Lung (1991) 169:305–309

Lung

Value of CD-1-Positive Cells in Bronchoalveolar Lavage Fluid for the Diagnosis of Pulmonary Histiocytosis X

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Abstract. Pulmonary histiocytosis X is characterized by an accumulation of CD-1-positive histiocytosis X cells in the lung, which also can be found in the bronchoalveolar lavage fluid (BALF). However, it has recently been demonstrated that CD-1-positive cells can also be detected in BALF of patients with other interstitial lung diseases and in healthy smokers. We therefore examined the frequency of CD-1-positive cells in a pool of patients with different pulmonary disorders, according to their smoking habits and diagnoses. We have studied the bronchoalveolar lavage in patients with pulmonary histiocytosis X (n = 6), sarcoidosis (n = 88), and in 97 patients with other miscellaneous lung disorders by using the immunoperoxidase method to detect CD-1-positive cells on glass slides.

All patients with histologically proven histiocytosis X displayed more than 5% CD-1-positive cells, whereas patients with other pulmonary disorders showed no more than 3.6% CD-1-positive BAL cells. The dividing line of 5% CD-1-positive cells was not influenced by patients' smoking habits. The identification of CD-1-positive cells in BALF appears to be useful in diagnosing pulmonary histiocytosis X.

Key words: Histiocytosis X—Bronchoalveolar lavage.

Introduction

Pulmonary histiocytosis X is a rare disease; its prevalence has been shown to be less than 2% of all forms of interstitial lung diseases [6, 7, 9, 11]. One important feature of pulmonary histiocytosis X is the increase in Langerhans'

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cells, which can be found in the bronchoalveolar lavage fluid (BALF) [1, 4, 5, 8]. Langerhans' cells express a specific CD-1 antigen, recognized by the monoclonal antibody anti-T6 [4–6, 12, 16].

An increased number of CD-1-positive cells in BALF has been proposed to be of diagnostic significance for patients with histiocytosis X. This proposal is, however, based only on a few observations [4, 5, 12] and is limited by the detection of Langerhans' cells in the BALF of healthy smokers [3] and of patients with pulmonary diseases other than pulmonary histiocytosis X [4, 21]. We recently observed 6 patients with pulmonary histiocytosis X with histologically confirmed diagnoses by examination of open lung biopsies. In all patients the level of CD-1-positive cells in BALF was at least 5% of all cells. This was considerably higher than the numbers of CD-1-positive cells occasionally observed in nonsmokers or smokers with other interstitial lung or airway diseases.

Since the definition of a diagnostic usable number of CD-1-positive cells in patients with suspected histiocytosis X could preclude the need for more invasive diagnostic procedures (e.g., open lung biopsy), we measured the number of CD-1-positive cells in BALF of a larger population of smoking and nonsmoking patients with a variety of bronchopulmonary diseases to compare it with data obtained from our patients with histiocytosis X.

Patients and Methods

Bronchoalveolar lavage was performed for diagnostic reasons in 191 patients with various bronchopulmonary diseases via a flexible bronchoscope.

In all patients, diagnosis was based on history, clinical examinations, conventional x-ray examinations and occasionally computer tomography, lung function measurements, and, in cases of airway diseases, allergy testing and measurement of bronchial responsiveness to histamine.

Bronchoscopic examination was followed by BAL of the middle lobe or the lingula using 5 aliquots of 20 ml sterile physiological saline according to standard techniques [15]. In patients with suspected interstitial lung disorders, BAL was followed by transbronchial biopsies and in patients with suspected sarcoidosis by biopsies of the airway mucosa of the lower lobes.

If these procedures did not yield morphologic confirmation of the suspected diagnosis, open lung biopsy was performed. The bronchoalveolar lavage cells were processed immediately as described elsewhere [2, 21]. Three times 10^5 cells were coated onto glass slides by cytocentrifugation, fixed in pure acetone, and stained by immunoperoxidase method using a specific murine monoclonal antibody to detect CD-1-positive lavage cells (T-6, Dako, Hamburg, Germany). Cells were subsequently incubated with secondary and third peroxidase-conjugated monoclonal antibodies (Dianova, Hamburg, Germany). Specific binding was visualized by diaminobenzidine substrate. Counterstaining was performed with hemalaun. At least 1000 cells from each patient were counted to determine CD-1-positive cells.

Results

The numbers of CD-1-positive cells detected in BALF of patients with various lung disorders are shown in Table 1. All patients with histiocytosis X showed at least 5% CD-1-positive lavage cells, with a range of 5–7%. All of these

Table 1. Numbers, diagnoses, and smoking habits of patients investigated for the percentage of CD-1-positive cells in BALF

Diagnoses	Number	Smoker (%)	Patients with CD-1-Positive Cells ≥5%	CD-1-Positive Cells X/range (%)
Histiocytosis X	6	100	100	6.3/(5–7)
Sarcoidosis	88	20	0	0.2/(0–3.6)
Hypersensitivity pneumonitis	11	9	0	≤0.1/(0–0.1)
Lung fibrosis				
Idiopathic	20	20	0	≤0.3/(0–2.3)
CDV	15	20	0	≤0.1/(0–1)
Airway diseases				
Chronic bronchitis	15	33	0	≤0.1/(0–0.6)
Bronchial asthma	24	25	0	≤0.1/(0–0.2)
Miscellaneous[a]	13	31	0	≤0.1/(0–0.2)

[a] Pulmonary involvement due to Hodgkin's disease and tumor (n = 9), asbestosis (n = 1), miliar tuberculosis (n = 1), toxic bronchitis (n = 1)

CDV, collagen vascular diseases

patients were smokers. The amount of CD-1-labeled cells in the lavage of the 88 patients with pulmonary sarcoidosis of different radiographic stages ranged from 0 to 3.6% (mean, 0.2%) of total lavage cells. CD-1-positive cells could not be detected or did not exceed 2.3% of all cells examined (mean, 0.1%) in the lavage fluid of 97 patients with miscellaneous lung disorders. Only 15 of 191 patients (7.85%) revealed more than 1% CD-1 positive lavage cells. Among these, we found all patients with histiocytosis X, 5 patients with sarcoidosis, 3 of 20 patients with idiopathic pulmonary fibrosis and 1 patient with vasculitis. The percentage of smokers in this group of patients was 66.6% (10 of 15 patients), whereas smokers made up only 26% of all other patients.

Discussion

The diagnosis of pulmonary histiocytosis X requires the histologic demonstration of specific histiocytosis X cells in biopsy specimens of the lung [12, 19, 20]. The diagnostic sensitivity of transbronchial biopsies by flexible bronchoscopy for histiocytosis X has been proven to be unsatisfactory [20]. The diagnosis of histiocytosis X has been mostly made by open lung biopsy [6, 10, 20]. The demonstration of a phenotypic marker of high predictive value, reacting with a distinct population of bronchoalveolar lavage cells in histiocytosis X as postulated for the CD-1 monoclonal antibody [4, 5, 13, 14, 17, 18], could be a useful alternative among the diagnostic procedures for histiocytosis X. In our study, the demonstration of more than 4% CD-positive lavage cells reaches a sensitivity and specificity of 100%. There was a clear dividing line between the CD-1 ratio in patients with histiocytosis X (5–7%) and the elevated CD-1 levels in patients

with other interstitial lung disorders (1–3.6%). Smoking, which is correlated with an accumulation of Langerhans' cells on the epithelial surface of the lower respiratory tract [3] and with elevated CD-1 levels in bronchoalveolar lavage [4, 21], did not influence this result.

A similar study by Xaubet et al. [21] revealed a clear identification of CD-1 cell ratios in patients with histiocytosis X (n = 3) and other interstitial lung disorders as well as in a control group (n = 67). Furthermore, an elevated level of CD-1 labeled cells in bronchoalveolar lavage correlated with smoking habits but did not affect the clear difference between histiocytosis X and other diagnoses. Similar results were obtained by Chollet et al. [4]. Patients with histiocytosis X (n = 18) showed 1.8–25% CD-1-positive lavage cells. There were 2.8% and 2.5% CD-1-positive cells in 1 patient with bullous lung disease and in another with idiopathic pulmonary fibrosis, respectively. The CD-1 ratio of all other 111 patients with miscellaneous lung disorders and 3 control subjects was 1.5% or less.

In all the studies the diseases that demonstrated the highest quantity of elevated or overlapping CD-1 levels in the lavage fluid (e.g., idiopathic pulmonary fibrosis or sarcoidosis) were diagnosed by transbronchial biopsies and bronchoalveolar lavage. Only a few cases still required open lung biopsy [7, 20]. Thus, for the exclusion of these differential diagnoses in suspected cases of histiocytosis X, both transbronchial biopsies and bronchoalveolar lavage appear sufficient in most cases. Although pulmonary histiocytosis X is a disease with low prevalence among interstitial lung disorders, CD-1-positive cells should be evaluated in all patients with clinical and radiographic findings consistent with histiocytosis X.

Acknowledgments. This work was supported by Landesversicherunganstalt, LVA, Freie und Hansestadt Hamburg.

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Accepted for publication 4 June 1991