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Quantitative Determination of Immunoglobulin in Sputum from Chronic Obstructive Lung Disease

Yoshio Kimura

Second Devision, Department of Internal Medicine, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan.

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Abstract. Quantitative analysis of IgG, IgA and IgM in serum and sputum from patients with chronic obstructive lung disease was carried out by radial immunodiffusion method. The concentration of IgE in the sera and sputa was also determined by radioimmunosorbent test. The mean IgA level in the sputa showed no significant difference between the control, bronchial asthma, chronic bronchitis and pulmonary emphysema groups, but the sputum from patients with pulmonary emphysema had a tendency to give higher mean values of IgA. The mean value of IgE in the sputum was not significantly different among four groups, but the number of sputa with measurable IgE concentration was much greater in chronic obstructive lung disease group. There was no significant difference of the mean IgG level in the sputa between the four groups, but there was a tendency to increase the mean IgG level in sputa from the bronchial asthma group. IgM was detectable in a few samples. Results of the present study suggested that the increased IgA and IgE in sputa might play a role in causative and/or pathophysiological mechanism in cases of chronic obstructive lung disease.

Key Words: immunoglobulin pulmonary emphysema, bronchial asthma, chronic bronchitis

Introduction

Tomasi and his associates[1] have shown that immunoglobulin contents in various external secretions are quite different from those in serum. It has been reported that the major class of immunoglobulin in external secretions is IgA[2]. Since the discovery of IgE[3], many investigators[4,5] have measured the serum level of this immunoglobulin in various allergic disorders. It has been also proven that IgE is present in certain external secretions such as nasal secretions[6], colostrum and sputum[7]. Although there have been many studies concerning serum immunoglobulin levels in patients with chronic obstructive lung disease (COLD)[8-10], studies concerning sputum levels of immunoglobulins from patients with COLD are rare [11]. The aim of present study was to compare levels of various immunoglobulin classes in sputa obtained from patients with COLD in an effort to clarify the pathophysiology of COLD.

Materials and Methods

Forty-one sputum samples were obtained from

patients with COLD and normal adult cigarette smokers. Samples included 14 sputa from patients with bronchial asthma, 12 from patients with chronic bronchitis, 8 from patients with pulmonary emphysema and 7 normal adult cigarette smokers as control (Table 1).

Table 1 Characteristics of study groups.

	NUMBER OF PATIENTS	AGE	
		MEAN	SD
CONTROL	7	36.4 (25~50)	4.2
BRONCHIAL ASTHMA	14	57.7 (28~78)	3.9
CHRONIC BRONCHITIS	12	63.4 (32~78)	4.4
PULMONARY EMPHYSEMA	8	70.5 (56~84)	3.2
TOTAL	41		

The diagnosis of COLD was made on the basis of the criteria of a report of the American College of Chest Physicians and American Thoracic Society joint committee[12].

Sputum specimens were expectorated into plastic cups and were homogenized in a glass tissue grinder with 10 times volume of 0.01M ethylenediaminetetraacetate (EDTA), pH 8.0. The samples were stirred at 4°C overnight and then centrifuged at 3,000 × g for 30 min. The supernatants were subjected to the assay.

Blood samples were taken simultaneously with expectoration of sputum by phlebotomy, and the sera were separated within 1 h. The sputum and serum samples were frozen at −20°C until use.

Total protein concentration was determined by the method of Lowry[13]. The concentration of albumin, IgG, IgA and IgM was estimated by single radial immunodiffusion according to the method of Mancini[14] with slight modification using standard sera obtained from a commercial source. Since the use of a serum standard (7S) for IgA underestimates the levels of secretory IgA (11S) by approximately threefold, the results for IgA in sputum were multiplied by 3[15].

IgE concentration was determined by radioimmunosorbent test obtained from a commercial source (Phadebas IgE RIST kit).

Local immunoglobilon production in the sputum was estimated by means of clearance calculations [16], which have been considered suitable for estimation of such production in other mucous membrane. The following formula was used:

$$local\ production = Ig(Sp) - \frac{Alb(Sp)}{Alb(Se)} \times Ig(Se)$$

where Ig(Sp) means the concentration of the immunoglobulin in question in the collected sputum and Ig(Se) means the serum concentration of the same immunoglobulin. Alb(Sp) and Alb(Se) are the albumin concentrations in the sputum and serum, respectively, in the same individual. Since albumin is not produced in the place where the sputum is excreted, ratio Alb(Sp)/Alb(Se) can be regarded as an index of the ultrafiltration of the macromolecular substance from serum to sputum.

Results

The mean value of the total protein, albumin, IgG, IgA, IgM and IgE in the sputa are illustrated in Table 2. Albumin, IgG and IgA in the sputa were detected in all samples and only 6 of 41 sputa (14.6%) gave a positive ring on the IgM immunoplates. IgE in the sputum specimens was detected in 25 of 34 samples (73.5%) from patients with COLD and in 2 of 7 samples (28.6%) from the control group. Namely, frequency of sputum with measurable IgE concentration in COLD was higher than that in control.

Since the mean value of IgA concentration in the sputum was 208.5±27.1 mg/dl (mean±SE) and that of IgG in the sputum was 40.6±4.2 mg/dl, it can be said that IgA is the predominant immunoglobulin in the sputum.

Table 3 lists the local production of each immunoglobulin, calculated by the formula. The apparently high local production of IgA and IgE in the patients with COLD is worthy of note. IgG also appeared to be produced locally, but to a smaller extent.

Fig. 1-a shows the value of IgG concentration in each group. There was no significant difference between the control, bron-

Table 2 Concentration of IgG, IgA, IgM, IgE, albumin and total protein in the sputum.

	CONTROL		BRONCHIAL ASTHMA		CHRONIC BRONCHITIS		PULMONARY EMPHYSEMA		TOTAL	
	MEAN SE (RANGE)	POSITIVE CASE (%)	MEAN SE (RANGE)	POSITIVE CASE (%)	MEAN SE (RANGE)	POSITIVE CASE (%)	MEAN SE (RANGE)	POSITIVE CASE (%)	MEAN SE (RANGE)	POSITIVE CASE (%)
TOTAL PROTEIN (mg/dl)	351.4 38.7 (220~500)	7 (100)	431.4 69.2 (150~1030)	14 (100)	370.0 52.7 (110~820)	12 (100)	391.3 78.4 (190~790)	8 (100)	392.8 33.7 (110~1030)	41 (100)
ALBUMIN (mg/dl)	29.3 15.0 (8~119)	7 (100)	41.6 11.5 (16~183)	14 (100)	40.5 13.2 (12~180)	12 (100)	32.0 6.57 (14~67)	8 (100)	38.5 6.30 (8~183)	41 (100)
IgG (mg/dl)	26.3 1.73 (12~33)	7 (100)	45.8 7.25 (11~110)	14 (100)	38.2 7.10 (11~82)	12 (100)	44.3 11.9 (11~108)	8 (100)	40.6 4.18 (11~110)	41 (100)
IgA (mg/dl)	136.3 35.2 (36~306)	7 (100)	172.6 42.1 (51~675)	14 (100)	202.0 27.4 (48~372)	12 (100)	318.6 91.7 (129~756)	8 (100)	208.5 27.1 (36~756)	41 (100)
IgM (mg/dl)		0	19.5 6.25 (13.5~26)	2 (14.3)	35.3 9.25 (5.7~44.5)	3 (25)	(58)	1 (12.5)	28.9 7.92 (13.5~58)	6 (14.6)
IgE (IU/ml)	5.6 3.72 (0~24)	2 (28.6)	74.1 27.9 (0~400)	13 (92.9)	24.6 11.4 (0~145)	9 (75)	44.3 11.7 (0~70)	6 (75)	54.6 14.0 (0~400)	30 (73.2)

Table 3 Locally produced IgG, IgA and IgE concentrations in the sputum.

	CONTROL	BRONCHIAL ASTHMA	CHRONIC BRONCHITIS	PULMONARY EMPHYSEMA
	MEAN SE (RANGE)	MEAN SE (RANGE)	MEAN SE (RANGE)	MEAN SE (RANGE)
IgG (mg/dl)	21.6 2.2 (14.4~29.2)	27.5 6.9 (5~98.8)	19.1 6.8 (0~61.1)	28.0 12.4 0~99.9)
IgA (mg/dl)	134.9 35.1 (35.2~305.2)	170.1 41.5 (49.4~664.2)	200.6 29.5 (45.9~368.7)	314.9 91.4 (119.3~746.3)
IgE (IU/ml)	7.4 3.7 (0~22.9)	70.9 28.0 (0~399.2)	15.2 12.8 (0~142.0)	29.4 11.3 (0~68.9)

chial asthma, chronic bronchitis and pulmonary emphysema groups, but the sputum from patients with bronchial asthma had a tendency to give the higher mean value of IgG. Fig. 1-b shows the local production of IgG in the sputum in each groups. There was no significant difference between any of the matched groups. However, the local production of IgG was inclined to increase in pulmonary emphysema group.

As shown in Fig. 2-a, there was no significant difference of IgA levels in the sputa between the four groups, but there was a tendency to increase the IgA levels in sputa from the pulmonary emphysema group. Fig. 2-b shows the local production of IgA in each groups. There was no significant difference between the four groups, but there was also a tendency to increase the local production of IgA in sputa from the pulmonary emphysema group.

As shown in Fig. 3-a, there was no significant difference of IgE levels in the sputa between the four groups, but the sputa from patients with bronchial asthma had a tendency to increase the IgE levels. Additionally, the number of sputa with measurable IgE concentration was much greater in COLD

group. The local production of IgE in each groups appears in Fig. 3-b, there was no significant difference among the four groups .In the bronchial asthma group, the local production of IgE in the sputa tend to have higher level.

Discussion

In the present study comparison of levels of various immunoglobulin classes in sputum from patients with COLD was undertaken in an effort to clarify the pathophysiology of COLD. In bronchial asthma group, there was a tendency to increase in IgE concentration and the local production of IgE in sputum. In pulmonary emphysema group, both IgA concentration and the local production of IgA in sputa tended to have higher level. Furthermore, the local production of IgG in sputum from the pulmonary emphysema group showed a tendency to higher level. In chronic bronchitis group, there was no characteristic findings of local production of immunoglobulin, but IgG concentration in sputum was inclined to higher level. Since IgM was detectable only in a few sputa, no discussion was made for IgM in the sputum.

Sahöstrom and Tukiaimen[17] have reported protein levels in sputa from asthmatic patients. Albumin concentrations were between 0.85-3.40 g/l, IgA concentrations were between 0.51-1.80 g/l and IgG concentrations were between 0.50-2.20 g/l. Ishizaka and Newcomb[7] determined the levels of

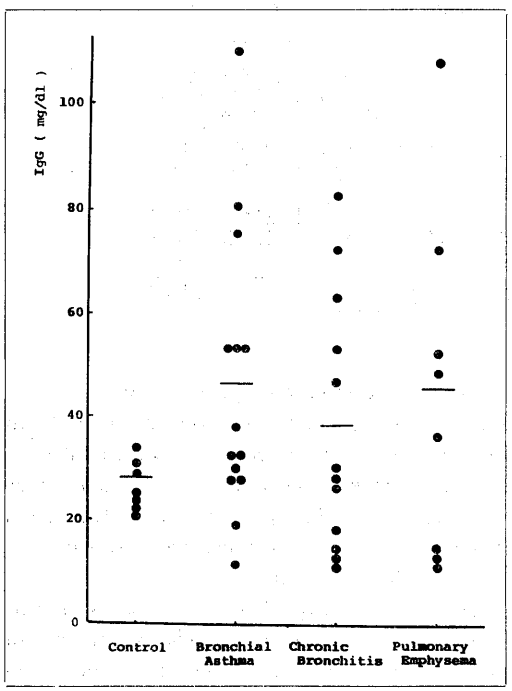

Fig. 1-a. Sputum IgG levels in control, bronchial asthma, chronic bronchitis and pulmonary emphysema. Bar indicates the mean value.

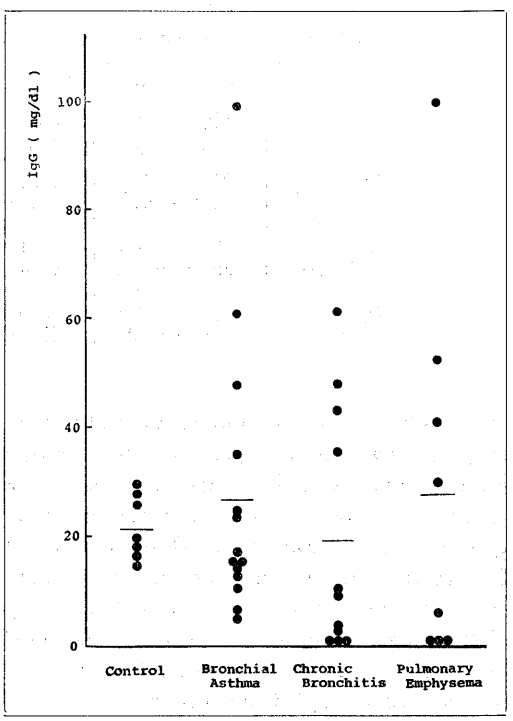

Fig. 1-b. Locally produced IgG levels in sputum in control, bronchial asthma, chronic bronchitis and pulmonary emphysema. Bar indicates the mean value.

IgG, IgA and IgE in sputa from asthmatic children and normal cigarette smokers. IgG levels were between 0.06-1.09 mg/ml, IgA levels were between 0.12-1.93 mg/ml, and IgE levels were between 0.05-0.54 μg/ml. The protein concentration of the sputum observed in this investigation was understandable.

Tomasi et al[2]. reported that the IgA found in external secretion was the secretory form consisting of a dimer of serum type IgA, together with two additional non-immunoglobulin moieties, known respectively as secretory component and J chain, and secretory IgA played as a primary defense mechanism at all mucosal surfaces. Immunofluorescent examination with anti-heavy-chainantiserum of the bronchial mucosa have shown that IgA-containing cells predominate at these sites in the lamina propria[18].

Ishizaka and his coworkers[7] have reported that IgE was present in sputum from some asthmatic patients. Deuschl and Johansson[16] measured IgE, IgA and IgG concentrations in tracheobronchial secretions in various pulmonary diseases. Their calculations indicated that IgE, IgA and IgG were produced locally in the tracheobronchial mucosa. They mentioned especially for IgA and IgE, whose presence in the mucosa seemed to be predominantly due to local production. Newcomb et al[19]. reported that IgE both in secretions and in sera was chemically and immunologically identical. However, the role of IgE in bronchial mucosa is not clear.

The concentration of serum immunoglobulins in patients with COLD have been reported[8-10]. Yamamoto and his group[9] reported

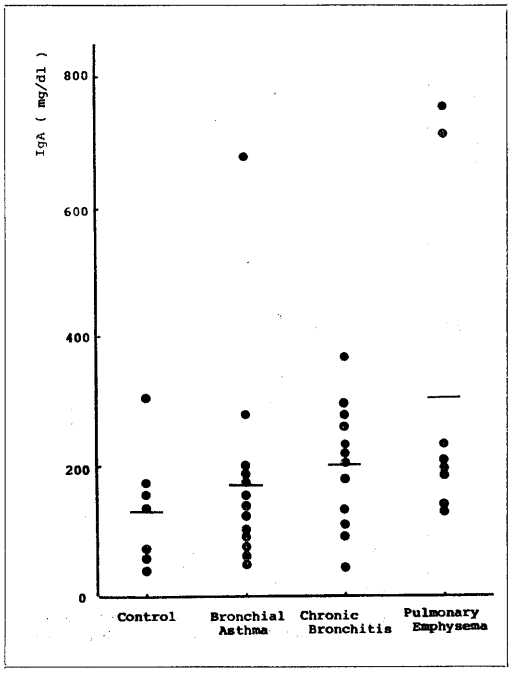

Fig. 2-a. Sputum IgA levels in control, bronchial asthma, chronic bronchitis and pulmonary emphysema. Bar indicates the mean value.

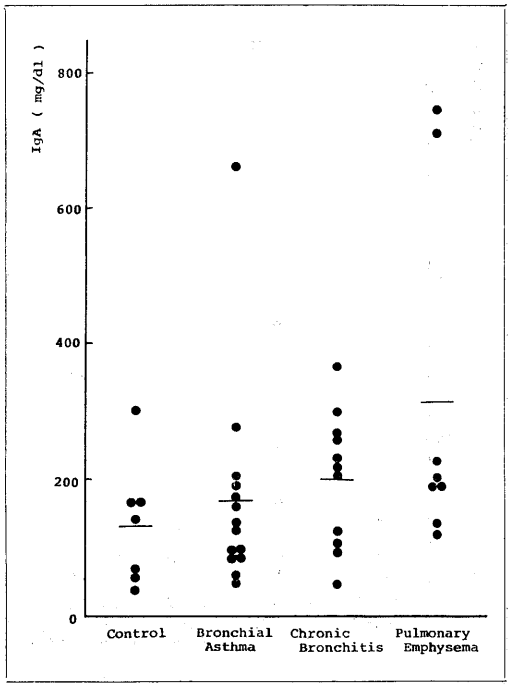

Fig. 2-b. Locally produced IgA levels in sputum in control, bronchial asthma, chronic bronchitis and pulmonary emphysema. Bar indicares the mean value.

that IgM and IgG levels of the groups of pulmonary emphysema patients were of the same order as those of normal subjects, but the IgA levels in the pulmonary emphysema group were significantly higher than the normal subjects. Biegel et al[8]. reported that serum IgA levels were significantly higher in pulmonary emphysema patients than that of the same aged control group. Moreover, Biegel et al[8]. discussed about the pathogenesis of pulmonary emphysema that the destruction of collagen and elastin on alveolar walls could be brought by a difference in sol-gel structure of the fibrous proteins involved, due to a change in hydrophilic properties and subsequent inability to adapt to the imposed forces of wear and tear. So, they considered that irreversible changes in hydrophilic properties of the elastin and collagen fibers appeared, when the tissue fluids showed a constant excess of the immunoglobulin under specially IgA.

Medici and Buergi[20] reported that the IgA content in sputum from chronic bronchitis was related to damage of the bronchial epithelium and the degree of inflammation using the lactic dehydrogenase, deoxyribonucleotic acid fiber, and fibrinogen in the sputum as criteria of inflammation. The mean value of samples with little signs of inflammation was 52 mg/ml, while that of severe inflammatory processes was 105 mg/ml. They mentioned that the resistance of the respiratory tract to infection was directly related the presence of IgA.

Callerame and his coworkers[21] reported that in chronic respiratory-tract diseases, the number of IgE-producing cells in the

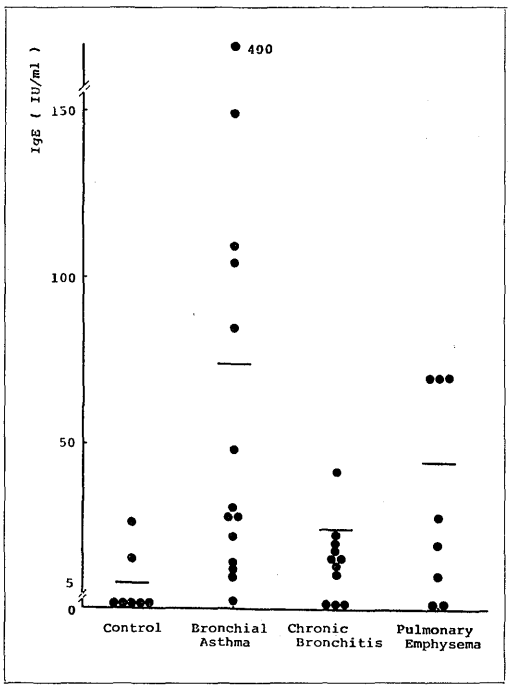

Fig. 3-a. Sputum IgE levels in control, bronchial asthma, chronic bronchitis and pulmonary emphysema. Bar indicates the mean value.

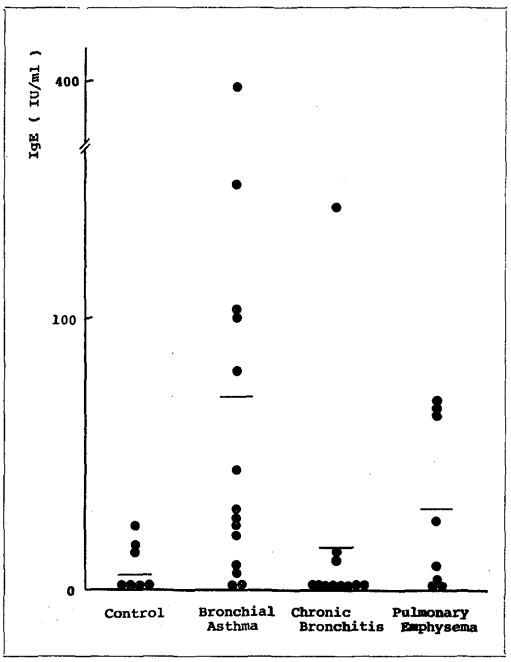

Fig. 3-b. Locally produced IgE levels in sputum in control, bronchial asthma, chronic bronchitis and pulmonary emphysema. Bar indicates the mean value

submucosa of bronchus tended to rise at a rate disproportionate to that in other classes. It is likely that IgE immunoglobulins play a role in the inflammatory response, and one can be attracted to postulate that this may sometimes be a critical protective role. On the other hand, Yamamoto et al[9]. measured the serum IgE levels in patients with COLD and discussed that the elevated IgE levels in patients with pulmonary emphysema would suggest the possibility that an atopic disease may one of the causative factors for pulmonary emphysema.

In this study, the cause of few cases of elevated IgA concentration in sputum in pulmonary emphysema should be related to alveolar destruction of the defense mechanism of the bronchial mucosa. Frequency of measurable IgE concentration in the sputa in COLD patients was higher than that in the control group. This might be the result of recurrent bronchopulmonary infections in COLD.

Findings of this study suggested that immunological mechanisms might role in causative and/or pathophysiological factors in cases of COLD.

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