial biopsy specimens or open lung biopsy specimens. For clinical grading, lung function tests were performed (vital capacity [VC] diffusing capacity for carbon monoxide [Dco], P02, at rest and after exercise) (Table 1). All patients underwent bronchoalveolar lavage (BAL). The control group consisted of 10 healthy donors (nonsmokers, 6 men, 4 women) for BAL and an additional 23 healthy donors for serum samples (14 women, 9 men, 19 nonsmokers, 4 smokers) between 25 and 50 years old. The study was cleared by the Ethics Committee of the Medical Faculty, University of Munich, Germany.

Bronchoalveolar Lavage

After informed consent was obtained, BAL was performed by instilling 180 ml of 0.9 percent saline solution in 20-ml aliquots into the lingula or middle lobe and by withdrawing the fluid immediately. Total cell counts were determined and cytospin smears were prepared for cytologic and immunocytochemical analysis. Differential cell counts of 200 cells were made (Wright-Giemsa staining) (Table 2).
Table 1—Patient Characteristics and Lung Function

| Patient/ | Smoker (s) | X-Ray Staging* | PO2 at Rest $|$ | PO2 at After 25% Work $|$ |
|----------|------------|----------------|----------------|---------------------------|
| Sex/Age, yr | Nonsmoker (ns) | VC† | Dco† | | |
| 1/M/37 | s | II | -38 | -72 | 16 | 29 |
| 2/M/34 | ns | III | -27 | -42 | 6 | 18 |
| 3/F/34 | ns | II | -11 | -46 | 9 | 10 |
| 4/M/33 | ns | II | -20 | -26 | -1 | 2 |
| 5/M/33 | s | II | -23 | -60 | +3 | -25 |
| 6/M/35 | ns | II | +9 | -19 | +4 | ND |
| 7/M/39 | ns | II | -35 | -53 | -10 | -20 |
| 8/F/45 | ns | III | -64 | -53 | -4 | -26 |
| 9/F/71 | ns | II | -6 | ND | +16 | 0 |
| 10/M/35 | ns | II | +32 | -9 | +1 | 4 |
| 11/M/30 | ns | II | +2 | -40 | +8 | -1 |

Mean ± SD M=8 38.9±13

Sarcoidosis F=3
Mean ± SD M=6 34±7 10 ns
Control F=4 Range: 26-48

*II = enlargement of hilus lymph nodes and pulmonary opacities; III = pulmonary opacities.
†Deviation from predicted value (%).
‡Deviation from predicted value (mm Hg).
§Deviation from predicted value at rest (mm Hg).
ND = not determined.

Immunocytochemistry

For immunocytochemical staining, monoclonal antibodies (MoAB) were used in conjunction with the alkaline phosphatase antialkaline phosphatase technique. T-cell subsets were identified with CD4 and CD8 MoABs (Dakopatts, Hamburg, Germany). For detection of CD14, we used the antibody M4 (Coulter Electronics, Krefeld, Germany) at saturating concentrations along with the respective CD8 isotype control.

Cytometry

Staining of AMs for CD14 results in a broad distribution of staining intensities with the difficulty of discriminating weakly positive and negative cells by eye. Therefore, these cells were evaluated by cytometry. For these analyses, 100 cells or more per specimen were studied using a microscope (Zeiss UEM, objective magnification × 25, num. sp. 0.55, optovar 1.6, filter 0G 550 nm) and CCD-TV camera (Hamamatsu C.3077-01). Each cell was segmented semiautomatically or interactively and was measured with an image analysis system (SAMBA, Dynatech Laboratories, Inc) and the MINT 5 program. This program performs a standardized measurement for optical density (OD), including shading correction. The OD readings were corrected by subtracting the background of the respective slides. The selected images were digitized into 512 × 512 pixels resulting in pixel distances of 0.273 μm. Transmission was transformed pixelwise into extinction and digitized into 256 channels (8 bit). Mean specific staining intensity was determined by subtraction of the mean OD for the control (CD8) staining from the mean OD for the specific (CD14) staining. Percent positive cells was determined using channel-by-channel subtraction of isotype control histograms from histograms for specific staining.

Table 2—Cytologic and Immunologic Features

<table>
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<tr>
<th>Case No.</th>
<th>Total Cell Recovery x10$^4$</th>
<th>AM, %</th>
<th>Lymphocytes, %</th>
<th>Neutrophils, %</th>
<th>Eosinophils, %</th>
<th>CD4/CD8 Ratio</th>
<th>My4/OD</th>
<th>sCD14, mg/L</th>
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Mean ± SD Sarcoidosis
Mean ± SD
Control donors (n=10)

*ND = not determined; OD = optical density.
†n = 23; 9 = M, 14 = F; 19 nonsmokers, 4 smokers.

Lung Function Impairment in Pulmonary Sarcoidosis (Pforte et al)
Assay for Soluble CD14 in Serum (ssCD14)

The sCD14 in serum samples of patients with sarcoidosis and healthy control subjects was determined with an enzyme-linked immunosorbent assay, using two MoAbs as described.[13]

Statistics

For comparison of patients and control groups, Kruskall-Wallis test was used. Correlations were analyzed using Pearson's test.

RESULTS

Clinical evaluations of the patient groups can be found in Table 1. All patients had lung function impairment ranging from slight to severe changes. Results of differential cell count exhibited a lymphocytic alveolitis, and CD4/CD8 ratio was elevated (Table 2).

CD14 Staining on AMs

In healthy control subjects, staining of AMs with the CD14 antibody My4 gave a weak signal, while My4 staining on AMs of patients with sarcoidosis gave a stronger signal on almost all cells (Fig 1, top). CD8 staining gave only a slight background staining of AMs, but it gave a positive staining of a proportion of T cells (Fig 1, bottom). Single-cell cytometry, which allows the evaluation of staining intensity, expressed mean specific OD (mean OD for My4 - mean OD for CD8) revealed for 10 control donors a specific mean OD of 92 ± 87, while in patients with sarcoidosis (n = 11), the specific mean OD was 297 ± 180 (p = < 0.004). Determination of positive cells gave 21.5 ± 18.7 percent positive AMs in healthy control subjects and 33.9 ± 17.1 percent in patients with sarcoidosis (not significant).

Soluble CD14

Cells of the monocyte lineage may shed the CD14 cell surface molecule and ssCD14 can be detected in serum. In the control group, ssCD14 was 3.2 ± 0.7 mg/L with no significant difference between smokers and non-smokers. In sarcoidosis all patients had serum levels above the control range with an average of 5.3 ± 1.6 mg/L (p = 0.0001) (Fig 2). There was no correlation between ssCD14 and CD14 in AMs.

Effect of Steroid Treatment

In one patient (No. 2), there was the opportunity to look at CD14 expression on AMs and at ssCD14 levels before and after a period of therapy with corticosteroids.

FIGURE 1. Expression of CD14 on alveolar macrophages in patients with sarcoidosis and healthy control subjects. Cytospin preparations of lavage cells were stained by APAAP technology with a CD14 monoclonal antibody (top) and with a CD8 control antibody (bottom).

FIGURE 2. Serum levels of sCD14 in patients with sarcoidosis and healthy control subjects.
Expression of CD14 on AMs was reduced from 478 to 243 channels and ssCD14 was reduced from 4.5 mg/L to 3.0 mg/L after 10 weeks of therapy. During the same time, results of lung function tests improved (Fig 3) in that PO2 at rest and PO2 after exercise increased.

**Correlation Between Cellular CD14 Expression and Lung Function Parameters in Patients With Sarcoidosis**

Correlation analysis revealed a significant inverse correlation between specific mean OD for My4 and VC (p = 0.02) (Fig 4). Impressive was the negative correlation between specific mean OD for My4 and PO2 at rest (p = 0.0005) and also for PO2 after exercise (deviation from predicted value of PO2 at rest) (p = 0.02). Since the degree of lung function impairment forms the basis for the therapy decision also in patients with pulmonary sarcoidosis, there was a positive correlation between My4 expression and the indication for therapy (p = 0.0003).

**Correlation Analysis Between ssCD14 and Lung Function Parameters in Patients With Sarcoidosis**

An inverse correlation could be detected for ssCD14 levels and Dco (p = 0.006) (Fig 5) and a borderline for ssCD14 and VC (p = 0.052), while no correlation was found with PO2 at rest or after labor.

**Discussion**

The increase of CD4-positive T cells is a salient feature in lavage samples of patients with sarcoidosis and these lymphocytes show some degree of activation as evidenced by expression of Ia-like antigens and of low levels of IL-2 and IL-2 receptor. It is reasonable to assume that these T cells are involved in interaction with AMs in an immune reaction against an agent still to be defined. Absolute numbers of lymphocytes and CD4/CD8 ratio in lavage, however, did not correlate with any clinical parameter. This lack of correlation does not dispute the importance of T cells in the pathophysiologic
condition of sarcoidosis, but it indicates that other cellular elements may be crucial to the disease.

In this study, we could demonstrate a marked increase of CD14 expression in AMs from patients with sarcoidosis compared with healthy control subjects. It is unclear at present whether this increase is dependent on the influx of monocytes from peripheral blood to the site of inflammation as suggested by Hance et al10 or whether it is related to a local activation of AM, which induces CD14 expression in the lung.

A striking finding in this study was the positive correlation between CD14 expression on AMs and lung function impairment, especially reduction of VC and grade of hypoxemia at rest and after labor in patients with sarcoidosis. Thus, it might be hypothesized that CD14 expression on AMs reflects the degree of inflammation, which influences lung volumes and gas exchange.

The expression of CD14 on AMs is obviously influenced by application of corticosteroids since in one patient, whose condition clinically improved after 10 weeks of therapy, the number of CD14+ cells in BAL was reduced as well as the levels of sCD14 (Fig 3). These preliminary data need to be confirmed in additional patients.

As in other cell surface molecules, there exists a soluble form of CD14, which can be found in supernatants of resting and activated monocytes.18,19. In this study, we could detect an impressive increase of sCD14 in patients with sarcoidosis compared with healthy control subjects. The patients, exhibiting extremely high levels for sCD14 (Table 2), had serious lung function impairment (Table 1, patients 1 and 8). Correlation analysis revealed an inverse correlation between sCD14 levels on VC and Dco (Fig 5). Although both cell-associated CD14 and soluble CD14 gave clear correlations to lung function tests in patients with sarcoidosis, there was no significant correlation between expression on AM and sCD14 levels. This indicates that generation of sCD14 is not directly determined by its cell-associated expression but that other elements like proteases govern this process independent of the degree of CD14 expression. The independence of sCD14 and CD14 on AMs is also stressed by the finding that both correlate with different aspects of lung function (CD14 on AMs with VC and Po2, sCD14 with VC and Dco). In addition, in patients with EAA, we can show a similar concomitant decrease or increase of CD14 on AMs and of sCD14 with allergen avoidance or exposure (Pforte et al, Upregulation of CD14 in exogenous allergic alveolitis, submitted). This suggests that CD14 behaves similarly in these two types of alveolitis.

It remains to be shown whether for effective monitoring of patients with sarcoidosis, determination of cell-associated CD14 is required or whether measurement of soluble CD14 in serum is sufficient. In previous studies in sarcoidosis, we have seen a concomitant increase of cellular IL-2R and soluble IL-2Rα and of cellular ICAM-1 and soluble ICAM-1 (Pforte. Respiration, in press) ICAM-1 expression on AMs and in serum in EAA. We suggest that these soluble receptors may be derived from AMs in the lung and that their increase in serum reflects the degree of inflammation in the lung. A multivariate study to analyze the value of these different soluble receptors is currently under way.

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REFERENCES


4 Haslam PL, Parker D, Townsend PJ. Increases in HLA-DQ, DP, DR and transferrin receptors on alveolar macrophages in sarcoidosis and allergic alveolitis. Chest 1990; 97:651-61


16 Pinkston F, Bitterman PB, Crystal RG. Spontaneous release of