T-lymphocyte Alveolitis and B-lymphocyte Alveotitis: A New Classification of Interstitial Pneumonitis Based on Broncho-alveolar Lavage Findings*

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SUMMARY For various types of interstitital pneumonitis, lymphocytes were collected by broncho-alveolar lavage (BAL). These lymphocytes were studied to determine whether T- or B- cells were more activated. Activated T-cells were calculated by counting the number of T-cells which formed rosettes with non-neuramidase treated sheep red blood cells (SRBC) at 37°C; activated B-cells, by counting the spontaneous immunoglobulin-secreting cells (IgSC). Activated T-cell levels were significantly higher in patients with sarcoidosis, chronic beryllium disease (CBD) and hypersensitivity pneumonitis (HP) compared with those of controls. While there were significant increases in activated T-cells during the active phase of idiopathic pulmonary fibrosis (IPF) and interstitial pneumonitis associated with collagen vascular disease (IP with CVD), the increase in IgSC levels was even more marked in patients with these diseases. These findings suggest that interstitital pneumonitis might more effectively be classified into two groups. If so, one should be termed T-lymphocyte alveolitis inclusive of sarcoidosis, CBD and HP, while the other should be termed B-lymphocyte alveolitis inclusive of IPF and IP with CVD.

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Many diseases are included within the interstitital lung disease group.[1] Reports have shown that some of these diseases in their pathogenesis are related to immunological mechanisms.[2] Recently, following the introduction of the new BAL technique,[3] we now possess the ability to collect cells, which may be related to inflammatory and immune processes, directly from local lesions in the lungs.[4] Assuming that diseases with increased lymphocyte levels confirmed by BAL are related to the immune process (i.e., whether they are afferent or efferent in nature), we attempted to analyze the nature and the function of these increased lymphocyte levels from a pathophysiological point of view. In the present preliminary study, we investigated which lymphocyte, T-cell or B-cell, was more greatly activated in BAL fluid.

SUBJECTS AND METHODS

Patients

Seventeen patients with sarcoidosis, four with CBD, seven with HP, 10 with the inactive stage of IPF and five with the active stage of IPF, and eight patients with IP with CVD (three with systemic lupus erythematosus, three with rheumatoid arthritis and two with progressive systemic sclerosis) were investigated. Nine individuals (seven of them healthy, one with localized lung cancer, and one with a benign lung tumour) were also investigated as controls. The results of these two groups were compared. For all subjects, diagnosis had been made recently, and they had not yet received corticosteroid therapy. In those with IPF, each patient was judged as being in an active or inactive stage based on clinical signs and chest radiographic findings six months after the BAL examination.

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Collection of BAL cells

An Olympus 4B2 fibre-bronchoscope was wedged in the subsegmental bronchus of the right middle lobe or in the left lingula, and lavage was taken four times with 50 millilitres of saline each, totalling 200 millilitres.[3] In the patients with a tumour, lavage was performed in the unaffected side of the lung. BAL fluid was centrifuged at 200 xG for 10 minutes. The cells it contained were collected and stained with May-Giemsa staining. Thereafter, the percentage of macrophages, lymphocytes and neutrophils in the BAL fluid was computed.

Enumeration of activated T-cells[5]

An equivalent amount of 1% non-neuraminidase treated SRBC solution was added to the BAL cells (2 x 10^6 cells/ml). The suspension was incubated at 37°C for 5 minutes, centrifuged at 250 xG for 10 minutes, and further incubated at 37°C for 15 minutes. More than 1,000 cells were counted, and the number of lymphocytes which formed rosettes with four or more SRBC, relative to the total number of lymphocytes, was then computed.

Enumeration of IgSC[6]

The number of IgSC was counted by reverse haemolytic plaque assay using protein A-coated SRBC. 10μl of BAL cells (2 x 10^6 cells/ml), 50 μl of 25% protein A-coated SRBC, 25 μl of diluted complement (guinea pig serum), and 25 μl of diluted anti-human immunoglobulin rabbit serum (Fuji Zoki, Tokyo, Japan) were mixed and transferred to a Cunningham chamber, followed by incubation at 37°C. After 12 hours, the amount of haemolytic plaque was determined, and then, the number of IgSC per 10^6 lymphocytes. In order to correct for the effect of the presence of neutrophils or macrophages in BAL cells, the results of IgSC computation were corrected by computing the number of peroxidase-stain positive cells and non-specific esterase-stain positive cells. Intergroup difference were examined using Student's t-test.

RESULTS

The ratio of lymphocytes and neutrophils to total cells was computed; measurements of activated T-cells and IgSC were then taken in BAL fluid collected from patients with various types of interstitital pneumonitis. The results were compared with those of the controls as shown in Table 1 and Figure 1.

The mean percentage of lymphocytes to the total number of cells was highest at a value of 80.6 per cent in patients with HP, followed by 52.0 per cent in those with CBD, and 31.3 per cent in those with sarcoidosis. It should be noted that, although some previous reports[7-10] have claimed that the major change in IPF is in an increased level of neutrophils, the results of our study indicate that only five out of the 15 patients with this disease showed 5 per cent or more of neutrophils. By contrast, the mean level of lymphocytes in patients in the active stage was 9.6 per cent, significantly higher than that for the control group.

In those patients with HP, the mean value of activated T-cells to

Table 1 BAL cell findings collected from patients with various types of interstitial pneumonitis

Diseases	No. of cases	Lymphocytes (%)	Neutrophils (%)	Activated T-cells (%)	Spontaneous immunoglobulin secreting cells/10^6 lymphocytes IgM	IgA	IgG	Total
Control	9	3.7 ± 1.3*	0.1 ± 0.1	2.9 ± 0.4	143 ± 84	340 ± 121	266 ± 81	749 ± 279
Sarcoidosis	17	31.3 ± 7.0 ($p<0.02$)	0.9 ± 0.7	32.6 ± 7.2 ($p<0.01$)	1,608 ± 622	1,438 ± 437	3,551 ± 888 ($p<0.02$)	6,597 ± 1,491 ($p<0.01$)
Chronic beryllium disease	4	52.0 ± 9.0 ($p<0.001$)	0.1 ± 0.1	18.0 ± 11.4 ($p<0.05$)	255 ± 109	1,003 ± 260 ($p<0.05$)	605 ± 276	1,953 ± 305 ($p<0.05$)
Hypersensitivity pneumonitis	7	80.6 ± 7.7 ($p<0.001$)	0.7 ± 0.5	48.5 ± 10.3 ($p<0.001$)	172 ± 86	2,194 ± 1,370	652 ± 472	3,018 ± 1,902
Idiopathic pulmonary fibrosis								
Inactive stage	10	3.7 ± 1.0	4.0 ± 2.7	15.1 ± 6.6	2,508 ± 1,305	7,076 ± 3,977	7,593 ± 3,558	17,177 ± 7,697
Active stage	5	9.6 ± 1.9 ($p<0.05$)	6.0 ± 3.8	12.8 ± 3.6 ($p<0.001$)	4,104 ± 1,221 ($p<0.001$)	5,340 ± 1,221 ($p<0.005$)	15,964 ± 7,268 ($p<0.02$)	25,414 ± 9,034 ($p<0.001$)
Interstitial pneumonitis associated with collagen vascular disease	8	31.8 ± 9.9 ($p<0.01$)	4.7 ± 1.9	14.3 ± 4.4 ($p<0.05$)	3,325 ± 1,088 ($p<0.01$)	9,718 ± 2,234 ($p<0.001$)	28,978 ± 4,616 ($p<0.001$)	42,030 ± 6,503 ($p<0.001$)

*Mean ± S.E.

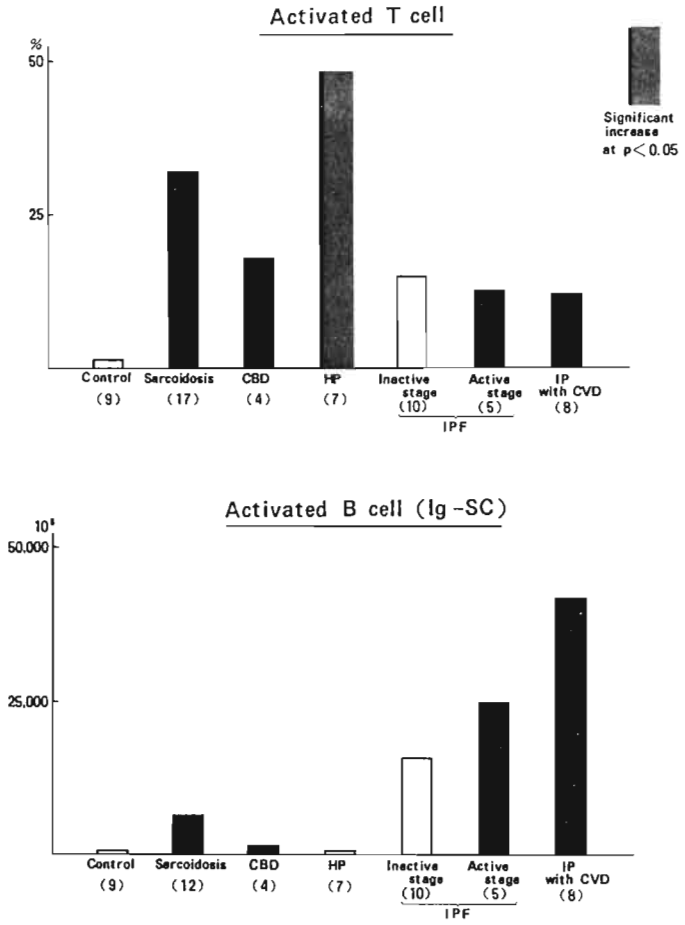

Fig. 1 Activated lymphocytes in BAL fluids collected from patients with various types of interstitial pneumonitis.

total lymphocytes was as high as 48.5 per cent. This level was over 16.7 times that of the control level value of 2.9 per cent. Activated T-cell levels were also significantly higher, at 32.6 per cent in patients with sarcoidosis, and 18.0 per cent in those with CBD. IgSC per 10^6 lymphocytes totalled 6,597 in patients with sarcoidosis, which was 8.8 times higher than the 749 for the control group, while the total of 1,953 in CBD patients was 2.6 times higher than that of the control group. Individual components of immunoglobulins (i.e., IgM, IgA and IgG) were also examined. Only IgG was significantly higher in the patients with sarcoidosis, and only IgA in those with CBD; no significant rise in activated B-cells was noted in those with HP.

The levels of both activated T-cells and IgSC were significantly increased during the active stage of IPF and IP with CVD. IgSC, IgM, IgA and IgG all increased in patients with the aforementioned diseases; furthermore, they differed with respect to the pattern of IgSC rise compared with sarcoidosis and CBD. The total number of IgSC was 8.8 times higher than that of controls in cases of sarcoidosis; 2.6 times higher, in CBD as stated above. The increase in the total IgSC level was much more marked in the active stage of IPF (33.9 times) and IP with CVD (56.1 times). In the inactive stage of IPF, the mean number of both activated T-cells and B-cells increased. However, there was a wide variation among individual patients, so no significant results were obtained for this groups.

The results of the present study suggest that interstitial pneumonitis might more effectively be classified into T-lymphocyte alveolitis (i.e., sarcoidosis, CBD and HP) and B-lymphocyte alveolitis (i.e., IPF and IP with CVD).

DISCUSSION

Several previous reports have demonstrated that lymphocytes in BAL fluid increased in patients with sarcoidosis and HP.[4,7,10] Just as in these diseases, lymphocyte levels were also higher in patients with CBD in that epithelioid-cell granulomas were formed. While some reports are available stating that IPF is neutrophil alveolitis,[7-11] in the present study, patients having only a short history of the disease were investigated, especially those who had not yet received any corticosteroid therapy. In these patients, neutrophils did not necessarily exhibit any increase, but increased lymphocytes were rather more noteworthy. Lymphocyte levels increased in BAL fluid collected from patients with sarcoidosis, CBD, HP and IP as stated above. These results suggest that these diseases may be related to the immunological mechanism, whether they are derived from afferent or from efferent limbs. This hypothesis is also supported by published evidence other than that provided by BAL.

The problems involved with using this indicator for determining the level of activated lymphocytes is of special importance. It is reported that rosette formation by T-cells and SRBC is affected by various factors. However, evidence is available that T-cells transformed and activated by mitogen form stable rosettes with SRBC even at 37°C.[12] Based on this evidence, stable rosette cells were selected as the indicator for activated T-cells. On the other hand, spontaneous IgSC was chosen as the indicator for activated B-cells for the same reason reported in previous investigations.[13]

In conclusion, the results of the present study suggest that the immunological diseases with increased lymphocytes in BAL fluid that we studied may be divided into two groups depending on whether T- or B- cells are mainly activated. One is termed T-lymphocyte alveolitis; the other, B-lymphocyte alveolitis.

It has been demonstrated in patients with sarcoidosis that monocyte chemotactic factors and various other lymphokines are produced from activated T-cells, and as a result, epithelioid-cell granulomas are formed.[14] In this disease, B-cells are also stimulated, and thus, hypergammaglobulinaemia results.[15] As in cases of sarcoidosis, epitheliod-cell granulomas may also be formed in cases of CBD and HP by a similar mechanism. It has been shown that B-cells are also significantly activated in sarcoidosis and CBD. However, this activation may possible result from stimulation by T-cell products, and probably does not represent a major change.

It is quite obvious that the levels of IgSC (i.e., activated B cells) were markedly increased in cases of IP. The fact that immunoglobulins and complements are deposited on the alveolar wall or that immune complexes are detected in BAL fluid as well as in the blood in cases of IPF and IP with CVD proves that these disorders are immune complex diseases.[16,17] It is not difficult to accept the idea that the increase of IgSC in BAL fluid in our study is related to the immune complex disease theory. However, not only B-cells but also T-cells were definitely activated in cases of IP. Questions concerning which is the major change and which type of cell is changed first should be pursued in further studies.

We propose the idea of classfying interstitial pneumonitis into T-lymphocyte alveolitis and B-lymphocyte alveolitis. Whether this classification is effective or not will be known only after more specific information is obtained as to how the lungs are affected by the products of activated T-cells and activated B-cells.

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