Cholesterol crystals in BAL fluid from patients with idiopathic pulmonary fibrosis

E. FIREMAN*, S. SPITZER†, J. GRIEF*, S. KIVITY* AND M. TOPILSKY‡

Departments of *Pulmonary Diseases and Allergy Unit and †Internal Medicine 'H', Tel-Aviv Sourasky Medical Center, and ‡Department of Pulmonary Diseases, Beilinson Hospital, Petah Tiqwa, Sackler School of Medicine, Tel-Aviv, Israel

Introduction

Abnormalities in pulmonary surfactant phospholipids from patients with idiopathic pulmonary fibrosis (IPF) correlate with the nature of the histopathological and clinical impairment (1). This report presents the recovery of cholesterol crystals in the bronchoalveolar lavage (BAL) fluid in two cases of IPF. This evidence may suggest the direct involvement of cholesterol crystals in tissue damage or of their being a marker for altered composition of lung lining fluid.

Patients and Methods

Two patients suffering from severe IPF were studied. Patient 1 was a 62-year-old male who was a non-smoker and who had had progressive dyspnoea since 1991. There was no clubbing, but crepitations were present at both lung bases. X-rays and computerized topographic (CT) scanning of the chest showed widespread bilateral interstitial changes.

Patient 2 was a 45-year-old male, also a non-smoker, who had had progressive dyspnoea and a non-productive cough since 1986. Clubbing was present and crepitant rales were audible at both lung bases. Chest X-rays showed reticular interstitial infiltrates. Transbronchial biopsies yielded lung tissue with mural thickening and intra-alveolar fibrinous exudate. Some bronchioles and alveolar ducts had become obliterated while others were only disrupted. These compensatory dilatations produced a typical honeycomb appearance. The fibrotic tissue and the honeycombed area formed a hyperplastic or metaplastic epithelial cover of the airspaces. All these findings were consistent with the diagnosis of pulmonary fibrosis.

Neither patient was treated before BAL. Pulmonary function tests (PFT) were done using a spirometer and a body plethysmograph (MasterLAB-Herich, Jaeger GmbH Co., KG, Germany). Bronchoalveolar lavage was performed by conventional methods (2). The recovered fluid was processed as described previously (3).

Fluorocytometric analysis was performed on a scanner (Fac-Scan) with an Ar+ laser (Becton-Dickinson) as described previously (4). Cells were pelleted (2 ml of solution, 10⁶ cells ml⁻¹) and lipid extraction was performed according to Folch et al. (5). For the analysis of cholesterol and phospholipids, the methods of Abbe1 et al. (6) and Bartlett (7) were used. Light and light-polarized microscopy (Olympus Optical Co. Ltd, Tokyo, Japan) were used to perform the morphological identification of crystals. Two control subjects were included; non-smokers who underwent bronchoscopy due to persistent cough with no X-ray or clinical evidence of interstitial lung disease.

Results

Pulmonary function tests showed a severe restrictive impairment (TLC=50% of predicted in both patients) and reduction of DLCO (Patient 1=46% and Patient 2=25% of predicted). Differential counts of cells yielded a high percent of neutrophils (Patient 1=18% and Patient 2=35%) with mild lymphocytosis (Patient 1=32% and Patient 2=22%). There was a differential pattern of T-cell subsets; the helper/suppressor ratio was above the normal range (3-9) in Patient 1, while it was low (0-8) in Patient 2. Cholesterol crystals were recovered from the BAL fluid from each patient. Ghosts of some symmetric extracellular structures were initially identified by a
Table 1  Phospholipid and cholesterol content of BAL cells

<table>
<thead>
<tr>
<th></th>
<th>Phospholipids (µM)</th>
<th>Cholesterol (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>1.3</td>
<td>0.307</td>
</tr>
<tr>
<td>Patient 2</td>
<td>0.5</td>
<td>0.282</td>
</tr>
<tr>
<td>Control 1*</td>
<td>0.16</td>
<td>0.066</td>
</tr>
<tr>
<td>Control 2*</td>
<td>0.16</td>
<td>0.070</td>
</tr>
</tbody>
</table>

*Determinations of phospholipids and cholesterol were performed in BAL cells of two patients who underwent bronchoscopy due to persistent cough (foreign body).

Discussion

The data presented in this report show the presence of cholesterol crystals in the BAL of two patients with a clinical course and histological findings compatible with IPF. The percent neutrophils in BAL cells was high, but a different pattern was seen in the lymphocyte subsets. Patient 1 had a high percent of CD4+ cells, while Patient 2 had a high percent of CD8+ cells. These findings were compatible with the authors' earlier conclusions (4) that a high or normal percentage of helper T-lymphocytes in BAL of fibrotic patients correlates with a better response to corticosteroid therapy. In fact, the DLCO capacity of Patients 1 improved by 25% after treatment with corticosteroids, although Patient 2 did not improve (data not shown).

The bronchoalveolar cells extract obtained from both patients showed a high content of cholesterol and other phospholipids. This material consisted of pulmonary surfactants and a complex mixture of cells including macrophages, lymphocytes and their secretory product. Studies on this lining fluid in humans (8) revealed it to have suppressive effects on lymphocyte proliferation. Other studies showed that exogenous cholesterol increases the antigen-presenting function of monocytes in extrinsic allergic alveolitis by modulating the HLA-DR region products (9). It is, therefore, possible that alterations in the lipid composition of the lung lining fluid reported in these patients may have led to reduced immunosuppressive properties and favoured the development of localized immune response and tissue damage.

In the present patients, the foamy macrophages may have been the main source of the cholesterol crystals, as was shown in extrinsic allergic alveolitis (10), and in patients suffering carmustine-induced pulmonary fibrosis (1). On the other hand, extracellular cholesterol crystals were associated with the presence of lipid-laden macrophages and smooth muscle cells in arteriosclerotic plaques (12). Since macrophage foam cells are usually more numerous and closer to the surface than are lipid-laden macrophages (13), it is reasonable to have obtained foamy macrophages among cells recovered from the washed alveolar areas. As for the transbronchial biopsy,

Plate 1  Ghosts of some symmetric extracellular structures first identified by Giemsa stained cytospin slides. Foamy macrophages were also seen.

Plate 2  The same structures as in Plate 1 were finally identified as being cholesterol crystals using a fresh preparation with a polarized light microscope.
it may be that the sample was not taken from distal areas which had airway disease obstruction associated with lipid-laden macrophages.

In conclusion, the proteolitic enzyme activity may contribute to the development of severe tissue damage in susceptible regions of the lung in IPF. This may be due to high levels of matrix-degrading proteinases (14) secreted by foamy macrophages which contain substantial amounts of cholesterol esters (15).

References