Secretory IgA(S-IgA) Levels in Sera from Patients with Diffuse Panbronchiolitis

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The serum S-IgA levels of 33 patients with diffuse panbronchiolitis (DPB) were compared with those of 13 patients with chronic bronchitis (CB) and 24 patients with bronchiectasis (BE), to obtain information on differences in the pathologic states in DPB and other chronic bronchial diseases. The S-IgA level was elevated in all three bronchial diseases, being significantly higher in DPB than in CB, and intermediate in BE. Persistent bacterial infections developed in most of the patients with DPB and two-thirds of those with BE, but in few of those with CB. Serum S-IgA levels were especially high in patients expectorating *Pseudomonas aeruginosa*-positive sputum, who constituted two-thirds of the patients with DPB and about one-third of those with BE. The highest levels over (100 $\mu\text{g/ml}$) were observed in far-advanced patients with DPB who expectorated *P. aeruginosa*-positive sputum. The increase in the serum level of IgA was less than that of S-IgA in all three diseases. These results indicate that the marked elevation of the serum S-IgA level in patients with DPB is due to extensive, chronic infection of the airways of the lungs, especially the peripheral airways, and that serum S-IgA is a useful marker for determining the clinical stage and the pathologic state of patients with diffuse peripheral airway diseases.

Key Words: Serum Secretory-IgA, Diffuse panbronchiolitis, Chronic bronchitis, Bronchiectasis

Secretory-IgA (S-IgA) is secreted locally from the airway walls onto mucous membranes (1-7), and is believed to play an important role in protecting the mucous membranes from infection (1-2, 8). Previous studies have suggested that its secretion from the bronchial walls is greater in the central airways than in the periphery ones (5-7).

S-IgA is normally present in serum at very low concentrations. Higher S-IgA concentrations have been found in patients with various diseases (9-14), but there have been few studies on the effects of chronic airway diseases on the serum S-IgA level.

Diffuse panbronchiolitis (DPB), which is common in Japan, is a chronic airway disease. Homma et al. have proposed that this disease is distinct from chronic bronchitis and that the main regions affected in DPB are the respiratory bronchioles (15-17). Most patients with DPB have the complication of chronic bacterial infection of the airways.

In this study, we compared the serum S-IgA levels of patients with DPB, chronic bronchitis (CB) and bronchiectasis (BE), to determine the effect of chronic airway infection on the serum S-IgA level,

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and to obtain information on differences in the pathologic states in DPB and other chronic airway diseases.

SUBJECTS, MATERIALS AND METHODS

Subjects

The subjects studied were as follows:

Eighteen healthy men aged 50 to 65 yrs (mean 53 yrs) who were non-smokers. They showed no abnormalities on physical examination, chest radiography, a complete blood count, serum biochemical analysis or forced expiratory spirometry.

Patients with chronic airway diseases consisted of 33 (8 men and 25 women) with DPB, aged 23 to 75 yrs, 13 (6 men and 7 women) with CB, aged 51 to 77 yrs, and 24 (6 men and 18 women) with BE, aged 42 to 71 yrs. All the patients regularly expectorated various amounts of sputum.

The diagnosis of DPB was based on the criteria proposed by the Committee for the Diagnosis of DPB, sponsored by the Ministry of Public Health Welfare of Japan (18). The diagnosis of CB was based on the criteria proposed by the American Thoracic Society (19). BE was diagnosed on the basis of radiographic evidence for airway dilatation associated with persistent sputum production. The diagnosis of DPB and BE were confirmed by computer tomography of the chest.

Data on the patients (age, sex, duration of disease and lung function) are summarized in Table 1.

Collection of serum

Venous blood was obtained from normal volunteers and patients after they had fasted overnight, and the serum was separated by centrifugation at 3,000 rpm at 4°C.

Collection of sputum

Sputa were collected in sterile plastic containers and subjects to routine bacteriological examination. The patients were classified according to the result of repeated bacteriological tests on their sputa as follows: Group I — Patients whose sputa contained mainly nonpathogenic normal bacterial flora; Group II — Patients whose sputa contained *Haemophilus influenzae*, *Streptococcus pneumoniae* or *Klebsiella pneumoniae*; and Group III — Patients whose sputa contained *Pseudomonas aeruginosa* continuously for at least half a year. *H. influenzae*, *S. pneumoniae*, *K. pneumoniae* and other bacteria were often present in the sputa from Group III, and *P. aeruginosa* was often present in the sputa from Group II.

Measurement of protein components

(1) S-IgA Serum S-IgA concentrations were measured by a micro enzyme-linked immunosorbent assay (ELISA), by a modification of the sandwich method described by Nakagawa (20). The immunoglobulin fraction of rabbit antiserum against the human secretory component (SC) was purchased from DAKOPATTS a/s, and further purified by DEAE-Sephacel column chromatography to isolate the IgG antibody. Alkaline phosphatase-conjugated goat antiserum to human IgA (specific to the α chain) was obtained from Tago, Inc. Purified human S-IgA from colostrum (Behringwerke, AG, Marburg), with a purity of approximately 96% was used as a standard.

Polystyrene microplates (Nunc-Immunoplate 1, Nunc Inc) were coated with anti-SC IgG antibody (coating). The plates were treated with 1% bovine serum albumin to prevent their non-specific binding of protein. A volume of 200 μ l of standard S-IgA

Table 1. Summary of data for patients examined

Group	n	Sex male/female	Age (yr) mean (range)	Duration of disease (yr) mean (range)	FEV _{1.0} /FVC, % mean $\pm$ SD
CB	13	6/7	68.7 (51-77)	5.7 (3-10)	64.4 $\pm$ 11.1
DPB	33	8/25	53.0 (23-75)	9.6 (2-40)	58.4 $\pm$ 11.3
BE	24	6/18	55.6 (42-71)	12.6 (1-22)	61.5 $\pm$ 13.6

Abbreviations CB: chronic bronchitis,
DPB: diffuse panbronchiolitis
BE: bronchiectasis

solution (2-250 $\mu\text{g/ml}$) or serum sample, diluted 1,000-fold with physiological saline, was introduced into the plates. Then alkaline phosphatase-labelled goat anti-human α chain antibody was added, and finally the concentration of alkaline phosphatase remaining on the plates was measured from the absorption at 405 nm with p-nitrophenyl-phosphate as substrate. Serum S-IgA levels were calculated from a standard curve obtained with colostrum S-IgA.

(2) Serum Monomer IgA (IgA) levels

Serum IgA concentrations were measured by a nephelometric immunoassay, with goat anti-human α chain antiserum in a nephelometer (NephrotekTM, Kyoto Daiich Co) (21). Standard serum and the antiserum were obtained from Kallestadt. IgG concentrations in the serum were measured by the same method.

Measurement of pulmonary function

Data on pulmonary function data ($\text{FEV}_{1.0}/\text{FVC}, \%$) were obtained with a Fudac 800, an apparatus capable of measuring general lung functions (Fukuda Electric Co).

Statistical analyses

Results are expressed as means $\pm$ SD. The significance of differences between values for groups was analyzed by the unpaired Wilcoxon's test, and p values of 0.05 or less were considered to be significant. Correlations between serum S-IgA levels and other parameters were assessed using Spearman's rank correlation coefficient.

RESULTS

1. Serum S-IgA concentration

The S-IgA concentration in serum samples of healthy controls ranged from 4 to 12 $\mu\text{g/ml}$ (mean value, $8.1 \pm 1.8 \mu\text{g/ml}$).

The S-IgA levels in all three groups of patients were higher than those of the healthy controls; the level was highest in the DPB group, and the levels in the CB and BE groups were similar as shown in Table 2. All sera from CB patients contained S-IgA at levels of below 35 $\mu\text{g/ml}$, and all sera from the BE patients had levels of below about 80 $\mu\text{g/ml}$, but 9 of 28 sera from the DPB patients had levels of over 80 $\mu\text{g/ml}$, as shown in Fig. 1.

And as shown in Fig. 1, about 60% of the sera from the DPB patients belonged to group III, 30% to group II and 10% to group I, respectively. Of the sera from BE patients 30%, 20% and 45% belonged to groups I, II and III, respectively. Most of the sera from the CB patients belonged to group I.

Fig. 1 shows that in general, the serum S-IgA levels were lowest in group I and highest in group III. All sera in group I had levels of below 30 $\mu\text{g/ml}$, and most of those in group II had levels of below 40 $\mu\text{g/ml}$. In group III, the serum S-IgA levels were higher in DPB patients than in BE patients. Sera with S-IgA levels of over 100 $\mu\text{g/ml}$ were found only in DPB patients in group III.

2. Serum IgA concentration

The IgA level in the serum samples of healthy

Table 2. Concentrations of S-IgA, IgA and IgG in sera of patients with chronic bronchial diseases.

Group	n	S-IgA ($\mu\text{g/ml}$)		IgA (mg/dl)		IgG (mg/dl)		p*	p**
NC	17	8.1 $\pm$ 1.8		221 $\pm$ 51		1,146 $\pm$ 138			
CB	13	19.8 $\pm$ 7.8	a	250 $\pm$ 94	ns	1,265 $\pm$ 339		ns	
DPB	33	53.5 $\pm$ 48.3	a	371 $\pm$ 168	a	1,341 $\pm$ 645	b	ns	ns
p***		a		ns		a			
BE	24	31.5 $\pm$ 20.0	a	346 $\pm$ 111	a	1,624 $\pm$ 319	b	a	a

Values are means $\pm$ SD
p*: difference from normal controls
p**: difference from CB group
p***: difference between DPB and BE group
a: $p < 0.01$, b: $p < 0.05$, ns: not significant
NC: normal controls

Fig. 1. Relation of bacteria in sputum and serum S-IgA levels in patients with chronic bronchial diseases.

Patients were classified into groups I, II, or III according to the bacterial contents of the sputa, as described in the Results.

Horizontal lines show mean values.

H. Inf: *Haemophilus influenzae*, S. pneum: *Streptococcus pneumoniae*, K. pneum: *Klebsiella pneumoniae*

controls ranged from 120 to 298 mg/dl (mean value of 221 ± 51 mg/dl).

Fig. 2 shows that the sera of, 3 of 13 CB patients, 23 of 33 DPB patients and 15 of 24 BE patients had levels of over 300 mg/dl, the upper limit for the healthy controls, the levels of most other samples from the patients being similar to those of the healthy controls. As shown in Table 2, the mean serum IgA level of the CB patients was not significantly higher than that of the healthy controls, but the mean levels of the DPB and BE patients, which were very similar, were significantly higher than the mean for the healthy controls ($p < 0.01$), and slightly higher than that of the CB patients ($p < 0.05$) (Table 2).

The differences between the IgA levels of groups I, II and III were in general smaller than those between their S-IgA levels, as shown in Figs. 1 and

Fig. 2. Relation of bacteria in sputum and serum IgA levels in patients with chronic bronchial diseases.

2. The sera of DPB patients in group III had the highest IgA levels. Thus, the sera of DPB patients in group III exhibited higher IgA levels than those in group II ($p < 0.01$), while the sera of BE patients in groups III and II had similar IgA levels.

Table 2 also shows the serum IgG levels in each group. The IgG levels in the CB and DPB patients were not significantly higher than those of the healthy controls, but the IgG levels in the BE patients were much higher.

3. Correlations between serum levels of S-IgA and other parameters

A significant positive correlation between the serum S-IgA and IgA levels was found in patients with DPB ($r = +0.681$, $p < 0.01$) and with CB ($r = +0.484$, $p < 0.01$), as shown in Fig. 3, and a slightly positive correlation was found between the levels in BE patients ($r = +0.238$, $p < 0.05$), (data not shown). The regression line for the DPB group is to the right of that for the CB group.

No significant correlation was found between the serum S-IgA and IgG levels ($r = -0.072$, -0.007 and -0.023 for DPB, BE and CB patients, respectively).

Fig. 3. Correlations of serum S-IgA and IgA levels in patients with DPB and CB.

●: patient with DPB, ○: patient with CB

DISCUSSION

In this study we found that the serum S-IgA concentration was increased in patients with chronic bronchial diseases. The increase was significantly more in patients with DPB than in those with CB or BE.

Bacteriological examination of the sputa of the patients indicated that almost all the DPB patients and two-thirds of the BE patients had persistent bronchial infections, while most of the CB patients did not, and that two-thirds of the DPB patients and about half the BE patients had bronchial infections or colonies of *P. aeruginosa*. There are reports that most patients with advanced DPB have chronic bacterial infections of the airways (15-16), and that many patients with far-advanced cystic fibrosis (22) and DPB (15-16) are infected by *P. aeruginosa*. The main pathogenic bacteria infect the airways of DPB patients are *P. aeruginosa*, *H. influenzae*, *S. pneumoniae* and *K. pneumoniae* (23). In general, inflammatory and destructive changes of the airway are thought to be more severe in patients infected with *P. aeruginosa* than in those infected with other bacteria, partly because bronchial infection and colonization of *P. aeruginosa* occurs more readily in patients with destructive lesions in the airways, and partly because there are few effective drugs for *P. aeruginosa*. Fig. 1 shows that the elevation of the serum S-IgA level was more marked in patients in-

fected with *P. aeruginosa* than in those infected with other bacteria, such as *H. influenzae* and *S. pneumoniae*, or in those not infected with bacteria.

Tanimoto classified DPB into three stages (I)-(III) on the basis of clinical and pathological findings (24). Groups I, II and III in the present study correspond roughly to his stages (I), (II) and (III), respectively. This our results on serum S-IgA levels in DPB patients suggest that the serum S-IgA level is useful for estimating the clinical stage of DPB patients.

The S-IgA level in the serum of patients with chronic bronchial diseases may be increased in two ways: by increase in release of S-IgA from the airways or other organs into the blood stream and by decrease in removal of S-IgA from the blood stream. At least the former mechanism probably occurred in our patients, because S-IgA is actively synthesized in the lower airway walls, and these patients had various kinds of lesions of the lower airways. Immunohistological studies have suggested that secretion of S-IgA from the lower airways is more active in the central than in the peripheral airways (5-6). But S-IgA is probably also secreted from the peripheral airways, as IgA-containing cells have been observed in small, round bronchi and bronchioles (5), and as bronchoalveolar lavage fluid, which mainly contains components from the peripheral airways and alveolar regions, contains S-IgA as a predominant component of the total IgA (4, 25). As mentioned above the incidence of inflammatory and destructive lesions in the lower airways, especially in those of the periphery, is thought to be much greater in DPB patients than in CB patients. Thus, the higher serum S-IgA levels in DPB patients, than in CB patients, may be partly because S-IgA in the airways passes into the blood stream more easily in DPB patients than in CB patients, even though the levels of S-IgA in the airways of patients with DPB and CB may be very similar.

We previously reported that there was no significant difference between the S-IgA levels in the sputum samples from patients with DPB and CB (26). But we could not determine the exact amount of synthesis of S-IgA in the bronchial walls only from the S-IgA level in sputum samples, because the S-IgA in the bronchial lumen is destroyed by various kinds of proteases especially when there is marked

infection (8). Bronchial IgA (S-IgA) secretion has been suggested not to be impaired in mild to moderate CB, but to be impaired in far-advanced CB due to the damage of the bronchial epithelium (6, 27). A characteristic of DPB is infiltration of lymphocytes and plasma cells into the peripheral airways (15-17), and Satoh et al. (28) observed hyperplasia of bronchus-associated lymphoid tissue (BALT), and the presence of IgM, IgA and IgG-containing cells in the BALT in the peripheral airways of DPB patients who had high levels of serum IgA. These results suggest that, in DPB, the syntheses of IgA and S-IgA in the peripheral airways is not always impaired, but rather increased to above the control level.

In this study, the extent of increase in the serum concentration of IgA over the normal level was less than that of S-IgA in all three bronchial diseases. The serum IgA level was sometimes elevated and sometimes not, the elevation being greater in patients with DPB and BE than in those with CB. There are reports that the serum IgA level is elevated or remains unchanged in patients with CB (29-32) and BE (32-33). Hirayama (34) observed that increase in the serum IgA level was greater in patients with DPB than in those with CB. In the present study we found a slight correlation between the serum S-IgA and IgA levels, but no correlation between the serum S-IgA and IgG levels in patients with DPB, CB and BE. This correlation was thought to be partly because systemic IgA production is enhanced in patients with chronic bronchial diseases, who have high serum S-IgA levels. The shift of the regression line for values in DPB patients to the right of the line for values for the CB group is partly because bronchial S-IgA is more easily released into the blood stream of DPB patients than into that of CB patients.

The mean serum S-IgA level of BE patients was intermediate between those of DPB and CB patients, indicating that there is a common pathologic change leading to increase in the S-IgA level in the lower airways of DPB and BE patients. Of the patients expectorating *P. aeruginosa*-positive sputum, DPB patients had significantly higher S-IgA levels than BE patients (Fig. 1). There are reports that in advanced case of DPB, dilatation of proximal terminal conducting bronchioles resembles bronchiectasis,

and that inflammatory changes including bacterial infection and bronchiectatic changes extended throughout the lungs from the peripheral airways to the central airways (15-17). Therefore, in general, the area of bronchial lesions may be larger in DPB patients than in BE patients. The higher serum S-IgA levels in patients with DPB than in those with BE may thus be partly due to the larger area of bronchial lesions in the patients with DPB.

In group III, the serum S-IgA levels of about half of the DPB patients were similar almost to those of BE patients (Fig. 1). Further studies are necessary to clarify the relationship between the pathophysiology of DPB and serum S-IgA levels in this disease, and the mechanism of increase in the serum S-IgA level in chronic bronchial diseases.

We concluded from our results that the extent, area and location of chronic inflammation, especially of chronic infections of the airways, are closely related with the extent of increase of the serum S-IgA level in chronic bronchial diseases, and that measurement of the serum S-IgA level is useful in diagnosing and examining the pathologic states of diffuse peripheral airway diseases.

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