THE BRONCHOALVEOLAR LAVAGE. ITS ROLE IN PNEUMOLOGICAL INVESTIGATION

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INTRODUCTION

Extensive information regarding the role of different lymphocyte subsets in pulmonary host defense mechanisms has recently been accumulated. The present knowledge of the complex network of interactions between cellular components of the pulmonary immune system largely arises from the evaluation of cell populations retrieved from the bronchoalveolar lavage (BAL) fluid. In particular, it has been documented that when an unwanted antigen enters and a cascade of intercellular communications occurs between accessory cells and effector T lymphocytes.

Taking advantage of the BAL in the last decade it has been possible to characterize lymphocytes recovered from the lung of patients with pulmonary disorders using a variety of monoclonal antibodies (mAb). In particular, flow cytometry analysis has played an important role in the study of the phenotypic characteristics of human lymphocytes in healthy and in immune mediated lung diseases.

TECHNICAL CONSIDERATIONS

BAL is an useful and safe method for sampling cellular and biochemical components from the respiratory tract. Methods for handling BAL cell suspensions, the evaluation of total and differential cell counts and flow cytometry analysis of pulmonary T cell subsets will be referred according lo the technical guidelines and recommendations proposed by the Task Group on BAL of the European Resporatory Society[(1)].

BAL is performed under local anesthesia with a flexible fiberscope. Coughing during lavage should be prevented as it can cause contamination with the blood of BAL fluid and loss of instilled fluid. The use of sedating compounds such as diazepam or meperidine together with atropine as premedication for fiberscopy may minimize this minor sideeffect. Concerning the site of lavage sampling, the middle lobe or the lingula are used as standard sites. The fluid used to carry out BAL must be a pyrogenfree saline solution (isotonic 0.9% NaCl), warmed to body temperature (37ºC). Saline flud is warmed since it causes less coughing and bronchospams, less deterioration of lung function and, therefore, a better fluid recovery and increased cell yields. It can be instilled through the fiberscope as a bolus with a syringe or, better, by hydrostatic fluid from a reservoir fo 300 mI. The fluid is usually recovered with the help of a suction trap. Filtration of BAL is recommended as it preferentially removes brochial epithelial cells. After measuring the recovered volume and performing total cell counts, cytospins are prepared for differential counting. After air-drying, the slides are stained with an appropriate differential white cell stain (May-Grümwald-Giemsa or Diff-Quick stains). The differential counts are made by means of a light microscope and at least 300-500 cells should be counted. The following percentages can be expected in normal non-smoker individuals: lymphocytes <10%, neutrophils <3%, eosinophils 4.5%, alveolar macrophages > 90%. Following differential cell count, the slides should be screened at low magnification for the presence of unusual morphological features (dust particles, microorganisms, tumor cells, hemosiderin laden macrophpages, etc.). When unusual features are detected, special stains can be undertaken: PAS, iron or silver stains. A high percentage of epithelial cells (>5%) is indicative of BAL sample

contamination by bronchial cells. On cells recovered from the BAL, the follow methods can be applied: cellular analysis by flow cytometry, using a variety of monoclonal antibodies, and molecular analysis techniques including Southem hybridization, a diagnostic methology which allows to detect and characterize a single gene among tens of thousands of other genes, Nothem hybridization, which allows the detection and qualitative analysis of a gene's mRNA transcripts, and polymerase chain reaction (PCR), which provides the identification of DNA sequences that would otherwise pass undetected due to such low concentrations.

LUNG LYMPHOCYTES IN HUMAN DISEASE STATES

B cell immunocompetence in lung diseases

The pulmonary B cell system may be affected in sevetal pulmonary disorders[2] Due to an in situ aberrant immunoglobulin production, the levels of IgG and IgA are increased in the BAL fluid of HIV infected subjects. Sarcoidosis is also characterized by a polycIonal increase in BAL immunoglobulins. This finding is in keeping with the helper nature of the lymphocyte infiltration characterizing this discase. Furthermore, BAL of patients with hypersensitivity pneumonitis (HP) contains large quantities of precipitating antobodies against the allergens which are involved in the inflamatory process. Goodpasture's syndrome and idiopathic pulmonary fibrosis represent other two examples of pulmonary disorders that are associated with a local release of immunoglobulins and deposition of immune complexes. Finally, the production of antineutrophil cytoplasmic autoantibodies (ANCA) within the respiratory tract has been claimed to aecount for in the immunopathogenesis of pulmonary involvement during Churg-Strauss syndrome and Wegener's granulomatosis[3].

Lung T cell subsets in interstitial lung diseases

Lymphocytic alveolitis occuring in Interstitial Lung Diseases (ILDs) can be further defined according to specific membrane antigens related to the T cell subset involved in the inflamatory process. BAL lymphocytes usually belong to the CD4 T cell subset in patients with sarcoidosis, tuberculosis, berylliosis and asbestosis, whereas an intralveolar accumulation of with HP, HIV and human T cell leukemia virus 1 (HTLV-1) infection, silicosis, collagen vascular diseases, amiodarone pneumonitis, blastornycosis, and graft versus host disease (GVHD) (Table 1)[1,4-17].

A number of groups have recently proposed the use of BAL analysis in the differential diagnosis of ILDs. According to this method, patients with ILD are subdivided into patients with high-intensity CD4 or CD8 alveolitis. For example, it has been suggested that when the CD4/CD8 ratio is > 3.5 the diagnosis of sarcoidosis can be made by BAL since 50 to 60% of sarcoid patients show this phenotypic pattern[15]. Actually, the phenotypic profile of the lymphoid constituents of alveolitis does not permit to discriminate between patients with sarcoidosis, berylliosis or asbestosis or other diseases characterized by a CD4 alveolisis. Furthermore, rare patients with sarcoidosis show a CD8 alveolitis[16]. Again, as a consequence of the therapy or the different clinical phases of ILD, the phenotypic profile of alveolitis can change during the course of ILD. In other words, the immunological analysis of BAL cells may provide an additional tool to formulate the diagnosis, but a final conclusion can not be achieved only on the basis of the phenotypic evaluation of BAL T cell subsets.

More recently, some investigators focused their attention on the possibility of using the number of T cells expressing a function-related phenotype cells to monitor ILD. For instance, it is known that the production of IL-2 is genetically determined in the lung of patients with sarcoidosis, since the gene for IL-2 is specifically tumed on in the CD4+/HLADR+ subpopulation[18]. In this context, the evaluation of CD4+/HLA- DR+ cells couId theoretically give a rough idea of the activation state of the cell subset which accounts for the production of IL-2. Using the two colour immunofluorescence techniques it has been demonstrated that the increase in the CD4+/HLA-DR+ cells is high1y statistically significant in the active phase of the disease with respect to the control population, with the significance

being less evident or even lacking in the split and inactive stages of the disease[19]. Furthemore, a positive correlation has been recently demonstrated between the expression of HLA-DR and CD25 antigens by sarcoid CD4 cells the reduction in diffusing capacity, expressed as both DLCO and KCO [20]. Thus, the determination of CD4+ HLA-DR+ cells is considered a useful indicator for the evaluation of the activation state of lymphoid component of alveolitis during the clinical follow-up of these patients.

Besides sarcoidosis, the clinical utility of determining cell components of BAL fluid has recently bcen a matter of active investigation in other ILDs. We reported that a high count of BAL CD8+ lymphocytes can be demonstrated in both symptomatic and asymptornatic HP patients who are regularly exposed to their inciting antigens[17]. By contrast, the CD8 cell count recovers to normal levels in individuals who are not further exposed to specific antigens which account for the development of HP alveolitis. This suggests that serial measurements of BAL cell counts may be of value to monitor the clinical course of HP.

Lung T cefis during HIV infection

The morphological and immunologic evaluation of BAL cytospin provides useful information also during ILD characterizing HIV infection. Several patients with AIDS develop a CD8 alveolitis which is sustained by three populations of CTL: MHC restricted CTL, T cells bearing CD56 and CD57 NK-related markers and α+/δ+T cells[21-23]. While MHC restricted CTL are involved in the clearance of HIV-1 antigen and HIV-infected cells, NK subsets play a crucial role in the host's defenses against the development of opportunistic infections[24]. The pulmonary helperrelated CD4+ lymphocytes are virtually absent in the lung of all HIV infected patients, although patients with earlier infection generally tend to have more CD4 cells than those with opportunistic infections.

In view of the frequent fatal outcome of pulmonary complications during HIV disease, several groups recently attempted to identify pulmonary abnormalities that predict the clinical outcome of pulmonar involvement during HIV infectionll. The presence of gas exchange abnormalities and the accumulation of neutrophils in the bronchoalveolar lavage (BAL) have been suggested to be strongly correlated with imminent mortality in HIV-seropositive patients[26-28]. More recently, we have demonstrated that patients with a low number of BAL CD4 cells have poor prognosis. It is interesting to note that in patients with opportunistic infections circulating CD4 counts do not represent a sensitive measure of mortality, as in most patients the recent CD4 count is <50 cells/mm^3 (Agostini et al, in preparation). These data are in agreement with previous results showing that the rate of peripheral blood CD4 cell decline can prediet survival, but only before CD4 counts reach 100 cells/mm^3 [29]. Thus, with disease progression the potential significance of precise CD4 counting in BAL is increasing while the CD4+ T cell count in the peripheral blood has a negligible effect on predicting survival.

Lung T cells in patients with asthma

Taking advantage of BAL studies, it has recently been demonstrated that allergic and non allergic asthma show distinct pattems of T-cell activation and cytokine production. In patients with allergic asthrna, a direct relationship between activated T lymphocyte numbers[30], levels of IL-2, soluble IL-2R and eosinophil count in BAL fluid has been dernostrated[33-34]. Moreover, helper T cells accumulating in the lung of these patients show an increased expression of the IL-2R p55 chain[30-32], since the degree of disease is in correlation with the activation state of the CD4 infiltratc[35]. A selective recruitment of CD4 cells in the lung probably takes place in response to the bronchial allergenic challengell. Furthermore, CD4 cells in asthrna definitely show a Th2 cytokines pattem. In fact, increased levels of IL-3, IL-4, IL-5, IL-10 and GM-CSF have been found in supematants from enriched BAL lymphocyte preparations from asthmatic patienst. The cytokines are likely to promote the IgE synthesis and eosinophil activation at sites of pulmonary inflammation(32,37-39).

Different data have been observed in non allergic asthmatic patients. BAL CD8+ T cells are increased and both CD4+ and CD8+ T cells show an increased expression of CD25 and HLA-DR[38]. In addition, an increased production of cytokines with inflammatory properties may be demonstrated in the airways of patients with non atopic asthma, including IL-1, IL-5, IL-6 and GM-CSF[40]. Interestingly, in both types of asthma, a close correlation between BAL IL-5 levels and eosinophilia may be found, thus supporting the notion that this cytokine accounts for the tissue eosinophilia characterizing these diseases[41]

Lung T cells in lung transplant recipients

Different T cells subsets are involved in acute and chronic rejection of lung allograft. Oncoming rejection episodes are an expression of a specific reactivity directed towards donor class 11 MHC antigens and mediated by CD4+/CD29+/CD45RA-[42]. On the other hand, CD8+ lymphocytes prevail in the BAL of patients with obliterative bronchiolitis[43]. Immunological analysis of BAL in transplant recipients has recently been used in clinical monitoring of allograft rejection. In fact, a high percentage of recipients who exhibit a positive PLT (primed lymphocyte test for donor specific allo-reactivity) develop rejection episodes despite negative transbronchial biopsy histology, further highlighting the relevance of monitoring immune lung cells in these conditions.

Pulmonary involvement in collagen vascular diseases

The presence of an alveolitis has been demostrated in several collagen vascular disease (CVD) characterized by diffuse multisystem immune-mediate inflammatory lesions, including patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS), derrnatornyositis/polymyositis, mixed connective tissue disease, Sjögren's syndrome and ankylosing spondylitis.

In particular, taking advantage of the results obtained from BAL in patients with SLE, it has been suggested that a cell-mediated immune response takes place in the lungs of these patients, involving activated CD8+ T lymphocytes and CD56+/CD16+ NK cells. The T cells response is associated with an up-regulation of the local production of oxygen radicals and an impaired pulmonary diffusing capacity. Interestingly, the pulmonary inflarnmatory process seems to be independent of general SLE disease activity[44].

Interstitial involvement which characterizes the lungs of PSS patients is generally indistinguishable from cryptogenic fibrosing alveolítis. Both conditions are characterized by the presence of a BAL neutrophilia and/or eosinophilia, while the alveolar lymphocytosis is quite uncommon. Conceming other CVD, a lymphocytic and neutrophilic alveolitis has been reported as a possible cause of respiratory failure in patients with dermatopolymyositis and pulmonary involvement, while an accumulation of BAL CD8+ T cell is a characteristic finding in several patients with Sjögren's syndrome.

Taken together, the data suggest the potential role of BAL in monitoring ILD in patients with CVD. Despite this, whether sequential analysis of BAL cells may he1p the clinical management of these patients has not yet been established.

BAL in the diagnosis of malignant lymphomas

Primary malignant lymphornas arising from BALT are usually characterized by an indolent clinical course, broad cytological spectrum (centrocyte-like), lymphoepithelial lesions, and admixture with reactive germinal centers characterizes low grade-B-cells lymphoma type. The lowgrade mucosa-associated lymphoid tissue lymphornas lack the bcl-2 gene rearrangement, typical of follicular cell lymphomas[45-46] By contrast, high grade lymphomas are rare in the lung[47] and often represent the transformation of low grade B cell lymphoma. Noteworthy, recent data support that the majority of pulmonary lesions, which in the past were defined as "pseudolymphorna" and some inflitrative disease of the lung as the lymphoid interstitial pneumonia (LIP), are true low-grade lymphomas[48-50]. T cell-lymphomas are also rarely found in the lung.

Immunohistology techniques, gene rearrangement analysis and irranunophenotypic study of BAL lymphocytes represent the gold standards in establishing the diagnosis of pulmonary malignant lymphoma and are sometimes helpful to differentiate the pulmonary involvernent of a systernic disease from the inflammatory infiltrate.

CONCLUSIONS

The analysis of the cellular components of the BAL has undoubtedly improved our diagnostic possibilities in the definition of the different stages of immune-mediated pulmonary diseases. Furthermore, the comprehension of the immune system's role in the pathogenesis of lung darnage provided important clues for elucidating the pathogenesis of several pulmonary diseases. Advances in the clinical management of these patients will depend on the ability to identify and define, on a molecular basis, the complex mechanisms which account for the local immune response during hypersensitivity reactions. For instance, the evaluation of BAL cells could represent a model for studying the molecular basis of host inflammatory response against persistent antigens and ideal approach for testing the efficacy of new immunosoppresive and immunomodulatory strategies in the control of the immune mechanims taking place in the lung microenvironment. lt would also prove interesting to attempt to alter the local immunocompetence using drugs or cytokines which selectively modulate the number and activity of specific T-cell or measure the efficacy of our therapeutic intervention and prevent the spontaneous exacerbations of intersticial lung disease.

We are still a long way from these goals, but it is likely that they are achievable. With the advances that have marked the field of immunology in recent years we will likely be witness of an accellerated phase in the translation of major advances of basic science into clinical applications. Hopes in this regard are associated with the current revolution in the arca of molecular biology which if expected to improve our knowledge of the complex mechanisms which regulate the pulmonary immune system.

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