Workshop on
Bronchoalveolar lavage: new insights in research and clinical application

April 7-8, 1989
Medical Center of Rehabilitation VERUNO (Novara) - Italy

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Cryptogenic fibrosing alveolitis: pathogenetic mechanisms and therapeutic approaches

P.L. Haslam

Cryptogenic fibrosing alveolitis (CFA) remains one of the most serious of interstitial lung diseases. Prognosis is poor (mean survival 3–5 yrs) and progression highly variable for reasons unknown. Corticosteroids remain the main drugs used in treatment of CFA although response is only achieved in about 20%.Bronchoalveolar lavage (BAL), by furthering information on cellular mechanisms in the lungs of CFA patients, should supply better prognostic indicators to assess and monitor disease activity and, thus, rationalize the approach to therapy and use of drugs. There have been no controlled trials of corticosteroid therapy in CFA but many reports of subjective improvement in 40–70% of cases and objective improvement in only 11–30%. Factors favouring steroid response were younger age and shorter duration of disease [1]; thus emphasizing the importance of early diagnosis and treatment. Survival curves were significantly better in patients with marked cellular infiltrates in their lung biopsies than in those with a mixed cellular/fibrotic picture or predominance of fibrosis [2]. This favours the hypothesis that early disease is associated with influx of inflammatory cells in the lungs preceding the development of fibrosis. Subsequent studies of BAL also support this hypothesis.

Immunological mechanisms are thought to be involved in the pathogenesis of CFA. Most patients have polyclonal increases in serum immunoglobulin levels; >60% have circulating auto-antibodies (anti-nuclear antibodies or rheumatoid factors); lymphocyte sensitization to these auto-antigens and to collagen has been reported; and fibrosing alveolitis frequently occurs in association with systemic “auto-immune” connective tissue diseases including rheumatoid arthritis and systemic lupus erythematosus [3].

Little evidence was found of immune complex deposits within the lungs but increased levels of circulating soluble immune complexes were frequently observed [4] and have also been noted in BAL fluids. Their levels were found to be significantly higher in patients with shorter duration of disease, indicating that their formation is another feature of early stage disease [5]. Many patients have been reported to show a better response to steroids [6].

BAL provides a safe, repeatable and minimally invasive method of sampling inflammatory cells and components from the air spaces of the lungs. This has extended information on components within the lungs of CFA patients and changes that occur with treatment. Use of BAL in the initial assessment of CFA patients has supplied additional prognostic indicators.

Lymphocytosis occurs in only 17% of CFA patients and counts are low, yet these patients have a significantly better chance of responding favourably to corticosteroids [7, 8]. Levels of lymphocytes show a significant correlation with levels of soluble immune complexes in serum and lavage [9] implying that increases in lavage lymphocytes are also a feature of earlier stage disease. By contrast, increases in neutrophils, which are the most striking feature in lavage of CFA patients, tend to be higher in those who fail to respond to steroids [7, 8]. Patients who have increased eosinophils as well as neutrophils in lavage have a poor chance of responding to steroids and show the highest correlation with progressive disease prior to treatment [7, 8]. We observed that CFA patients with increased lavage eosinophils did well on cyclophosphamide (100 mg per day) combined with prednisolone (20 mg per alternate day) and 69 such patients showed objective maintained response [10].

The repeatability of BAL makes it ideal for monitoring changes in pulmonary inflammatory cells and components during disease progression and therapy. We reported our findings for 32 CFA patients with a mean follow-up period of 4 yrs [10]. Of 15 patients on high-dose prednisolone, those showing maintained objective response had a significant overall decrease in inflammatory cells, whilst the non-responders had a significant increase. The most significant changes were in neutrophil counts. Of 11 patients on cyclophosphamide and low-dose prednisolone, the only significant change was a decrease in eosinophils. Hence, cyclophosphamide may be effective in a different sub-group or at a different stage of disease to prednisolone and initial BAL cell counts may be useful in selecting the most appropriate drug therapy for an individual. Serial BAL studies may also be useful to monitor the effects of different drug dosages in suppression of inflammation.

Increases in lavage lymphocytes and soluble immune complexes have thus emerged as features of early stage disease in CFA. By contrast, increases in lavage eosinophils show a significant lack of association with lymphocytosis and are associated with progressive disease prior to treatment [7, 8]. Many of the eosinophils show features of degranulation and significantly elevated levels of eosinophil cationic protein were identified in lavage samples of CFA patients compared with controls [11]. These have been shown to be highly cytotoxic to many cell lines in tissue culture, including epithelium, suggesting that their release may be a factor contributing to the marked damage to type I and II pneumocytes, which is a feature of the histology of CFA. The possibility that mast cells might be involved in the mechanisms of CFA was investigated, since they are a potent source of eosinophil chemotactic factor. Mast cells were not significantly increased in BAL fluid of CFA patients but
were increased in lung biopsy samples, especially in areas of dense fibrosis. There were also significantly increased levels of histamine, a mediator derived from mast cells, in the BAL samples [12]. Histamine levels showed a significant correlation with percentages of eosinophils and neutrophils in BAL samples and with higher grades of fibrosis in lung biopsies from the same patients. We conclude that increased eosinophil and histamine levels are features of later stage disease in CFA. Evidence that histamine enhances fibroblast proliferation [13] suggests that this may contribute to the fibrogenic process.

Clearly many factors released in chronic inflammation may contribute to CFA. Current information is probably limited to secondary rather than primary factors in pathogenesis. Evidence suggests that factors released from activated alveolar macrophages (AMs) may stimulate fibroblast proliferation in CFA. Betterman et al. [14] demonstrated that AMs from CFA patients spontaneously release fibronectin and AM-derived growth factor for fibroblasts. The former acts as a competence factor priming fibroblasts for response, whilst the latter acts as a progression factor promoting fibroblast proliferation. Two other mediators derived from AMs, interleukin 1 and tumour necrosis factor, independently augment fibroblast proliferation induced by fibronectin and AM-derived growth factor. However, Elias [15] showed that when present together these factors synergistically stimulate production of prostaglandin E2 by fibroblasts, which suppresses proliferation.

The minority of patients with lavage lymphocytosis appear to be at an earlier stage of disease and show a more favourable response to corticosteroids but biopsy studies show that numerous lymphocytes are present in the interstitial tissues of most patients with CFA. B-lymphocytes are readily detectable in the centres of lymphoid follicles but the majority of lymphocytes scattered throughout the interstitium are T-lymphocytes, with variable numbers of T-helper/inducer and T-suppressor/cytotoxic phenotypes. One of the mediators produced by activated T-lymphocytes, gamma interferon, has recently been shown not only to stimulate the growth of quiescent human lung fibroblasts but to inhibit rapidly growing cells [16]. The role of lymphocytes and their products in the pathogenesis of CFA and the propensity toward auto-antibody production awaits elucidation.

BAL studies have shown that many untreated CFA patients have significantly reduced proportions of phosphatidylglycerol relative to the other phospholipid components of pulmonary surfactant [17]. These changes do not predict response to corticosteroids but levels of phosphatidylglycerol return to normal in patients responding to prednisolone. Such changes may reflect the extent of damage to the alveolar epithelium in CFA, in particular to the type II pneumocytes which produce surfactant phospholipids. Defective surfactant function could be a secondary factor in pathogenesis.

Corticosteroids are used in CFA treatment because of their anti-inflammatory effect. Second-line drugs are commonly immunosuppressives, in particular cyclophosphamide or azathioprine. Poor prognosis of CFA patients has led to investigation of other agents, mainly by increasing knowledge of pathogenetic mechanisms. These agents include the immunosuppressives cyclophosphamide and A and chloroquine, drugs which might act more directly on fibroblasts or collagen such as colchicine and prednisolone, the anti-viral drug ribavirin and, as a final recommendation, heart/lung or single lung transplantation.

New knowledge of mechanisms in CFA and major advances in recombinant DNA technology, leading to production of new immunotherapeutic agents, are opening many avenues of research to combat this serious disease.

References
Cellular immune responses in the lung of hypersensitivity pneumonitis

G. Semenzato, L. Trentin

Bronchoalveolar lavage (BAL) of hypersensitivity pneumonitis (HP) patients is studied in the initial phases of disease. In subacute or chronic phases the pattern may be different.

In this brief report we will summarize the work that has been performed in our laboratory on cell suspensions recovered from the BAL of HP patients.

Cellular recovery from BAL of HP patients is fivefold that in controls, and mostly represented by lymphocytes [1]. Immunological evaluation demonstrated that few BAL lymphocytes express B-cell related markers, most being represented by T-lymphocytes [2]. Analysis of subsets revealed that CD8+ lymphocytes are the predominant cells retrieved, hence the CD4/CD8 ratio is extremely low (around 0.5 vs 1.8 controls). Although the % of cells bearing the proliferation associated markers (T9 and CD25 antigens) is low, a significant difference with respect to controls exists in absolute numbers. An increase of lymphocytes bearing HLA-DR determinants was also demonstrated.

Table 1: Phenotypic analysis of cytotoxic cells recovered from the BAL of HP patients

<table>
<thead>
<tr>
<th>Group</th>
<th>CD57</th>
<th>CD56</th>
<th>CD16</th>
<th>TCRβ1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>ml x 10^3</td>
<td>%</td>
<td>ml x 10^3</td>
</tr>
<tr>
<td>HP patients</td>
<td>31.2</td>
<td>122.5</td>
<td>40.1</td>
<td>88.0</td>
</tr>
<tr>
<td>±3.7</td>
<td>±28.6</td>
<td>±39.4</td>
<td>±17.1</td>
<td>±1.5</td>
</tr>
<tr>
<td>Controls</td>
<td>9.7</td>
<td>1.0</td>
<td>4.9</td>
<td>0.5</td>
</tr>
<tr>
<td>±1.5</td>
<td>±0.2</td>
<td>±1.5</td>
<td>±0.1</td>
<td>±1.6</td>
</tr>
<tr>
<td>p &lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>ns</td>
</tr>
</tbody>
</table>

CD57: HNK-1; CD56: N901; CD16: NK-15; BAL: bronchoalveolar lavage; HP: hypersensitivity pneumonitis.

The pattern of reactivity with monoclonal antibodies defining cells with cytotoxic phenotype shows a significantly increased number of cells positive for HNK-1 (CD57) and NKH-1 (CD56) reagents in the lavage of HP patients with respect to controls (table 1) [1]. The number of CD56 and CD57 cells co-expressing T-cell markers is predominant over those lacking these determinants. The pattern of expression of these markers in controls is statistically different (fig. 1). Other markers strictly defining natural killer cells are lacking on the surface membrane of BAL cells [1]. Thus, the alveolitis in HP patients is mostly represented by CD3+, CD8+, CD57+, CD56+, CD16- non-major histocompatibility complex (MHC) restricted cytotoxic lymphocytes.

It is important to differentiate the pattern of BAL in HP from that in other disorders known to be associated with lymphocytosis. In sarcoidosis, tuberculosis and berylliosis the BAL lymphocytes express the helper-related phenotype. BAL lymphocytes from patients with interstitial pneumonia associated with collagen vascular diseases, silicosis, histiocytosis X, AIDS and amiodarone pneumonitis express the suppressor/cytotoxic phenotype. The presence of an alveolitis characterized by CD3+/CD8+/CD56+/CD57+/CD16- phenotype suggests HP. Introduction of more specific reagents may help to differentiate some of the above disorders.

Since the lung contains a separate compartment for cytotoxic cells that react to foreign antigens, the presence of cytotoxic lymphocytes in the BAL of HP patients may fit with the pathogenesis of this disease, notably sensitization via the upper respiratory tract by extrinsic antigens.