Tryptase concentrations in bronchoalveolar lavage from patients with chronic eosinophilic pneumonia

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ABSTRACT
In order to characterize BAL (bronchoalveolar lavage) in CEP (chronic eosinophilic pneumonia) and to investigate the possible role of mast cells and tryptase in the pathogenesis of this interstitial disease, cells and tryptase levels were determined in BAL of patients with CEP and in a group of healthy controls. The results show that a statistically significant increase in tryptase concentration was found in patients with CEP compared with the healthy controls. This is the first report that shows an increase in tryptase levels in CEP and could reflect higher mast cell activation as well as larger mast cell populations in the lungs of these patients. These results strongly support the involvement of mast cells and eosinophils in the immunopathogenesis of CEP.

INTRODUCTION
CEP (chronic eosinophilic pneumonia) is a rare ILD (interstitial lung disease) of unknown aetiology. It is characterized by chronic respiratory symptoms, alveolar and blood eosinophilia and chest imaging evidence of peripheral pulmonary infiltrates [1]. BAL (bronchoalveolar lavage) is useful in the diagnosis of CEP, as it can replace biopsies in a large number of cases and also enables the study of pathogenetic processes [2]. As a rule, BAL shows an increase in eosinophils; however, there is evidence that other cells, such as lymphocytes and mast cells, can play a role in CEP pathogenesis [2]. Although the role of mast cells in the fibroproliferative response, particularly in CEP, is unclear [3], an abundance of mast cells (often found in close apposition to lung fibroblasts) has been found in the parenchyma of patients with other ILDs, such as sarcoidosis [4], asbestosis [5] and pulmonary fibrosis [6]. Activated mast cells selectively produce tryptase, a serine protease stored in the granules as a tetrameric enzyme bound to heparin [7]. Elevated levels of tryptase have been reported in serum of patients with acute anaphylaxis or systemic mastocytosis [8], in nasal lavage fluid of allergic patients and in induced sputum and BAL of patients with asthma and ILD [9]. Tryptase enhances defence against bacterial infections [10], stimulates fibroblast, epithelial and smooth muscle cell proliferation, and has proinflammatory functions, including oedema formation and accumulation of eosinophils [11]. The enzyme stimulates the synthesis of type I collagen [12] and promotes vascular permeability and tissue remodelling during lung inflammation through selective proteolysis of matrix proteins, activation of proteinase-activated receptors and matrix metalloproteinases [13]. Walls et al. [14] reported an increase in tryptase concentration in BAL of patients with ILD and postulated that this enzyme may be involved in the pathogenesis of sarcoidosis and pulmonary fibrosis. This hypothesis was later corroborated by Eklund et al. [15].

On the basis of the above preliminary findings and evidence of increased eosinophils and mast cells in allergic disorders such as CEP [8], the aim of the present study...
was to evaluate, for the first time, whether mast cells are involved in the pathogenesis of CEP. Since tryptase is a specific marker of mast cell activation, concentrations of this enzyme were determined in BAL of CEP patients and compared with values measured in healthy controls.

**MATERIALS AND METHODS**

**Subjects**

The study population consisted of 12 patients with untreated CEP and six healthy controls. BAL was performed with the informed consent of patients for diagnostic or clinical purposes (healthy subjects underwent bronchoscopy and BAL after an episode of haemoptoe, in order to exclude pulmonary disease). CEP was diagnosed according to the criteria described by Carrington et al. [1]. The patients underwent clinical, radiological and functional tests, including single-breath DLCO (diffusing capacity of the lung for carbon monoxide). Patients with CEP underwent a total serum IgE test, screening for serum autoantibodies and tests for parasitic infections to exclude other possible causes of pulmonary and peripheral eosinophilia. Three patients with CEP had a history of asthma. The main symptoms at onset were dyspnoea, cough and malaise in all patients. The average values of serum eosinophilia and serum total IgE were 17.01 ± 14.02 % and 218.45 ± 273.28 k-units/l respectively. Chest X-rays showed diffuse ‘ground glass’ areas in seven patients and peripheral consolidation zones with nodular infiltrates in five cases. Lung function tests were normal in four cases, with a restrictive deficit in four patients and with an obstructive deficit in two cases. All subjects gave their written informed consent for participation in the study.

**BAL and phenotype analysis**

BAL was obtained by instillation of four 60 ml aliquots of saline solution by fibrobronchoscope (Olympus IT-10) [16,17]. The first sample was kept separate from the others and was not used for immunological tests. Cells were separated by centrifugation and the fluid fraction frozen for tryptase assay. Differential cell counts were performed. Lymphocyte phenotype was analysed by flow cytometry (Facs-Calibur, Becton Dickinson) using anti-CD3, -CD4 and -CD8 monoclonal antibodies (Becton Dickinson). To count mast cells, slides were stained with 0.5 % Toluidine Blue in 0.5 mol/l HCl for 30 min, and 500 or more consecutive cells were counted, as described previously [18]. Mast cells were identified on the basis of their affinity for Toluidine Blue and their morphology.

**Assay of tryptase concentration**

BAL samples of CEP patients were concentrated 5-fold and those from healthy controls were concentrated 10-fold (Microcon YM-10 Centrifugal Filter Unit; Amicon). Concentrations of tryptase in BAL samples were determined by a modification of a commercially available assay (UniCAP-Tryptase Fluoromunnoassay; Pharmacia Upjohn), which is currently used to determine tryptase in serum, plasma and nasal fluid [9]. Anti-trypase antibodies, covalently coupled to ImmunoCAP, were added to the BAL samples and incubated to form a complex. Unbound antibodies were then removed by washing and the bound complex was incubated with a developing agent. The reaction was stopped and fluorescence was measured in the eluate. This method has been used previously to determine tryptase release from tissues and mucosal mast cells [19]. This fluoro-immunoenzymatic method is technically similar to an RIA [14], and it allowed tryptase to be detected at concentrations as low as 1 ng/ml. This methodology was used to study BAL by preconcentration of samples and by determining fluorescence against standard solutions (range of concentrations, 0.1–1.0 ng/ml) added to samples before analysis (method of internal additions). The percentage recovery of an exogenous peak in the tryptase assay was 97 %. Each sample was assayed in triplicate on seven different occasions, and intra- and inter-assay coefficients of variation were below 2.5 % and 3.4 % respectively.

**Statistical analysis**

The results are means ± S.D. Their distribution was normal as determined by the Shapiro–Wilk’s test. Comparisons between the two groups were performed by Student’s t test. Pearson’s coefficient of rank correlations was calculated to assess relationships between variables. A P value < 0.05 was considered to be significant. Statistical software (SPSS 11.5 for Macintosh) was used for all data analysis.

**RESULTS**

The clinical findings in CEP patients and healthy subjects are shown in Table 1.

Table 1  Clinical findings in patients with CEP and controls  
<table>
<thead>
<tr>
<th></th>
<th>Patients with CEP</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (male/female)</td>
<td>12 (5/7)</td>
<td>6 (3/3)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.9 ± 16.3</td>
<td>42.1 ± 13.5</td>
</tr>
<tr>
<td>TLC (%)</td>
<td>91 ± 22</td>
<td>107.5 ± 10.1</td>
</tr>
<tr>
<td>FEV 1 (%)</td>
<td>89.9 ± 24.3</td>
<td>101.2 ± 7</td>
</tr>
<tr>
<td>DLCO (ml/min/mmHg)</td>
<td>85.0 ± 24.3</td>
<td>–</td>
</tr>
<tr>
<td>DLCO/VA</td>
<td>88.3 ± 12.8</td>
<td>–</td>
</tr>
</tbody>
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Table 2 shows the cellular composition of BAL from CEP patients and controls. All cell populations are shown...
with the exception of mast cells. Lymphocyte immuno-
phenotyping revealed a CD4/CD8 ratio of 1.0 ± 0.6 in
CEP and 2.1 ± 1.1 in controls. The percentages of eosino-
phils (Table 2) and mast cells (Figure 1a) were significantly
higher ($P < 0.01$) in BAL of CEP patients than in samples
from healthy controls. Mast cells varied in shape (from
oval to round) and size. Partially degranulated mast cells
and clusters of granules outside the membranes were also
observed.

The highest concentrations of mast cell tryptase were
measured in BAL of CEP patients, whereas very low
levels were observed in healthy subjects (Figure 1b). The
difference between tryptase concentrations in CEP pa-
tients and controls was significant ($P = 0.005$). No rela-
tionships were found between the percentage of mast cells
or tryptase concentrations and lung function parameters
such as FEV$_1$ (forced expiratory volume in 1 s), FVC
(forced vital capacity) and DLCO.

**DISCUSSION**

Alveolar inflammation in patients with CEP is associated
with an increase in eosinophils and lymphocytes. Besides
these cells, other cells, such as mast cells, are probably in-
volved in the pathogenesis of CEP [20]. Consistent with
other studies [1,2,21], we found an increase in the per-
centages of eosinophils and mast cells in BAL, maintaining
the hypothesis that mast cells contribute to the develop-
ment of lung fibrosis in CEP. Mast cells are likely to promote
the secretion of fibroblast growth factors, such as trypt-
ase. This is the first study to report a statistically signi-
ficant increase in tryptase levels in BAL of CEP patients,
which is associated with a higher than normal percentage
of mast cells. This finding reflects a higher degree of
mast cell degranulation, which may be indicative of en-
hanced cell activation.

As with the findings by Eklund et al. [15], we failed
to find any correlation between the number of mast cells
in BAL and lung function parameters. However, in both
of these studies the number of patients was rather low and
both populations included patients with lung function
parameters only slightly lower than predicted values.
Jarjour et al. [22] reported an increase in tryptase and
histamine in BAL of patients with allergic asthma and al-
lergic rhinitis. They found a positive correlation between
histamine levels and the percentage of eosinophils in
BAL and between histamine concentrations and FEV$_1$
values. However, as in the present study, they did not
find a statistically significant correlation between tryptase
activity in BAL and lung function parameters [22].

In conclusion, the results of this preliminary study
suggest that mast cells and tryptase, as well as eosinophils,
play a prominent role in the immunopathogenesis of CEP.

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