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CASE REPORT

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Intriguing bronchoalveolar lavage proteome in a case of pulmonary langerhans cell histiocytosis

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Data Collection B
Statistical Analysis C
Data Interpretation D
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ABCDEF 1,2 Bijar Ghafouri*
ABDEF 3 H. Lennart Persson*
ADEF 2 Christer Tagesson

1 Rehabilitation Medicine, Department of Medical and Health Sciences (IMH), Faculty of Health Sciences, Linköping University, Pain- and Rehabilitation Centre, County Council of Östergötland, Linköping, Sweden
2 Occupational and Environmental Medicine, Department of Clinical and Experimental Medicine (IKE), Faculty of Health Sciences, Linköping University, Centre of Occupational and Environmental Medicine, County Council of Östergotland, Linkoping, Sweden
3 Division of Pulmonary Medicine, Department of Medical and Health Sciences (IMH), Faculty of Health Sciences, Linköping University, Department of Respiratory Medicine, Centre of Surgery and Oncology, County Council of Östergotland, Linkoping, Sweden

* Bijar Ghafouri and H. Lennart Persson contributed equally

Corresponding Author: Bijar Ghafouri, e-mail: bijar.ghafouri@liu.se

Background: Pulmonary Langerhans cell histiocytosis (PLCH) is a rare interstitial lung disease associated with tobacco smoke exposure. New insights into its pathogenesis and how it differs from that of chronic obstructive pulmonary disease (COPD) may be provided by proteomic studies on bronchoalveolar lavage fluid (BALF).

Case Report: We present the BALF proteome in a biopsy-proven case of PLCH and compare it with typical proteomes of COPD and of the healthy lung. The BALF proteins were separated by two-dimensional gel electrophoresis (2-DE) and the protein patterns were analyzed with a computerized 2-DE imaging system. As compared to the healthy subject and the COPD case, the PLCH case showed a strikingly different 2-DE pattern. There was much more IgG (heavy chain) and orosomucoid, and less α_1-antitrypsin, surfactant protein-A, haptoglobin, cystatin-S, Clara cell protein 10, transthyretin and gelsolin. Moreover, no apolipoprotein-A1, pro-apolipoprotein-A1, amyloid P, calgranulin A, or calgranulin B was detected at all.

Conclusions: This case of PLCH presents with an extreme BALF proteome lacking significant amounts of protective and anti-inflammatory proteins. Thus, the intriguing BALF proteome opens up new lines of research into the pathophysiology of PLCH and how its pathogenesis differs from that in COPD.

Key words: BALF • COPD • inflammation • lung • tobacco smoke

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Background

Pulmonary Langerhans cell histiocytosis (PLCH), also called Langerhan's cell granulomatosis or pulmonary histiocytosis X, is a very rare interstitial lung disease that primarily affects young adults [1,2]. Although the pathogenesis remains obscure, tobacco smoke is believed to be an etiologic factor. This is because nearly all individuals affected have a history of current or prior cigarette smoking [1,2], and because PLCH cases have been reported to recover after smoking cessation [3]. A much more common disease also associated with cigarette smoking is chronic obstructive pulmonary disease (COPD). It remains to be clarified how cigarette smoke may cause two such very different conditions. New insight into the pathogenesis of COPD has recently been provided by detailed proteomic studies on bronchoalveolar lavage fluid (BALF) [4]. We here report an extreme BALF proteome with a significant lack of protective and anti-inflammatory proteins in a case of biopsy-proven PLCH.

Case report

A 61-year-old female Caucasian presented in 2009 with a four-month history of nonproductive cough and dyspnea on exertion. She was a smoker, starting at the age of 15 years and averaging 15 cigarettes a day. She had epilepsy and was therefore under continuous treatment with phenytoin since many years. Magnetic resonance tomography (MRT) of the brain was normal. Before admission, she was investigated because of weight loss but no cause was found. Cough and dyspnea persisted, but otherwise the patient was well. Physical examination was unremarkable. BMI was 20.5 kg/m^2. Chest X-ray showed multiple ill-defined nodules mostly in the upper lobes and sparing the costophrenic angels. High-resolution computed tomography (HRCT) scan of the chest showed abnormalities typical for PLCH (Figure 1A and 1B). Routine laboratory tests including total cell count were normal except a moderate elevation of γ-GT and ALP secondary to on-going treatment with phenytoin. Anti-neutrophil cytoplasmic

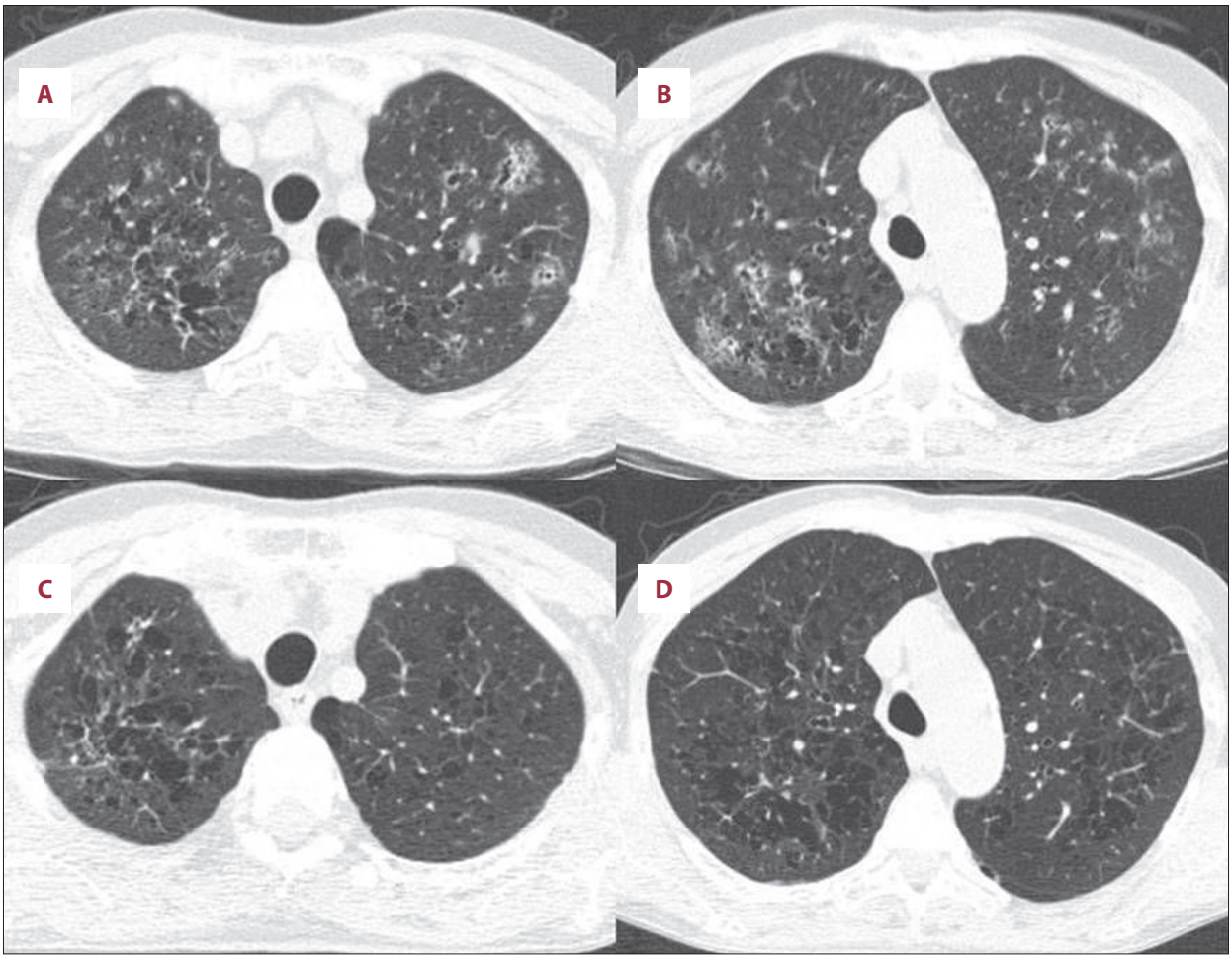

Figure 1. Radiologic findings on serial HRCTs performed at the time of admission (**A, B**) and 10 months later following 4 months of successful smoking cessation (**C, D**). Abnormalities suggestive of PLCH are a combination of multiple cysts and nodules predominantly in the middle and upper lung zones.

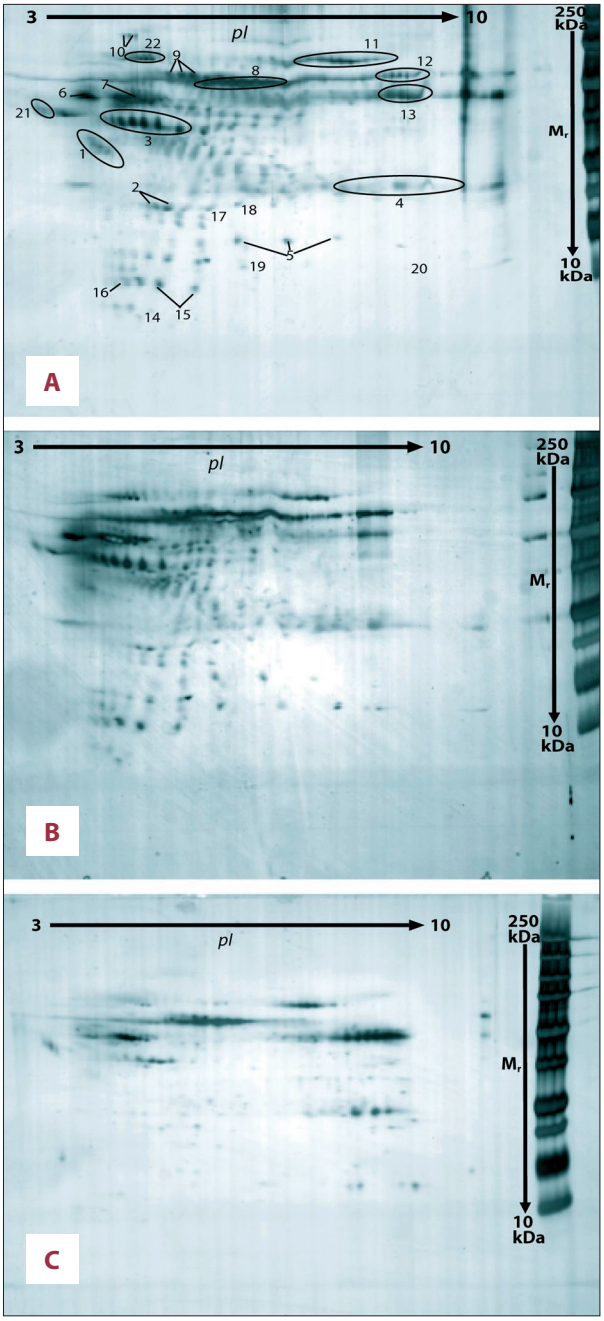

Figure 2. 2-DE images of the BALF proteome in a healthy subject (**A**), a case of COPD (**B**), and a case of PLCH (**C**). 50 µg proteins were separated by isoelectric focusing (pI 3–10) in the first dimension and by SDS-PAGE (M_r 10–250 kDa) in the second dimension. The proteins were visualized with silver staining and the typical BALF proteins identified by pattern matching. **1** surfactant protein A, **2** apolipoprotein A1, **3** haptoglobin, **4** immunoglobulin G light chain, **5** haptoglobin fragment, **6** α_2-HS-glycoprotein, **7** α_1-antitrypsin, **8** albumin, **9** hemopexin, **10** gelsolin, **11** transferrin, **12** lactoferrin, **13** immunoglobulin G heavy chain, **14** CC10, **15** transthyretin, **16** cystatin S, **17** pro-apolipoprotein, **18** amyloid P, **19** calgranulin B, **20** calgranulin A, **21** orosomucoid, **22** immunoglobulin A.

antibody (ANCA) test was negative. Pulmonary function testing showed FEV_1 2.22 L (86% predicted) and FVC 3.16 (97% predicted). There was no improvement following β_2-inhalation. Oxyhemoglobin saturation was 97% at rest. No microbes were detected. Cytopathologic examination revealed 99.5% macrophages (normally 85.2±1.6% in healthy never-smokers). Staining for CD1a and S-100 on BALF-cells were not conclusive. There were no signs of malignancy. BALF T-lymphocyte CD4/CD8 ratio was normal (1.4). Multiple transbronchial biopsy specimens directed against the radiological changes in the right upper lobe revealed inflammation and focal fibrosis, but also Langerhan's cells staining positive for CD1a. Based on pathology and typical radiologic abnormalities the diagnosis of PLCH was made. The patient was advised to stop smoking. No other therapy was given.

A second HRCT was performed six months after the first one. Overall, this HRCT showed a significant progression. The patient was still smoking because smoke cessation had lasted only for a very short time. Symptoms were the same and lung volumes were unchanged. The patient was again firmly advised to stop smoking, but no other treatment was started. Following a 4-month period of smoking cessation, the patient was evaluated by a third HRCT (performed 10 months after the first one). This time the HRCT showed significant improvement (Figure 1C and 1D). In line with the radiologic findings, the patient had no longer any chest complaints. FEV_1 was unchanged while FVC was slightly improved (102% of predicted).

BAL was performed by standardized washing. The protein content in BALF of the PLCH patient was analyzed by two dimensional gel electrophoresis (2-DE) and compared with typical proteomes of COPD and of the healthy human lung (unexposed to tobacco smoke). Figure 2 shows 2-DE images of the different BALF proteomes. About 340 protein spots were detected in the BALF from the healthy lung (Figure 2A); most of the proteins had a molecular weight of 30–70 kDa and an isoelectric point (pI) between 4.5 and 7.0. There were large amounts of albumin, transferrin, and immunoglobulins together with a great number of other proteins at varying concentrations, some of them occurring in different isoforms. In the BALF from the COPD human lung (Figure 2B), about 250 spots were detected and the overall 2-DE image was rather similar to that from the healthy subject. By contrast, in the BALF from the PLCH case (Figure 2C) only 140 spots were detected and the overall 2-DE image was clearly different. Most strikingly, there were very large amounts of IgG (both heavy and light chain) and only minute amounts of low molecular weight proteins (10–25 kDa) with pI between 4 and 6.

Discussion

The BALF proteome from this PLCH case was strikingly different from all other BALF proteomes that we have encountered. Thus, there was much more IgG (heavy chain) and orosomucoid, and much less alpha-1-antitrypsin (ATT), surfactant protein A (SP-A), haptoglobin, cystatin-S, Clara cell secretory protein (CC10), transthyrethin, and gelsolin. A non-specific increase in IgG in BALF from PLCH patients has been demonstrated previously [5] and our early studies showed that smokers had significantly higher levels of immunoglobulins in their BALF than non-smokers [6]. Increased levels of orosomucoid in BALF of humans seem not to have been reported before; however, orosomucoid levels were increased in blood of humans with an intense inflammatory reaction in the airways [7]. As to the decreased amounts of ATT, such decreases have previously been found in patients with sarcoidosis [8] and in healthy volunteers given intrapulmonary lipopolysaccharide [9], a major constituent in cigarette smoke [10]. It has been suggested that AAT is a concentration-dependent modulator of the inflammatory response, and that decreased ATT may dispose for inflammation [11,12].

Aside from ATT, there were also small amounts of SP-A, haptoglobin, cystatin-S, CC10, transthyrethin, and gelsolin. Thus, these proteins appeared in much less quantities in BALF from the PLCH case. Decreased amounts of SP-A were recently found in lung tissue from smokers and rats chronically exposed to cigarette smoke, and decreased SP-A was also found in BALF of healthy volunteers given intrapulmonary lipopolysaccharide [9]. As to CC10, several previous investigations have demonstrated lower levels in BALF of smokers than nonsmokers [7,11–14], and significantly decreased BALF CC10 levels were also detected in smokers compared with nonsmokers in a recent proteomic study [15]. Moreover, reduced production of CC10 and decreased CC10 levels in BALF appear to be features of environmentally induced lung inflammation in horses [13] and rats [14].

None of the normally occurring BALF proteins, apo-lipoprotein-A1, pro-lipoprotein-A1, amyloid P, calgranulin A, and calgranulin B was detected in BALF from the PLCH case, *i.e.*, the amount of each of them was below the limit of detection (1–10 ng). The precise functions of these proteins are not completely understood and so the biological implications of their apparent lack in BALF are uncertain. It can be hypothesized, however, that at least some of them may have a role in protecting the respiratory tract from oxidative stress and inflammation. Apo-lipoprotein-A1 is known to have anti-inflammatory properties [16] and both calgranulin A and B are emerging as anti-inflammatory proteins with postulated roles in the defense against oxidants [17,18]. Further studies are required to clarify whether a lack of anti-inflammatory proteins in BALF may play a role in PLCH.

Conclusions

This unusual case demonstrates that the BALF proteome in PLCH may differ considerably not only from that in healthy subjects but also from that in COPD. A previous study of the BALF proteome in PLCH [19] reported much more discrete alterations than in our case. The reason for this is unclear, but it might well be that our patient was more seriously ill at the time of investigation. This notion is supported by the great number of inflammatory cells found in the BALF. Moreover, the proteolytic activity due to inflammation has been reported to be increased in the PLCH lung [19], which might explain the great loss of anti-inflammatory and protective proteins and the low concentration of protein found in the BALF of the present PLCH case. Thus, the intriguing BALF proteome as shown in the present case opens up new lines of research into the pathophysiology of PLCH and how its pathogenesis differs from that in COPD.

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