Interleukin-8 in bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis or idiopathic pulmonary fibrosis

Fumitaka Ogushi, Kenji Tani, Koji Maniwa, Waka Ichikawa, Hiroya Tada, Tetsuya Kawano and Saburo Sone

Third Department of Internal Medicine, The University of Tokushima School of Medicine, Tokushima, Japan

Abstract : This study was designed to clarify the contribution of IL-8 as a specific neutrophil chemotactic factor in the human respiratory tract in various pulmonary diseases. The neutrophil chemotactic activity(NCA), neutrophil counts and IL-8 concentration in the bronchoalveolar lavage fluid (BALF) obtained from normal volunteers (NV), control patients (CP), patients with diffuse panbronchiolitis (DPB) and patients with idiopathic pulmonary fibrosis (IPF) were examined. Neutrophil counts, NCA and IL-8 concentration in BALF obtained from patients with DPB or IPF was significantly higher than that from NV or CP. The IL-8 concentration correlated with neutrophil count and also correlated with NCA in BALF from patients with IPF, whereas there was no correlation between these factors in BALF from DPB. These results suggest that the contribution of IL-8 to neutrophil accumulation of the lower respiratory tract is different between IPF and DPB. J. Med. Invest. 44 : 53-58, 1997

Key Words : *interleukin-8, bronchoalveolar lavage fluid, diffuse panbronchiolitis, idiopathic pulmonary fibrosis*

INTRODUCTION

Neutrophils are thought to play a central role in combating bacterial infection in the lower respiratory tract (1,2). They are also thought to be involved in the pathogenesis of various pulmonary diseases, such as pulmonary emphysema, idiopathic pulmonary fibrosis (IPF) and cystic fibrosis(3-5). Neutrophils are rare in the alveolar space or respiratory tract of normal persons(6). Therefore, neutrophils are thought to be attracted to disease lesions from the peripheral circulation by various neutrophil chemotactic factors(NCFs). NCFs have been characterized in the respiratory tract and are thought to be responsible for neutrophil infiltration in pulmonary diseases. Different NCFs, such as cell-derived NCF(7-12), complement derived NCF(13), and bacterium-derived NCF(14)may correspond to various pathophysiologic states of the lung. We have already reported that different types of NCFs exist in bronchoalveolar lavage fluid (BALF) obtained from patients with chronic airway diseases and IPF, suggesting that the main NCF, which neutrophils accumulate in the lung, may differ with each disease(15).

IL-8, previously referred to as monocyte-derived neutrophil chemotactic factor or neutrophil activating peptide-1, is a 72 amino acid polypeptide that has potent neutrophil activating and chemotactic activities(9,16,17). IL-8 has been demonstrated in a number of inflammatory disease states, including rheumatoid arthritis(18), sepsis(19)and adult respiratory distress syndrome(20). IPF is characterized by the accumulation of neutrophils and mononuclear cells, followed by the progressive deposition of collagen within the interstitium and subsequent destruction of lung air spaces(4). Although the mechanism of cellular injury is unclear, neutrophils are thought to play important roles. Diffuse panbronchiolitis(DPB), a lung disease in which chronic inflammation is restricted to the respiratory bronchioles, is common in Japan(21). As the disease progresses, the inflammatory changes extend to the proximal airways and inflammation due to bacterial infection is superimposed(22), and it is thought that neutrophil accumulation is responsible for tissue damage. Thus, in IPF and DPB, neutrophils which accumulate in the disease site are thought to play important roles in the pathogenesis of the disease. On the other hand, various types of NCF may cause the accumulation of neutrophils in these diseases. In this study, to examine whether IL-8 was responsible for accumulation of neutrophils in the lower respiratory tract, we measured IL-8 concentration in BALF and examined the correlation between IL-8 concentration and neutrophil chemotactic activity in BALF obtained from patients with IPF or DPB.

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[1] Address correspondence and reprint requests to Fumitaka Ogushi, M.D., Ph.D., Third Department of Internal Medicine, The University of Tokushima School of Medicine, 3-18-15, Kuramoto-cho, Tokushima, Japan

MATERIALS AND METHODS

Subjects

Four groups of subjects were studied : patients with DPB, patients with IPF, normal volunteers, and control patients.

The DPB group consisted of ten patients with DPB (6 males and 4 females). Patients with DPB were diagnosed clinically on the basis of the clinical, functional and radiological criteria proposed by the Committee for Diffuse Panbronchiolits (part of the Interstitial Pulmonary Research Project of the Ministry of Public Welfare of Japan) (21,22). All demonstrated an increased volume of sputum. The presence of bacteria in the sputa of patients was examined at the time BAL was performed and 2 Hemophilus influenzae,1 Streptococcus peumoniae, and 2 Pseudomonas aeruginosa were detected. The results of the others showed normal flora. These patients were not treated with erythromycin at that time. The average age of the ten patients was 46±14 yrs (all data are presented as mean±SD). Four patients were former smokers and six non-smokers.

The IPF groups consisted of 11 patients. The diagnosis of IPF was based on clinical symptoms, chest roentgenograms, pulmonary function tests, and histologic findings that showed interstitial fibrosis compatible with IPF in more than two specimens obtained by repeated transbronchial biopsy from different parts of the lung. No patient had evidence of sarcoidosis, hypersensitivity pneumonitis, collagen-vascular diseases, or a history of significant occupational exposure or glucocorticoid treatment. The average age of this group was 57±13 yrs and all were men. Five did not smoke and the others were former smokers.

The normal volunteer (NV) group consisted of 7 healthy volunteers who were without symptoms and had no abnormal findings on chest roentgenogram or pulmonary function testing. Their average age was 22±1 yr ; all were men, 4 were non-smokers, 3 were smokers of less than 3 pack years.

The control patients (CP) group consisted of 9 patients who had a localized lung lesion such as lung cancer. No patient received any treatment. Their average age was 56 ±13 yrs ; all were men, 2 were non-smokers, 3 were ex-smokers and 4 were current smokers. BAL was performed on the non-affected lung, and the BALF obtained was used as an age-matched control for the patients with DPB and IPF.

Informed consent was obtained from every NV and patient.

Bronchoalveolar Lavage

As described previously (23), BAL was performed with a flexible bronchofiberscope in the subsegmental bronchus, B^4 or B^5 of the right lung of NV, for the DPB and IPF groups, and non-affected lungs of CP. Fifty milliliters of sterilized saline was introduced gently by syringe through the bronchoscope and recovered after one deep inspiration. This procedure was repeated three times and the BAL fluids were pooled, filtered through three layers of gauze and centrifuged at 200 Xg for 10 min to separate the cells from the fluid. The fluid was stored at -70℃ for the assays of neutrophil chemotactic activity and IL-8 concentration. The cells were washed twice with phosphate buffered saline (PBS) and counted in a hemocytometer. Differential cell counts were performed using May-Giemsa and non-specific esterase staining.

Measurement of Neutrophil Chemotactic Activity

Neutrophil chemotactic activity (NCA) in the BALF was measured using the membrane filter method and a 48-well microchemotactic chamber (Neuro Probe, Inc., Bethesda, MD) as described by Falk and coworkers (24). Neutrophils were purified from the peripheral blood of a normal volunteer by the sedimentation method of Böyum (25) and suspended at a concentration of 3×10^6 per ml in PBS containing 0.4% bovine serum albumin (BSA) (Sigma). A 30 μl aliquot was placed into the lower well separated by a membrane filter with 3 μm diameter pores, and the upper well was filled with 50 μl of neutrophil suspension. The chamber was kept standing for 30 min at 37℃ under 5% CO_2 in air. Then, the filter was removed and stained with Diff-Quik (Harleco, Gibbstown, NJ). The cells that migrated through the filter to the other side were counted in five microscopic fields and NCA was expressed as the average cell number per high power field (1 X 1000). In each experiment, a negative control was assessed using saline. A positive control was assessed using 10^{-6}-10^{-9}M N-formyl-Met-Leu-Phe (fMLP ; Sigma).

Measurement of IL-8 in BALF

The IL-8 concentration in BALF was measured using an ELISA kit (Research and Diagnostic System, Minneapolis, MN). A standard curve was sufficient to measure concentrations of IL-8 from 4.7 pg/ml to 1200 pg/ml. The IL-8 concentration was expressed as pg/ml in BALF.

Statistical Analysis

Results are expressed as the mean±SD. The significance of differences between values was evaluated by Student's t-test. Correlation was examined by Spearman's correlation coefficients method. p values of 0.05 or less were considered to be significant.

RESULTS

Cell Numbers in BALF

The total cell and neutrophil counts in BALF obtained from the control groups and patients with IPF and DPB are shown in Table 1. The mean total cell count in BALF from patients with DPB and IPF was higher ($p<0.05$) than in NV. The neutrophil counts in BALF from patients with DPB and IPF were also higher ($p<0.05$) than in NV and CP. The mean neutrophil count in BALF from CP was slightly, but not significantly, higher than that in BALF from NV. The neutrophil count in patients with DPB was higher than that from patients with IPF ($p<0.05$).

Neutrophil Chemotactic Activity in BALF

NCA in BALF obtained from control groups and patients with DPB and IPF are shown in Table 2. NCA in BALF obtained from patients with DPB and IPF was significantly higher than that from CP or NV ($p<0.05$). NCA in BALF from CP was also higher than that from NV ($p<0.05$). NCA in the BALF from patients with DPB was

Table 1. Neutrophil Count in Bronchoalveolar Lavage Fluid*

	n	Cell Number in BALF	
		Total Cells ($\times 10^5$/ml)	Neutrophils ($\times 10^3$/ml)
Normal volunteers	7	1.9±0.9	1.7±1.9
Control patients	9	2.6±0.9	2.6±1.7
Patients with DPB	10	6.6±0.4†¶	289±130‡¶
Patients with IPF	11	3.5±1.1†	25±15‡

Definition of abbreviations : BALF=bronchoalveolar lavage fluid, DPB=diffuse panbronchiolits, IPF=idiopathic pulmonary fibrosis
*Values are expressed as the mean±SD
† P<0.05 compared to normal volunteers
‡ P<0.05 compared to normal volunteers and control patients
¶ P<0.05 compared to patients with IPF

Table 2. Neutrophil Chemotactic Activity in Bronchoalveolar Lavage Fluid*

	n	Neutrophil Chemotactic Activity (cells/hpf)
Saline		4±1
FMLP (10^{-7}M)		68±5
Normal volunteers	7	1±32
Control patients	9	20±7
Patients with DPB	10	56±16†‡
Patients with IPF	11	39±9†

Definition of abbreviations : hpf=high power fluid
For other definitions, see Table 1.
*Values are expressed as the mean±SD
† P<0.05 compared to normal volunteers and control patients
‡ P<0.05 compared to patients with IPF

higher than that in BALF from patients with IPF (p<0.05).

Concentration of IL-8 in BALF

The concentrations of IL-8 in BALF from normal individuals and patients with DPB and IPF are shown in Fig 1. The concentration of IL-8 in BALF from NV and CP was 37±17 pg/ml and 53±18 pg/ml, respectively (not significantly different). The concentrations of IL-8 in BALF from patients with DPB and IPF were 357±301 pg/ml and 102±35 pg/ml, respectively. In both patient groups, the level was significantly higher than in control groups (p <0.05). Further, the IL-8 concentration from patients with DPB was higher (p<0.05) than that from patients with IPF.

Correlation between the IL-8 concentration and neutrophil count or NCA in BALF

There was no correlation between the IL-8 concentration and neutrophil count in BALF obtained from patients with DPB (Fig.2 A). On the other hand, a significant correlation (p<0.01) was found between the IL-8 concentration and neutrophil count in BALF from patients with IPF (Fig 2 B). There was no correlation between the IL-8 concentration and NCA in BALF obtained from patients with DPB (Fig 3 A). However, a significant correlation (p< 0.01) was found between the IL-8 concentration and NCA in BALF obtained from patients with IPF (Fig.3 B).

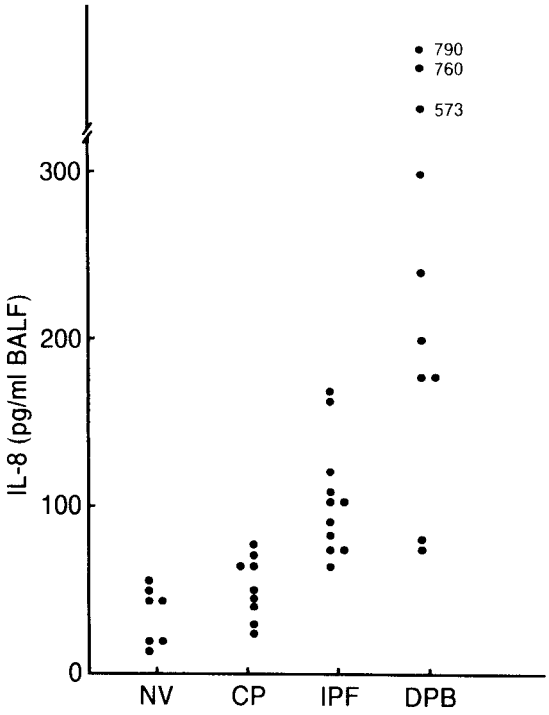

Fig. 1. Concentration of IL-8 in BALF obtained from normal individuals and patients with IPF or DPB. The results are expressed as pg/ml of IL-8 in BALF. Definition of abbreviations, NV : normal volunteer, CP : control patients. For other definitions, see Table 1.

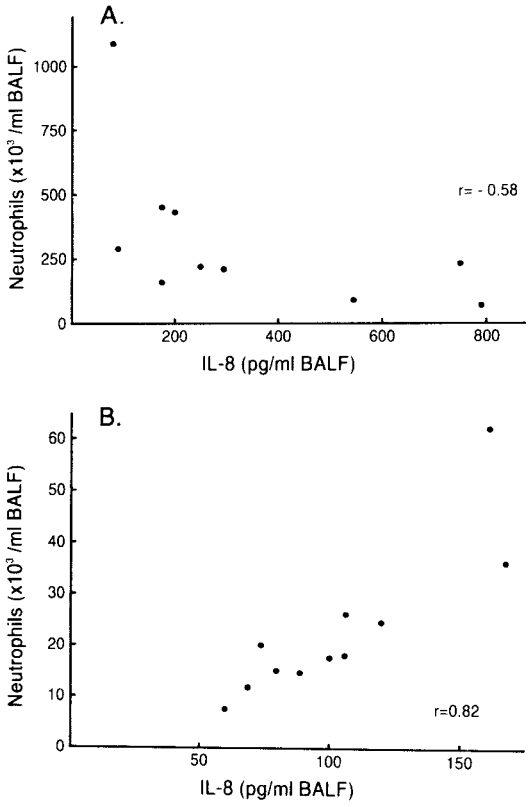

Fig. 2. Correlation between concentrations of IL-8 and neutrophil counts in BALF obtained from patients with DPB (A) or IPF (B). The results are expressed as pg/ml of IL-8 in BALF and the neutrophil count per ml of BALF. For a definition of abbreviations, see Table 1.

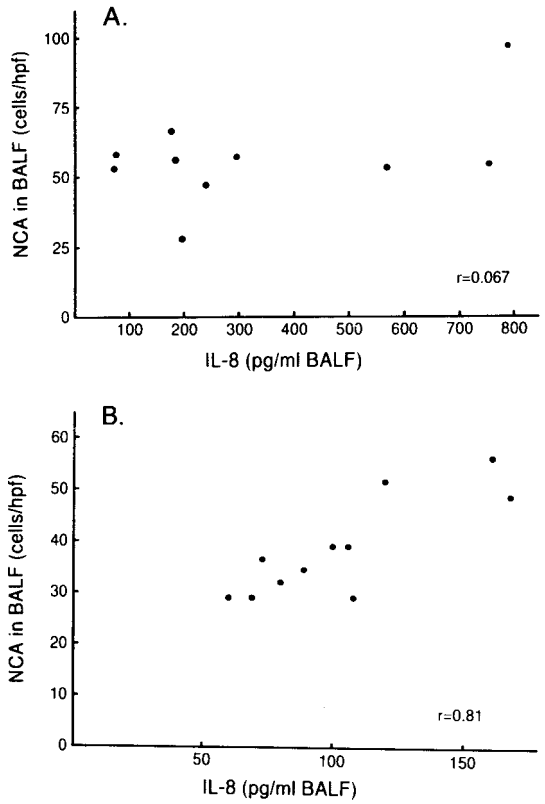

Fig. 3. Correlation between concentrations of IL-8 and neutrophil chemotactic activity in BALF obtained from patients with DPB(A) or IPF (B). Neutrophil chemotactic activity is expressed as cells per high power field and IL-8 concentration is expressed as pg/ml of BALF.
For a definition of abbreviations, see Table 1.

DISCUSSION

Neutrophils that accumulate in the lower respiratory tract are thought to be involved in the pathogenesis of various pulmonary diseases. Neutrophil accumulation is caused by various NCFs, which are derived from bacteria (14), serum(26), complement(13)and cells(7-12). Various NCFs have been detected in BALF from patients with pneumonia(27), adult respiratory distress syndrome(28), IPF(15, 29)and cystic fibrosis(5).

Recently, IL-8, which is a polypeptide with specific neutrophil chemotactic activity, has been cloned. IL-8 is produced by monocytes/macrophages (9, 16, 30), fibroblasts(10, 31), epithelial cells(11, 32)and mast cells(12)in response to an exogenous or endogenous signal. IPF and DPB are chronic inflammatory diseases which are characterized by the accumulation of neutrophils in the lower respiratory tract(15, 29, 33). However, the disease site in DPB differs from that in IPF. In patients with DPB, the airways, particularly the peripheral airways and intermediate zones are thought to be narrowed by inflammation of the bronchiolar walls(21,22). In IPF, the disorders are invariably associated with inflammation of interstitium(4).

In the present study, we examined whether the contribution of IL-8 to neutrophil accumulation into the disease site may differ between DPB and IPF. To test this hypothesis, we measured the IL-8 concentration in the BALF of patients with IPF and DPB, and compared these IL-8 concentrations to the NCA levels in the BALF of these patients. NCA in BALF from patients with DPB and IPF was higher than that from NV or CP. There is no correlation between the IL-8 concentration and NCA in the BALF of patients with DPB. We have noticed that BALF from patients with chronic airway disease contained different types of NCF such as coplement, IL-8 and LTB_4 (15). Therefore, the discrepancy between IL-8 concentration and NCA in BALF from DPB patients may be explained as follows. That is, chief NCFs, which contribute to the accumulation of neutrophils in the lower respiratory tract may differ with the disease state of DPB. Furthermore, NCF may be inactivated by various inhibitors in BALF from DPB patients(34, 35). On the other hand, there is a correlation between the IL-8 concentration and BALF NCA in patients with IPF. These results suggested that IL-8 is an important NCF in the lower respiratory tract of the IPF lung. IL-8 in the BALF of IPF patients is thought to be produced by various lung cells, such as alveolar macrophages, endothelial cells, and fibroblasts. The alveolar macrophages seem to be of primary importance among lung cells. Increased numbers of activated alveolar macrophages have been found by BAL and within the alveolar space and wall in IPF(36, 37). Lynch et al. have reported that BAL cells from patients with IPF constitutively express mRNA for IL-8,however, BAL cells from healthy subjects failed to express constitutively mRNA of IL-8(38). Carre et al. showed using reverse transcription polymerase chain reaction that expression of the IL-8 gene by alveolar macrophages is increased in IPF and the level of IL-8 mRNA correlated with the number of neutrophils per mililiter in the BALF. They also demonstrated that the IL-8 level in BALF reflected the pattern of IL-8 mRNA expression by alveolar macrophages(39). Thus, alveolar macrophages appear to be the major cellular source of the IL-8 in BALF obtained from patients with IPF. Therefore, IL-8 derived from alveolar macrophages is thought to be an important NCF in the lower respiratory tract of the IPF lung and may contribute to the involvement of neutrophils in the pathogenesis of IPF. Moreover, Nakamura et al. demonstrated that alveolar macrophages of patients with IPF were primed for IL-8 production(40).

In the present study, we demonstrated that the IL-8 level was elevated in the BALF of patients with DPB or IPF when compared with the control. There was a correlation between IL-8 concentration and NCA in BALF from patients with IPF, but not DPB. These results suggest that the NCF increases specifically according to the particular pathophysiologic state and that NCF is produced in response to a specific pathogen or disease state.

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