Elevated Level of Soluble Interleukin-2 Receptor in Bronchoalveolar Lavage Fluid from Sarcoidosis Patients

Fumitaka OGUSHI, Saburo SONE, Sukh M. SINGH, Kenji TANI, Toshio OZAKI, Susumu YASUOKA, Takeshi OGURA and Mituo HONDA*

Pulmonary sarcoidosis is a granulomatous disorder of unknown cause, characterized by an accumulation of active T lymphocytes in the lung. We measured the levels of soluble interleukin-2 receptor (IL-2R) in bronchoalveolar lavage fluid (BALF) of patients with active pulmonary sarcoidosis and normal subjects by a sensitive enzyme-linked immunosorbent assay. Soluble IL-2R was detectable in BALF from 6 of 11 patients with sarcoidosis but in only 1 of 8 normal control subjects, the mean levels of IL-2R in BALF of the two groups being 2.8 ± 0.9 U/ml and 0.1 ± 0.1 U/ml, respectively (p < 0.01). A slight correlation was found between the soluble IL-2R level and the number of CD4-positive cells. Sarcoidosis patients were classified by radiographical staging. Soluble IL-2R was not detectable in the BALF of any stage I patients, but was found in the BALF of 6 of the 7 stage II and stage III patients. These results suggest that in the clinical management of patients with sarcoidosis, measurement of soluble IL-2R in BALF is useful for evaluating the activity of the lung disease is sarcoidosis.

Key words: Enzyme-linked immunosorbent assay, Disease activity in sarcoidosis, Lymphocyte subsets

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levels of soluble IL-2R in BALF from sarcoidosis with parenchymal involvement (Radiographic stage II or stage III) were compared with those from sarcoidosis without parenchymal involvement (stage I). The measurement of soluble IL-2R in lavage fluid was found to be clinically useful for the evaluation of the activity of lung disease in sarcoidosis.

METHODS

These studies were performed after approval by the Institutional Human Investigations Committee and in advance, informed consent was obtained from all volunteers and patients after they had been given a full explanation of the procedures.

Study population

Studies were made on 8 healthy non-smoking individuals (8 males of 23 ± 3 yr old) and 11 patients with untreated sarcoidosis (5 males and 6 females of 42 ± 14 yr old). Three patients were cigarette smokers, eight patients were non-smokers. Data is presented as mean ± SE, and statistical comparisons were made by the two-tailed Student's t-test. The normal volunteers were free of lung disease as judged by their history, physical examination, chest X-ray examination, and lung function tests. The patients with sarcoidosis showed typical clinical and chest radiographic features and evidence of non-caseating epitheloid cell granuloma by biopsy. From chest radiographic findings the patients were classified as stage I (hilar adenopathy), II (hilar adenopathy and pulmonary infiltration) or III (pulmonary infiltration only). Disease activity was evaluated according to clinical features and laboratory findings, including the percentages of lymphocytes and CD4-positive cells in BALF, the positivity of the $^{67}$Ga lung scinti-scan and the level of activity of serum angiotensin converting enzyme (SACE). All patients showed abnormalities in $^{67}$Ga lung scinti-scan features, SACE levels and/or bronchoalveolar lavage lymphocytes. The cell profiles in BALF are shown in Table 1. The total number of cells, and the percentages of lymphocytes were higher in all the patients than in normal subjects. The ratio of CD4-positive cells to CD8-positive cells was more than 4.0 in all cases.

Bronchoalveolar lavage

BAL was performed using a flexible fiberoptic bronchoscope as described previously (12). Briefly, the bronchoscope (Olympus Model BFIT 20) was wedged into a segmental or subsegmental bronchus of the middle lobe. Lavage was performed three times with 50 ml of saline solution. The BALF was centrifuged at 250 x g for 10 min to collect cells. The total cell number was counted in a haemocytometer, and cell differentiation was performed by May-Giemsma and non-specific esterase stainings. The percentages of lymphocytes expressing CD4 or CD8 markers were determined by an established flow cytometric procedure. The supernatant was kept at −70°C until measurement of soluble IL-2R.

Measurement of soluble IL-2R in BALF

Soluble IL-2R was assayed by ELISA described previously (13). Briefly, polystyrene microwell plates (Nunc, Inter Med, Roskilde, Denmark) were coated with monoclonal antibody IgG and incubated overnight at room temperature. The plates were then washed with 0.15 M phosphate-buffered saline (PBS) containing 0.1% Tween 20, and unbound sites

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cells (× 10⁶)</th>
<th>AM</th>
<th>Ly</th>
<th>Neut</th>
<th>Eo</th>
<th>Ba</th>
<th>CD4/CD8 ratio</th>
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<tr>
<td>NV</td>
<td>10.4 ± 1.6</td>
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<td></td>
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<td></td>
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<td>(n = 8)</td>
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<tr>
<td>Sar</td>
<td>37.7 ± 3.6*</td>
<td>53.6 ± 6.4*</td>
<td>39.0 ± 6.0*</td>
<td>2.2 ± 0.9</td>
<td>0.5 ± 0.2</td>
<td>0.1 ± 0.03</td>
<td>8.3 ± 1.0</td>
</tr>
<tr>
<td>(n = 11)</td>
<td></td>
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AM, alveolar macrophages; Ly, lymphocytes; Neut, neutrophils; Eo, eosinophils; Ba, basophils. The values are means ± SEs. *p<0.001 vs corresponding values for NV group.
were saturated with PBS containing 1% bovine serum albumin. The plates were then washed and incubated for 60 min at 37°C. The plates were then washed and incubated with biotinylated monoclonal antibody IgG for 60 min at 37°C. The plates were washed again and incubated with streptavidin-β-D-galactosidase. The absorbance was measured using excitation and emission wavelengths of 360 and 450 nm, respectively. The levels of soluble IL-2R were calculated from a standard curve, and expressed as units of soluble IL-2R per ml of BALF.

RESULTS

Concentration of soluble IL-2R in BALF obtained from normal subjects and sarcoidosis patients

The levels of soluble IL-2R in BALF of normal subjects and sarcoidosis patients are compared in Fig. 1. The mean level of soluble IL-2R in the BALF of the patients (2.8 ± 0.9 U/ml) was significantly higher than that in the normal subjects (0.1 ± 0.1 U/ml) (p < 0.01). Soluble IL-2R was detected in the BALF of about half of the patients with sarcoidosis, but in only one normal subject, in whom the level was very low.

Correlation of the soluble IL-2R concentration and the number of CD4-positive cells in BALF from sarcoidosis patients

A slight correlation was found between the soluble IL-2R concentration and the number of CD4-positive cells in BALF from sarcoidosis patients (Fig. 2; r = 0.48, p < 0.05).

Comparison of soluble IL-2R levels in BALF and stage of sarcoidosis

Sarcoidosis patients were staged on the basis of radiographical findings. Soluble IL-2R was not detected in the BALF of any patients with stage I sarcoidosis, but was detected in the BALF of 6 of the 7 patients with stage II or III sarcoidosis (Fig. 3). Four patients with parenchymal involvement were followed up for more than 1 yr. In three patients with detectable IL-2R in BALF, no change in chest X-ray examination was found, but in a patient with no detectable IL-2R in BALF, an abnormal shadow of the lung field improved.

Fig. 1. Soluble IL-2R levels in BALF from normal volunteers (NV) and patients with sarcoidosis (Sar). Significance of difference between groups: p < 0.01.

Fig. 2. Correlation between soluble IL-2R levels and CD4 positive cells in BALF.

Fig. 3. Comparison of soluble IL-2R levels in BALF from patients with stage I, II and III sarcoidosis.
Correlation of soluble IL-2R levels in BALF with parameters of disease activity

The correlation of soluble IL-2R levels in the BALF and the parameters of disease activity were examined. No correlation was found between the soluble IL-2R levels and the results of \(^{67}\text{Ga}\) lung scanning or the serum ACE levels (data not shown).

**DISCUSSION**

Sarcoidosis is a disease characterized by the accumulation in affected tissues of activated, proliferating helper/inducer T lymphocytes, a process that leads to granuloma formation, distortion of the tissue architecture and organ dysfunction. In active pulmonary sarcoidosis these changes of the lung increase and the helper/inducer T cell ratio in the BALF increases (1, 2, 6, 7). These T lymphocytes at the site of disease are known to proliferate spontaneously and release IL-2, a T cell growth factor (8). As reported by Muller-Quernheim et al (14), in active sarcoidosis, the activation of the IL-2 gene in T cells occurs at the site of disease, not throughout the body. Moreover, in patients with active sarcoidosis, evaluation of inflammatory cell populations in affected organs has demonstrated an increase in T cells that express IL-2R (15–17). The membrane-associated IL-2R is composed of two different cell surface glycoproteins, termed alpha and beta chain (18–20). T cells can release a soluble form of IL-2R of smaller molecular weight than the alpha chain expressed on their cell surface (9). Moreover, a soluble form of their receptor has been found in human sera and in the supernatant of stimulated lymphocytes (9). This finding in a certain disease state has indicated that abnormal levels of secreted IL-2R are associated with excessive lymphocyte activation related to a significant immunologic disorder (21, 22). Therefore, we examined whether measurement of soluble IL-2R in BALF could be used to evaluate the disease activity of sarcoidosis. Lawrence et al have reported studies on soluble IL-2R in samples of serum and concentrated BALF (11). They found that measurement of soluble IL-2R in sera was more useful than that in BALF. As we were interested in whether the measurement of soluble IL-2R at the site of disease, that is in BALF in sarcoidosis patients, would be useful in the estimation of disease activity, we measured soluble IL-2R levels in BALF samples that had not been concentrated. Sarcoidosis patients were classified as stage I and stages II+ III from chest radiographic findings, and soluble IL-2R levels in BALF were compared between stage I and stage II+ III groups. Using a sensitive ELISA, the concentration of soluble IL-2R in BALF from sarcoidosis patients was higher than that in BALF from normal subjects. However, soluble IL-2R in BALF was detected in only 6 of 11 patients with sarcoidosis and the values overlapped those for normal subjects. Nevertheless, all sarcoidosis patients who had high levels of soluble IL-2R in their BALF also showed radiographical abnormality of the lungs, that is, stage II or stage III parenchymal involvement, and increased proportions of lymphocytes and CD4-positive cells in their BALF. Therefore, high levels of soluble IL-2R in the BALF were proposed to indicate activity of lung disease in sarcoidosis.

There are several possible explanations for high levels of soluble IL-2R in BALF from patients with sarcoidosis. There is evidence that IL-2R expression can be up-regulated by IL-2 (23–26). Therefore, although the cause of sarcoidosis is unknown, probably T cells are activated at the site of disease in these patients and release a great amount of IL-2, and then IL-2R is expressed on the surface of the T cell. On the other hand, a increased in vitro production of IL-1 and IFN-\(\gamma\) by T lymphocytes and macrophages from BALF of patients with active sarcoidosis has been reported (27, 28). Agostini et al have reported that IL-2R positive macrophages are increased by these cytokines in the active sarcoid lung (29). Therefore, an increase in IL-2R positive cells at the site of disease might be associated with high disease activity. Moreover, as soluble IL-2R is known to be released by activated lymphocytes (9) and monocytes/macrophages (30), soluble IL-2R in BALF might be released by these cells. In this study, increased levels of soluble IL-2R were observed in the BALF from patients with sarcoidosis. The function of soluble IL-2R in BALF is unknown. As soluble IL-2R is reported to bind IL-2 efficiently (31), soluble IL-2R released from mononuclear cells may have an immunoregulatory function, inhibiting inflammation by binding to free IL-2 at the site of disease.

The evaluation of soluble IL-2R in BALF may be an effective method for estimating the different phases of sarcoidosis, and may provide a basis for
a new approach in the treatment of these patients.

REFERENCES


