Differential Diagnostic Value of Plasma Cells in Bronchoalveolar Lavage Fluid*

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The aim of this study was to investigate whether the demonstration of plasma cells (PC), which are normally absent in bronchoalveolar lavage (BAL) fluid, facilitates differentiation among pulmonary disorders. Initial BAL fluid samples of 1,260 patients were analyzed. In 83 of these, PC were found. Of these 83, 47 were obtained from individuals suffering from extrinsic allergic alveolitis (EAA). The number of PC in BAL fluid from EAA patients was found related to the time between antigen exposure and BAL. Drug-induced pneumonitis appeared to be another disorder with a high percentage of cases with PC in the BAL fluid (35.7 percent). Therefore, we conclude that determination of PC in BAL fluid has differential diagnostic value in discriminating among interstitial lung diseases of various origins. However, the exact role of BAL fluid and PC and the link to clinical manifestations of these diseases needs further investigation. ***(Chest 1993; 103:1720-24)***

EAA = extrinsic allergic alveolitis; PC = plasma cells

Bronchoalveolar lavage (BAL) fluid provides a sample of cells present in the intra-alveolar space. Many disorders have specific features evidenced in BAL fluid.[1-5] By the identification of the cellular constituents, inflammatory processes with predominant lymphocytes (*eg*, extrinsic allergic alveolitis [EAA] and sarcoidosis) can be distinguished from those in which neutrophils or alveolar macrophages predominate (*eg*, idiopathic pulmonary fibrosis).[1,4-8] Alveolar macrophage and neutrophil activation, and subsequent production of cytokines have been implicated in the pathogenesis of tissue destruction and fibrosis. The presence of lymphocytes in BAL fluid equivocally reflects immune stimulation.[5] To date, most interest has been focused on the identification of BAL fluid T lymphocytes and the CD4/CD8 ratio in various pulmonary disorders.[1,5] Interestingly, few data are available on the presence of B lymphocytes and plasma cells (PC). Under normal circumstances, PC are not found in blood or in BAL fluid.[9-12] Following antigenic stimulation, *eg*, vaccination and viral and bacterial infections, an increase in PC in both blood and lymph is observed.[13-17] The occurrence of a low percentage (0.1 to 2.0 percent) of PC in BAL fluid in EAA patients already has been reported by Costabel et al[9] (1985). However, little attention has been paid to this observation. These authors claim that the presence of PC in BAL fluid is a highly diagnostic criterion for EAA, since no PC were found in BAL fluid from sarcoidosis patients.[9] Other reports describe the inconsistent finding of low numbers of B lymphocytes or PC in the interstitium of the lung in patients with interstitial infiltrates associated with collagen vascular disease or malignant lymphomas.[1,13]

The aim of this study was to investigate whether the presence of PC in BAL fluid has predictive value for the diagnosis of specific pulmonary disorders.

Methods and Materials

Patients

Retrospectively, the initial BAL fluid samples of 1,260 patients with a great variety of pulmonary diseases were selected for study out of all BAL fluid analyses (n = 2,008) performed over a ten-year period between 1980 and 1990.

Bronchoalveolar Lavage

Bronchoalveolar lavage was performed as previously reported during fiberoptic bronchoscopy.[3] At the same time, blood samples were drawn for differential cell counts. In short, the procedure is as follows. After premedication with atropine and sometimes diazepam and codeine, and local anesthesia of the larynx and bronchial tree with 0.5 percent tetracaine, BAL was performed by standardized washing of the right middle lobe with four aliquots of 50-ml of sterile saline solution kept at room temperature. Lavage fluid samples, kept on ice in a siliconized specimen trap, were centrifuged (10 min, 350 g). Cells were washed twice, counted and suspended in minimal essential medium (Gibco, Grand Island, NY) supplemented with 1 percent bovine serum albumin (Organon, Teknika, Boxtel, the Netherlands). Preparations of the cell suspension were made in a cytocentrifuge and stained with May-Grünwald-Giemsa (Merck, Darmstadt, Germany). At least 1,000 cells were counted. The PC were morphologically identified in a routine May-Grünwald-Giemsa-stained slide.

Data Analysis

Patients with PC in the BAL fluid were divided into groups according to various categories of pulmonary disorders (Table 1). The EAA population was divided into two groups: In the first group (EAA-group A; n = 69), the diagnosis of EAA was based on clinical

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Table 1—*Occurrence of Plasma Cells in Bronchoalveolar Lavage Fluid in Various Pulmonary Diseases*

Disorders	Σn*	n	%
Interstitial lung diseases			
EAA	69	36	52.1
Suspected of having EAA	26	11	42.3
Pneumonitis induced by drugs†	14	5	35.7
Idiopathic pulmonary fibrosis	47	1	2.1
Idiopathic pulmonary fibrosis (not proven by biopsy)	68	0	0
Sarcoidosis	401	5‡	1.2
Bronchiolitis obliterans and organizing pneumonia	7	1	14.2
Chronic eosinophilic pneumonia	26	3	11.5
Pulmonary manifestations of collagen-vascular diseases	100	7	7.0
Occupation-induced disorders	40	0	0
Other interstitial lung diseases	18	0	0
Microbial pulmonary diseases			
Pulmonary aspergillosis/allergic bronchopulmonary aspergillosis	12	2	16.7
Pneumonia/infiltration	100	4	4.0
Tuberculosis	14	0	0
Obstructive diseases			
Bronchitis/COPD	126	0	0
Neoplasms/Malignancies			
Malignant lymphomas	21	3	14.3
Hematologic malignancies	23	3	13.0
Neoplasms of the lung	38	2	5.3
Other disorders			
Pulmonary circulatory disorders	18	0	0
Diagnosis unclear	16	0	0
Digestive tract disorders	3	0	0
Control subjects	73	0	0
Total	1260	83	6.6

*Σn = total number of cases; n = number of cases with PC in BAL fluid; % = n/Σn × 100.

†Amiodarone, nitrofurantoin or methotrexate.

‡Patients exposed to EAA-inducing antigens (birds).

information, radiologic picture, the presence of precipitins in the blood, pulmonary function, and disappearance of symptoms after avoidance of antigen exposure. The second group (EAA-group B; n = 26) consisted of patients who satisfied the aforementioned diagnostic criteria with the exception of the serologic parameter. For further analysis, nonsmoker EAA patients from the first group (n = 61) were selected. All EAA patients were frequently exposed to birds. No patient was receiving corticosteroid or other treatment either at the time of or before the lavage. The patients were divided into four categories, based on the time period between termination of antigen exposure and initial BAL: group 1, less than 24 h; group 2, 2 to 7 days; group 3, 8 to 30 days; group 4, 1 to 12 months. Furthermore, patients with PC-positive BAL fluid were separated from those without PC in BAL fluid. Provocation was realized by providing contact between the patient and the birds, followed by BAL within 24 h. The first BAL fluid sample was used for statistical analysis. If a patient had BAL after provocation as well, this BAL fluid sample was included instead of the initial lavage fluid sample.

The control group consisted of 37 nonsmoker control subjects without any pulmonary history or chest x-ray film abnormalities.

Statistical Analysis

The data are presented as the mean ± SEM. The Mann-Whitney U test was used to evaluate the statistically significant differences between each category of EAA patients with or without PC in the BAL fluid and the control group. The probability values less than 0.05 were considered to be significant.

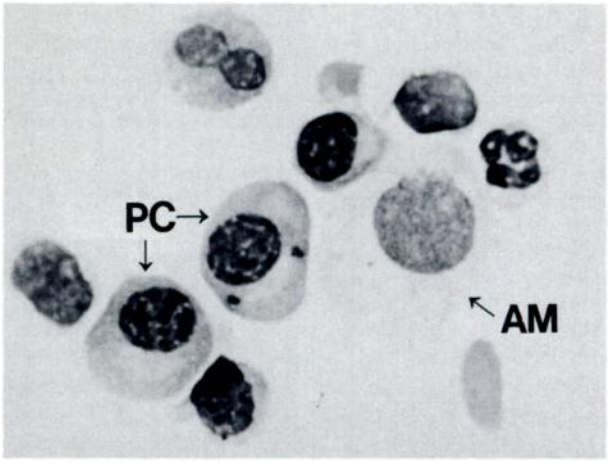

FIGURE 1. Photomicrograph of bronchoalveolar lavage fluid showing PC; AM = alveolar macrophage (May-Grünwald-Giemsa stain, original magnification × 100).

RESULTS

In 6.6 percent (n = 83) of the cases, PC were detected in the BAL fluid (Fig 1, Table 1). Patients with PC in BAL fluid were most frequently found among those with verified EAA (EAA-group A; 36 of 69 [52.1 percent]). Also, patients with mere clinical symptoms of EAA (EAA-group B; 11 of 26 [42.3 percent]) and those with drug-induced pneumonitis (5 of 14 [35.7 percent]) showed PC in BAL fluid (Table 1). Among the remaining interstitial lung diseases, PC were found in 7 of 100 patients with pulmonary manifestations of collagen-vascular diseases, 3 of 26 patients suffering from chronic eosinophilic pneumonia, 1 of 47 patients with idiopathic pulmonary fibrosis as well as 1 of 7 patients with bronchiolitis obliterans and organizing pneumonia. In contrast, PC in BAL fluid was demonstrated in only 5 of 401 patients with histologically verified sarcoidosis (1.2 percent) of whom all were known to be frequently exposed to birds. A minor number of patients with PC in BAL fluid was found among those with microbial pulmonary diseases and malignancies (Table 1).

The relative number of PC in BAL fluid varied from 1 to 144 (per 1,000 cells counted), *ie*, 0.1 to 14.4 percent of the total cell count. The highest percentage of PC of the total cell count (144 percent) was found in a patient with non-Hodgkin's lymphoma. In the BAL fluid of all the other patients (n = 82), the PC varied from 1 to 39. The overall mean percentage of PC was 0.74 ± 0.06 in the 83 patients who had PC in BAL fluid (data not shown). No PC were found in peripheral blood.

In the nonsmoker serologically verified EAA patients (EAA-group A) with PC in BAL fluid (34 of 61), the overall mean PC count of the total cell count was

Table 2—*Total Cell and Differential Cell Count in Bronchoalveolar Lavage Fluid of Extrinsic Allergic Alveolitis Patients and Control Subjects**

	EAA (n = 61)†	Control Subjects (n = 37)
Total cell count × 10^4/ml	36.3 ± 3.3	10.7 ± 1.2
Percentage of total cells		
Alveolar macrophages	37.6 ± 2.1	87.0 ± 1.0
Polymorphonuclears	4.7 ± 0.8	1.3 ± 0.2
Lymphocytes	54.3 ± 2.4	10.8 ± 0.9
Plasma cells	0.37 ± 0.01	0.0 ± 0.0
Eosinophils	2.3 ± 0.01	0.8 ± 0.40
Mast cells	0.76 ± 0.09	0.10 ± 0.02

*Data are expressed as mean ± SEM.
†All values of EAA patients were found to be significantly different from those of control subjects ($p<0.01$)

0.67 ± 0.13. Total and differential cell counts of 61 EAA patients and 37 control subjects are listed in Table 2. Both total cell count and the various cellular components in BAL fluid of the EAA patient population differed significantly from those of the control subjects.

There are statistically significant differences between the EAA patient group in whom PC were and were not found in BAL fluid. In a follow-up study, we will further analyze these differences.

Taking into account the critical time relationship between the appearance of PC in the alveolar space and the moment of final antigen exposure, the timing of BAL is essential for detection of these cells. The PC mainly seemed to appear in the alveolar space during the first week following termination of antigen exposure (group 1 and 2) in 26 of 44 EAA patients (59.1 percent), with a relatively high percentage of PC of the total cell count (0.76 ± 0.15) demonstrated in the BAL fluid (Fig 2). Accordingly, the highest percentage of PC (3.9 percent) was found in one patient belonging to group 2. After avoidance of the antigen exposure for more than a week (groups 3 and 4), a lower number of PC (0.30 ± 0.12) was detected in only 8 of 17 (47.1 percent) of EAA patients (Fig 2). To compare, the mean PC count was 0.12 ± 0.02 in the PC-positive sarcoidosis patients ($p<0.05$, data not shown).

Discussion

Plasma cells are seldom found in BAL fluid. Morphologically, these cells can be identified in a routine May-Grünwald-Giemsa-stained slide. The incidence of PC in BAL fluid has been reported in a number of pulmonary disorders, especially in EAA.[9] In the present study, PC were found in the BAL fluid obtained from 36 of 69 patients with serologically proven EAA and in 11 of 26 patients with clinical manifestations of the disease. Since nearly no BAL fluid PC were found in other interstitial lung diseases, such as sarcoidosis and idiopathic pulmonary fibrosis, both of which may be difficult to differentiate from EAA due to similarities in clinical presentations, our results suggest that the presence of PC are highly specific for EAA or EAA-like pulmonary disorders. These findings are in agreement with Costabel et al[9]

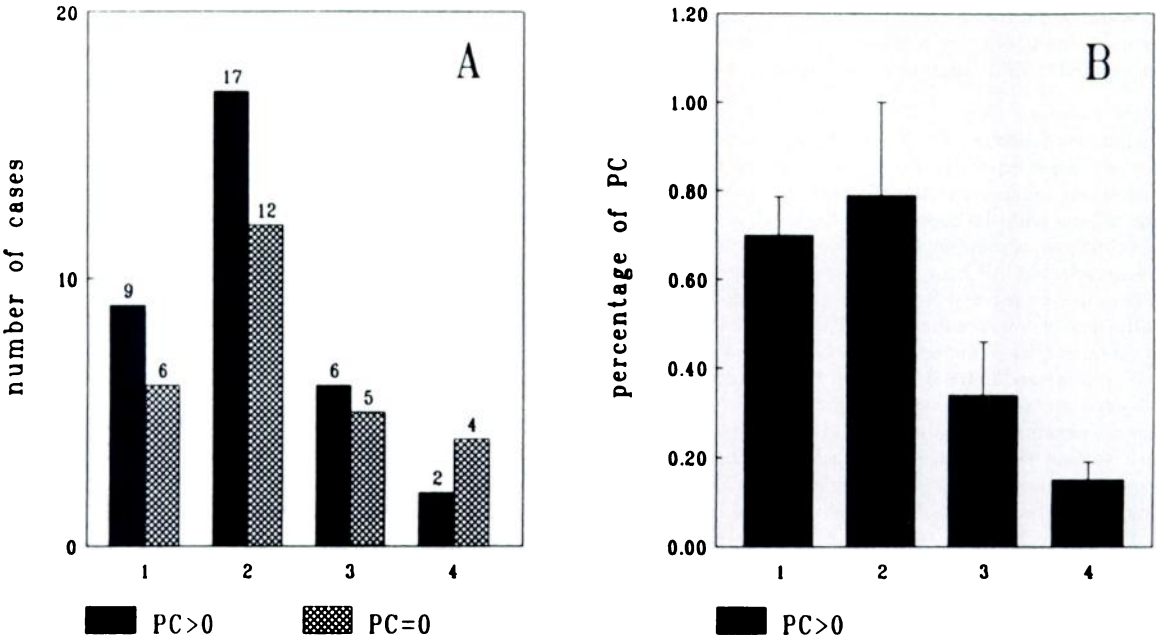

FIGURE 2. The effect of time elapsed between termination of antigen exposure and initial BAL on the occurrence of PC in BAL fluid. *A*, The number of EAA patients with PC versus number of EAA patients without PC in BAL fluid (group 1: <24 h; group 2: 2 to 7 days; group 3: 8 to 30 days; group 4: 1 to 12 months). *B*, The relative number of PC in BAL fluid from EAA patients with PC in the BAL fluid only. Data in 2B are expressed as percentage PC ± SEM of total cell count in BAL fluid.

(1985). There were only 5 of 401 patients with sarcoidosis and PC in the BAL fluid and they frequently were exposed to birds, which was believed to be a potential EAA-inducing antigen. Although these findings seem controversial, these individuals may be susceptible to EAA-inducing antigens and, as a consequence, have PC in the BAL fluid.

In the pathogenesis of EAA, several immune mechanisms are involved.[7,18-28] Alveolitis is characterized by an accumulation of inflammatory cells at the distal structures of the lung.[7,8,26] Our results confirmed this, showing an increase in lymphocytes, polymorphonuclear neutrophils, eosinophils, and mast cells with a simultaneous decrease in alveolar macrophages in the BAL fluid samples from EAA patients. The presence of PC supports the concept of local production of antibodies. Antigen-antibody complexes may lead to lung damage due to the interaction with other constituents of the interstitium of the lung.[10,29] The relative number of PC in BAL fluid samples was related to the time latency from termination of antigen exposure and performance of BAL. The PC mainly seem to appear in the alveolar space during the first week following termination of antigen exposure. When BAL is performed within the first week after antigen contact, a relatively high percentage of PC of the total cell count can be demonstrated in BAL fluid. After avoidance of exposure to the antigen for more than a week, the number of PC was found to gradually decrease in EAA patients. Therefore, the presence of PC is considered to be a feature of recent antigen exposure. These findings are in line with the etiology of EAA as an inflammatory reaction induced by repeated exposure to extrinsic antigens in susceptible individuals and the reported involvement of immunoglobulins, locally secreted by PC.[5,8,18-22] In view of this, PC may be expected to occur in BAL fluid samples from patients with drug-induced pneumonitis, a disease of similar pathogenesis as EAA, which was indeed confirmed by the present data.[27-35] The increased number of eosinophils found in the BAL fluid of these patients similarly points to hypersensitivity states.[27,35,36] Recently, eosinophilia has been described in a patient with aspergillosis.[35] In our patient population, we also found two aspergillosis patients with PC in the BAL fluid. A patient with eosinophilic pneumonia with PC in BAL fluid was described.[36] In three cases with chronic eosinophilic pneumonia, PC were detected in BAL fluid. These findings suggest that in inflammatory pulmonary diseases with an antibody-mediated component involved, the presence of PC may coincide with high numbers of eosinophils in BAL fluid. The absence of PC in the BAL fluid of some EAA patients remains unclear. Apparently, other mechanisms as well are involved in the pathogenesis of allergic alveolitis.[4,5,7] In accordance with previous reports, PC were found in BAL fluid obtained from patients with malignant lymphomas, hematologic malignancies, and pulmonary manifestations of collagen-vascular diseases.[1,37] In conclusion, counting PC in BAL fluid has differential diagnostic value, especially in disorders with an increased absolute and relative number of lymphocytes based on allergic, inflammatory processes with an antibody-mediated component involved. This patient group is characterized by an increased number of eosinophils as well. In discriminating among interstitial lung diseases of various origins, the demonstration of PC in BAL fluid is highly suggestive of the diagnosis of EAA. Furthermore, a relatively high number of PC in BAL fluid of EAA patients suggests recent antigen exposure. The exact role of BAL fluid PC in EAA patients and the link to the clinical manifestation of the disease needs further investigation.

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