Characterization of p40 and IL-10 in the BALF of Patients with Pulmonary Sarcoidosis

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ABSTRACT

This study investigated cytokine protein levels (interleukin-12 p70 [IL-12p70], p40, and IL-10) in bronchoalveolar lavage fluid (BALF) from patients with pulmonary sarcoidosis (n = 59), healthy control subjects (n = 17), and patients with idiopathic pulmonary fibrosis (IPF) (n = 30). The relationship between cytokine levels and clinical course of sarcoidosis was also examined. Overall, p40 was far more abundant than IL-12p70. p40 levels (pg/ml, mean ± SEM) were significantly higher in the BALF from patients with sarcoidosis (2.97 ± 3.69) than in IPF patients (0.83 ± 1.57) and healthy subjects (0.78 ± 1.00). Size exclusion chromatography indicated that p40 detected in BALF from sarcoidosis patients corresponded to p40 monomers or (p40)2 homodimers. Further, p40 levels were associated with (paralleled) the clinical course of sarcoidosis, with the highest levels detected in BALF from patients with persistent disease. Higher p40 levels were also found in the BALF from sarcoid patients who required corticosteroid treatment compared with patients with spontaneous regression (3.51 ± 3.83 vs. 2.01 ± 3.43, p = 0.03). IL-10 concentrations paralleled p40 changes. No similar association was found for IL-12p70 levels. In conclusion, this report shows that the BALF from patients with sarcoidosis contains elevated levels of p40, (p40)2, and IL-10 protein but not of IL-12p70. The present data also suggest that BALF p40 concentrations may be indicative of the sarcoidosis clinical course.

INTRODUCTION

INTERLEUKIN-12 (IL-12) IS A HETERODIMERIC disulfide-linked cytokine with an apparent molecular mass of 70 kDa composed of a 40 kDa (p40) and a 35-kDa (p35) subunit, each encoded by a distinct gene.1,2 Although the single p40 or p35 chains can be produced separately, only the p70 heterodimer has full IL-12 activity.3 IL-12p70 mediates its biologic activities through a specific, high-affinity receptor that also comprises two subunits, IL-12Rβ1 and IL-12Rβ2. p40 conveys receptor-binding activity, and p35 mediates IL-12 biologic activity.4,5 IL-12 is produced mainly by macrophages, monocytes, and dendritic cells (DCs) and plays a key regulatory role in promoting T helper lymphocyte polarization. It enhances Th1 and inhibits Th2 development mainly by inducing high levels of interferon-γ (IFN-γ) production by natural killer (NK) and Th1 cells, as well as by acting directly on these T lymphocyte populations.6 IL-12, therefore, regulates host responses against infection with parasites, viruses, and bacteria and in the rejection of tumor cells. Importantly, IL-12-producing cells generally express far more p40 than p35 chain, and the p40 subunits form homodimers (p40)2. (p40)2 binds with high affinity to the IL-12R and has been shown to be a potent IL-12 antagonist.7 Moreover, based on studies with IL-12p35−/− and IL-12p40−/− mice, an IL-12 agonist function for (p40)2 has also been suggested.7–9

IL-10 is a noncovalently linked homodimeric cytokine produced by a large variety of Th2 cells, monocytes/macrophages, B lymphocytes, and NK cells. IL-10 has been reported to exert anti-inflammatory and immunosuppressive activities,10,11 for example, by reducing the synthesis of proinflammatory cytokines, such as IL-1, IL-6, IL-8, and tumor necrosis factor-α (TNF-α), downregulating IFN-γ in T cells,12 and also reducing free radical release13 and nitric oxide (NO) synthesis14 by macrophages. Importantly, IL-10 counteracts Th1 type immune responses by inhibiting the production of Th1 cytokines.

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Sarcoidosis is a multiorgan granulomatous disorder most frequently affecting the lungs that results from the accumulation of CD4+ T lymphocytes and a Th1 immune response. Pulmonary sarcoidosis either resolves spontaneously or becomes a chronic condition, with rare progression to fibrosis. The current view on the immunopathogenesis of sarcoidosis is that the granulomatous response is initiated by one or more microbes behaving in a noninfectious fashion in genetically predisposed individuals. Uptregulation of cell adhesion molecules, chemokines, and Th1 cytokines, including IL-12, is a characteristic feature of the disease process. Although a number of studies on mRNA and protein levels implicate IL-12 as an important mediator of Th1 polarization in pulmonary sarcoidosis, there has been limited and incomplete information about the respective participation in sarcoidosis of IL-12p70 and p40. In contrast to IL-12, IL-10 has not been extensively investigated in sarcoidosis. A recent report by Bingisser et al. indicates increased IL-10 secretion from sarcoid alveolar macrophages (AM) in vitro and suggests a possible role for this inflammatory and immunosuppressive cytokine in sarcoidosis.

In this study, we have, therefore, determined IL-10 and IL-12 protein levels in bronchoalveolar lavage fluid (BALF) from patients with sarcoidosis and compared these with levels in healthy subjects and in patients with idiopathic pulmonary fibrosis (IPF). Further, we investigated the relative contribution of the p40 and IL-12p70 forms to the changes of IL-12 in the disease, and we also characterized under which form p40 was implicated in pulmonary sarcoidosis. Finally, we examined which of the investigated cytokine parameters was associated with clinical and laboratory characteristics of sarcoidosis development and progression.

MATERIAL AND METHODS

Study population

The study population consisted of 106 subjects referred to the University Hospital in Olomouc (Czech Republic). Two patient groups were considered, 59 patients with sarcoidosis and 30 patients with IPF. The control group consisted of 17 subjects who, at the time of presentation, showed no clinical signs of lung inflammation and had no lung disease in their medical history. No individual with abnormal BALF cytology, immunology, or microbiology was included in the control group. The diagnosis of sarcoidosis was based on typical clinical features, including granulomas and a lymphocytic CD4+ cell population in BALF and was compatible with the criteria of the international statement on sarcoidosis. According to international consensus on the treatment of sarcoidosis, a total of 37 patients required corticosteroid treatment during the course of their disease because of (1) initial chest x-ray stage III presentation, (2) progressing and/or symptomatic stage II, or (3) persisting (>6 months) stage I or II disease. The clinical course of sarcoidosis was evaluated after 2 years from the time of presentation. Of the 59 sarcoidosis patients, 13 recovered without treatment (category 1), 23 patients recovered after corticosteroid treatment (category 2), 7 patients had persistent disease despite treatment (category 3), recurrence of the disease was observed in 7 patients (category 4), and 9 patients could not be classified into these clinical categories.

The diagnosis of IPF was based on the criteria of the ATS/ERS International Consensus Statement: typical clinical features and abnormalities on chest high-resolution computed tomography (HRCT) scans, abnormal lung function tests with reduced lung diffusing capacity for carbon monoxide (DLCO) or restrictive pulmonary deficit or both, exclusion of other known causes of interstitial lung disease (ILD), and confirmatory surgical biopsy (in 21 of 30 patients). In 9 individuals without surgical biopsy, the BAL or transbronchial lung biopsy excluded other diagnoses. The study was performed with the approval of the Ethics Committee of the Medical Faculty and University Hospital, Olomouc.

Bronchoalveolar lavage (BAL)

BAL was performed at the time of the first presentation before initiation of a possible treatment. BAL was conducted according to our standard procedure by infusing five successive 20-ml aliquots of 0.9% saline, which were immediately aspirated by gentle suction. The first aliquot, considered representative of a bronchial wash, was discarded. The four subsequent aliquots were pooled and used for cell population determinations and cytokine measurements. After centrifugation, the BALF supernatant was stored in several aliquots (1.5 ml) at −20°C until analysis, and they were not concentrated or submitted to multiple freeze-thaw manipulations. Differential cell counts of BALF were performed after May-Grünwald-Giemsa staining of cytoospin slides (200 cells counted), and BALF CD4/CD8 T lymphocyte ratio was determined by FACS analysis using monoclonal antibodies (mAbs) against CD4+ and CD8+ (Opticlon CD4 FITC/CD PE, Immunotech, Marseille, France).

Cytokine measurements

The concentration of IL-10 was measured with an ultrasensitive cytokine-specific ELISA (Biosource International, Camarillo, CA, Ref: KHC0103). Because of the limited volume of BALF available, IL-10 could be measured only in part of the samples (n = 88). No correction for BALF dilution was applied. The concentration of total IL-12 (p40+p70) and IL-12p70 proteins in BALF was measured by specific ELISA (Biosource International, Refs: KHC0121 and KAC1568, respectively). The total IL-12 ELISA recognizes both the IL-12p70 protein and the p40 subunit. The IL-12p70 ELISA recognizes exclusively the IL-12p70 heterodimer. p40 concentrations were calculated by subtracting IL-12-p70 from total IL-12.

Size exclusion chromatography

BALF samples from selected patients with high p40 concentrations (12–16 pg/ml) but no detected IL-12p70 were concentrated 10-fold with ultrafree-4 filter units (Millipore, Bedford, MA) and analyzed on a Superose 12 column (Amersham Pharmacia Biotech, Roosendaal, The Netherlands). The chromatography was calibrated with ovalbumin (40 kDa) and human transferrin (80 kDa). The presence of p40 in the eluted
fractions was detected by the total IL-12 ELISA. Previous studies have shown that this procedure does not disrupt IL-12p70.

**Statistical analysis**

Comparisons between the groups were performed by non-parametric ANOVA (Kruskal-Wallis test) complemented by a Dunn’s multiple comparison test, chi-square, or trend test as appropriate. Associations between pairs of variables were assessed by simple linear correlations. Statistical significance was considered as \( p < 0.05 \).

**RESULTS**

**Characteristics of study populations**

General characteristics of the patients and control subjects are given in Table 1. Women were relatively more represented among sarcoidosis patients than in the IPF and control groups. On the average, patients with IPF were significantly older, and smokers were relatively less frequent among sarcoidosis patients than in other groups.

The mean total cell count in BALF was not significantly different in sarcoidosis and IPF patients compared with control subjects. The mean percentage of AMs was significantly reduced in sarcoidosis and IPF patients compared with control subjects. Lymphocytic alveolitis was found in patients with sarcoidosis with a highly significant increase of the CD4+ T cells and accompanying reduction of the CD8+ lymphocyte subset, resulting in an elevated CD4/CD8 ratio. Increased mean percentages of neutrophils and eosinophils were noted in IPF patients.

**BALF cytokine levels**

Cytokine levels are reported in Table 2. Total IL-12 and p40 levels were on the average significantly higher in sarcoidosis patients than in control and IPF subjects (\( p < 0.0001 \)). IL-12p70 could only be detected in some samples, the proportion of which was not different among the three groups. Overall, a very significant correlation (\( r^2 = 0.998, p < 0.0001 \)) was found between the individual levels of total IL-12 and p40, which, together with the very low levels of IL-12p70 detected, indicated that most of the protein measured in BALF was p40. The very significant preponderance of p40 was found in all groups. We, therefore, investigated whether the p40 detected in

| Table 1. Characteristics of Study Population and BAL Cellularity |
|------------------|------------------|------------------|------------------|
|                  | Normal subjects  | Patients with sarcoidosis | Patients with IPF | p value |
| Subjects, n      | 17               | 59               | 30               |        |
| Sex, F/M         | 5/12             | 36/23            | 11/19            | 0.0198 |
| Age, years       | 44 ± 18a A       | 46 ± 11 A        | 56 ± 10 B        |        |
| Smokers (%)      | 41               | 17               | 33               | 0.0658 |
| BAL cells        |                  |                  |                  |        |
| Total cells, millions | 2.21 ± 1.38 A   | 2.36 ± 1.03 A   | 2.53 ± 0.75 A   |        |
| Macrophages, %   | 92 ± 5 A         | 70 ± 15 B       | 75 ± 22 B       |        |
| Lymphocytes, %   | 7 ± 5 A          | 26 ± 12 B       | 15 ± 17 A       |        |
| CD4, %           | 39 ± 10 A        | 60 ± 18 B       | 37 ± 17 A       |        |
| CD8, %           | 34 ± 8 A         | 20 ± 13 B       | 33 ± 16 A       |        |
| Neutrophils, %   | 0.5 ± 0.6 A      | 1.5 ± 5.8 A     | 7.3 ± 13.9 B    |        |
| Eosinophils, %   | 0.02 ± 0.08 A    | 0.25 ± 0.69 A   | 2.77 ± 8.01 B   |        |

*Arithmetic mean ± SEM (values with the same letter are not statistically different).

**Table 2. BALF Cytokine Measurements**

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects</th>
<th>Patients with sarcoidosis</th>
<th>Patients with IPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12 total, pg/ml</td>
<td>0.79 ± 1.00a A</td>
<td>3.02 ± 3.68 B</td>
<td>0.83 ± 1.57 A</td>
</tr>
<tr>
<td>IL-12p40, pg/ml</td>
<td>0.78 ± 1.00 A</td>
<td>2.97 ± 3.69 B</td>
<td>0.83 ± 1.57 A</td>
</tr>
<tr>
<td>IL-12p70, n</td>
<td>2 A</td>
<td>12 A</td>
<td>9 A</td>
</tr>
<tr>
<td>IL-12p70, pg/ml</td>
<td>0.173 ± 0.133f</td>
<td>0.267 ± 0.265 A</td>
<td>0.229 ± 0.131 A</td>
</tr>
<tr>
<td>IL-10, pg/ml (n)</td>
<td>2.86 ± 2.78 (15)</td>
<td>6.02 ± 4.67 (50) B</td>
<td>4.15 ± 3.83 (23) A</td>
</tr>
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</table>

*Arithmetic mean ± SEM (values with the same letter are not statistically different).

| Number of samples detected (>0.023 pg/ml). |
| Arithmetic mean ± SEM of detected values. |
| (n), number of samples measured. |
BALF of sarcoidosis patients corresponded to p40 monomers or p40 homodimers. To address this issue, concentrated BALF of 2 sarcoidosis patients with high total IL-12 concentrations but little or no IL-12p70 were analyzed by size exclusion chromatography. Ovalbumin (±40 kDa) and transferrin (±80 kDa) were added as molecular weight markers. p40 protein concentrations were measured on the eluted fractions. As indicated in Figure 1, p40 was eluted as a homodimer in patient A (i.e., close to transferrin) and as p40 monomer in patient B (i.e., close to ovalbumin). Subsequent analyses were, therefore, mainly focussed on p40.

p40 and IL-10 BALF levels were not affected by smoking habits except in the group of IPF patients in which p40 tended to be higher in nonsmoking patients (pg/ml, mean ± SD, 1.20 ± 1.82 vs. 0.10 ± 0.17, p = 0.06).

Relationship of cytokine levels with BALF cell populations

In order to explore possible associations among IL-10, p40, and BALF cellular profile we examined if cytokine levels were associated with relative or absolute numbers of BALF lymphocytes, macrophages, and polymorphonuclear leukocytes. When considering all subjects together, a striking positive correlation was found between the p40 concentration and the number of lymphocytes but not of macrophages (r² = 0.184, p < 0.00001 and r² = 0.005, p = 0.486, respectively). As shown in Figure 2, a correlation between p40 and the proportion of lymphocytes was also observed (r² = 0.169, p < 0.0001), and a marked elevation of BALF p40 was mainly apparent in sarcoidosis patients, with more than 20% BALF lymphocytes. BALF lymphocyte numbers were also positively correlated with p40 levels in IPF patients (r² = 0.254, p = 0.009) but not in controls. Correlation analyses considering all subjects together showed a significant positive correlation between p40 levels and CD4⁺ lymphocyte numbers (r² = 0.074, p = 0.010). No similar correlation was found in the subgroups. BAL CD8⁺ lymphocyte numbers were significantly positively correlated with p40 concentrations in sarcoidosis patients only (r² = 0.112, p = 0.012). BAL CD4⁺/CD8 ratios were significantly positively correlated with p40 levels in IPF (r² = 0.196, p = 0.039) but not in sarcoidosis patients. BAL neutrophil numbers were significantly positively correlated with p40 levels in sarcoidosis patients only (r² = 0.150, p = 0.003). No significant association was found between the p40 concentrations and BAL eosinophils or total cell number.

A significant positive correlation was found between IL-10 levels and the number of lymphocytes in the BALF of all patients (r² = 0.086, p = 0.008) and in the BALF of patients with sarcoidosis (r² = 0.097, p = 0.033). A significant positive correlation existed also between IL-10 levels and the number of CD4⁺ lymphocytes in the BALF of all patients (r² = 0.084, p = 0.014). No association could be found between IL-10 levels and neutrophils, eosinophils, CD8⁺ counts, or total cell number (p > 0.05). A weak positive correlation was found between p40 and IL-10 levels in the whole study population (r² = 0.063, p = 0.018) but not in any of three subgroups.

Relationship between cytokines and clinical course of sarcoidosis

To explore possible clinical relevance of BALF cytokine measurements, we compared p40 and IL-10 levels in patients with a distinct clinical course. p40 levels were significantly higher in the BALF of patients who required corticosteroid treatment during disease evolution in comparison with patients with spontaneous disease resolution (3.51 ± 3.83 vs. 2.01 ± 3.43 pg/ml; p = 0.034). The same tendency was observed for
BALF IL-10 levels, but the difference did not attain significance (6.95 ± 5.24 vs. 4.29 ± 2.91 pg/ml, p = 0.055). Importantly, p40 levels in BALF paralleled the clinical course of the disease (trend test, p = 0.0135) (Fig. 3). The highest levels were observed in the subgroup of patients with persistent disease (as evaluated after 2 years from presentation). IL-10 levels in BALF did not show any significant association with the clinical course of the disease. IL-12p70 levels were not related to any of the clinical parameters.

**DISCUSSION**

This study aimed at investigating IL-12, its components, and IL-10 in BALF of patients with sarcoidosis or IPF and in control healthy subjects. The main finding is that the majority of IL-12-related proteins measured in BALF were not p70 but were represented by p40 homodimers or monomers. p40 levels were significantly increased in the BALF of sarcoidosis patients in comparison to control subjects or IPF patients and were associated with the clinical course of the disease. IL-10 BALF levels were also higher in sarcoidosis patients than in the other groups.

The first question to be considered is the biologic plausibility of the observed predominance of p40 in sarcoidosis and how it fits with the previously published data, which seem to associate this disease with an upregulation of biologically active IL-12p70. Positive immunostaining for IL-12p70 and increased *in vitro* production of IL-12p70 by BALF cells have indeed been reported in sarcoidosis patients. Also, by using a polyclonal anti-IL-12p70 antibody, the production of IL-12 was reported to regulate the expression of IFN-γ, the prototypic Th1 cytokine. However, specific p40 expression was not examined in this study. In other studies that have examined *in vivo* IL-12 expression in the BALF of sarcoidosis patients, authors have measured total IL-12 without differentiating between p40 and p70 proteins. They reported an increased production of p40 protein, which was assumed to reflect biologically active IL-12p70 or reported overexpression of p40 at the transcript level. Other authors have reported an increased proportion of BAL cells expressing mRNA for p40 in sarcoidosis patients. Altogether, these observations invite the possibility of an overproduction of p40 in sarcoidosis, not necessarily associated with the upregulation of IL-12p70, and are compatible with our observation. The authors of previous studies have attributed the preponderance of p40 vs. p70 protein in BALF or in serum to the lability of the bioactive heterodimeric IL-12p70 protein on freeze-thaw manipulations. However, this artifact appears unlikely in the present study because our samples were not thawed and refrozen on multiple occasions. Furthermore, the disulfide link that associates the p40 and p35 monomers is generally recognized as a stable chemical bond, and other dimeric molecules involving disulfide bonds (e.g., transforming growth factor-β [TGF-β], CC10) are readily detected in BALF. In addition, the fact that the (p40)₂ form was identified in BALF implies the contribution of other mechanisms than a simple degradation of IL-12p70. Finally, a specific upregulation of p40, not IL-12p70, has been reported in other diseases, such as endometriosis and systemic lupus erythematosus and multiple sclerosis. Therefore, the presence of biologically relevant concentrations of p40, independently of IL-12p70, appears as a plausible hypothesis.

The next issue to be discussed is the exact form and the possible biologic significance of this increased expression of p40 in sarcoidosis. The elevated levels of p40 appeared relatively specific for sarcoidosis patients and correlated with T lymphocyte numbers. Because p40 is mainly produced by antigen-presenting cells (APCs) and not lymphocytes, it is likely that this association reflects a biologic activity of p40 to stimulate the recruitment and proliferation of lymphocytes in the lungs of sarcoidosis patients. The exact mediator of this effect needs to be identified.

Several studies have shown that in addition to IL-12p70, APCs also express homodimeric (p40)₂, which *in vitro* and *in vivo* competes with IL-12p70 for binding to the high-affinity IL-12R and behaves as a potent IL-12p70 antagonist. In human macrophages, p40 alone or in the form of homodimers has been reported to exert immunosuppressive activity and to...
contribute to Th2 polarization.\(^{(38–40)}\) Of note, it has been shown that a switch to Th2-type T cells may occur in patients with sarcoidosis evolving toward lung fibrosis.\(^{(41)}\) An overexpression of p40 in epithelial cells has also been reported in a mouse model of airway inflammation and in human subjects with asthma.\(^{(42)}\) We reported a selective overexpression of p40, but not IL-12p70, associated with a Th2 polarization in the lungs of silicotic mice\(^{(43)}\) and contributing to the recruitment of alveolar macrophages.\(^{(44)}\) Taken together, these recent observations suggest a role for p40 alone and for its homodimeric form in the lung immune response that might be abnormally programmed during inflammatory disease. The relative congruence between IL-10 and p40 BALF levels reported in the present study is consistent with the hypothesis that sarcoidosis is accompanied by the upregulation of an anti-inflammatory and immunosuppressive molecule that might be mediated by (p40).\(^{(45)}\) Increased expression of IL-10 by AM and monocytes has been suggested to contribute to a downmodulating mechanism accompanying the alveolitis in sarcoidosis.\(^{(27)}\) In contrast, Moller et al.\(^{(19)}\) reported lower IL-10 concentrations in the BALF of sarcoidosis compared with that of IPF patients and controls.

Under certain conditions, p40 can also function as an IL-12p70 agonist. Using both mice deficient in IL-12p70 and administration of antibodies blocking p40 or (p40), several authors have elegantly demonstrated that p40 contributed to Th1 polarization and production of IFN-\(\gamma\) in experimental models of cardiac allograft rejection\(^{(7)}\) as well as bacterial and viral infection.\(^{(9,45)}\) In addition, transgenic mice overproducing p40 in basal keratinocytes spontaneously developed an inflammatory skin disease that is also caused by injection of IL-12p70.\(^{(46)}\) We must, therefore, consider that p40 levels measured in the BALF of sarcoidosis patients may have promoted, to some extent, the Th1 immune response associated with this disease.

IL-23 is another recently discovered composite cytokine consisting of p40 and a p19 subunits covalently linked that shows both overlapping and unique functions compared with IL-12. IL-23 binds to IL-12R\(\beta1\), the p40-specific component of the IL-12 receptor. IL-23 is expressed in several tissues, including DCs, and stimulates the proliferation of human CD4\(^+\) naive and memory T cells.\(^{(47)}\) Similar to IL-12, human IL-23 stimulates IFN-\(\gamma\) production by phytohemagglutinin A (PHA) blast T cells, and the biologic activity of IL-23 can be neutralized by blocking anti-p40 antibodies, indicating that studies using anti-p40 antibodies (or conceivably polyclonal anti-IL-12 antibodies) have addressed the role of both IL-12 and IL-23. Transgenic animals overexpressing IL-23 displayed infiltrates of lymphocytes and macrophages in skin, lung, liver, pancreas, and the gastrointestinal tract.\(^{(48)}\) Together, these observations indicate that IL-23 might exert, conceivably in association with IL-12, important biologic functions in immunity and immunopathology. In view of its activity in stimulating the production of IFN-\(\gamma\) and the proliferation of lymphocytes, IL-23 appears as a plausible mediator of increased expression of p40 in sarcoidosis patients. This hypothesis can, however, be tested only after the reagents to measure human IL-23 become available.

Importantly, our study had clinical correlates. BALF p40 levels paralleled the clinical course of sarcoidosis and were associated with clinically more apparent disease. Patients who required corticosteroid treatment had significantly higher p40 levels at presentation than those in whom sarcoidosis resolved spontaneously. p40 has been associated with the severity of other immune-mediated inflammatory diseases, such as systemic lupus erythematosus,\(^{(32)}\) multiple sclerosis,\(^{(34,35)}\) and endometriosis.\(^{(33)}\)

In conclusion, this report shows that the BALF of patients with sarcoidosis contains elevated levels of p40 monomers and homodimers and of IL-10 protein. Further studies, aimed at elucidating the exact nature and function of these proteins in sarcoidosis, will allow confirmation of the possible clinical significance of p40 BALF measurements.

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