CORRELATION OF LOWER RESPIRATORY TRACT INFLAMMATION WITH CHANGES IN LUNG FUNCTION AND CHEST ROENTGENOGRAMS IN PATIENTS WITHUNTREATED TROPICAL PULMONARY EOSINOPHILIA

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ABSTRACT
Forty-one patients with untreated tropical pulmonary eosinophilia (TPE) were studied to determine whether there was any relationship between lower respiratory tract inflammation and either changes in lung function or abnormalities in chest roentgenograms. Total number of inflammatory cells in bronchoalveolar lavage (BAL) fluid, consisting of alveolar macrophages, lymphocytes, eosinophils and neutrophils, had significant negative correlations with transfer factor (TLCO) (r=-0.319, p<0.001), transfer coefficient (KCO) (r=-0.32, p<0.05) and total lung capacity (TLC) (r=-0.25, p<0.05). The absolute count of eosinophils in BAL fluid had a significant negative correlation with TLC (r=-0.43, p<0.01) and KCO (r=-0.3, p<0.05), but not with forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) or TLC. There was no correlation between the types of cells recovered in BAL fluid and changes in chest radiographs as assessed by the ILD classification.

INTRODUCTION
Studies of bronchoalveolar lavage (BAL) in patients with acute untreated tropical pulmonary eosinophilia (TPE) have shown that there is an intense eosinophilic alveolitis and this is associated mainly with a reduction in single-breath transfer factor (TLCO) in most patients. A proportion of patients have obstructive, restrictive and combined ventilatory defects in addition. Although there were studies correlating the lower respiratory tract inflammation as assessed by BAL, to changes in lung function in patients with sarcoidosis and idiopathic pulmonary fibrosis, such a study has not been reported in patients with tropical pulmonary eosinophilia. As eosinophils have been shown to produce toxic mediators which are injurious to lung parenchyma, leading to chronic progressive tissue damage with interstitial fibrosis in bronchopulmonary aspergillosis, the impairment in lung function, reflecting underlying lung injury, may be a direct result of the inflammatory reaction in the lower respiratory tract of patients with acute TPE. A study was therefore undertaken to define precisely the respective roles of distinct cell components in alveolar sites (macrophages, lymphocytes, eosinophils and neutrophils) implicated in the pathophysiological changes observed in TPE.

SUBJECTS AND METHODS
Forty-one consecutive patients (38 males and 3 females, age 15–52 years) with symptoms of one week to six months’ duration, and fulfilling the diagnostic criteria of respiratory symptoms such as cough, dyspnoea and nocturnal wheezing, pulmonary infiltrates and peripheral blood eosinophilia > 2000 cells/mm3 were included in the study. Each individual was evaluated by a detailed history, physical examination, a full plate PA chest radiograph, total and differential leucocyte counts and absolute eosinophil counts in peripheral blood. stool examinations were done to exclude any infestation with intestinal helminths. Informed consent was obtained from all study subjects. A chest physician (VKV), experienced in reading chest radiographs for nearly 15 years, read all chest radiographs randomly at the end of the study, using the modified ILD classification.

Lung Function Tests:
Lung function tests were carried out using transfer test Model C (PK Morgan Ltd. Chatham, UK). Forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) were determined from the best 2 of 3 acceptable forced vital capacity manoeuvres varying by not more than ±5%. The largest FVC and FEV1 values at BTPS were recorded, even if the two values did not come from the same curve. Total lung capacity (TLC) was measured by closed circuit helium dilution method by obtaining, in each patient, two functional residual capacity (FRC) measurements which did not vary by more than ±10%. The mean value of these two measurements was used to determine FRC at BTPS. The single-breath diffusing
Bronchoalveolar lavage:

BAL was performed with a flexible fibreoptic bronchoscope as previously described and all bronchoscopies were done under local anaesthesia. Briefly, five 20 ml aliquots of normal saline at room temperature were instilled into each lobe and recovered immediately using suction with 50-100 mm water negative pressure, the bronchoscope being wedged in a sub-segmental bronchus of the right middle lobe, lingula and left lower lobe. The fluid was filtered through gauze to remove mucus and was pooled. An aliquot was used for filter preparations. The total number of cells was estimated on a haemocytometer and expressed as cells/dl. Filter preparations were made on pooled lavage fluid (uncentrifuged cells) as reported by Saltini et al and were stained using haematoxylin-eosin stain. A minimum of 400 cells were counted on each slide by two experienced observers (VKV, KS) and recorded independently. Both observers agreed to within 5% of all lavage analysis and the mean value was used for analysis. BAL was also performed on 17 normal non-smoking individuals as controls. None of these subjects had respiratory symptoms or abnormal physical findings and all had normal chest radiographs and normal pulmonary functions.

All data are expressed as mean ± SEM. Results between groups were compared using two tailed students’ ‘t’ test. For correlations, we used Pearson’s product moment correlation.

RESULTS

The mean age of the study subjects was 23.9 ± 1.1 years (range 15-52 years). The mean total number of inflammatory and immune effector cells (Table I) in the lower respiratory tract in TPE patients was 160.3 ± 17.1 cells x 10^6/dl (range 24-575 cells x 10^6/dl); this was significantly higher compared to normal subjects (p<0.001). Except for two patients, all others had total number of cells greater than 40x10^6/dl. The mean eosinophil percentage (50.0 ± 4.5, range 1-99%) was also significantly higher compared to normals (p<0.001) and only two patients had eosinophils of less than 10% in the lower respiratory tract. The mean proportion of alveolar macrophages fell significantly (p<0.001) to 43.9 ± 4.2% (range 1 to 91%). One patient with symptoms for only one week had a normal percentage of eosinophils in BAL fluid had a negative correlation with various pulmonary function measurements. The correlations of BAL cellular constituents to various pulmonary function measurements are given in Table II. We found that total number of cells in BAL fluid had a negative correlation with TCL (r=-0.352, p<0.05). TLCO (r=-0.519, p<0.001) and KCO (r=-0.312, p<0.05). Similarly, total number of eosinophils in BAL fluid had a negative correlation with TLCO (r=-0.430, p<0.01, Fig 2) and KCO (r=-0.300, p=0.05). However, the total number of alveolar macrophages in BAL fluid had a significant negative correlation with FVC (r=-0.343, p<0.001).

Among the 41 patients, who had TLCO measured, 36 (88%) had results of less than 85% of the predicted. A reduction in both TLC and FVC of less than 85% of predicted was seen in 18 (44%) patients. FEV1/FVC% was less than 75% in 14 (34%) patients. TLCO had a positive correlation with FVC (r=0.551, p<0.001). FEV1/FVC% had a positive correlation with TLCO (r=0.557, p<0.0001) and KCO (r=0.533, p<0.0001).

The correlations of BAL cellular constituents to various pulmonary function measurements are given in Table II. We found that total number of cells in BAL fluid had a negative correlation with TLC (r=-0.352, p<0.05). TLCO (r=-0.519, p<0.001) and KCO (r=-0.312, p<0.05). Similarly, total number of eosinophils in BAL fluid had a negative correlation with TLCO (r=-0.430, p<0.01, Fig 2) and KCO (r=-0.300, p=0.05). However, the total number of alveolar macrophages in BAL fluid had a significant negative correlation with FVC (r=-0.343, p<0.001).

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Table II
Correlations of BAL cells with Pulmonary function in TPE

<table>
<thead>
<tr>
<th></th>
<th>Total cells x10^6/dl</th>
<th>Macrophages x10^6/dl</th>
<th>Lymphocytes x10^6/dl</th>
<th>Eosinophils x10^6/dl</th>
<th>Neutrophils x10^6/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>FVC (% Pred.)</td>
<td>-0.287</td>
<td>NS</td>
<td>-0.343</td>
<td>&lt;0.05</td>
<td>-0.171</td>
</tr>
<tr>
<td>FEV1 (% Pred.)</td>
<td>-0.251</td>
<td>NS</td>
<td>-0.341</td>
<td>&lt;0.05</td>
<td>-0.110</td>
</tr>
<tr>
<td>TLC (% Pred.)</td>
<td>-0.352</td>
<td>&lt;0.05</td>
<td>-0.305</td>
<td>&lt;0.05</td>
<td>-0.315</td>
</tr>
<tr>
<td>TLCO (% Pred.)</td>
<td>-0.519</td>
<td>&lt;0.001</td>
<td>-0.287</td>
<td>NS</td>
<td>-0.108</td>
</tr>
<tr>
<td>KCO (% Pred.)</td>
<td>-0.312</td>
<td>4.05</td>
<td>-0.097</td>
<td>NS</td>
<td>0.129</td>
</tr>
</tbody>
</table>

NS: Not Significant

DISCUSSION

A significant increase in the proportion and total number of eosinophils along with a significant rise in total number of inflammatory and immune effector cells in the lower respiratory tract of patients with untreated tropical pulmonary eosinophilia corroborates our earlier observation of eosinophilic alveolitis in 8 acute tropical pulmonary eosinophilia patients. Although there was a reciprocal significant reduction in proportion of alveolar macrophages, the total number of alveolar macrophages was significantly higher as a result of a significant rise in total number of inflammatory cells. Thus, the inflammation in the lower respiratory tract in patients with untreated TPE is due to an abnormal accumulation of both alveolar macrophages and eosinophils, resulting in macrophage-eosinophilic alveolitis. Similar two cell interactions leading to alveolitis have been described in other interstitial lung diseases as well. Activated macrophages and eosinophils are capable of producing spontaneously various toxic mediators resulting in injury and fibrosis to lung parenchyma. The electron microscopic demonstration of severe degranulation with loss of both the core and peripheral portion of granules of eosinophils recovered from the lower respiratory tract of TPE patients, suggests that these cells are possibly activated and may be responsible for pathological changes seen in these patients. One of our study subjects, who had a one-week history of symptoms, showed no increase in total number of inflammatory cells or proportion of different cell types in lavage fluid. He had normal pulmonary function including measurements of diffusing capacity. This suggests that in the very early stages of the illness there may not be any influx of eosinophils into the lower respiratory tract. The finding of a reduced single-breath diffusing capacity in 88% of patients suggests that the alveolitis resulting from the abnormally accumulated inflammatory cells can cause injury to lung parenchyma. The significant correlation of TLCO to lung volumes such as TLC, FVC and FEV1 further suggests that inflammatory changes in the lower respiratory tract are severe enough to produce reductions in lung volumes as well. Similar observation of a significant correlation of TLCO with FVC has been reported in sarcoidosis. In this study, we could not...
find any correlation between absolute number of inflammatory cells recovered by BAL fluid and chest roentgenogram findings or lung function abnormalities. Similar findings have been described in other interstitial lung diseases as well[10,11]. There is no correlation between BAL findings and chest radiographic changes because radiographic abnormalities reflect both inflammatory and fibrotic changes in the lung, while BAL detects only pulmonary inflammation[12]. Hence the lack of correlation between BAL findings and chest radiographic changes in our study subjects with less than 6 months of symptoms suggest the possibility that early fibrosis, in addition to pulmonary inflammation, has occurred in TPE.

The findings of a significant negative correlation of total number of inflammatory cells and absolute eosinophil count in lavage fluid in both TLCO and KCO suggest that the alveolitis, causal by the abnormal accumulation of inflammatory cells and eosinophils in particular, may be responsible for the impairment of diffusing capacity. Similarly, a significant correlation of BAL fluid eosinophilia with a reduced diffusing capacity of the lung, but not with vital capacity or forced expiratory volume has been reported in idiopathic pulmonary fibrosis[22]. However, Watters et all[23] reported that BAL fluid eosinophils had a negative correlation with FVC in idiopathic pulmonary fibrosis, while a negative correlation of BAL fluid lymphocytes with diffusing capacity had been reported in sarcoidosis[24].

Interestingly, the total number of alveolar macrophages had a significant negative correlation with lung volumes such as TLC, FVC and FEV1, and the absolute lymphocyte count had a significant negative correlation with TLC. Since the recovery of macrophages and lymphocytes was related, as evidenced by a positive correlation between these two types of cells, the reaction in lung volumes in untreated TPE may be due to cytotoxic products liberated by these cells[21]. The findings of a significant negative correlation of the eosinophil count to TLCO and KCO, and also the absolute counts of macrophages and lymphocytes IO lung volumes indicate that there may be a dissociation of pathophysiological changes produced by these cells in untreated TPE. The changes may be due to a variety of toxic mediators produced by activated inflammatory cells and the modes of action of these mediators may vary at different sites. Further immunological studies with serial lavage are required to answer these questions.

References