Enhanced Expression of Interleukin-18 Receptor α Chain by CD4+ T Cells in Sarcoidosis*

Yanqiu Zhou, MD; Etsuro Yamaguchi, MD; Yoshinobu Fukui, MD; Satoshi Konno, MD; Yukiko Maeda, MD; Koji Kimata, PhD; and Masaharu Nishimura, MD

Study objectives: To investigate the expression of interleukin-18 receptor α chain (IL-18Rα) in BAL and peripheral blood (PB) T cells in patients with sarcoidosis compared with control subjects, to evaluate the relationship between the expression and clinical manifestations, and to clarify the mechanisms of altered expression.

Subjects and methods: The study subjects consisted of 21 patients with sarcoidosis and 8 normal control subjects. The expression of IL-18Rα was examined by flow cytometry. Results: The proportions of BAL CD4+ and PB CD4+ T cells expressing IL-18Rα were significantly increased in patients with sarcoidosis compared to control subjects. BAL CD4+ T cells expressed IL-18Rα in a higher proportion than did paired CD8+ T cells in patients with sarcoidosis but not in control subjects. Greater proportions of BAL CD4+ T cells and BAL CD8+ T cells than of their PB counterparts expressed IL-18Rα in both patients and control subjects. CD4+ T cells were more sensitive to the induction of IL-18Rα by cytokines in vitro, such as interleukin (IL)-2, IL-12, and tumor necrosis factor-α than were CD8+ T cells. Increased expression of IL-18Rα by BAL T cells commonly observed in patients and control subjects was associated with the expansion of CD45RO+ cells in BAL T cells. However, there were no significant correlations between the expression of IL-18Rα by any cell populations and BAL findings, serum angiotensin-converting enzyme activities, radiograph stages, or clinical courses.

Conclusion: The overexpression of IL-18Rα predominantly by CD4+ T cells in sarcoidosis emphasizes crucial roles played by T-helper type 1 cells in the IL-18/IL-18Rα system in sarcoidosis.

(CHEST 2005; 128:2497–2503)

Key words: BAL; CD4+ T cell; interleukin-18; interleukin-18 receptor α chain

Abbreviations: IFN = interferon; IL = interleukin; IL-18Rα = interleukin-18 receptor α chain; PB = peripheral blood; Th1 = T-helper type 1; TNF = tumor necrosis factor

Sarcoidosis is a systemic granulomatous disease of unknown etiology. The current consensus regarding the pathogenesis of sarcoidosis is that it results from exposure of genetically susceptible hosts to particular environmental factors. Accumulated evidence indicates that the immunologic changes associated with pulmonary sarcoidosis are characterized by the expanded pool of CD4+ T cells and activated monocyte/macrophage lineage cells. Previous studies have revealed that the profile of cytokines and their receptors in inflamed lesions of sarcoidosis belongs to T-helper type 1 (Th1) cells, given that lung CD4+ T cells produce interleukin (IL)-2 and interferon (IFN)-γ, and express the Th-1–associated chemokine receptors CXCR-3 and CCR-5. IL-18 can induce potent IFN-γ expression in the presence of an IL-12 costimulus. In addition, IL-18 exerts broad immunopotentiating actions, such as enhancement of expression of the Fas ligand and induction of the production of granulocyte macrophage colony-stimulating factor.
age-colony stimulating factor, IL-1β, tumor necrosis factor (TNF)-α, and IL-8. Increased expression of these molecules as well as of IL-18 has been reported in sarcoidosis.

The signals triggered by IL-18 are mediated by its specific receptor. The IL-18 receptor complex consists of two chains: interleukin-18 receptor α chain (IL-18Rα) and β signal-transducing chain. The amino acid sequence of IL-18Rα is analogous to the previously described human IL-1 receptor-related protein. The importance of the IL-18Rα in IL-18 signal transduction was demonstrated by transient transfection of the receptor into COS-1 cells, which imparted IL-18 responsiveness and activation of the nuclear factor-κB–driven luciferase reporter gene. IL-18Rα is expressed in a broad range of cell types; however, precise cell types, proportions of cells expressing the receptor, and the intensities of the expression are likely to collectively restrict ligand-acting sites and determine the global direction of immune responses.

The expression of IL-18Rα at the single-cell level has been reported for sarcoidosis without control data or in a very limited number of patients. In the present study, we compared the expression of IL-18Rα in patients with sarcoidosis with that in healthy control subjects, and investigated the mechanism of enhanced expression of IL-18Rα by CD4+ T cells in sarcoidosis.

Materials and Methods

Twenty-one patients who underwent bronchoscopy for diagnostic evaluation of suspected pulmonary sarcoidosis were included in the study (Table 1). The diagnosis of sarcoidosis was established when patients had biopsy evidence of noncaseating epithelioid cell granulomas in any organ and compatible clinical manifestations such as bilateral hilar and/or mediastinal lymph node enlargement with or without lung parenchymal infiltrates, eye lesions, and skin lesions. None of the patients had evidence of an alternative diagnosis such as mycobacterial, fungal, or parasitic infection, or of exposure to any inorganic material known to cause granulomatous disease, and there was no evidence of such an exposure in histologic sections. Eight healthy volunteers with no history of any pulmonary diseases or any evidence of such an exposure in histologic sections. Eight healthy volunteers with no history of any pulmonary diseases or any parasitic infection, or of exposure to any inorganic material known to cause granulomatous disease, and there was no evidence of such an exposure in histologic sections.

Table 1—Demographic Data of Study Subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Sarcoidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female gender</td>
<td>5/3</td>
<td>3/18</td>
</tr>
<tr>
<td>Age, yr</td>
<td>21 (20–22)</td>
<td>56 (20–73)</td>
</tr>
<tr>
<td>Radiograph stage</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>1</td>
</tr>
<tr>
<td>BAL findings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cells, ×10⁶</td>
<td>13.1 (9.0–36.6)</td>
<td>22.1 (10.2–53.4)</td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>80.7 (51.8–90.3)</td>
<td>60.2 (27.5–89.5)</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>17.9 (8.7–43.3)</td>
<td>38.7 (9.5–72.5)</td>
</tr>
<tr>
<td>CD3</td>
<td>89.6 (83.0–97.4)</td>
<td>94.0 (44.7–98.2)</td>
</tr>
<tr>
<td>CD4</td>
<td>55.9 (15.4–69.7)</td>
<td>75.2 (41.1–89.2)</td>
</tr>
<tr>
<td>CD8</td>
<td>34.1 (19.3–82.4)</td>
<td>16.6 (5.4–24.9)</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>1.68 (0.19–3.51)</td>
<td>4.65 (1.66–10.79)</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>1.1 (0.0–2.5)</td>
<td>0.3 (0.0–4.1)</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>0.5 (0.0–2.7)</td>
<td>0.0 (0.0–1.0)</td>
</tr>
<tr>
<td>Pulmonary function tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vital capacity, % predicted</td>
<td>115.4 (80.4–135.2)</td>
<td>92.4 (71.8–105.2)</td>
</tr>
<tr>
<td>FEV₁/FVC ratio, %</td>
<td>83.8 (77.1–96.8)</td>
<td>81.9 (62.1–98.0)</td>
</tr>
<tr>
<td>DLCO/VA, % predicted</td>
<td>93.8 (79.8–139.0)</td>
<td>83.9 (59.8–109.0)</td>
</tr>
</tbody>
</table>

*pData are presented as median (range) unless otherwise indicated. DLCO/VA = diffusion capacity of the lung for carbon monoxide corrected by alveolar volume.

Cytokine Measurements

Levels of TNF-α, IL-2, and IFN-γ were measured by specific enzyme-linked immunosorbent assay kits (Biosource Europe;
Nivelles, Belgium [TNF-α and IL-2]; and Biotrak System; Amersham Biosciences; Little Chalfont, UK [IFN-γ]).

**Statistical Analysis**

Horizontal bars in figures are medians. The difference in the percentages of IL-18Rα+ cells between patients with sarcoidosis and healthy control subjects was assessed using the Mann-Whitney U test. For all pairwise comparison, the Wilcoxon signed-rank test was used. We used the Spearman correlation coefficient for testing correlations between the levels of cytokines and the proportions of IL-18Rα+ cells or between the proportions of PB IL-18Rα+ cells and those of BAL IL-18Rα+ cells in sarcoidosis. All statistical analysis was performed using software (StatView for Windows 5.0; SAS Institute; Cary, NC). Differences with a p value < 0.05 were considered significant.

**RESULTS**

The proportions of BAL CD4+ and PB CD4+ T cells expressing IL-18Rα were significantly increased in patients with sarcoidosis compared with healthy control subjects, whereas those of BAL CD8+ T cells were significantly decreased in the patient group (Fig 1). The histograms of IL-18Rα+ cells were unimodal, and increased proportions of the cells expressing the receptor resulted from an overall rightward shift of the histograms (not shown). However, there was no significant difference in the percentage of PB CD8+ T cells expressing IL-18Rα between patients and control subjects.

Intraindividual comparison revealed that BAL CD4+ T cells in patients with sarcoidosis expressed IL-18Rα in a higher proportion than did BAL CD8+ T cells (Fig 1). However, there was no such a difference in the control subjects. Meanwhile, significantly higher proportions of BAL CD4+ and BAL CD8+ T cells expressed IL-18Rα than did their blood counterparts in both patients and control subjects (Fig 2). There was no significant correlation between the proportions of BAL IL-18Rα+ cells and those of PB IL-18Rα cells in patients with sarcoidosis (data not shown).

In order to clarify the mechanism of increased expression of IL-18Rα by CD4+ T cells in patients with sarcoidosis, PB mononuclear cells were cultured in the absence or presence of cytokines for 48 h and measured for IL-18Rα expression. The expression of IL-18Rα was significantly enhanced by IL-2, IL-12, and TNFα in CD4+ T cells compared with that in CD8+ T cells cultured in the absence of cytokines, whereas that in CD8+ T cells was enhanced solely by IL-2 (Fig 3).

We measured levels of IL-2, IFN-γ, and TNF-α for BAL fluid and serum in patients with sarcoidosis...
to explore the in vivo effects of overexpression of IL-18Rα. There was a significant positive correlation between IL-2 levels in BAL fluid and the proportion of IL-18Rα+ cells in BAL CD4+ T cells (Fig 4, top, A). Serum IFN-γ levels were positively correlated with the proportion of IL-18Rα+ cells in BAL CD4+ T cells (Fig 4, center, B). We also found a significantly positive correlation between serum TNF-α levels and the proportion of IL-18Rα+ cells in PB CD4+ T cells (Fig 4, bottom, C). There were no significant correlations in any other combinations of cytokine levels and the proportion of IL-18Rα+ cells (data not shown).

We then measured the expression of IL-18Rα according to CD45RO expression, a putative surface marker for memory T cells, in eight patients with sarcoidosis. CD45RO+ lymphocytes had significantly increased expression of IL-18Rα compared with CD45RO− lymphocytes for BAL CD4+, BAL CD8+, and PB CD4+ T cells, but not for PB CD8+ T cells (Fig 5). A significantly greater proportion of BAL lymphocytes than of their blood counterparts expressed CD45RO (p = 0.0117 and 0.0173 for CD4+ T cells and CD8+ T cells, respectively; not shown). Similarly, the proportion of CD45RO+ cells in CD4+ T cells was significantly higher than that in CD8+ T cells for BAL lymphocytes (p = 0.0117, not shown) but not for blood lymphocytes (not shown). These results indicated that the increased expression of IL-18Rα by BAL T cells, especially by CD4+ T cells, in sarcoidosis is at least partly explainable by the expansion of cells expressing CD45RO.

There were no significant correlations between the expression of IL-18Rα by any cell populations and BAL findings, serum angiotensin-converting enzyme activities, or radiograph stages (not shown). The proportions of IL-18Rα+ cells did not correlate significantly with disease duration before BAL was done or prospective clinical courses after BAL.

**DISCUSSION**

IL-18 has now been well characterized as a co-stimulatory factor for IL-12–mediated Th1 differentiation.11 While the expression of IL-12 receptor by inflammatory cells in sarcoidosis has been demonstrated,19 comparative studies on IL-18Rα expression between patients with sarcoidosis and control subjects have been lacking except for one study18 done in only three patients. The most important finding in the present study was that the proportion of blood and alveolar CD4+ T cells expressing IL-18Rα in patients with sarcoidosis was significantly increased compared with that in control subjects, whereas such an overexpression was not observed for CD8+ T cells.
IL-18 directly induces IFN-γ production in synergy with IL-12,29 and indirectly augments IFN-γ production by enhancing IL-12 receptor expression.21 Also, IL-12 induces the expression of IL-18 receptor.22,23 In view of the increased expression of IL-12, IL-12 receptor, and IL-18 in sarcoidosis,10,19,24 our present study revealed another important component that leads to increased expression of IFN-γ by CD4+ cells, and all these components complete a mutual potentiating system that characterizes the Th1-dominant immune responses in sarcoidosis (Fig 6). The presence of a positive correlation between serum IFN-γ and the proportion of IL-18Rα+ cells in BAL CD4+ T cells suggests that the overexpression of the receptor is actually functional in sarcoidosis.

Regarding the mechanism of the overexpression of IL-18Rα, we hypothesized that it was related to the presence of abundant cytokines in sarcoidosis.25–27 Indeed, we found that blood CD4+ T cells showed enhanced expression of IL-18Rα in response to IL-2, IL-12, and TNF-α following 48-h culture, whereas weak induction of the receptor was observed for CD8+ T cells solely following culture with IL-2 compared with that for CD4+ T cells. Consistent with this, serum levels of TNF-α were significantly correlated with the proportion of IL-18Rα+ cells in PB CD4+ T cells but not in PB CD8+ T cells. These results indicate that IL-18Rα, as has been reported for IL-12,23 is differentially regulated by cytokines between the two main subsets of human T cells.

Another possible mechanism may involve the roles of T-cell receptor engagement in the process of IL-18Rα induction. It has been reported that co-stimulation with IL-12 and phytohemagglutinin or T-cell receptor stimulation is required for the optimal induction of IL-18Rα by human or murine T cells.23,28 Since CD4+ T cells are predominantly accumulated in the alveolar surface and at the center of epithelioid cell granulomas, it is possible that those CD4+ T cells but not CD8+ T cells are stimulated with causative antigens of sarcoidosis, if any, leading to optimal expression of IL-18Rα (Fig 6). Consistent with this, the usage of a specific T-cell receptor variable region by CD4+ lung T cells has been demonstrated in subgroups of patients with sarcoidosis.29,30

We could confirm the previous finding that a significantly higher proportion of BAL T cells expressed IL-18Rα than did their blood counterparts in patients with sarcoidosis.4 However, this phenomenon was not specific to sarcoidosis, since similar increased expression by BAL T cells was observed for control subjects. In order to clarify the mechanisms of this finding, we focused on CD45RO, a critical positive regulator of T-cell receptor.31 Differential expression of CD45 splice variants has frequently been used to distinguish between “ naïve” CD45RB and “memory” CD45RO T cells. However, this phenotypic distinction rather parallels the state of activation of a given T cells and changes in response to cytokines and mitogenic stimuli.31 Induced CD45RO+ cells then produce abundant cytokines and express several surface antigens and cytokine receptors.32,33

Previous studies34,35 have shown that a higher proportion of BAL T cells than of blood T cells express CD45RO in healthy control subjects as well as in patients with sarcoidosis or chronic beryllium disease. We could verify this phenomenon for sarcoidosis, and found that a higher proportion of BAL CD4+ T cells than of BAL CD8+ T cells were CD45RO+. Importantly, the proportion of IL-18Rα+ cells in CD45RO+ cells was significantly increased compared with that in CD45RO- cells. These results collectively indicate that increased expression of IL-18Rα by alveolar T cells, and especially by CD4+ T cells, is associated with the
accumulation of CD45RO+ lymphocytes in the alveolar lumen (Fig 6). However, it is unlikely that the accumulation of CD45RO+ cells can totally account for enhanced expression of IL-18Rα in sarcoidosis compared with control subjects, since it has been reported that there is no significant difference in the proportions of CD45RO+ cells between patients with sarcoidosis and control subjects.34

The reduced expression of IL-18Rα by BAL CD4+ T cells in sarcoidosis is in sharp contrast to that of BAL CD4+ T cells. Reports on similar phenomena have been scanty. Distinct regulatory mechanisms may exist in the sarcoid lung and await further studies.

In conclusion, increased expression of IL-18Rα by CD4+ T cells from both lung and blood in sarcoidosis indicates the importance of the IL-18/IL-18 receptor system, as well as that of CD4+ T cells in the inflammatory process of sarcoidosis and mirrors the systemic nature of the disease. Further examination of the relationship between receptor expression and cytokine production at the single cell level to identify more specific T-cell subsets should help to further elucidate the pathogenic mechanisms of sarcoidosis.

ACKNOWLEDGMENT: The authors thank Dr. Nobuyuki Hizawa for valuable discussion and Dr. Daisuke Takahashi for help with BAL.

REFERENCES
12. Parnet P, Garka KE, Bonnert TP, et al. IL-1Rrp is a novel receptor-like molecule similar to the type I interleukin-1 receptor and its homologues T1/ST2 and IL-1R AcP. J Biol Chem 1996; 271:3967–3970
28. Smeltz RB, Chen J, Hu-Li J, et al. Regulation of interleukin (IL)-18 receptor a chain expression on CD4(+) T cells during


