Value of ICAM-1 Expression and Soluble ICAM-1 Level as a Marker of Activity in Sarcoidosis*

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Background: The natural course of sarcoidosis is variable, but no single parameter has been generally accepted as a good marker for disease activity. Adhesion molecules are required for the migration of inflammatory cells; thus, they may be markers of activity in sarcoidosis.

Methods: In 16 patients with active sarcoidosis and 11 with inactive disease (10 were male, 17 were female; mean age [± SD], 39.6 ± 11.0 years; mean follow-up, 21 ± 16 months), the expression of adhesion molecules on cells obtained with BAL (measured by flow cytometry) and the level of soluble intercellular adhesion molecule 1 (sICAM-1) in the serum and BAL fluid (BALF) were measured at the time of diagnosis and during the follow-up. The changes in serum sICAM-1 level and ICAM-1 expression on cells obtained with BAL were compared with the clinical course of the disease.

Results: In patients with active disease, the ICAM-1 on alveolar macrophage (AM) (relative linear median fluorescence intensity [RMFI], 3.21 ± 1.55) and sICAM-1 levels in serum (575 ± 221 ng/mL) and BALF (47.3 ± 19.3 ng/mL) were higher than those for patients with inactive disease (RMFI, 1.67 ± 0.66; p = 0.0034; serum, 263 ± 98.5 ng/mL; p = 0.0001; BALF, 27.5 ± 19.0 ng/mL; p = 0.0209). In the patients with active disease, ICAM-1 on AM and serum sICAM-1 decreased (RMFI, 1.51 ± 0.84; 284 ± 118 ng/mL, respectively) after steroid therapy, but no significant change was noted in patients with inactive disease. We also found that the initial ICAM-1 on AM and serum sICAM-1 had a significant correlation with the degree of improvement in pulmonary function tests after the therapy. The disease relapsed in four patients after the discontinuation of steroids, and the serum sICAM-1 level was elevated again at the time of relapse.

Conclusion: Our data suggest that the serum sICAM-1 level and the ICAM-1 expression on AM may be good markers of disease activity and also a predictor of outcome in sarcoidosis.

(CHEST 1999; 115:1059–1065)

Key words: activity; BAL; ICAM-1; sarcoidosis; sICAM-1

Abbreviations: ACE = angiotensin-converting enzyme; AM = alveolar macrophage; BALF = BAL fluid; ICAM-1 = intercellular adhesion molecule 1; IL = interleukin; MFI = median fluorescence intensity; MoAb = monoclonal antibody; RMFI = relative linear median fluorescence intensity; sICAM-1 = soluble intracellular adhesion molecule-1; TNF-α = tumor necrosis factor-α

Sarcoidosis is a multisystemic, chronic, noncaseating, granulomatous disease of unknown origin, predominantly involving intrathoracic organs.1 The natural course of the disease is variable, from spontaneous remission to continuous progression resulting in significant morbidity or death in 25% to 30% of patients.2 Corticosteroid is the most widely used drug for sarcoidosis to prevent ocular and pulmonary fibrosis and to overcome abnormal calcium metabolism. However, long-term use of this drug has caused serious side effects.3 If the treatment is started too late in the fibrotic phase, no patient will improve. Therefore, it is important to know at the time of diagnosis whether the patient’s disease will remit spontaneously or deteriorate without therapy. Radiologic findings or pulmonary function test results are known to show a poor correlation with the disease progression. Several different parameters, such as
gallium scan, serum angiotensin-converting enzyme (ACE) level, technetium 99m-DTPA scanning, total lymphocyte count or T4/T8 lymphocyte ratio in BAL fluid (BALF), serum lysozyme, or soluble interleukin 2 (IL-2) receptor levels have all been studied, but the results were often contradictory and none was proved to be a satisfactory marker.4–14

Recently, it has been found that the activation of adhesion molecules is required for the migration of the inflammatory cells to the loci of disease,15–18 and the expression of adhesion molecules and their ligands is increased in various types of chronic inflammatory diseases.19–21 Among the adhesion molecules, intercellular adhesion molecule 1 (ICAM-1), present on endothelial cells, is a ligand for β2-integrin on neutrophils, lymphocytes, and other leukocytes, and plays an important role in the migration of these cells. Recently, ICAM-1 was found to be present not only on endothelial cells, but also on various kinds of cells including macrophages and epithelial cells.22 Sarcoidosis is a kind of granulomatous inflammation caused by activated macrophages and lymphocytes, and the expression of ICAM-1 was reported to be increased on alveolar macrophages (AM), epithelioid cells, and giant cells in sarcoidosis.23–27 The soluble components of ICAM-1 (sICAM-1) are thought to be shed from the cell surface during binding to ligands, so its concentration in serum or extracellular fluid may reflect the degree of cell infiltration or activation.28 There have been several studies on adhesion molecules and sICAM-1 in sarcoidosis, but the results are controversial.24–27,29–33 There are only two reports showing the change in serum sICAM-1 levels with follow-up clinical data. The purpose of this study is to test the possibility of adhesion molecules as a marker of disease activity of sarcoidosis, not only by measuring the expression of adhesion molecules on cells obtained with BAL and the serum sICAM-1 level at the time of diagnosis, but also by comparing them with the clinical follow-up data.

Materials and Methods

Subjects

We studied 27 patients who were diagnosed as having sarcoidosis by compatible clinical findings and biopsy (transbronchial lung biopsy, 23; mediastinoscopic biopsy, 5; skin biopsy, 4; peripheral lymph node, 2; liver, 1). The mean (± SD) follow-up period was 21.3 ± 16.3 months. The activity of the disease was assessed by the criteria of Boudouin and du Bois.2 Active disease was defined as (1) newly developed significant respiratory symptoms such as dyspnea and cough with decreased lung function at the time of diagnosis, or (2) newly evolving or progressing radiologic abnormality or pulmonary function during the follow-up. By these criteria, only patients with active pulmonary involvement were categorized as the active group and this is appropriate for our study, because we looked for the usefulness of the adhesion molecules on cells obtained with BAL and BALF as well as at the serum level. Sixteen patients had active sarcoidosis by these criteria and 11 patients had the inactive disease. All patients had lung involvement. The other clinical features of the subjects are shown in Table 1. As a control group, nine healthy normal volunteers (doctors and medical students) without respiratory symptoms or atopic diseases were studied.

Table 1—Demographic Features of the Subjects*

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<th>Demographic Features</th>
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<th>Active</th>
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<td>11</td>
<td>16</td>
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<tr>
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<td>6:10</td>
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<td>Age, yr</td>
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<tr>
<td>Nonsmoker</td>
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<td>4</td>
<td>1</td>
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<tr>
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<td>7:4:0</td>
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<td>Uveitis</td>
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<td>2</td>
<td>2</td>
<td></td>
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<tr>
<td>Hypercalcemia†</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Peripheral lymph node</td>
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<td>1</td>
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<td>Liver</td>
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<td>1</td>
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<tr>
<td>Pericardial effusion</td>
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<td>FVC, % pred</td>
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<td>97.7 ± 11.9</td>
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<td>DLCO, % pred</td>
<td>84.5 ± 22.1</td>
<td>91.5 ± 11.1</td>
<td>79.0 ± 23.9‡</td>
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</table>

* M = male; F = female; DLCO = diffusing capacity of the lung for carbon monoxide. Values given as mean ± SD.
† Patients with liver involvement.
‡ 24-h urine calcium > 200 mg.
§ p < 0.05 compared with inactive sarcoidosis.
Methods

At the time of diagnosis, in addition to the detailed history and physical examination, routine pulmonary function tests (predicted value by Morris34), including the diffusion capacity (Sensor Medics; Yorba Linda, CA); chest radiology with high-resolution CT; slit lamp examination; measurement of serum ACE level, serum calcium level, and 24-h urine calcium excretion; and BAL with 50-mL aliquots (repeated five times) were performed. BALF was centrifuged at 2,000 rpm for 10 min, and the supernatant was stored at −70°C until the measurements were taken. Total cell count was done with a hemocytometer and a differential count was performed on the cytospin slide with Diff-Quick stain (Baxter; MacGaw Park, IL) after counting 300 cells. T4 and T8 lymphocytes and the IL-2 receptor–positive lymphocytes were measured by flow cytometry (FACScan, Becton Dickinson; Franklin Lakes, NJ) after incubation with the appropriate monoclonal antibody (MoAb). Adhesion molecule expression on cells obtained with BAL was measured by flow cytometry after reacting 50 μL of 1 × 107 BAL cells/mL with phycoerythrin-labeled MoAb for ICAM-1 (Becton Dickinson) and CD-18 at 4°C for 40 min. After gating the AM or lymphocytes on the dot plot, fluorescence intensity of 10,000 cells was displayed on a histogram in arbitrary units. From the given median channel number, the data were transformed to calculate the linear median intensity of the entire AM population. To obtain the ICAM-1 expression on AM, we calculated the relative linear median fluorescence intensity (RMFI), which is the ratio of the linear median fluorescence intensity (MFI) emitted by the bound monoclonal anti-ICAM-1 antibody to the MFI of the corresponding isotype control antibody.35 This calculation corrects for the variation in cell autofluorescence between patients.

Soluble ICAM-1 levels in serum and BALF were measured by the enzyme-linked immunosorbent assay (ELISA) kit (R&D System; Minneapolis, MN). For the correction of the dilution effect in BALF, some researchers used the ratio of solutes (eg, sICAM-1) to albumin, but it has been shown that this standardization caused more variation of the data.36 Particularly in the inflamed state, the ratio to albumin is lower than in the noninflamed state, so we have used the measured value of sICAM-1 without any correction.

Statistical Analysis

A nonparametric method was used. The Mann-Whitney U test was used for the comparison between the two groups, and the Wilcoxon signed-rank test was performed to evaluate the significance of the changes after treatment. The Spearman rank correlation coefficient was calculated for the correlation between two parameters. All statistical analyses were performed with the StatView program (Abacus Concepts; Berkeley, CA) for the Macintosh computer.

Results

BALF Findings

The total number of cells, the number of AM, the percentage and number of lymphocytes, and the CD4/CD8 lymphocyte ratio in BALF of patients with sarcoidosis were significantly higher than those of the control group (Table 2). None of these parameters, however, could differentiate active from inactive sarcoidosis.

Expression of Adhesion Molecules

ICAM-1 expression of the AM of patients with active sarcoidosis was significantly higher (RMFI, 3.21 ± 1.55) than not only that of the normal control group (0.94 ± 0.17), but also that of patients with the inactive disease (1.67 ± 0.66; p = 0.0034). The sICAM-1 level in serum was also significantly elevated (575 ± 221 ng/mL) in patients with the active disease, compared with those with the inactive disease (263 ± 95.5 ng/mL; p = 0.0001) and with the normal control group (190 ± 38.9 ng/mL). The patients with the active disease had a significantly higher sICAM-1 level in BALF than both the normal group and the inactive disease group (p = 0.0209) (Table 3). The ICAM-1 expression on AM had a significant correlation with sICAM-1 levels in serum (p = 0.0002; ρ = 0.633) and BALF (p = 0.0001; ρ = 0.698). In addition, there was a significant correlation between sICAM-1 levels in serum and BALF (p = 0.0002; ρ = 0.637). The ICAM-1 expression on AM correlated significantly with the total cell number (p = 0.0001; ρ = 0.677) and percentage, and the number of AM (p = 0.0001; ρ = 0.772) in BALF, suggesting the role of ICAM-1 to be in the accumulation of AM in the alveolar space. A similar

| Table 2 | Comparison of BAL Findings Between Active and Inactive Sarcoidosis* |
|---------|-------------------|-----------|----------|----------|
| BAL Findings | Control Group | Total | Inactive | Active |
| Total cell, ×10⁶/mL | 0.88 ± 0.43 | 2.49 ± 1.24† | 2.49 ± 1.61 | 2.49 ± 0.95 |
| AM, % | 88.3 ± 7.6 | 53.7 ± 19.4† | 61.8 ± 23.2 | 48.3 ± 14.9 |
| No., ×10⁴/mL | 7.79 ± 3.16 | 12.1 ± 5.66† | 12.5 ± 5.9 | 11.9 ± 5.7 |
| Lymphocyte, % | 11.0 ± 7.3 | 43.0 ± 20.3† | 34.4 ± 22.8 | 48.8 ± 17.0 |
| No., ×10⁴/mL | 11.6 ± 10.4† | 10.7 ± 14.8 | 12.3 ± 6.5 | 12.3 ± 6.5 |
| Neutrophil, % | 0.5 ± 0.4 | 1.9 ± 4.1 | 1.0 ± 0.8 | 2.5 ± 5.1 |
| T4/T8 | 1.52 ± 0.96 | 6.19 ± 4.70† | 6.20 ± 4.57 | 6.42 ± 4.90 |
| IL-2 receptor | 8.62 ± 8.88 | 8.17 ± 4.51 | 9.23 ± 5.99 | 7.44 ± 3.19 |

* Values given as mean ± SD.
†p < 0.05 compared with control group.
correlation was noted between the sICAM-1 in BALF and the cell number and the pattern of the BALF (data not shown).

Changes in ICAM-1 Expression in the Follow-up Period

Sixteen active patients had all been treated with oral steroids, and the initially high serum sICAM-1 level decreased from $575 \pm 6221$ ng/mL to $284 \pm 118$ ng/mL with clinical improvement. Ten patients with inactive disease were followed up without therapy, and a chest radiograph showed improvement of bilateral hilar enlargement in six patients and no changes in four patients. Their sICAM-1 levels in serum were slightly reduced from $248 \pm 89$ ng/mL to $207 \pm 56$ ng/mL, which was not statistically significant. One patient with inactive disease was given oral steroids at the dermatology service, and her serum sICAM-1 level decreased from 380 ng/mL to 202 ng/mL. BAL was repeated in six patients with active disease after the steroid treatment, and ICAM-1 expression on AM was also reduced from $3.68 \pm 1.71$ to $1.51 \pm 0.837$. Four patients relapsed after steroid treatment was discontinued because of severe side effects. The serum sICAM-1 levels changed in parallel with the clinical course of the patients, and increased at the time of relapse in all four patients. Their sICAM-1 levels in serum were slightly reduced from $248 \pm 89$ ng/mL to $207 \pm 56$ ng/mL, which was not statistically significant. One patient with inactive disease was given oral steroids at the dermatology service, and her serum sICAM-1 level decreased from 380 ng/mL to 202 ng/mL. BAL was repeated in six patients with active disease after the steroid treatment, and ICAM-1 expression on AM was also reduced from $3.68 \pm 1.71$ to $1.51 \pm 0.837$. Four patients relapsed after steroid treatment was discontinued because of severe side effects. The serum sICAM-1 levels changed in parallel with the clinical course of the patients, and increased at the time of relapse in all four patients. In one patient, serum ICAM-1 level came down after readministration of steroid with relief of symptoms (Fig 1). Finally, to assess whether ICAM-1 levels can predict the patient’s future outcome, we analyzed the relationship between initial ICAM-1 level and change in pulmonary function tests. There was a significant correlation between the initial ICAM-1 expression on AM and the degree of improvement in FVC after steroid therapy (Fig 2). The initial sICAM-1 level in serum also had a significant correlation with the change in FVC ($p = 0.0023$; $r = 0.611$) and diffusing capacity of the lung for carbon monoxide ($D_{lco}$; $p = 0.0218$; $r = 0.469$). These data suggest that sICAM-1 levels in serum, as well as the ICAM-1 expression on AM, may be used as a marker of activity in sarcoidosis and also as a predictor of prognosis. Serum ACE levels were significantly higher in those patients with sarcoidosis than in the control group, but they cannot distinguish active sarcoidosis from the inactive form. Nor was there any significant correlation between serum ACE levels and serum sICAM-1 levels.

**DISCUSSION**

Our findings of increased ICAM-1 expression on AM and increased sICAM-1 in serum and BALF in active sarcoidosis and not in inactive disease suggest that ICAM-1 can be a marker of disease activity. Furthermore, the change in serum sICAM-1 levels, in parallel with the clinical course of the patients, strongly supports this possibility. The fact that initial ICAM-1 expression on AM and serum sICAM-1...
levels had a significant correlation with the degree of improvement in lung function after the therapy suggests the possibility that ICAM-1 can be used as a predictor of the patient’s outcome. Because all of our active patients were treated, we could not confirm that the high initial value really indicates that the disease would progress without treatment. This possibility needs to be confirmed by observation of the patient’s course without treatment.

The association of adhesion molecules with sarcoidosis was first reported by Melis et al., who found increased expression of ICAM-1 and its ligand, leukocyte function-associated antigen-1 (LFA-1), on AM of patients with pulmonary sarcoidosis. Later, other investigators reported increased expression of ICAM-1 on AM, epithelioid cells, giant cells, and endothelial cells in sarcoid tissue with immunohistochemistry. Moreover, enhanced ICAM-1 expression in AM obtained from BAL was observed. In addition, Striz et al. found that ICAM-1 on AM was correlated with the total number of cells, percentage of AM, and IL-2 receptor expression in BALF, suggesting the role of ICAM-1 in the pathogenesis of sarcoidosis. Others found ICAM-1 on AM was increased only in clinically active sarcoidosis, but in the paper by Striz et al., 7 of 11 patients with inactive sarcoidosis were on steroid therapy at the time of the study, in contrast to only 1 patient with active sarcoidosis being on steroids. Our data also showed decreased ICAM-1 expression on AM after steroid treatment. Dalhoff et al. noted a significant correlation between ICAM-1 and spontaneous tumor necrosis factor-α (TNF-α) secretion from AM. However, serum sICAM-1 was normal in their report. There were some differences in the technique of measuring ICAM-1 expression. Dalhoff et al. used the semiquantitative ELISA method for measurement of ICAM-1 expression on AM, but others measured the percentage of positive cells in an immunostain of cells obtained with BAL. Because ICAM-1 is constitutively expressed on normal macrophages at a low level and is upregulated by various stimuli such as IL-1 or TNF-α, it may sometimes be difficult to evaluate the positivity on the immunostained slide, which may explain the wide range of positive cells in normal controls (42% and 42.7% by Striz et al. and Melis et al., respectively, and 10.8% by Shijubo et al.). We therefore used flow cytometry, which can quantify the expression levels as MFI, and we eliminated the problem of autofluorescence of AM by using the ratio of MFI emitted from the ICAM-1 antibody to the MFI from the idiotype control antibody. With this technique, we confirmed the increased ICAM-1 on AM in patients with active disease, compared with inactive sarcoidosis, and its correlation with the number of total cells, AM, and lymphocytes in BAL. We also found that it reduced after steroid therapy, supporting the theory that ICAM-1 expression is related to the activity of the disease. BAL is such an invasive test that it is difficult to repeat the test several times to evaluate the disease course or the response to therapy. Therefore, measuring the parameter in serum is clinically more useful.

sICAM-1 is the extracellular domain of ICAM-1 and is thought to be shed from the cell membrane after binding to its ligand. There are several studies on serum sICAM-1 levels in sarcoidosis, but their results are controversial. Several groups have reported increased serum sICAM-1 levels in patients with sarcoidosis compared to normal groups. In contrast, other investigators found no significant difference in serum sICAM-1 levels between patients with sarcoidosis and normal control subjects. The reason for this discrepancy is not
certain, but Baeumer et al.29 and Ishii and Kitamura30 studied patients with active disease and found increased sICAM-1 levels. Other researchers compared all the patients with sarcoidosis with a control group, but did not analyze the data for active disease separately, which may be the reason they could not find any difference. In this study, we have looked for the difference in serum sICAM-1 levels between patients with active sarcoidosis and those with inactive disease and found it significantly higher in active disease. We also noted that sICAM-1 levels were reduced significantly after steroid therapy with symptomatic improvement, and were elevated again in four patients at the time of relapse. In patients with inactive disease, whose clinical symptoms showed no change, serum sICAM-1 levels were also stable during the follow-up. Ishii and Kitamura30 also observed the change of sICAM-1 levels during the follow-up course in only a small portion of their subjects. Similar to our patients, Shijubo et al.33 have also recently observed that serum sICAM-1 levels changed in concordance with the clinical course in four patients. They also reported that serum sICAM-1 levels of patients with progressive disease were higher than those of patients whose disease regressed spontaneously. In our study, serum sICAM-1 levels were elevated, not only in patients with active pulmonary sarcoidosis, but also in patients with active skin lesions (but not in patients with just mild hypercalciuria). All these findings, together with our own results, strongly support the claim that sICAM-1 is a marker of activity. In addition, our finding of the significant correlation between initial serum sICAM-1 and ICAM-1 levels on AM, with the degree of clinical improvement, supports the possibility of using ICAM-1 as a predictor of clinical course. In the report of Shijubo et al.33 however, the disease progressed in five patients even though the initial sICAM level was low. More studies in larger groups of patients are needed.

Shijubo et al.24 and Ishii and Kitamura30 reported that sICAM-1 was also elevated in BALF. Ishii and Kitamura30 observed a significant correlation between the serum and BALF sICAM-1 levels, but in the patients of Shijubo et al.24 no such correlation was noted. We found a significant correlation between not only serum sICAM and BALF sICAM-1 levels, but also sICAM-1 levels and ICAM-1 expression on AM. The sICAM-1 in BALF is not just a simple transudation from serum, but is mainly produced locally, because the sICAM-1 to albumin ratio in BALF was much higher than that of serum (data not shown). Although the exact source of sICAM-1 in BALF is not certain, its significant relationship with ICAM-1 on AM suggests that AM may be a major cellular source. But it may be from other cells, such as vascular endothelial cells, epithelial cells, fibroblasts, or epithelioid cells. The correlation between these three parameters (sICAM-1 in serum and BALF, and ICAM-1 on AM) may be related to the fact that they represent one process, ie, disease activity. The number of patients is small in our study, because sarcoidosis is still a very rare disease in Korea, even though the incidence is slowly increasing.

In conclusion, our results strongly suggest that ICAM-1 expression on AM and serum sICAM-1 level can be used as a marker of disease activity in pulmonary sarcoidosis. We think the serum sICAM-1 level is clinically useful because it is measured by a noninvasive technique and can also reflect the extrapulmonary status.

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