Cell Profile and Elastase Activity in Diffuse Panbronchiolitis Investigated by Bronchoalveolar and Bronchial Lavage

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To examine the mechanism of tissue damage which causes bronchiolectasis in diffuse pan-bronchiolitis (DPB), the cellular components, elastase and its main inhibitor, alpha 1-protease inhibitor (α 1-PI) were measured in bronchoalveolar and bronchial lavage fluid (BALF and BLF) from 14 DPB patients. A predominant increase in the neutrophil count was observed in DPB. Elastase activity in BALF and BLF was about 1,000-fold higher in the DPB group than in the control group. An inhibitor study and a positive correlation between elastase activity and the neutrophil count in both lavage fluids from the DPB group indicated that the activity was mainly that of neutrophil elastase. Western blot analysis of α 1-PI showed that most of the α 1-PI in the lavage fluids from DPB group was degraded. These results indicated that neutrophil infiltration increases the level of elastase in the DPB lesions; this increase seems to be closely related to tissue damage.

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Key words: chronic bronchitis, alpha 1-protease inhibitor, neutrophils

Introduction

Diffuse panbronchiolitis, a lung disease in which chronic inflammation is restricted to the respiratory bronchioles (1), is common in Japan. In its initial stage it is characterized by thickening of the walls of respiratory bronchioles, with infiltration of lymphocytes and histiocytes, and extension of inflammatory changes toward peribronchiolar tissues (1). As the disease progresses, the inflammatory changes extend to the proximal airways and inflammation due to bacterial infection is superimposed. In the advanced stage, secondary ectasia of proximal terminal bronchioles (bronchiolectasis) is observed and in the final stage further bronchiectasis occurs (1, 2).

Elastase released from the inflammatory cells is related to the pathogenesis of lung diseases such as cystic fibrosis (3) and emphysema (4), causing degradation of various types of lung tissue components. However, the exact mechanism of the tissue damage leading to bronchiolectasis and bronchiectasis in diffuse panbronchiolitis is not fully clarified.

Here, we report a study of the mechanism of tissue damage in diffuse panbronchiolitis and the contribution of

protease and antiprotease imbalance to the pathogenesis of diffuse panbronchiolitis. For this, we examined the cell profile, elastase activity and the level of its main inhibitor, alpha 1-protease inhibitor in both bronchial and bronchoalveolar lavage fluids from patients with diffuse panbronchiolitis.

Materials and Methods

Four groups of subjects were studied: 1) The normal volunteer group consisting of 21 healthy male nonsmokers, 20 to 26 years old (mean: 23 years). 2) The control patient group consisting of 33 nonsmokers (13 males and 20 females), 35 to 67 years old (mean: 53 years), who had a localized lung lesion, but no detectable lesion in the airway on bronchoscopical examination. 3) The diffuse panbronchiolitis group consisting of 14 patients with this disease (8 males and 6 females) of 22 to 59 years old (mean: 45 years), who were all nonsmokers.

Diffuse panbronchiolitis was diagnosed clinically on the basis of the clinical, functional and radiological criteria proposed by the Committee for the Diagnosis of Diffuse Panbronchiolitis [part of the Interstitial Pulmonary Disease Research Project of the Ministry of Public

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Welfare of Japan] (1, 5). Homma et al (1) classified this disease into the following three stages based on the clinical findings, sputum bacteriology, and arterial blood gas analysis: Stage 1 clinical signs of bronchospasm and hypoxemia; stage 2 bronchospasm, pulmonary infection, and hypoxemia; stage 3, the terminal stage, bronchospasms, pulmonary infection due to *Pseudomonas aeruginosa*, hypoxemia, hypercapnia, and right heart failure. All of our patients were at stage 2. The backgrounds of the patients are summarized in Table 1. They had continuously expectorated 30 to 80 ml of purulent sputum per day for at least one year. The sputa of all the patients contained one or more pathogenic bacterium, such as *Hemophilus influenzae*, *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. *Pseudomonas aeruginosa* was found only intermittently in 3 patients. The chest radiograms of the patients showed diffuse nodular shadows, especially in the lower lung fields. 4) The chronic bronchitis group consisted of 12 patients (10 males and 2 females) with slight to mild chronic bronchitis, 50 to 76 years old (mean: 60 years), who were all smokers and had a mean one-second forced expiratory volume/forced vital capacity % of 69 and a mean % vital capacity of 95. Diagnosis of chronic bronchitis was based on the criteria of the American Thoracic Society (6). None of the patients showed marked bacterial infection of the airway at the time of the tests.

Method of lavage of the respiratory tract

Informed consent was obtained from all volunteers and patients after they had been given a full explanation of the procedures. Bronchoalveolar lavage and bronchial lavage were carried out using a flexible bronchofiberscope (Olympus BF, 1 T, Olympus Corporation) as reported previously (7, 8). For bronchoalveolar lavage, subsegmental or segmental lavage with 50 ml of saline was repeated 3 times in the right middle lobe or lingula, and

infused saline was recovered manually with a syringe (7). For bronchial lavage, the tip of the bronchofiberscope was inserted into a lower lobe bronchus without wedging, and 25 ml of saline was infused into the bronchus three times and recovered with an aspirator into a mucus trap at a negative pressure of 50 mmHg (8).

In all of the normal volunteers and 10 of the patients with diffuse panbronchiolitis, bronchoalveolar lavage was done on the right side and bronchial lavage on the left side at the same time. Twenty-two control patients and 4 of the patients with diffuse panbronchiolitis were subjected only to bronchoalveolar lavage. Twelve control patients and all of the patients with chronic bronchitis were subjected only to bronchial lavage.

The combined bronchoalveolar lavage fluid and bronchial lavage fluid from each subject were filtered through single layer of gauze and centrifuged at $250\times g$ for 10 minutes at 4°C to precipitate cells. The precipitated cells were suspended in 2–5 ml of RPMI-1640 medium, and total cell and differential cell analyses were performed as described previously (7, 8). The $250\times g$ supernatants were stored at -80°C and used for the following biochemical analysis.

Elastase activity was measured by a modification of the spectrofluorometric method of Oshima et al (9) with Suc-Ala-Pro-Ala-7-methylcoumaryl-4-amide (Peptide Institute, Osaka) as the substrate. The assay mixture contained 100 μM substrate, 0.2 ml of sample, 100 mM Tris-HCl buffer (pH 8.3) and 100 mM NaCl in a total volume of 1.5 ml, and incubation was carried out at 37°C for 1 hour. The fluorescence of the 7-amino-4-methylcoumarin (AMC) produced was measured at 440 nm with excitation at 380 nm in a Hitachi fluorescence spectrometer (Model F-3010). One unit of activity was defined as the amount that generated 1 pmol of AMC per minute.

The elastase inhibitors, phenylmethylsulfonyl fluoride and ethylenediamine tetraacetic acid were purchased from Sigma (St. Louis, MO). Purified neutrophil elastase was obtained from Elastin Products (Owensville, MO).

For assay of immunoreactive albumin and alpha 1-protease inhibitor, samples were diluted 1,000-fold (for albumin) and 100-fold (for alpha 1-protease inhibitor) with phosphate-buffered saline containing 0.5% Tween. These proteins were measured by the sandwich enzyme-linked immunosorbent assay described by Oellerichi (10). Rabbit and goat anti-serum albumin IgG antibody (Behringwerke AG, Marburg and Organon Teknika, West Chester), and rabbit peroxidase-labelled anti-goat IgG serum (Seikagaku Kogyo, Tokyo) were used for assay of albumin. Rabbit and goat anti-alpha 1-protease inhibitor IgG antibody (Behringwerke AG, and Organon Teknika) were used for assay of alpha 1-protease inhibitor. Standard serum was obtained from Behringwerke AG.

For analysis of the state of alpha 1-protease inhibitor

Table 1. Patient Profile of Patients with Diffuse Panbronchiolitis

No	Age (yr)	Sex	Duration of disease (yr)	FEV ₁ /FVC %	% Vital capacity	PaO ₂ torr
1	54	Male	4	57	49	70
2	59	Female	3	67	56	60
3	43	Female	8	72	57	72
4	22	Male	2	73	38	62
5	32	Female	10	67	55	82
6	55	Male	7	66	53	56
7	51	Male	1	68	54	67
8	42	Male	3	60	52	71
9	57	Male	10	62	38	62
10	35	Female	6	65	60	60
11	43	Female	5	67	68	69
12	45	Male	6	70	52	67
13	47	Female	15	63	53	67
14	48	Male	8	63	45	65
Mean 45.2			6.3	65.7	51.6	67.1
±SE ±2.7			±1.0	±1.1	±2.1	±1.7

by the western blot technique (11), 5 μ l samples of bronchial lavage fluids from the diffuse panbronchiolitis patients were treated with 2% 2-mercaptoethanol and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 7.5% acrylamide gel at 30 mA for 4–5 hours. The proteins were then transferred to polyvinylidene difluoride sheets (Millipore, Bedford, MA). The alpha 1-protease inhibitor bands were detected with goat anti-human alpha 1-protease inhibitor IgG antibody (Organon Teknika) and alkaline phosphatase-conjugated rabbit anti-goat IgG (Tago, Burlingame, CA). Purified alpha 1-protease inhibitor (Sigma, St. Louis, MO) was used as a source of native alpha 1-protease inhibitor. As further controls, alpha 1-protease inhibitor (Sigma) and neutrophil elastase were incubated at 37°C for 30 minutes in 0.10 M Tris-HCl-0.10 M NaCl (pH 8.3) with excess inhibitor or with excess elastase.

Statistical analysis

Results are expressed as mean $\pm$ standard error. The significance of differences between values for various groups was tested by the unpaired Student's t-test, and p values of 0.05 or less were considered to be significant. Correlations between elastase activities and neutrophil count (10^4 /ml) and alpha 1-protease inhibitor concentrations were assessed by calculation of Person correlation coefficients.

Results

I. Percentage recovery of infused saline on bronchoalveolar lavage and bronchial lavage

The percentage recovery of infused saline on bronchoalveolar lavage was significantly lower ($p < 0.01$) in

the diffuse panbronchiolitis group ($42.0 \pm 4.4\%$) than in the normal volunteer group ($66.1 \pm 1.1\%$) or the control patient group ($60.7 \pm 1.5\%$).

The percentage recovery on bronchial lavage was similar in the normal volunteer group ($43.9 \pm 2.2\%$), control patient group ($36.8 \pm 3.9\%$) and chronic bronchitis group ($41.6 \pm 3.6\%$), and tended to be higher in the diffuse panbronchiolitis group ($50.4 \pm 6.0\%$) than in the control patient group.

II. Cell profiles of bronchoalveolar lavage fluids and bronchial lavage fluids

The total cell counts (10^4 cells/ml) and cell profiles of both lavage fluids from control and diffuse panbronchiolitis patients are shown in Table 2. Both the total cell count and the cell profile of bronchoalveolar lavage fluids of the normal volunteer group were very similar to those of the control patient group. In the bronchoalveolar lavage fluids from these two control groups, alveolar macrophages and lymphocytes constituted about 90% and 8%, respectively, of the total cells and the percentage of neutrophils, eosinophils and other cells was below 1.5%. The mean total cell count was about 10-fold higher in the diffuse panbronchiolitis group ($p < 0.01$) than in either control group, showing increase of neutrophils to 66% and decrease of alveolar macrophages to 25%.

The total cell count in bronchial lavage fluid of the normal volunteer group was almost the same with that in the lavage fluid of the control patient group, and the cell profile of the normal volunteer group was essentially the same as that of the control patient group, although there were slight differences: the percentages of neutrophils and bronchial epithelial cells were higher,

Table 2. Cell Profiles of Bronchoalveolar Lavage and Bronchial Lavage Fluids

Sample	Group	No	Total cells 10^4 /ml	Percentage of total cells				
				AM	Ly	Neut	Eo	Others
BALF	NV	21	13.6	90.6	8.1	0.9	0.1	0.1
			± 1.2	± 1.1	± 1.0	± 0.1	± 0.02	± 0.02
	CP	22	14.9	89.1	7.6	1.4	0.4	0.7
			± 1.4	± 1.1	± 0.8	± 0.3	± 0.2	± 0.02
BLF	DPB	14	139.2	24.2	7.9	65.9	0.8	1.3
			± 4.3	± 4.1	± 1.6	± 4.6	± 0.4	± 0.5
	NV	21	17.7	59.9	5.0	10.5	0.9	22.6
			± 2.5	± 3.6	± 0.6	± 2.8	± 0.4	± 2.1
BLF	CP	12	17.4	33.0	3.3	19.8	0.4	44.4
			± 3.2	± 7.0	± 1.0	± 3.0	± 0.1	± 5.6
	DPB	10	416.8	9.4	3.6	81.8	0.7	4.3
			± 9.5	± 2.1	± 0.7	± 3.0	± 0.5	± 0.4
BLF	CB	12	17.5	45.5	3.9	18.0	1.8	32.9
			± 3.4	± 13.1	± 1.4	± 4.6	± 1.1	± 5.7

Values are means $\pm$ SE.

BALF: bronchoalveolar lavage fluid, BLF: bronchial lavage fluid, NV: normal volunteers, CP: control patients, DPB: diffuse panbronchiolitis, CB: chronic bronchitis, AM: alveolar macrophages, Ly: lymphocytes, Neut: neutrophils, Eo: eosinophils. Others consist of bronchial epithelial cells.

Table 3. Elastase Activities of Bronchoalveolar Lavage Fluid (BALF) and Bronchial Lavage Fluid (BLF)

Sample	Group	No	Units/ml		Units/ μ g albumin	
				p*		p*
BALF	NV	21	n.d.**		n.d.	
	CP	22	n.d.		n.d.	
	DPB	14	119.2 $\pm$ 30.2		3.331 $\pm$ 0.825	
BLF	NV	21	n.d.		n.d.	
	CP	12	1.0 $\pm$ 0.1		0.024 $\pm$ 0.002	
	DPB	10	1,083.3 $\pm$ 206.4	p < 0.01	22.558 $\pm$ 2.531	p < 0.01
	CB	12	14.5 $\pm$ 7.9	p < 0.05	0.326 $\pm$ 0.154	p < 0.05

Values are means $\pm$ SE, *Significance of difference from the value for the CP group by unpaired Student's t-test, **not detectable

and the percentage of alveolar macrophages was lower in the latter. In the diffuse panbronchiolitis group, the total cell count was about 20-fold those in the two control groups ($p < 0.01$), and showed an increase in neutrophils to about 80% of the cells with concomitant decreases in the other cells. The total cell count and cell profile of the chronic bronchitis group were very similar to those of the control groups.

III. Elastase activity (Table 3)

Elastase activity was not detectable in the bronchoalveolar lavage fluids of the normal volunteer and control patient groups, but was clearly detectable in all bronchoalveolar lavage fluids of the diffuse panbronchiolitis group although the activity varied in individual patients.

Elastase activity was also not detectable in the bronchial lavage fluids of the normal volunteer group, but very low elastase activity was detectable in the bronchial lavage fluids of the control patient group. The mean elastase activity per ml of lavage fluid and per μ g of albumin in the bronchial lavage fluid was about 1,000-fold and 15-fold higher, respectively, in the diffuse panbronchiolitis and chronic bronchitis groups than in the control patient group.

In the diffuse panbronchiolitis group, the mean elastase activity per μ g of albumin in the bronchial lavage fluids was about 7-fold higher than that in the bronchoalveolar lavage fluids ($p < 0.01$).

The effects of known elastase inhibitors on the elastase activities in the bronchial lavage fluids of the diffuse panbronchiolitis group were examined. In the present assay system, a commercial sample of purified neutrophil elastase activity was almost completely inhibited (95%) by 1 mM phenylmethylsulfonyl fluoride, a serine protease inhibitor, but was not inhibited by ethylenediamine tetraacetic acid, a metalloenzyme inhibitor (50 mM and 100 mM). The elastase activity in the bronchial lavage fluids of the diffuse panbronchiolitis group was also markedly inhibited (97%) by 1 mM phenylmethylsulfonyl fluoride, but not by 50 mM or 100 mM ethylenediamine tetraacetic acid.

The elastase activity (unit/ml) was positively correlated with the neutrophil count (10^4 /ml) in both bronchoalveolar lavage fluid ($n = 14$, coefficient of correlation = +0.909, $p < 0.01$) and bronchial lavage fluid ($n = 10$, coefficient of correlation = +0.849, $p < 0.01$) from the diffuse panbronchiolitis group.

Table 4. Alpha 1-protease Inhibitor (α 1-PI) Contents of Bronchoalveolar Lavage Fluid (BALF) and Bronchial Lavage Fluid (BLF)

Sample	Group	No	α 1-PI μ g/ml		α 1-PI ng/ μ g albumin	
				p value*		p value*
BALF	NV	21	1.25 $\pm$ 0.11	ns**	54 $\pm$ 5	ns
	CP	22	1.97 $\pm$ 0.47		58 $\pm$ 14	
	DPB	14	12.66 $\pm$ 2.87	p < 0.01	323 $\pm$ 30	p < 0.01
BLF	NV	21	1.38 $\pm$ 0.23	ns	46 $\pm$ 5	ns
	CP	12	2.07 $\pm$ 0.27		45 $\pm$ 5	
	DPB	10	32.34 $\pm$ 6.45	p < 0.01	648 $\pm$ 67	p < 0.01
	CB	12	11.15 $\pm$ 3.91	p < 0.05	162 $\pm$ 39	p < 0.05

Values are means $\pm$ SE, * Significance of difference from the value for the CP group by unpaired Student's t-test. ** not significant.

IV. Alpha 1-protease inhibitor content (Table 4)

The concentrations of alpha 1-protease inhibitor ($\mu\text{g/ml}$) and alpha 1-protease inhibitor/albumin ratio ($\text{ng}/\mu\text{g}$) were almost the same in the bronchoalveolar lavage fluids and bronchial lavage fluids of the normal volunteer and control patient groups. Both parameters were about 6-fold higher ($p < 0.01$) in the bronchoalveolar lavage fluids of the diffuse panbronchiolitis group than in those of the control patient group, and about 15-fold higher in the bronchial lavage fluids of the diffuse panbronchiolitis group ($p < 0.01$) and 5-fold higher in those of the chronic bronchitis group ($p < 0.05$) than in those of the control patient group.

In the diffuse panbronchiolitis group, the mean alpha 1-protease inhibitor/albumin ratio in the bronchial lavage fluids was about twice that of that in the bronchoalveolar lavage fluids ($p < 0.01$).

V. Western blot analysis of alpha 1-protease inhibitor in bronchial lavage fluid of the diffuse panbronchiolitis group

In Fig. 1, lane 1 shows native alpha 1-protease inhibitor (1-b, mol wt 54,000). After incubation of neutrophil elastase with excess alpha 1-protease inhibitor, a neutrophil elastase-alpha 1-protease inhibitor complex (2-a,

mol wt 78,000) and a cleaved 45,000 fragment of alpha 1-protease inhibitor (2-c) were formed (lane 2). The cleaved 45,000 fragment (3-c) with a small amount of the complex (3-a and 3-a') were found after incubation of alpha 1-protease inhibitor with excess elastase (lane 3) as reported by Goldstein and Doring (12); 3-a' represents a complex of neutrophil elastase and the cleaved alpha 1-protease inhibitor. In the bronchial lavage fluids, no intact alpha 1-protease inhibitor was detectable. Most of the alpha 1-protease inhibitor was detectable mainly in the position corresponding to the cleaved 45,000 fragment. There was a small amount of alpha 1-protease inhibitor in that corresponding to substances with a molecular weight of 78,000, the protease-alpha 1-protease inhibitor complex and also to substances of molecular weight 20,000–30,000 (4-d), the cleaved fragment.

Discussion

The airways, especially the peripheral airways and intermediate zones of patients with diffuse panbronchiolitis are thought to be narrowed by inflammation of the walls (1) and probably by excess fluid in the lumen. Therefore, it is likely difficult to obtain cells and substances from the lesions of the diffuse panbronchiolitis patients effectively by routine bronchoalveolar lavage (7, 13, 14), in which infused saline is recovered manually at a very low negative pressure. Therefore, in the present study, bronchial lavage in which infused saline is recovered at a higher negative pressure using an aspirator, was used. We have reported (15) that on bronchial lavage and bronchoalveolar lavage by the present method, the bronchial lavage fluid of normal volunteers contained much higher concentrations of the bronchial secretion components, such as airway mucus, S-IgA and lysozyme, than the bronchoalveolar lavage fluid. The cell profiles of bronchoalveolar lavage fluids from the normal volunteer and control patient groups were in good accordance with those reported by others (13, 14) and the percentages of neutrophils and bronchial epithelial cells were below 1–2%. In these control groups, the percentage of neutrophils was higher in the bronchial lavage fluid than in the bronchoalveolar lavage fluid. This result also is in accordance with other report (16). The presence of bronchial epithelial cells in bronchial lavage fluids of the control groups was due to recovery of infused saline at a higher negative pressure.

Analysis of the cells of both the bronchoalveolar lavage and bronchial lavage fluids of diffuse panbronchiolitis patients indicated predominant and marked increase of neutrophils in these lesions. These findings indicate that many neutrophils accumulate in the lesions of the diffuse panbronchiolitis patients. In contrast, reports of pathological examinations at autopsy, and studies of open lung biopsy and transbronchial lung specimens have indicated that characteristics of diffuse

Fig. 1. Western blot analysis of alpha 1-protease inhibitor ($\alpha 1$ -PI) in bronchial lavage fluids from patients with diffuse panbronchiolitis.

Lane 1: native $\alpha 1$ -PI, Lane 2: neutrophil elastase (50 $\mu\text{g/ml}$) incubated with excess $\alpha 1$ -PI (500 $\mu\text{g/ml}$) at 37°C for 30 min, Lane 3: $\alpha 1$ -PI (500 $\mu\text{g/ml}$) incubated with excess neutrophil elastase (250 $\mu\text{g/ml}$) at 37°C for 30 min, Lanes 4–6: bronchial lavage fluids from patients with diffuse panbronchiolitis.

Mol wt a: 78,000, b: 53,000, c: 45,000, d: 20,000–30,000

panbronchiolitis are infiltration of lymphocytes and foamy cells without significant infiltration of neutrophils into the respiratory bronchioles and more proximal airways (1). However, Kitagawa (17) reported that the foamy cells are the macrophages which have phagocytosed neutrophils. Thus the infiltration of foamy cells into the respiratory bronchioles indicates active infiltration of neutrophils into the lesions. Therefore, our above results are generally not inconsistent with the pathological findings.

Recently, Kindt et al (18) reported findings obtained by open lung biopsy and bronchoalveolar lavage fluid in 15 patients with bronchiolitis. In their patients, the percentage of neutrophils in the bronchoalveolar lavage fluid was significantly elevated (mean 54%), as in our diffuse panbronchiolitis patients. The disease entity they identified as bronchiolitis seemed, however, to be distinct from diffuse panbronchiolitis, because the histological findings in their biopsy specimens were consistent with those in bronchiolitis obliterans (19) or small airway disease (20), and showed acute or chronic bronchiolar inflammation with infiltration of neutrophils into the bronchiolar lumen and walls.

One possible cause of chemotaxis of neutrophils into the lesions in diffuse panbronchiolitis is bacterial infection, because all the diffuse panbronchiolitis patients had been expectorating purulent sputa containing pathogenic bacteria. It is still unknown, however, whether other factors were also related to the chemotaxis because the primary cause of diffuse panbronchiolitis is unknown.

Elastase activity was not detectable in the bronchoalveolar lavage fluids from the normal volunteer and control patient groups. Probably its activity would have been detectable if the bronchoalveolar lavage fluids from these groups had been concentrated 20- to 100-fold before the assay, as reported by previous investigators (21, 22). The much higher elastase activities in the bronchoalveolar lavage and bronchial lavage fluids from the diffuse panbronchiolitis group than in those from the two control groups clearly indicates that elastase activity is markedly elevated in the lesions of diffuse panbronchiolitis patients. Neutrophils (21–23), alveolar macrophages (21, 22, 24, 25) and bacteria (26, 27) are all possible sources of elastase. But in the diffuse panbronchiolitis group the elastase was probably derived mainly from neutrophils for the following reasons: 1) neutrophils were the main inflammatory cells in the lavage fluids, 2) there was a close correlation between the elastase activity and neutrophil count in the lavage fluids, and 3) neutrophil elastase is a serine protease (23), whereas the elastases released from alveolar macrophages (24, 25) and bacteria (26, 27) are metalloenzymes. The results of the inhibitor study indicated that elastase activity in the bronchial lavage fluids from diffuse panbronchiolitis patients is mainly due to neutrophil elastase.

The marked increase in elastase activity in the lavage

fluids from the diffuse panbronchiolitis group indicates that its activity exceeds its inhibitor activity in the lesions of diffuse panbronchiolitis patients. A previous study (28) showed that alpha 1-protease inhibitor is the main neutrophil elastase inhibitor in the bronchoalveolar lavage fluids of normal subjects, suggesting that it is the main protease inhibitor in the alveolar region and peripheral airways. In this work, we examined the content and state of alpha 1-protease inhibitor, because in diffuse panbronchiolitis the main lesions are located in the respiratory bronchioles, an intermediate zone between the airway and alveolar region. We observed increases in the immunoreactive alpha 1-protease inhibitor content in both bronchoalveolar lavage and bronchial lavage fluids from the diffuse panbronchiolitis group. These findings suggest an increase in alpha 1-protease inhibitor in the lesions of diffuse panbronchiolitis in response to inflammation. However, Western blot analysis indicated that most of the alpha 1-protease inhibitor was degraded to inactive lower molecular weight forms and the neutrophil elastase was closely related to the degradation. There are reports that alpha 1-protease inhibitor is inactivated by oxidation by substances released from neutrophils and macrophages (29, 30) and by hydrolysis with proteases (elastase) released from neutrophils and bacteria (12, 26). Probably neutrophils are related to inactivation of the alpha 1-protease inhibitor by oxidation as well as by the protease that they release in diffuse panbronchiolitis.

Neutrophil elastase is reported to be closely related to the pathogenesis of pulmonary emphysema (4) by hydrolyzing elastic fibers in the alveolus, and causing injury to bronchial epithelial cells (3, 31), degradation of immunoglobulin (IgG and S-IgA) (32–34), and enhancement of bronchial mucus secretion (35). Moreover, the elastase activities of sputum samples from patients with cystic fibrosis (36) and bronchiectasis (37), who have destructive lesions in the airways, are increased. Therefore it seems likely that in diffuse panbronchiolitis neutrophil elastase causes deterioration of the pathologic state and is related to damage of the peripheral airways.

From the present results and previous reports, we conclude that in diffuse panbronchiolitis, infiltration of neutrophils increases the elastase level in the lesions, and that this elastase is very important in the deterioration of the pathologic state, and tissue damage.

In the diffuse panbronchiolitis group, the increases in elastase activity and alpha 1-protease inhibitor content were greater in the bronchial lavage fluid than in the bronchoalveolar lavage fluid. Probably this is partly because the lesions are greater in the lower lung field than in the middle lobe, and partly due to the fact that components from the lesions of diffuse panbronchiolitis patients could be obtained more effectively by bronchial lavage than by bronchoalveolar lavage, and neutrophil

infiltration is thought to be more marked in the proximal airways than in the peripheral airways.

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