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Cellular Analysis in Bronchoalveolar Lavage Fluids in Infiltrative and Fibrotic Stages of Idiopathic Pulmonary Fibrosis

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SHINDOH, Y., SHIMURA, S., TOMIOKA, M., AIKAWA, T., SASAKI, H. and TAKISHIMA, T. *Cellular Analysis in Bronchoalveolar Lavage Fluids in Infiltrative and Fibrotic Stages of Idiopathic Pulmonary Fibrosis.* Tohoku J. exp. Med. 1986, **149** (1), 47-60 —— We performed cellular analysis of bronchoalveolar lavage fluids (BALF) from 20 patients in different stages of idiopathic pulmonary fibrosis (IPF) and compared the results with those from 16 controls and 10 sarcoidosis patients. Patients with IPF were divided into infiltrative and fibrotic groups as diagnosed by transbronchial lung biopsy and chest x-rays. Total cell numbers counted from BALF in IPF patients were not different from those from controls and were lower than in sarcoidosis patients ($p<0.01$). Lymphocytes in BALF were significantly higher in the infiltrative type IPF than in the fibrotic type ($p<0.05$), but there was no significant difference in fibrotic type IPF and control subjects. Numbers of neutrophils and eosinophils counts were not significantly different between infiltrative type and fibrotic type. The neutrophil count in fibrotic type IPF was higher than in control subjects ($p<0.01$) but not in infiltrative type IPF. ^{67}Ga scintiscanning uptake correlated with the lymphocyte population ($r=0.65$, $p<0.01$) but with neutrophils. These findings suggest that lymphocytes play a major role in the pathogenesis of the infiltrative type of IPF and that neutrophils are related to the development of fibrotic changes in IPF. ———— BAL; idiopathic pulmonary fibrosis; Gallium-67 scanning; lung biopsy

Idiopathic pulmonary fibrosis (IPF) is a fatal disorder characterized by interstitial and intra-alveolar infiltrates composed mainly of mononuclear cells, with smaller numbers of neutrophils and eosinophils. Although many of the clinical courses of IPF result from interstitial fibrosis, the inflammatory process seems to precede and to contribute to the development of fibrosis. The pathogenesis of IPF has been studied using bronchoscopic lavage assessments of parenchymal inflammation. However, there has been some controversy as to which cell population increases in bronchoalveolar lavage fluid (BALF) and plays

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the leading role in the pathogenesis of IPF. Reynolds et al. (1977) and Weinberger et al. (1978) have reported the increased neutrophils in BALF from IPF patients. Line et al. (1978) reported that Gallium-67 citrate uptake correlated with the percentage of neutrophils, but not with that of lymphocytes, eosinophils, or macrophages. They reported that since Gallium-67 citrate uptake correlated with the degree of interstitial cellularity and the degree of alveolar cellularity observed by morphologic studies in lung biopsy, the differential percentage of neutrophils would correlate with IPF activity. On the other hand, Ozaki et al. (1982) observed that the percentage of lymphocytes was increased in IPF with a shorter duration of symptoms. Haslam et al. (1980) have noted increased lymphocytes in BALF from IPF patients and that the number of lymphocytes is relevant to the effectiveness of corticosteroid therapy. Contributions of both neutrophils (Gadek et al. 1979; Hunninghake et al. 1981) and lymphocytes (Kravis et al. 1976) to the pathogenesis of IPF have been reported from cellular functional analysis of BALF. Therefore, it is necessary to determine whether lymphocytes or neutrophils play the primary role in the pathogenesis of IPF.

Since there have been conflicting reports, we have restudied the relationship between BALF cell counts and the different stages of IPF as diagnosed from histologic and roentgenographic findings. Gallium-67 citrate uptake was also compared with BALF cell counts and the different stages of IPF. We classified IPF into infiltrative and fibrotic types and observed the possible roles of inflammatory and immune cells in the pathogenesis of IPF.

Methods

Subjects examined. We studied 20 patients who were diagnosed as having IPF by clinical, roentgenographic and physiologic criteria (Crystal et al. 1976). In all of them, this diagnosis was histologically confirmed by transbronchial lung biopsy. These patients had no history of inhalation of inorganic or organic dusts and showed negative precipitanting autibodies when examined with 11 major commercialy available antigens (Hollister-Stier Labs., WA, USA). The patients consisted of 14 men and 6 women (59.9 ± 15.2 years of age, mean $\pm$ S.D.), 8 current smokers and 4 exsmokers. Four of them were being treated with 10 mg of corticosteroid diary. No IPF subjects showed pathological germs in the sputum capable of inducing pulmonary infection.

The control group consisted of 15 patients who had undergone bronchoscopy because of hemoptysis and/or contralateral localized abnormal shadows. They included 13 men and 2 women (54.2 ± 15.8 years of age, mean $\pm$ S.D.) and 9 current smokers and 2 exsmokers.

Ten patients with sarcoidosis (no pulmonary fibrosis) were also studied. They included 6 men and 4 women (33.4 ± 13.8 years of age, mean $\pm$ S.D.), 3 current smokers and none of them were receiving corticosteroid treatment.

Transbronchial lung biopsy (TBLB). Using a fibroptic bronchoscope, two to four specimens from the peripheral regions of different unilateral lobes were obtained from each patient. The specimens were embedded in paraffin, sectioned (4 μm thickness) and stained with hematoxyline-eosin, elastica-masson and periodic acid-Schiff. The TBLB samples, about 3×3 to 5×5 mm in size coming mainly from the alveolar region (Fig. 1) and obtained from different lobes, were used for the following histologic examinations.

Classification of IPF stage. Patients with IPF were classified into two groups

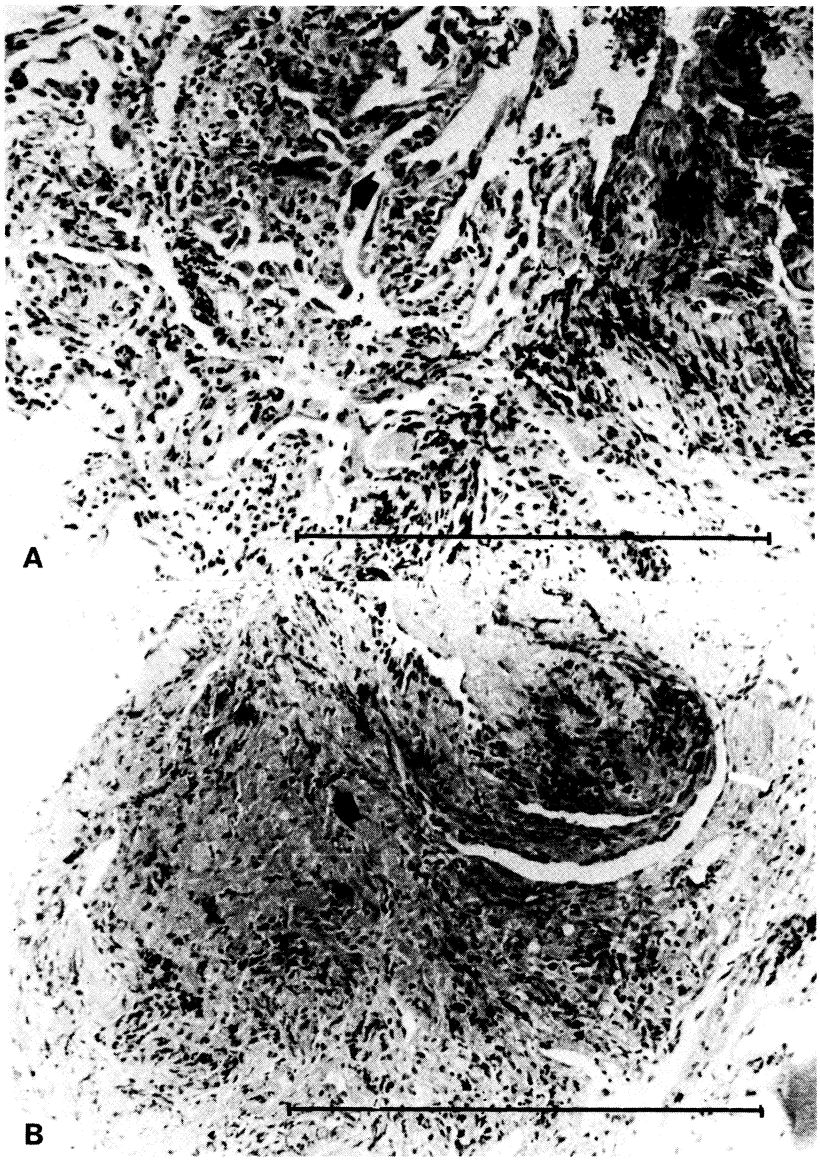

Fig. 1. Portions (one-third to quarter) of 4 m thick paraffin sections of TBLB samples from IPF patients with infiltrative type (A) and fibrotic type (B) IPF. Desquamative cells in alveoli (arrows), mononuclear cell infiltration and slight fibrosis in alveolar septums are seen in A. B shows massive fibrosis and cell infiltration in alveolar regions. Arrows indicate elastic layers of the alveolar septum. Hematoxyline-eosin stain. Bars represent 0.5 mm.

(infiltrative type: Fig. 1A and fibrotic type: Fig. 1B) according to histological findings from transbronchial lung biopsy, and into three groups (A, B and AB type), according to roentgenographic findings. Histologic findings were alveolar desquamation and interstitial inflammation (I) and alveolar septal fibrosis (II). The infiltrative type was defined by the presence of (I) with or without (II) and the fibrotic type was defined by the presence of (II). No subject showed histologic evidence only (I). Roentgenologic findings consisted of the ground glass pattern (I), and the reticulonodular pattern (II) and the reticular pattern on honey combing (III). Patients with (I) and/or (II) were considered to be A type and patients with (II) and/or (III) to be of B type. Patients with (I), (II) and (III) were considered to be of AB type (Crystal et al. 1976). Roentgenologic observations were made from chest x-rays taken within one week before bronchoscopic examination. Morphologic IPF types were identified by three pathologists independently without access to the patients names. Type identification by the three pathologists was required for final a decision. Roentgenologic IPF types were treated in a similar way and identified by three physicians.

Bronchoalveolar lavage. After premedication with atropine sulphate (0.5 mg/50 kg of body weight) and pentazocine (15 mg/50 kg), a fibroptic bronchoscope (FBS-6T, Machida Co., Tokyo) was introduced and wedged into a segmental or subsegmental bronchus of the middle or lingual lobe under local anesthesia with lidocaine. Then, 20 ml of 0.9% sterile saline was infused and immediately aspirated by a low negative pressure (-100 mmHg) to prevent collapse of the bronchus. This procedure was repeated five times. The fluids obtained were strained through one layer of surgical gauze and centrified for 8 minutes at $180 \times g$. The pellet of cells was used to count the total cell number and then smeared to determine the differential cell count by means of Wright-Giemsa and nonspecific esterase stains. BAL fluids were examined at the same time lung biopsies were performed on the opposite lung.

Gallium-67 scanning. Each patient was injected intravenously with 2 to 3 m Ci of ^{67}Ga citrate. Seventy two hours later, a whole body scan was recorded with a radioisotope camera (Sigma 410, Ohio Nuclear Co. OH, USA). The posterior scans were evaluated according to the method of Line et al (1978). Namely, the ^{67}Ga index was calculated by addition of the factors of area, intensity and texture of ^{67}Ga uptake in both lung fields. The area of ^{67}Ga uptake represents a portion (0 to 100 percent) of the total lung image. Intensity was estimated by the concentration of tracer and the depth of involved lung. Texture was also used to determine ^{67}Ga patchy or diffuse uptake. The ^{67}Ga index was methodologically calculated in the range of 0 to 400.

Pulmonary function tests. Vital capacity (VC), FEV_1, and FEV_1/VC were measured using a 13.5-L Benedict-Roth spirometer. Total lung capacity was determined by the closed circuit gas dilution method (Meneely et al. 1960). Transpulmonary pressure (the difference between mouth and esophageal pressure) was obtained using an esophageal balloon-catheter system coupled to a Hewlett-Packard 267 differential pressure transducer. Volume changes within the plethysmograph were measured with a Krogh respirometer equipped with a pressure compensated volume-displacement body plethysmograph. The esophageal pressure at TLC was defined as the maximal esophageal pressure (Pes max). To normalize lung volume, Pesmax was divided by TLC which is the coefficient of retraction (Pes max/TLC). The pulmonary diffusing capacity for carbon monoxide (DLco) was determined by the single breath technique (Ogilvie et al. 1957). The PaO_2, $PaCO_2$ and pH were measured using an IL meter 213. The VC, and DLco were expressed as percent of predicted values (Cotes 1979).

BAL, transbronchial lung biopsies, chest roentgenologic films, pulmonary function tests and Gallium 67 scans were studied within one week, in most cases, or at most, two weeks.

Statistical analysis. All data were statistically examined by one-way analysis of variance and by Student's t-test. Significance was accepted at $p < 0.05$.

Results

The stages of IPF defined by histologic and reontgenographic examination were well correlated (Table 1). Namely, infiltrative and fibrotic types determined histologically were correlated with A and B types determined by roentgenographically. The following parameters were compared with histologic and roentgenologic stages of IPF.

BALF findings in control, IPF and sarcoidosis patients: There was no difference in volume of fluid recovered between controls, IPF patients and sarcoidosis patients (Table 2). The total number of cells counted in the BALF of IPF patient was almost the same as that in controls (IPF: $6.9\pm1.2\times10^6$, Controls: $6.4\pm1.1\times10^6$), and total cell count from sarcoidosis patients was increased significantly ($16.1\pm4.9\times10^6$, $p<0.01$). In cell differentials, lymphocyte (controls: $8.3\pm6.7\%$, IPF: $22.5\pm6.0\%$), neutrophil (controls: $0.6\pm0.2\%$, IPF: $7.0\pm2.1\%$) and eosinophil (controls: $0.2\pm0.4\%$, IPF: $2.1\pm0.6\%$) from IPF showed significant increases in percentages, compared with controls (Table 1, Fig. 3) ($p<0.05$, $p<0.01$, $p<0.01$ respectively). Sarcoidosis patients showed a marked increase in the number of lymphocytes, as much as $53.7\pm6.4\%$. Fig. 2 summerizes the cell differentials of BALF from controls, IPF and sarcoidosis patients which apper in Table 2. Fig. 2 shows the ranges of cell differentials of the present subjects. Basophilic cells were less than 1.0% in each group.

BALF findings in infiltrative and fibrotic types: There was a significant difference in lymphocyte percentages from the infiltrative type (28.6 ± 8.1) and the fibrotic type (8.4 ± 2.0), classfied by the histologic stage of IPF ($p<0.05$) (Fig. 3). However, there was no difference in lymphocyte number between fibrotic type IPF and controls. There was no significant differences in other cell counts between infiltrative and fibrotic types. Although the percentage of neutrophils in histologically diagnosed fibrotic type IPF ($10.1\pm4.0\%$) was significantly higher than that in control patients (0.6 ± 0.8, $p<0.01$), there was no significant difference in percentage of neutrophils between infiltrative type IPF and controls. The

Table 1. *Classification of stages of idiopathic pulmonary fibrosis*

Histology		Cest x-ray				
		A type		A B type	B type	
		I	I・II	I・II・III	II	II・III
Intiltrative type	I・II	4	6	4	0	0
Fibrotic type	II	0	0	2	0	4

Cest x-ray I, Ground glass pattern; II, Reticulonodular pattern; III, Reticular pattern and honey combing. Histology I, Alveolar desquamation and inerstitial inflammation; II, Alveolar septal fibrosis.

TABLE 2. *Comparison of bronchoalveolar lavage data from patients in the diagnostic groups*

	Controls	IPF	Sarcoidosis
Recovery of fluid(%)	50.2±3.2	47.7±3.6	59.2±3.5
Total cells in lavage fluid ($\times 10^6$)	6.4±1.1	6.9±1.2	16.1±4.9**
Cell differentials			
Macrophage(%)	90.9±1.8	67.9±6.0**	44.2±6.4**
Lymphocyte(%)	8.3±6.7	22.5±6.0*	53.7±6.4**
Neutrophil(%)	0.6±0.2	7.0±2.1**	1.9±0.8
Eosinophil(%)	0.2±0.4	2.1±0.6**	0.2±0.1

IPF, idiopathic pulmonary fibrosis. Values are given in terms of mean±S.E.
* $p<0.05$, ** $p<0.01$ compared to controls.

percentage of eosinophils in histologically diagnosed infiltrative type IPF was significantly higher than in control subjects but not in fibrotic type IPF. From roentgenologic findings it was noted that although there is a tendency toward increased lymphocytes in A type and AB type, compared with B type, there were no significant differences between them. There was also no significant difference in neutrophil, eosinophil and macrophage counts (Fig. 4).

^{67}Ga indices and stages of IPF : The ^{67}Ga index ranged between 25 and 325 in patients with IPF. ^{67}Ga indices in fibrotic types and B types tended to be less than that in infiltrative types and B types but were not significantly different

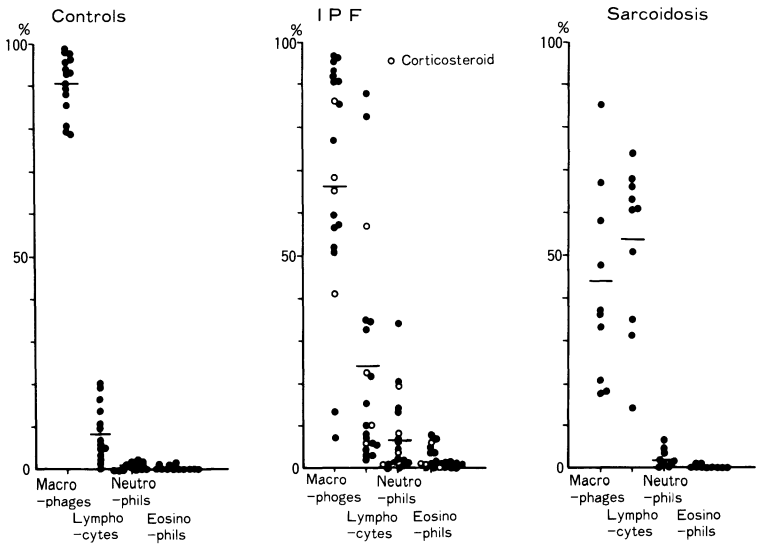

Fig. 2. Percentage of cells in the differential count from lavage fluids of controls, patients with idiopathic pulmonary fibrosis (IPF) and sarcoidosis. ●, no corticosteroid therapy ; ○, administration of corticosteroid.

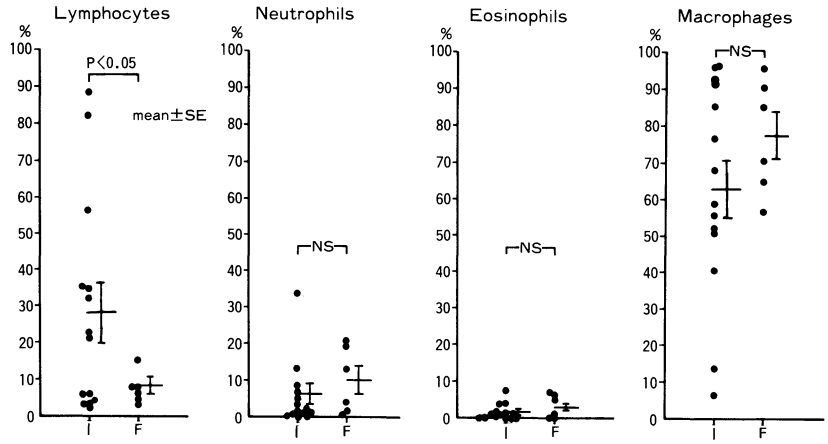

Fig. 3. Relation between BAL analysis and the histologic stages of IPF. Significant difference between the infiltrative type (28.6±8.1%) and fibrotic type (8.4±2.0%) was found in lymphocytes ($p<0.05$). I, infiltrative type; F, fibrotic type.

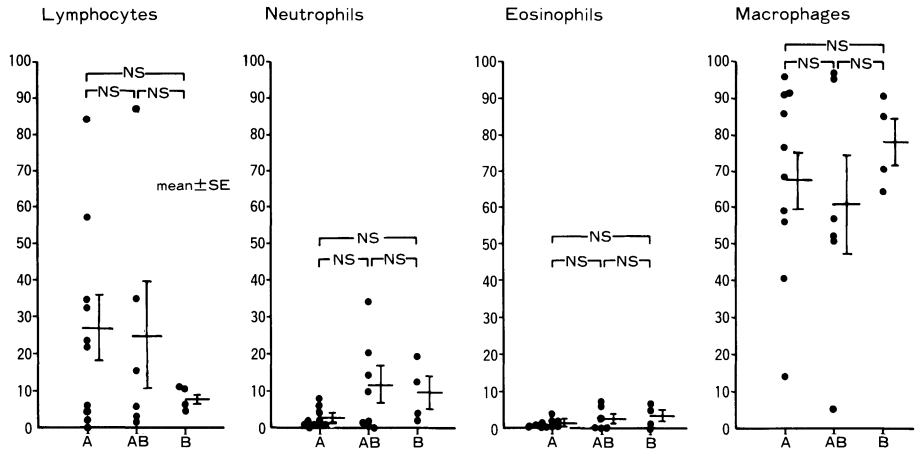

Fig. 4. Relation between BAL analysis and roentgenologic stages of IPF. No significant difference between A type, AB type and B type was found for any cell population.

(Fig. 5).

Pulmonary function and stage of IPF : The relationship between pulmonary functions (%VC, %DLco, Pao_2 and Pes max/TLC) and the two stages defined by histologically and roentgenologically finding, are shown in Figs. 6 and 7. The fibrotic type and the B type showed a significant increase in Pes max/TLC, compared to the infiltrative type and the A type (both $p<0.05$). %VC was significantly lower in the fibrotic type compared with the infiltrative type ($p<0.05$) in morphological analysis. Although not significant, the fibrotic type and

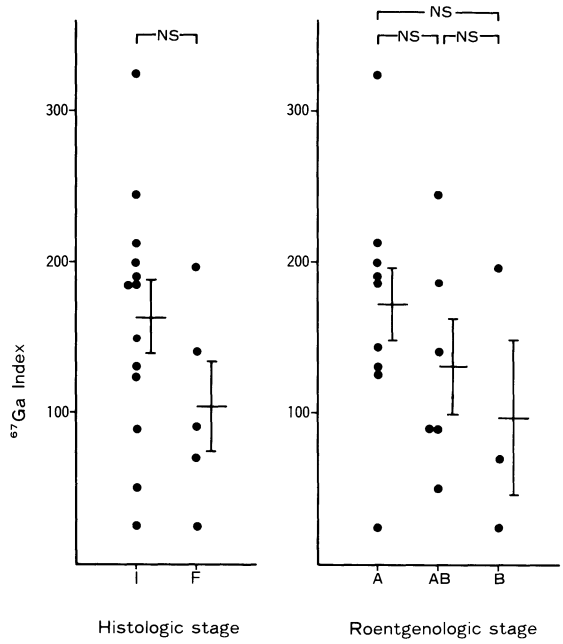

Fig. 5. Relation between ^{67}Ga indices and the stages of IPF: There were no significant differences between types.

B type tended to decreased %DLco, compared with the infiltrative type and the A type. There was no significant difference in PaO_2 between them.

^{67}Ga index and BALF findings: The relationships between the percentages of lymphocytes and neutrophils to the ^{67}Ga index are shown in Fig. 8. The ^{67}Ga index correlated significantly with the percentage of lymphocytes ($r=0.65$, $p<0.01$) and macrophages ($r=-0.60$, $p<0.01$). However, no significant correlation was found between the ^{67}Ga index and neutrophil or eosinophil counts ($r=-0.04$, $r=-0.03$, respectively).

Discussion

TBLB used for the present study may not be sufficient (compared with that obtained from open chest biopsy) for diagnosing the stage of interstitial lung disease because of the small tissue size (Levin et al. 1974). We used relatively larger samples (Fig. 1); small samples and samples of doubtful diagnostic value were discarded in the present study. Further, we obtained TBLB samples from different sites in the upper, middle and lower lung since the anatomical distribution of IPF in a patient is known sometimes to be heterogeneous. We found no systematic difference in appearances among the different biopsy sites and made the major determination of either infiltrative or fibrotic type based on appearances of the various samples. These multiple biopsies from different sites enhanced the usefulness of TBLB for the present study. Some patients were considered to be

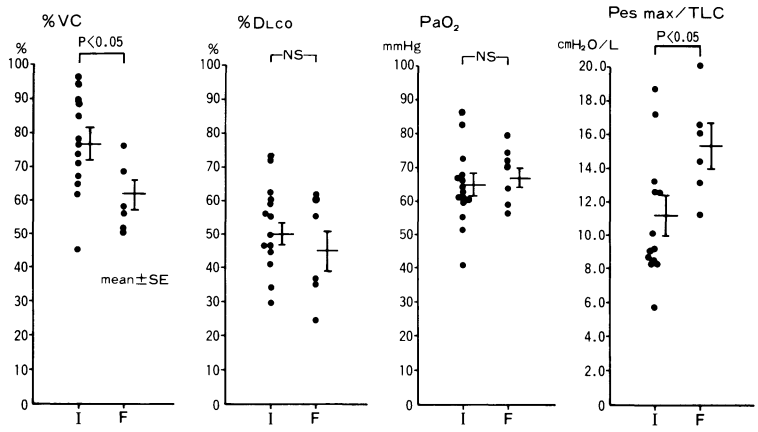

Fig. 6. Relation between pulmonary functions and histologic stages of IPF. Fibrotic type showed a significant decrease in %VC and in Pes max/TLC compared to the infiltrative type ($p<0.05$).

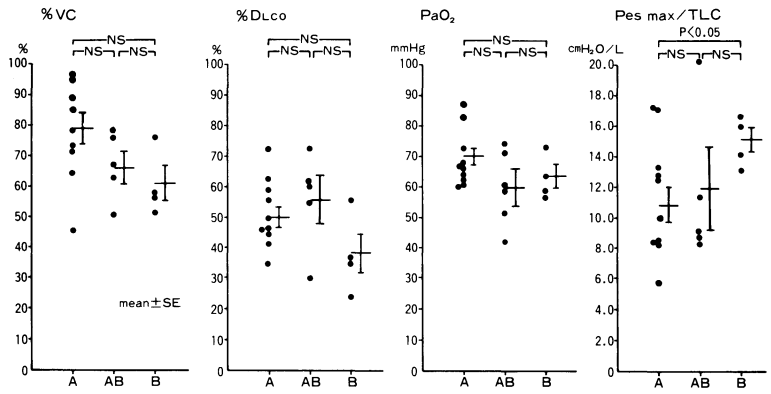

Fig. 7. Relation between pulmonary functions and roentgenologic stages of IPF : B type showed a significant increase in Pes max/TLC, compared to the infiltrative type ($p<0.05$).

at high risk for open chest biopsy because of advanced age, severe hypoxemia and advanced stage of IPF. Therefore histologic findings obtained from TBLB in our study do not involve end stages of IPF.

IPF patients in the present study were diagnosed according to Crystal et al.'s criteria (1976). The infiltrative type showed an increase in the number of lymphocytes in BALF which is also seen in the BALF of patients with hypersensitivity pneumonitis and sarcoidosis. However, the present IPF patients had no history of inhalation of inorganic dusts and showed negative precipitanting antibodies. After hospitalization (namely isolation from their living environments), they showed no significant improvements in chest x-ray, laboratory findings or symptoms with non-specific treatments except for corticosteroid ther-

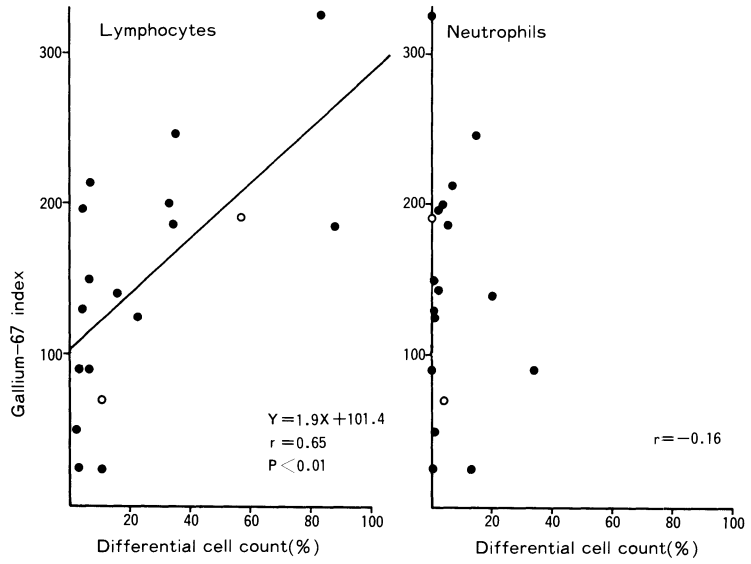

Fig. 8. Correlation between ^{67}Ga indices and BAL: The ^{67}Ga indices correlated significantly with the percentage of lymphocytes ($r=0.65$, $p<0.01$). However, no correlation between ^{67}Ga indices and neutrophils was found ($r=-0.16$).

apy. Further, the patients exhibited no clinical symptoms or laboratory indicative of sarcoidosis; i.e., the value of serum angiotensin-converting enzyme was normal and there were no extrapulmonary signs in the eyes, skin and lymphonode, etc. Of course, TBLB revealed no granuloma such as that seen in hypersensitivity pneumonitis and sarcoidosis. Therefore, it is unlikely that these patients suffered from hypersensitivity pneumonitis or sarcoidosis.

In the present study, BALF from IPF patients showed a significant increase in the number of lymphocytes, neutrophils and eosinophils. Lymphocytes were increased in infiltrative types of IPF, whereas neutrophils were increased in the fibrotic types, and eosinophils, although the percentages were small, were increased in the infiltrative types of IPF. These observations suggest that lymphocytes play an important role in the pathogenesis of the early stage of IPF. Abnormal immune complexes have been shown in blood from patients with IPF (Dreisin et al. 1978). Immunoglobulins and complements are deposited in lung parenchyma (Schwarz et al. 1978) and the levels of IgG in BALF of patients with IPF, thought to be drived from lung B lymphocytes, are increased, compared with normal levels (Reynolds et al. 1977; Weinberger et al. 1978). A cell-mediated autoimmune response to type I collagen suggests a role for T lymphocytes in the pathogenesis of IPF (Kravis et al. 1976). Experimental pulmonary fibrosis in animals has been used to study the pathogenesis of IPF. Thrall et al. (1982, 1984) demonstrated the importance of lymphocytes in the pathogenesis of

bleomycin induced fibrosis in rats. They compared lymphocyte populations in lung tissue and in bronchoalveolar lavage fluid in rats during the development of bleomycin-induced pulmonary fibrosis. In lung lavage fluid, they found a significant increase in lymphocytes shortly after a single intratracheal injection of bleomycin but no significant changes in the lymphocyte populations. Further, no lymphocytes were observed in the lavage fluid of control animals or in experimental animals long after administration of bleomycin. In lung tissues, the percentage of B lymphocytes was also increased in the early stages. These results are not contradictory to the present results.

On the other hand, Reynolds et al. (1977), Weinberger et al. (1978) and Crystal et al. (1981) have reported that the alveolitis of IPF is characterized by an increase in the number of neutrophils in BAL fluids, which are collected by the neutrophil chemotactic factor released by activated alveolar macrophages. We have also confirmed that the percentages of neutrophils in BAL fluids in IPF are increased in fibrotic types compared with normal subjects. We do not know the reasons for the discrepancy between the results of Crystal et al. and ours. Edwards and Carlile (1982) demonstrated in his morphometric study of the main, lobar, and segmental bronchi in IPF, that the quantity of gland and muscle was increased and the cause of these changes seemed due to proximal extension of repeated and persistent infection of the lung parenchyma. In our study, the patients with histologically diagnosed fibrotic type IPF had a longer duration of symptoms than patients with the infiltrative type, so that fibrotic type patients might have had repeated and persistent infections. There is the possibility that these infections in alveolar and lower airway tracts is related to the increase in neutrophils in BALF although no pathologically active germs were found in the sputum at the time of BAL examination. Crystal et al. (1981) observed that the percentage of lymphocytes was not increased in patients with IPF. However, other investigators have reported increased lymphocytes in BALF from patients with IPF, similar to the present results (Haslam et al. 1980; Ginns et al. 1982). Saltini et al. (1984) recently showed that cell differentials obtained by cytocentrifuge methods may underestimate the proportion of lymphocytes. Since, for cell differentials we used smear cell suspensions from BALF on glass, the number of lymphocytes in our results might be higher than those determined by the cytocentrifuge methods used by Crystal et al. (1981). Since no systematic differences in cell differentials between smokers and non-smokers or exsmokers have been observed, all of the data were analysed together. Some controversial correlations between the degree of fibrosis seen on open lung biopsy and the severity of disease estimated by chest film have been reported (Spencer 1967; Theros 1969). In the present subjects a positive correlation is observed between roentgenographic patterns and morphologic findings. Restrictive impairments were observed in the present fibrotic IPF patients and observed %VC are consistent with the fact that the coefficient of retraction (Pes max/TLC) is known to

correlate with the degree of fibrosis seen on open lung biopsy (Crystal et al. 1976). The duration of symptomes in the fibrotic type of IPF was longer than in the infiltrative type (Table 1).

^{67}Ga scanning is known to be useful in assessing the clinical activity of interstitial lung disease (Beaumont 1982 ; Line et al. 1978, 1981). Methods have been reported for quantifying ^{67}Ga uptake (Line et al. 1978), and we used these same methods to estimate the intensity and extent of ^{67}Ga uptake. The present study showed that ^{67}Ga indices correlate with the number of lymphocytes in BALF. Haslam et al. (1980) have reported that patients with more than 11% lymphocytes in BALF respond to steroid therapy, whereas patients with less than 11% lymphocytes do not. Rudd et al. (1981) showed that increased proportions of lymphocytes were associated with responsiveness to corticosteroids and exhibited good progress. This fact suggests that the ^{67}Ga index as well as the number of lymphocytes in BALF are important indices in the treatment of IPF and also support the observation of an increase in lymphocytes in the early stage of IPF. Line et al. (1978) demonstrated that, in patients with IPF, the ^{67}Ga index correlates with the degree of interstitial cellularity, the degree of alveolar cellularity and the differential percentage of neutrophils, but not with that of lymphocytes, eosinophils, or macrophages. In the present study, the ^{67}Ga indices in infiltrative type IPF tended to be higher than in the fibrotic type, as observed by Line et al. (1978). There may be a difference in the stage of the IPF of patients studied by Line et al. and those in our study. Their subjects had a longer duration of symptoms than the present subjects and 50 percent of their subjects were taking prednisolone. The effect of systemic corticosteroid administration has been demonstrated to decrease the absolute number of lymphocytes in the bronchoalveolar cell population (Domby and Whitcomb 1978). Furthermore, since Line et al. (1978) studied lung biopsy within one year from the time at which ^{67}Ga uptake had been assessed, and the type of IPF may have changed. In the present study, we performed BAL and ^{67}Ga uptake at the same time as lung biopsy. Therefore, direct comparisons are possible without any introducing a time lapse factor. The mechanism governing ^{67}Ga accumulation in the lung has not yet been determined. ^{67}Ga scanning also has been shown to be useful in the staging of sarcoidosis because ^{67}Ga uptake correlates with the number of T lymphocytes (Spencer 1967). Merz et al. demonstrated that lymphocyte affinity for ^{67}Ga in vitro was further increased in cells stimulated by photohemoagglutinin than in unstimulated cells (Merz et al. 1974). These results support the significant correlation between ^{67}Ga index and lymphocyte count (Fig. 8).

In conclusion, the present study suggests that in the early stages of IPF, the lymphocyte count increases in the alveolar regions and small airways, while in the advanced stages, mainly neutrophils increase, and eosiophils increase by small percentages in the alveolitis stages. Therefore, lymphocytes may play an important role in the pathogenesis of infiltrative type IPF and neutrophils may be

related to the development of fibrotic changes. Roth et al. (1981) have reported that sarcoidosis without lung fibrosis showed a marked increase in lymphocyte numbers in BALF similar to ours, and the development of lung fibrosis induced an increase in the number of neutrophils. This fact suggests that a mechanism similar to that which regulates inflammatory cells in the alveolar regions in sarcoidosis may also exist in IPF.

References

1) Beaumont, D., Herry, J.Y., Sapene, M., Bourguet, P., Larzul, J.J. & De Labarthe, B. (1982) Gallium-67 in the evaluation of sarcoidosis: Correlations with serum angiotensin-converting enzyme and bronchoalveolar lavage. *Thorax*, **37**, 11-18.
2) Cotes, J.E. (1979) Lung function: Assessment and application in medicine. In: *Assessment of Mechanical and Bellows Attributes of the Lung*, edited by J.E. Cotes, Blackwell Scientific Publications, Oxford, pp. 97-130.
3) Crystal, R.G., Fulmer, J.D., Roberts, W.C., Moss, M.L., Line, B.R. & Reynolds, H.Y. (1976) Idiopathic pulmonary fibrosis: Clinical, histologic, radiographic, physiologic, scintigraphic, cytologic, and biochemical aspects. *Ann. intern. Med.*, **85**, 769-788.
4) Crystal, R.G., Gadek, J.E., Ferrans, V.J., Fulmer, J.D., Line, B.R. & Hunninghake, G. W. (1981) Interstitial lung disease: Current concepts of pathogenesis, staging and therapy. *Amer. J. Med.*, **70**, 542-568.
5) Domby, W.R. & Whitcomb, M.E. (1978) The effect of corticosteroid administration on the bronchoalveolar cells obtained from guinea pigs by lung lavage. *Amer. Rev. resp. Dis.*, **117**, 893-896.
6) Dreisin, R.B., Schwarz, M.I., Theofilopoulo, A.N. & Stanford, R.E. (1978) Circulating immune complexes in the idiopathic interstitial pneumonias. *New Engl. J. Med.*, **298**, 353-357.
7) Edwards, C.W. & Carlile, A. (1982) The larger bronchi in cryptogenic fibrosing alveolitis: A morphometric study. *Thorax*, **37**, 828-833.
8) Gadek, J.E., Kelman, J.A., Fells, G., Weinberger, S.E., Horwitz, A.L., Reynolds, H.Y., Fulmer, J.D. & Crystal, R.G. (1979) Collagenase in the lower respiratory tract of patients with idiopathic pulmonary fibrosis. *New Engl. J. Med.*, **301**, 737-742.
9) Ginns, L.C., Goldenheim, P.D., Burton, R.C., Colvin, R.B., Miller, L.G., Goldstein, G., Hurwitz, C. & Kazemi, H. (1982) T-lymphocyte subsets in peripheral blood and lung lavage in idiopathic pulmonary fibrosis and sarcoidosis: Analysis by monoclonal antibodies and flow cytometry. *Clin. Immunol. Immunopath.*, **25**, 11-20.
10) Haslam, P.L., Turton, C.W.G., Lukoszek, A., Salsbury, A.J., Dewar,A., Collins, J.V. & Turnar-Warwick, M. (1980) Bronchoalveolar lavage fluid cell counts in cryptogenic fibrosing alveolitis and their relation to therapy. *Thorax*, **35**, 328-339.
11) Hunninghake, G.W., Gadek, J.E., Lawley, T.J. & Crystal, R.G. (1981) Mechanisms of neutrophil accumulation in the lungs of patients with idiopathic pulmonary fibrosis. *J. clin. Invest.*, **68**, 259-269.
12) Kravis, T.C., Ahmed, A., Brown, T.E., Fulmer, J.D. & Crystal, R.G. (1976) Pathogenic mechanisms in pulmonary fibrosis. Collagen-induced migration inhibition factor production and cytotoxicity mediated by lymphocytes. *J. clin. Invest.*, **58**, 1223-1232.
13) Levin, D.C., Wicks, A.B. & Ellis, J.H., Jr. (1974) Trnsbronchial lung biopsy via the fiberoptic brochoscope. *Amer. Rev. resp. Dis.*, **110**, 4-12.
14) Line, B.R., Fulmer, J.D., Reynolds, H.Y., Rorberts, W.C., Jones, A.E., Harris, E.K. & Crystal, R.G. (1978) Gallium-67 citrate scanning in the staging of idiopathic pulmonary fibrosis: Correlation with physiologic and morphologic features and bron-

choalveolar lavage. *Amer. Rev. resp. Dis.*, **118**, 355-365.
15) Line, B.R., Hunninghake, G.W., Keogh, B.A., Jones, A.E., Johnston, G.S. & Crystal, R.G. (1981) Gallium-67 scanning to stage the alveolitis of sarcoidosis: Correlation with clinical studies, pulmonary function studies, and bronchoalveolar lavage. *Amer. Rev. resp. Dis.*, **123**, 440-446.
16) Meneely, G.R., Ball, C.O.T., Kory, R.C., Callaway, J.J., Merrill, J.M., Mabe, R.E. & Roehm, D.C. (1960) A simplified closed circuit helium dilution method for the determination of residual volume of the lungs. *Amer. J. Med.*, **28**, 824-831.
17) Merz, T., Malmud, L., Mckusick. K. & Wagner, H.N., Jr. (1974) The mechanism of ^{67}Ga association with lymphocytes. *Cancer Res.*, **34**, 2495-2499.
18) Ogilvie, C.M., Forster, R.E., Blakemore, W.S. & Morton, J.W. (1957) A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J. clin. Invest.*, **36**, 1-17.
19) Ozaki, T., Nakayama, T., Ishimi, H., Kawano, T., Yasuoka, S. & Tsubura, E. (1982) Glucocorticoid receptors in bronchalveolar cells from patients with idiopathic pulmonary fibrosis. *Amer. Rev. resp. Dis.*, **126**, 968-971.
20) Reynolds, H.Y., Fulmer, J.D., Kazmierowski, J.A., Roberts, W.C., Frank, M.M. & Crystal, R.G. (1977) Analysis of cellular and protein content of broncho-alveolar lavage fluid from patients with idiopathic pulmonary fibrosis and chronic hypersensitivety pneumonitis. *J. clin. Invest.*, **59**, 165-175.
21) Roth, C., Huchon, G.J., Arnoux, A., Stanislas-Leguern, G., Marsac, J.H. & Chretien, J. (1981) Bronchoalveolar cells in advanced pulmonary sarcoidosis. *Amer. Rev. resp. Dis.*, **124**, 9-12.
22) Rudd, R.M., Haslam, P.L. & Turner-Warwick, M. (1981) Cryptogenic fibrosing alveolitis: Relationships of pulmonary physiology and bronchoalveolar lavage to response to treatment and prognosis. *Amer. Rev. resp. Dis.*, **124**, 1-8.
23) Saltini, C., Hance, A., Ferrans, V., Basset, F., Bitterman, P., Saltzman, L., Steel, L. & Crystal, R. (1984) Routine quantification of bronchoalveolar lavage cells by cytocentrifuge methods underestimates the proportions of lymphocytes present. *Amer. Rev. resp. Dis.*,**129**, Suppl. A64.
24) Schwarz, M.I., Dreisin, R.B., Pratt, D.S. & Stanford, R.E. (1978) Immuno-fluorescent patterns in the idiopathic interstitial pneumonias. *J. Lab. clin. Med.*, **91**, 929-938.
25) Spencer, H. (1967) Interstitial pneumonia. *Ann. Rev. Med.*, **18**, 423-442.
26) Theros, E.G. (1969) The value of radiologic-pathologic correlation in the education of the radiologist. *Amer. J. Roentgenol.*, **107**, 235-257.
27) Thrall, R.S. & Barton, R.W. (1984) A comparison of lymphocyte populations in lumg tissue and in Broncalveolar lavage fluid of rats and various times during the development of bleomycin-induced pulmonary fibrosis. *Amer. Rev. resp. Dis.*, **129**, 279-283.
28) Thrall, R.S., Barton, R.W., D'Amato, D.A. & Sulavik, S.B. (1982) Differential cellular analysis of bronchalveolar lavage fluid obtained at various stages during the development of bleomycin-induced pulmonary fibrosis in the Rat. *Amer. Rev. resp. Dis.*, **126**, 488-492.
29) Weinberger, S.E., Kelman, J.A., Elson, N.A., Young, R.C., Jr., Reynolds, H.Y., Fulmer, J.D. & Crystal, R.G. (1978) Bronchoalveolar lavage in interstitial lung disease. *Ann. intern. Med.*, **89**, 459-466.