

ORIGINAL ARTICLE

T-LYMPHOCYTE SUBSETS IN BRONCHOALVEOLAR LAVAGE: A FLOW-CYTOMETRIC ANALYSIS AND ITS IMPORTANCE IN THE DIFFERENTIAL DIAGNOSIS OF SARCOIDOSIS

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Background – The immunophenotyping of lymphocyte subsets in bronchoalveolar lavage (BAL) is of importance in the differential diagnosis of interstitial lung diseases. The main intent for lymphocyte population subtyping in BAL fluid is finding those diseases with increased lymphocytic cell infiltration in the affected organ such as sarcoidosis and asbestosis.

Methods – A dual color flow-cytometric analysis was performed to identify the percentages of total T (CD3+) lymphocytes, T-helper (CD4+) lymphocytes, cytotoxic (CD8+) T-lymphocytes, CD4/CD8 ratio, CD19+ B-lymphocytes, and CD16+56 natural killer cells in the BAL fluid of untreated patients with sarcoidosis, tuberculosis, and other interstitial lung diseases. Lymphocytes were gated on the basis of their expression of CD45 and their side scatter properties. T-lymphocyte subsets were analyzed in 37 patients with sarcoidosis, tuberculosis, nonsarcoidosis, and nontuberculosis. A *p* value of less than 0.05 was regarded as statistically significant for the results obtained.

Results – The results presented here show that a high percentage of CD3+ and CD4+ T-lymphocytes as well as a high CD4/CD8 ratio were observed in the BAL fluid of the patients with sarcoidosis when compared with other interstitial lung diseases. An analysis of CD4/CD8 ratio exhibited that a CD4/CD8 ratio of 4:1 had a positive predictive value of 95% in distinguishing sarcoidosis from other pulmonary diseases.

Conclusion – The high specificity of the procedure suggests that it may be used routinely for the analysis of lymphocytes in BAL fluid for the differential diagnosis of sarcoidosis.

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Keywords • CD4/CD8 ratio • sarcoidosis • tuberculosis

Introduction

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology that predominantly manifests in the lung.

In sarcoidosis, as in many other pulmonary diseases, there is a change in T-lymphocyte subsets.¹⁻³ Lymphocytic alveolitis is commonly found in the analysis of bronchoalveolar lavage

(BAL) fluid from sarcoidosis patients and is mainly related to an increase in CD4 T-helper lymphocytes. Therefore, an increased T-lymphocyte CD4/CD8 ratio in BAL fluid has been reported to be characteristic of sarcoidosis though it is not specific for the disease.^{4,5} It is also notable that an excess of CD8 cytotoxic T-lymphocytes is rarely observed in sarcoidosis.^{5,6}

The lymphocyte subsets distribution in BAL fluid has been reported to be of importance in the differential diagnosis of lung disorders such as sarcoidosis and berylliosis.^{1,4,7-10} The standard

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method of lymphocyte subtyping is indirect immunoperoxidase staining.¹¹ The peroxidase-anti-peroxidase technique is time consuming and the reliability of the results heavily depends on the number of cells counted and the experience of the observer.¹¹ Flow-cytometry permits rapid analysis of a large number of cells as well as counting small populations. The aim of the present study was to investigate the application of flow-cytometry for differentiating sarcoidosis from other interstitial lung diseases.

Patients and Methods

T-lymphocyte subsets were analyzed in 37 untreated patients with sarcoidosis, tuberculosis (TB), nonsarcoidosis, and nontuberculosis (non-TB) (18 women aged 29 – 62 years and 19 men aged 20 to 80 years). At the time of the study the patients were not taking any drugs. The diagnosis of pulmonary sarcoidosis was based on the histological examination of biopsy samples obtained from the lungs; the presence of non-caseating epitheloid cell granulomas; and typical clinical, radiological, and biochemical (high angiotensin-converting enzyme activity [ACE]) findings. Fifty-two percent of sarcoidosis patients were in stage I, 36% in stage II, and 12% in stage III.¹²

The samples obtained from the patients who were referred to Masih Daneshvari Hospital during years 2001 to 2002. BAL was performed according to the methods described in other references.^{13 - 15} The bronchoscope was passed transorally and wedged into a subsegmental bronchus. Three 50-mL volumes of sterile saline solution were infused into the lung and immediately recovered into sterile polycarbonate tubes.

Processing of BAL fluid

The pooled BAL fluid specimen was passed through sterile gauze. BAL fluid was centrifuged at 300 – 400 xg for 15 minutes. The pellet was resuspended in 2 mL of phosphate-buffered saline (PBS) for flow-cytometric analysis.

Flow-cytometric analysis of bronchoalveolar cells

Flow-cytometric analysis was performed using

a FACSCalibur flow-cytometry (Becton-Dickinson, Mountain View, California, USA). Pairs of monoclonal antibodies (FITC/PE conjugated), CD45/CD14 (LeucoGATE™), mouse IgG1/IgG2a (negative control), CD3/CD19, CD3/CD4, CD3/CD8, and CD3/CD16+56 (all from Becton-Dickinson, USA) were added to the BAL suspension and incubated for 30 minutes at room temperature. Then, 2 mL of FACS lysing solution was added followed by centrifugation for 5 minutes. The cells were then washed with PBS and subjected to flow-cytometric analysis. Analysis was performed using Cell-Quest program supplied by the manufacturer. Alveolar lymphocyte CD45FITC/SSC gating was found satisfactory. Data were collected for 15,000 cells in each measurement.

Statistical analysis

The results were analyzed using Pearson's regression model. For each subset of lymphocytes, the data were expressed as the mean \pm standard error of mean (SEM). The reproducibility of each subset was determined using Pearson's correlation coefficients. The differences of the mean were calculated by Student's *t*-test, using SPSS analysis with a *p* value of < 0.05 regarded significant.

Results

As shown in the Table, the mean percentages of total CD3+ T-lymphocytes in BAL fluid did not significantly vary among sarcoidosis, TB, nonsarcoidosis, and non-TB patients. Nonsarcoidosis and nontuberculosis patients consisted of 5 typical interstitial pneumonia cases, 1 adenocarcinoma, 1 silicosis, 1 alveolar cell carcinoma, and 2 interstitial pulmonary fibrosis cases. However, sarcoidosis patients, when compared with TB and non-TB patients, had a higher proportion of CD3+ CD4+ T-cells in their BAL fluid samples ($p < 0.001$). In addition, sarcoidosis patients had a lower proportion of CD3+ CD8+ T-cells than TB and non-TB patients ($p < 0.001$). Hence, the mean CD4/CD8 ratio was significantly higher in the patients with sarcoidosis than in pulmonary TB and non-TB patients ($p <$

Table. Mean \pm SEM percentage of T-lymphocyte subsets in BAL fluid.

Subjects	CD3+	CD4+	CD8+	Mean CD4/CD8	CD19+	CD16+56
Sarcoidosis (<i>n</i> = 19)	75.21 \pm 9	59.0 \pm 4.5	14.0 \pm 9.5	4.8 \pm 0.57	2.0 \pm 1.7	4 \pm 2.8
Tuberculosis (<i>n</i> = 9)	79.88 \pm 10.5	35.22 \pm 7.8	46.22 \pm 17.5	0.82 \pm 0.123	4.0 \pm 2.3	7 \pm 4.1
Nontuberculosis (<i>n</i> = 9)	61.37 \pm 6.5	32.37 \pm 4.8	40.25 \pm 5.2	0.98 \pm 0.166	3.0 \pm 1.9	4 \pm 2.45

n = number.

T-Lymphocyte Subsets in BAL

0.0001). Mean lymphocyte subset CD4+/CD8+ ratio was significantly lower in TB and non-TB subjects ranging from 0.81 to 1.0 in TB and non-TB patients, respectively.

In patients with sarcoidosis, the CD4/CD8 ratio ranged from 0.92 to 10.86 with a mean value of 4.8. The CD4/CD8 ratio was greater than 4 in 12 (63%) subjects. Five patients (26%) had a CD4/CD8 ratio less than 4 (range, 2 – 4) while in two others it was less than 1 (10.5%).

An analysis of CD4/CD8 ratios revealed that a CD4/CD8 ratio of 4:1 or greater had a positive predictive value of 95% in distinguishing sarcoidosis from pulmonary TB and non-TB patients with a sensitivity of 54%. A CD4/CD8 ratio of 1:1 had a 100% negative predictive value.

Discussion

Immunophenotyping of BAL fluid lymphocytes and their subsets has been reported as a useful means for the diagnosis and prognostic prediction of a number of interstitial lung diseases such as sarcoidosis, asbestosis, berylliosis, and other interstitial lung diseases. Hence, the aim of the present study was to use flow-cytometry for the evaluation of BAL fluid lymphocyte CD4/CD8 ratio for the differential diagnosis of sarcoidosis from nonsarcoidosis patients.

The data presented in this study indicate that neither the total percentages of T- and B-lymphocytes nor that of natural killer cells in BAL fluid can discriminate sarcoidosis from nonsarcoidosis patients. This is in agreement with the previous reports.⁹ Moreover, this investigation demonstrated a higher CD4/CD8 ratio (> 4.0, sensitivity 54% and specificity 95%) in BAL fluid samples of sarcoidosis patients when compared with those of TB and non-TB subjects. These results are in accordance with those studies^{2, 13 - 18} which likewise found an increase in CD4/CD8 ratio in the BAL fluid of patients with pulmonary sarcoidosis. Costabel et al⁹ showed that a CD4/CD8 ratio of 3.5 or greater had a sensitivity of 52% and a specificity of 97% for diagnosing sarcoidosis. Winterbauer et al¹⁵ showed that a ratio of 4.0 or greater distinguished sarcoidosis from other interstitial lung diseases with a sensitivity of 59% and a specificity of 96%. Thomeer and Demedts¹⁶ compared 42 sarcoidosis patients with 90 patients having other diffuse lung disorders and found that a CD4/CD8 ratio of greater than 4.0 had

a sensitivity of 55% and a specificity of 94% in discriminating sarcoidosis. Takahashi et al¹⁹ observed that, among their ocular sarcoidosis patients, 10 subjects (60%) had an increased CD4/CD8 ratio of greater than 3.5. All of these authors have concluded that an increased CD4/CD8 ratio in BAL fluid may prove diagnostic of sarcoidosis and obviate the need for confirmation by additional biopsy.

However, a study by Kantrow et al,²⁰ who found a variable CD4/CD8 ratio in BAL fluid in sarcoidosis patients, is at odds with ours and other reports. Kantrow et al doubted the usefulness of CD4/CD8 ratio determination in the BAL fluid based on the observation that this ratio is highly variable; they showed that only 42% in a population of 86 patients with biopsy-proven sarcoidosis had a ratio greater than 4.0, and that 12% had an inverted CD4/CD8 ratio below 1.0. That the CD4/CD8 ratio may be decreased in patients with sarcoidosis is not a new finding and has already been reported at a frequency of 10% by Costabel et al⁹ and Thomeer and Demedts,¹⁶ and also with a frequency of 10.5% in the present report.

This study also revealed a normal CD4/CD8 ratio in the majority of cases in pulmonary TB and other interstitial lung diseases. This was in marked contrast to the group of patients with active sarcoidosis who showed an elevated CD4/CD8 ratio. In this study, we showed that a CD4/CD8 ratio exceeding 4 has a specificity of 95% and a sensitivity of 54% in the differential diagnosis of sarcoidosis. Of 18 TB and other interstitial lung disease patients, none had a CD4/CD8 ratio > 3.5, which is the upper limit of normal in our laboratory. Ratios above 2.0 were not found in patients with pulmonary tuberculosis and other interstitial lung diseases. Additionally, HLA-DR+ expressing T-lymphocytes were significantly higher in both pulmonary tuberculosis and sarcoidosis (data not shown), suggesting activated T-lymphocytes as one of the immunopathogenetic roots of these pathologic conditions. Further studies are being carried out in our laboratory to investigate the role of other activation markers and T-lymphocyte cytokine production patterns in the immunopathogenesis of pulmonary sarcoidosis.

Summing up, evidence from the literature suggests that the determination of CD4/CD8 ratio in BAL fluid may be of diagnostic value in the differential diagnosis of sarcoidosis, perhaps obviating the need for biopsy in most cases.

References

- 1 Moodley PY, Dorasamy T, Venketasamy S, et al. Correlation of CD4/CD8 ratio and tumor necrosis factor (TNF) alpha levels in induced sputum with bronchoalveolar lavage fluid in pulmonary sarcoidosis. *Thorax*. 2000; **55**: 696 – 9.
- 2 James DG. Sarcoidosis. *Postgrad Med J*. 2001; **77**: 177 – 80.
- 3 Bergoin C, Lamblin C, Wallaert B. Biological manifestations of sarcoidosis [in French]. *Ann Med Interne (Paris)*. 2001; **152**: 34 – 8.
- 4 Costabel U. CD4/CD8 ratios in bronchoalveolar lavage fluid: of value for diagnosing sarcoidosis? *Eur Respir J*. 1997; **10**: 2699 – 700.
- 5 Crystal RG. Sarcoidosis. In: Harrison TR, Braunwald E, eds. *Harrison's Principles of Internal Medicine*. 15th ed. New York: McGraw-Hill; 2001: 1969 – 74.
- 6 Agostini C, Trentin L, Zambello R, et al. CD8 alveolitis in sarcoidosis: incidence, phenotypic characteristics, and clinical features. *Am J Med*. 1993; **95**: 466 – 72.
- 7 Nagai S, Izumi T. Bronchoalveolar lavage. Still useful in diagnosing sarcoidosis? *Clin Chest Med*. 1997; **18**: 787 – 97.
- 8 Costabel U. Consensus conference: activity of sarcoidosis. Third WASOG meeting, Los Angeles, USA, September 8 – 11, 1993. *Eur Respir J*. 1994; **7**: 624 – 7.
- 9 Costabel U, Zaiss A, Wagner R, et al. Value of bronchoalveolar lavage lymphocyte subpopulation for the diagnosis of sarcoidosis. In: Grassi C, Rizzato G, Pozzi E, eds. *Sarcoidosis and Other Granulomatous Disorders*. Amsterdam; New York: Excerpta Medica; New York, NY, USA: Sole distributors for the USA and Canada, Elsevier Science; 1988: 429.
- 10 Rossman MD, Kern JA, Elias JA, et al. Proliferative response of bronchoalveolar lymphocytes to beryllium. *Ann Intern Med*. 1988; **108**: 687 – 93.
- 11 Brandt B, Thomas M, von Eiff M, et al. Immunophenotyping of lymphocytes obtained by bronchoalveolar lavage: description of an all-purpose tricolor flow-cytometric application. *J Immunol Methods*. 1996; **194**: 95 – 102.
- 12 Deremee RA. The roentgenographic staging of sarcoidosis: historic and contemporary perspectives. *Chest*; **1983**: 128.
- 13 Kopinski P. Use of flow-cytometry in evaluation of cellular changes in interstitial lung diseases [in Polish]. *Folia Med Cracov*. 1997; **38**: 69 – 115.
- 14 Iida K, Kadota J, Kawakami K, et al. Analysis of T-cell subsets and beta chemokines in patients with pulmonary sarcoidosis. *Thorax*. 1997; **52**: 431 – 7.
- 15 Winterbauer RH, Lammert J, Selland M, et al. Bronchoalveolar lavage cell populations in the diagnosis of sarcoidosis. *Chest*. 1993; **104**: 352 – 61.
- 16 Thomeer M, Demedts M. Predictive value of CD4/CD8 ratio in bronchoalveolar lavage in the diagnosis of sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis*. 1997; **14 (Suppl 1)**: 36.
- 17 Hoheisel GB, Tabak L, Teschler H, et al. Bronchoalveolar lavage cytology and immunocytology in pulmonary tuberculosis. *Am J Respir Crit Care Med*. 1994; **149**: 460 – 3.
- 18 Kolopp-Sarda MN, Kohler C, De March AK, et al. Discriminative immunophenotype of bronchoalveolar lavage CD4 lymphocytes in sarcoidosis. *Lab Invest*. 2000; **80**: 1065 – 9.
- 19 Takahashi T, Azuma A, Abe S, et al. Significance of lymphocytosis in bronchoalveolar lavage in suspected ocular sarcoidosis. *Eur Respir J*. 2001; **18**: 515 – 21.
- 20 Kantrow SP, Meyer KC, Kidd P, et al. The CD4/CD8 ratio in BAL fluid is highly variable in sarcoidosis. *Eur Respir J*. 1997; **10**: 2716 – 21.