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Serum Secretory Leukoprotease Inhibitor Levels in Chronic Bronchitis and Sjögren's Syndrome

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Andoh, Y., Shimura, S., Saitoh, H., Sasaki, T., Mitsuhashi, H. and Shirato, K. *Serum Secretory Leukoprotease Inhibitor Levels in Chronic Bronchitis and Sjögren's Syndrome.* Tohoku J. Exp. Med., 1997, **182** (4), 319 325 —— Serum secretory leukoprotease inhibitor (SLPI) is synthesized and secreted by serous cells in airway glands, and the serum level is speculated to reflect airway gland hyperplasia. To test this hypothesis, we measured the serum SLPI in 38 clinically stable patients with chronic bronchitis (3F and 35M; 58±2 years, mean±S.E.M.) (group CB), 24 patients with Sjögren's syndrome (24F; 52±3 years) (group SG), and compared it with 12 healthy control subjects (6F and 6M; 54±3 years) (group CN) using an enzyme-linked immunosorbent assay (ELISA). The serum SLPI from group CB (105±8 ng/ml) was significantly higher than that from group CN (60±2 ng/ml) and further, it significantly correlated with sputum volume per day. Although the mean value of serum SLPI from group SG (64±5 ng/ml) was not different from that from group CN, serum SLPI significantly correlated with the duration of respiratory symptoms (cough and/or sputum) in group SG. In conclusion, serum SPLI level reflects airway gland hyperplasia, suggesting that SLPI measurement is a possible laboratory method to estimate airway glandular hyperplasia. —— serum secretory leukoprotease inhibitor; chronic bronchitis; Sjögren's syndrome; bronchial gland hyperplasia

Secretory leukoprotease inhibitor (SLPI) is a 12-kDa serine protease inhibitor (Lee et al. 1993) which has been called bronchial mucous proteinase (Carp and Janoff 1980) or antileukoprotease (De Water et al. 1986), and is produced locally and secreted by the serous cells of airway glands (De Water et al. 1986; Lee et al. 1993) which play a primary role in human airway secretion (Reid 1960). Kida et

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al. (1992) and Kramps et al. (1984) have reported that serum SLPI is increased in patients with pneumonia and with acute exacerbation of chronic obstructive pulmonary disease (COPD), respectively. Since various mediators and neutrophil-derived elastase released in inflamed airways all induce airway gland secretion (Shimura and Takishima 1994), elevated serum SLPI level in these patients is probably due to increased bronchial gland secretion. Meanwhile, these chemical mediators and substances induce not only glandular secretion but are also speculated to induce glandular hyperplasia which is a pathohistological finding characteristic to chronic obstructive pulmonary disease including chronic bronchitis (Reid 1960; Jamal et al. 1984) and bronchial asthma (Aikawa et al. 1992). Airway glandular hypertrophy produces persistent and significant amounts of sputum without any apparent infections in chronic airway diseases (Shimura and Takishima 1994). However, we have not yet any useful clinical tool for estimating airway glandular hyperplasia in these patients. The SLPI level is speculated to reflect airway gland hyperplasia in addition to glandular secretion in response to various stimuli by infections. To test this hypothesis, we measured serum SLPI in clinically stable patients with chronic bronchitis, a disease characterized by hyperplasia of airway glands (Reid 1960; Jamal et al. 1984). Further, the involvement of the airways in Sjögren's syndrome has recently attracted much attention. Newball and Brahim (1977) have found evidence of obstructive airway disease in patients with Sjögren's syndrome. In our previous study using autopsied lungs (Andoh et al. 1993), we found airway glandular hyperplasia which is similar to that of chronic bronchitis. Therefore, we measured serum SLPI in patients with chronic bronchitis and Sjögren's syndrome in this study.

Subjects and Methods

Subjects

The thirty-eight clinically stable patients with chronic bronchitis (3F and 35M; 58±2 years, mean±S.E.M.) (group CB), chosen for the present study, were all smokers (22 ex- and 14 current smokers) except for 2 female patients. The diagnosis of chronic bronchitis was made according to the criteria of the American Thoracic Society (1962). They all were chronic mucopurulent bronchitic patients according to the British Medical Research Council (1965) definition of chronic bronchitis. Patients with cystic bronchiectasis were excluded but we included 11 patents with diffuse, slight and tubular bronchiectatic changes by CT images which were judged to result from chronic bronchitis itself by their medical records (Nagaki et al. 1992). These patients did not show any significant exacerbation for 2 or more months before the SLPI measurement and were clinically stable without any findings of pneumonia. Any patient in group CB did not show abnormal values in CRP or peripheral blood leukocyte count at the measurement of serum SLPI. They were also confirmed to be free of any nasal diseases

including chronic sinusitis and allergic rhinitis by symptomatic, laboratory and radiographic findings. Twenty-four primary Sjögren's syndrome patients with cough and/or sputum (24F; 52±3 years) (group SG) were all non-smokers and chest radiographs revealed no diffuse abnormal reticulonodular shadows in any lung field. The diagnosis of Sjögren's syndrome was made from a combination of clinical, laboratory and histologic data, according to previously described criteria (Fox et al. 1986). Twelve control patients (6F and 6M; 54±3 years) (group CN) were all non-smokers and free of upper or lower respiratory symptoms or any pulmonary diseases.

Pulmonary function tests were performed one month within SLPI measurement. The average values of sputum volume per day for three days within one week of SLPI measurement were used for data analysis.

SLPI assay

Serum samples (25 μl) were diluted 21 times. The half-length SLPI (1/2 SLPI) was produced as previously reported (Stetler et al. 1989; Kida et al. 1992). The sandwich immunoassay for human SLPI was modified as follows: polystyrene balls, 6.3 mm in diameter were dipped in anti-1/2 SLPI in PBS (20 μg/ml) and incubated at 4°C overnight. Immunolization was terminated by rinsing the balls three times in PBS; next, the balls were coated with 1% bovine serum albumin and PBS at 4°C for 2 days. Recombinant human SLPI was used as the standard. Anti-1/2 SLPI-IgG fixed balls and 200 μl of serum sample or standard solution were placed in a glass tube. After incubation for 1.5 hour at 37°C, the balls were washed three times with saline, and 0.4 ml of 0.1 M phosphate-citrate buffer (pH 3.0) containing 0.045% tetramethylbenzidine and 0.017% hydrogen peroxide was added. The mixture was incubated at 37°C for 30 minutes, and the enzyme-substrate reaction was terminated by the addition of 1 ml of 0.1 N H_2SO_4. Absorbance was measured at 450 nm. We could measure 2.0 to 500 ng/ml of human SLPI by this assay.

Statistics

Data are expressed as mean±S.E.M. For group comparisons, Mann-Whitney test was used. The regression coefficient was also used for statistical analysis. Significance was accepted at $p<0.05$.

Results

All patients in group CB expectorated a large amount of sputum (35±3 ml/day; range, 7 to 72 ml/day), whereas patients in group SG experienced chronic cough but did not show persistent chronic sputum production of 10 ml or more per day. Macroscopic appearance of sputum samples from almost all patients in group CB showed "mucopurulent" except 5 patients (2 mucoid and 3 purulent sputum samples). All patients in group SG expectorated mucoid sputum.

Although pulmonary function tests were not available in 8 patients of group SG, those from the other 16 patients did not show any abnormal findings. In contrast, significant obstructive impairment ($FEV_{1.0}\% < 70\%$) was observed in 22 of 38 patients of group CB and the mean $FEV_{1.0}\%$ was $68 \pm 8\%$ ($n = 38$).

Serum SLPI concentrations from group CB (105 ± 8 ng/ml; range, 35 to 203 ng/ml) were significantly higher than group CN (60 ± 2 ng/ml; range, 40 to 76 ng/ml) ($p < 0.01$) (Fig. 1). Further, serum concentrations significantly correlated

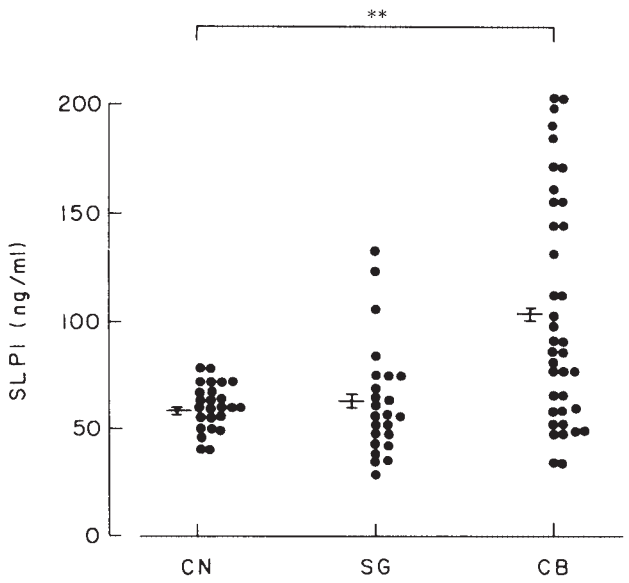

Fig. 1. Serum SLPI concentration (ng/ml) from healthy controls (group CN), Sjögren syndrome patients (group SG) and chronic bronchitis patients (group CB). The serum SLPI from group CB was significantly higher than that from group CN, while that from group SG was not. Bars represent mean ± S.E.. $^{**}p < 0.01$, comparison between groups CN and CB.

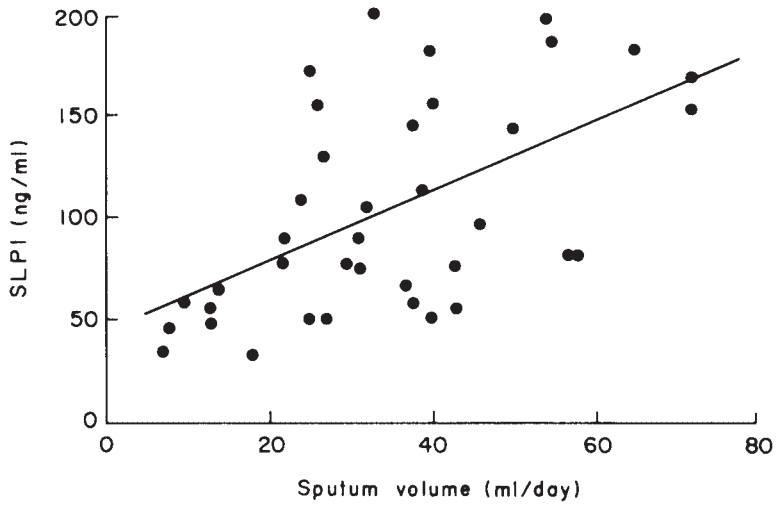

Fig. 2. Relationship between serum SLPI concentration (ng/ml) and sputum volume (ml/day) in chronic bronchitis patients (group CB). Serum SLPI level significantly correlated with the sputum volume ($r = 0.57$, $p < 0.001$).

with sputum volume in group CB ($r=0.57$, $p<0.001$) (Fig. 2). Exception of 5 patients who expectorated mucoid or purulent sputum did not alter the difference between groups CB and CN ($p<0.01$) or the relation between SLPI level and sputum volume ($r=0.55$, $p<0.01$). There was no significant relation between SLPI level and obstructive impairment ($FEV_{1.0}$, $r=-0.21$) or duration of symptoms (cough and sputum) (years, $r=0.18$) in group CB.

Serum SLPI concentrations in group SG (64 ± 5 ng/ml) were not different from those in group CN (Fig. 1). However, serum SLPI concentration significantly correlated with the duration of respiratory symptoms (cough and/or sputum) (years, $r=0.44$, $p<0.05$).

Discussion

Airway glandular hyperplasia is characteristic of chronic bronchitis, and airway submucosal gland volume or amount (i.e., Reid index [Reid 1960]) is known to correlate with sputum volume during clinical remission in chronic bronchitis (Jamal et al. 1984). SLPI is produced locally and secreted by serous cells of airway glands (De Water et al. 1986; Lee et al. 1993). Although SLPI gene was found to express in human airway epithelial cell line (Abe et al. 1991), superficial epithelium was not significantly or positively stained by a immunohistological experiment (Lee et al. 1993), which was confirmed recently by our experiment (Saitoh et al. 1996). The present study showed that the serum SLPI level in group CB was significantly higher than group CN, and further, it significantly correlated with sputum volume. We have not any direct evidence for the bronchial gland hyperplasia of patients in group CB. Taken together with these previously-reported findings, it is, however, possible that serum SLPI level reflects bronchial gland hyperplasia.

To exclude as much as possible the effects of airway infection or pneumonia (Kida et al. 1992), we chose clinically stable chronic bronchitis patients who showed no clinical or laboratory findings suggestive of exacerbation or pneumonia for 2 months or more before SLPI measurement. Serum SLPI level of group CN in the present study was similar to that of a previous report (Kida et al. 1992). Further, since nasal glands are also known to contain SLPI (Lee et al. 1993), we carefully excluded patients with nasal diseases including chronic sinusitis and allergic rhinitis. One third of serum SLPI values in group CB overlapped those in group CN, as did the Reid index (Thurlbeck and Angus 1964; Jamal et al. 1984). When pneumonia, airway infections and nasal diseases are excluded, serum SLPI over 80 (or 100) ng/ml may indicate airway gland hyperplasia, a laboratory finding suggestive for chronic bronchitis.

In our previous morphometric study using autopsied lungs (Andoh et al. 1993), we found that airway submucosal glands in Sjögren's syndrome showed significant hyperplasia compared to control subjects, which was similar to those from chronic bronchitis patients. The most likely explanation for the hyper-

plasia of airway secretory cells in Sjögren's syndrome is that some secretory disturbances in airway epithelial and gland cells, as in the exocrine gland cells, induced some defects of airway defensive mechanisms, repeated infections, chronic inflammation and resultant hyperplasia of secretory cells. Therefore, we included Sjögren's syndrome patients for the present study and found a significant relation between the duration of respiratory symptoms and serum SLPI concentration. However, the serum SLPI concentration in group SG was lower than that in group CB. This may be due to the large variation in the severity of airway changes and/or duration of respiratory symptoms (couch and sputum) of patients in group SG. Further, smaller number of neutrophils in the airways of group SG may be present than group CB since all sputum samples in group SG showed mucoid while almost all pateints in group CB expectorated mucopurulent sputum. Namely, it is possible that neutrophil-derived substances (elastase etc.) affected the serum SLPI level by the elevated secretion of airway submucosal glands in the present study. Although airway gland hypertrophy is also a histological finding of bronchial asthma, the symptoms of bronchial asthma patients, including sputum expectoration, often change. Namely, various unstable factors or stimuli in airways of bronchial asthma influence airway submucosal gland secretion, affecting serum SLPI. Therefore, we did not include asthma patients in the present study.

In conclusion, serum SLPI level reflects airway gland hyperplasia, suggesting that the SLPI measurement is a possible laboratory method to estimate airway glandular hyperplasia which is a pathological criteria of chronic bronchitis. Chronic bronchitis or its clinical features is frequently combined with almost all chronic lung diseases, affecting their clinical courses. Measurement of serum SLPI may be useful for the diagnosis of chronic bronchitis.

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