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Bronchoalveolar lavage for the diagnosis of Pulmonary Langerhans cell histiocytosis

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ABSTRACT

Background: The histologic diagnosis of Pulmonary Langerhans cell histiocytosis (PLCH) is invasive and can cause complications. To confirm the diagnosis of PLCH, guidelines therefore recommend measuring CD1a-positive bronchoalveolar lavage fluid (BALF) cells despite its poor sensitivity and specificity. Thus, an improved diagnostic accuracy of BALF cell analysis would be desirable.

Methods: Using four-colour flow cytometry, plasmacytoid and myeloid dendritic cells (DCs) were analysed in BALF of 10 newly diagnosed, untreated, smoking patients with PLCH, and compared with BALF DCs from 40 asymptomatic smokers and 21 never-smokers.

Results: Compared with controls, myeloid DCs (median: 0.79% of BALF leukocytes) and their subpopulation of Langerhans cells (median: 0.44% of BALF leukocytes) were not increased in PLCH. Patients with PLCH displayed a normal expression of the maturity marker CD83 on BALF myeloid DCs. However, the expression of the co-signaling molecule CD80 on BALF myeloid DCs was significantly lower than in both control groups, with the lowest expression found in more severe disease (presence of cysts > 2 cm in diameter). Based on receiver operating characteristic (ROC) curve analysis, a cut-off of 53% CD80-positive BALF myeloid DCs was optimal for the diagnosis of PLCH, yielding a sensitivity of 0.90 and a specificity of 0.90.

Conclusions: BALF Langerhans cells are not increased in PLCH. However, PLCH is characterised by a low expression of CD80 on BALF myeloid DCs. Due to its considerably higher sensitivity and specificity, this marker appears to be more appropriate to diagnose PLCH than the currently recommended marker CD1a.

1. Introduction

Pulmonary Langerhans cell histiocytosis (PLCH) is a rare, but frequently overlooked lung disease which affects almost exclusively smokers [1,2]. PLCH is characterised by an accumulation of activated Langerhans cells, a subgroup of myeloid dendritic cells (DCs), in granulomas (containing additional cells such as eosinophils and lymphocytes) that develop in distal bronchiole walls [2]. These granulomas are associated with a destruction of lung parenchyma and cyst formation (typically < 2 cm in diameter). More severe cases are characterized by larger, often confluent cysts, pulmonary fibrosis and traction emphysema. Symptoms of PLCH vary considerably since some patients are asymptomatic or suffer from dry cough only, while others develop severe dyspnea or a pneumothorax [1].

While lung biopsy is necessary for a definitive diagnosis, it is invasive and can lead to complications such as pneumothorax, bleeding and respiratory failure. Therefore, it may not be required in instances were imaging findings are highly characteristic [2]. Age at presentation and smoking history can also support the clinical diagnosis. Additionally, a microscopic analysis of bronchoalveolar lavage fluid (BALF) cells stained with the Langerhans cell marker CD1a is recommended (cut-off: > 5% CD1a-positive cells) [3,4], based on studies from the 1980s and 1990s [5–7]. Since then, the significance of CD1a-positive BALF cells for the diagnosis of PLCH has been challenged: (1) patients with PLCH often have < 5% CD1a-

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positive cells in BALF [5], (2) increased CD1a-positive BALF cells are also observed in other conditions including asymptomatic smokers [1], (3) CD1a can also be expressed on other cells such as alternatively activated ("M2") macrophages [8]. Therefore, both the sensitivity and the specificity of this marker have been questioned. Comprehensive analyses of BALF DCs in PLCH to improve this diagnostic technique, however, are lacking. It was the aim of this study, therefore, to phenotype BALF DCs (including the subpopulation of Langerhans cells) in PLCH for the first time, using an established and validated flow cytometric assay for human BALF DCs [9–14]. For this purpose, smokers with newly diagnosed, untreated PLCH were compared with control groups of asymptomatic smokers [10] and never-smokers [10,14].

2. Methods

2.1. Subjects

Patients referred to the Department of Pneumology at the University hospital of Rostock (Germany) between 2009 and 2015 were prospectively screened. Inclusion criteria were: (1) a characteristic high-resolution computed tomography compatible with the diagnosis of PLCH, (2) current smoking of ≥20 cigarettes per day. Exclusion criteria were: (1) any treatment with inhaled or systemic immunosuppressive drugs, (2) any previous diagnosis of PLCH or any other chronic pulmonary disease. Lung function testing using body plethysmography (Masterscreen, Jaeger, Carefusion, Hoechberg, Germany) and the analyses of BALF, blood and tissue specimens were part of the routine clinical assessment. The control group of never-smokers (n = 21) was pooled from two previously published control groups (n = 10 and n = 10) of asymptomatic never-smokers with normal lung function [10,14] and one additional never-smoker with normal lung function. The control group of asymptomatic current smokers (n = 40) is described elsewhere [10]. Briefly, these smokers were recruited using the following criteria: (1) age between 30 and 60 years, (2) smoking history of at least 10 pack years, (3) current smoking of at least 10 cigarettes per day, (4) no history of chronic cardiac, pulmonary or inflammatory diseases, (5) no regular oral or inhaled cardiac or pulmonary medication, (6) no signs of a respiratory tract infection within the last 2 weeks prior to bronchoscopy [10].

2.2. Analysis of blood and BALF dendritic cells

Blood DCs were quantified using freshly collected EDTA-blood as described [15,16]. Bronchoalveolar lavage was performed using a total of 100 ml prewarmed sterile saline as described [9–14]. BALF cells were isolated and analysed with four-colour flow cytometry using the previously published protocol [9–14], with antibodies shown in Table S1 in the online supplement. The laboratory personnel was not blinded to the origin of the samples (the allocation of the samples to one of the control groups or to the group of patients with PLCH was known). Myeloid DCs were defined as cells negative/dim for lineage markers (CD3, CD14, CD16, CD19, CD20, CD56) and positive for CD11c and HLA-DR ($CD11c^{+}HLA\text{-}DR^{+}lin^{neg/dim}$ cells), plasmacytoid DCs as cells negative/dim for lineage markers (CD3, CD14, CD16, CD19, CD20, CD56) and positive for CD123 and HLA-DR ($CD123^{+}HLA\text{-}DR^{+}lin^{neg/dim}$ cells) [9–14] (Fig. S1 in the online supplement shows the gating strategy). Myeloid DC surface molecule expression was quantified in histogram plots using isotype control antibodies for each marker (Fig. S2, online supplement). Langerhans cells were defined as CD1a-positive or Langerin-positive (CD207-positive) $CD11c^{+}HLA\text{-}DR^{+}lin^{neg/dim}$ cells.

2.3. Histologic and cytologic procedures

Tissue specimens were embedded in paraffin and the following stains were routinely performed: haematoxylin and eosin (H&E), periodic acid schiff (PAS), elastica and Prussian blue. Immunhistochemistry was performed with anti-Langerin antibodies (clone: NCL; Novocastra, Leica Biosystems, Wetzlar, Germany) using an autostainer link (DAKO, Hamburg, Germany). In the laboratory of U. Costabel in Essen (Germany), BALF cell cytospins were stained using an immonoperoxidase method and an anti-CD1a antibody (clone: MO721; DAKO, Hamburg, Germany). 600 cells were microscopically counted for CD1a positivity.

2.4. Statistical analysis

Statistical analysis was performed using SPSS Statistics (Version 20, IBM, Armonk, NY, USA). Parameters are expressed as medians (minimum − maximum). The Mann-Whitney-U test for unrelated samples was used for the comparison of groups, the Spearman's correlation coefficient for correlation analyses. Probability values of $p < 0.05$ were regarded as significant.

3. Results

3.1. Subject characteristics

Characteristics of the 10 patients with PLCH are shown in Table 1. There were no significant differences in age and gender distribution between patients with PLCH and controls (Table 2). Patients with PLCH smoked more cigarettes per day (median: 35 cigarettes/day) than the subjects in the control group of smokers (median: 20 cigarettes/day)(Table 2). Eight PLCH patients (No. 1-5, 7, 8, 10) displayed cysts with a diameter between 1 and 20 mm, two patients (No. 6, 8) had larger, irregular and partially confluent cysts with a diameter > 20 mm. The diagnosis PLCH was histologically confirmed in 5 patients. In the remaining patients, the diagnosis was based on typical computed tomography findings and a typical history, and further supported by radiological improvement after smoking cessation. In patient No. 4, the computed tomography of the chest revealed (in addition to nodules and cysts typical for PLCH) a tumor in the left lower lobe (4 cm in diameter). In this patient, non-small cell lung cancer with ipsilateral mediastinal lymph node involvement was diagnosed and a left pneumonectomy was performed. Macroscopic and microscopic analysis of the resected left upper lobe showed no intrapulmonary metastases, but typical features of PLCH (Fig. 1A–C). Staining with an anti-Langerin (CD207) antibody revealed that Langerhans cells were mainly present in the marginal area of the granulomas, but only rarely in the alveoli and airways distant from the granulomas (Fig. 1D).

3.2. DC concentrations in blood and BALF

General blood and BALF characteristics are shown in Table 3. Microscopic analysis of CD1a-stained BALF cytospins (performed in 6 out of 10 PLCH patients) showed a median of 3.6% CD1a-positive cells (minimum: 3.3%, maximum: 8.4% CD1a-positive cells in BALF). The percentage of myeloid DCs among blood leukocytes was decreased in PLCH, but there was no difference in total blood concentrations of myeloid DCs between the groups (Table 3). There were no differences in percentages or concentrations of blood plasmacytoid DCs between the groups (Table 3). The percentages and concentrations of BALF plasmacytoid DCs were lower in patients with PLCH as compared with control smokers, but did not differ between patients with PLCH and never-smokers (Table 3). The percentage of myeloid DCs (Table 3) and CD1a-positive or