Serum proteins in sputum of patients with asthma

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In a previous article[1] it was shown that saliva from both normal and allergic patients contained γ_{1A}-globulin but neither γ_2- nor γ_{1M}-globulin. Similar findings were recently reported by Chodirker and Tomasi.[2] It has also been found that saliva from allergic patients contains reaginic antibody.[1] In view of increasing evidence that reagin may be associated with γ_{1A}-globulin,[3-5] distribution of γ_{1A}-globulin in bronchial mucus as well as in the mucous membrane of the respiratory tract may be important in the pathogenesis of asthma.

The presence of several serum proteins in bronchial mucus has been reported[6]; in some cases, almost all serum proteins have been detected. In our experiments, serum proteins in bronchial mucus from patients with asthma were studied with particular interest in γ_{1A}- and γ_2-globulins. The results indicate that the γ_{1A}-globulin/γ_2-globulin ratio in bronchial mucus is much higher than in serum.

MATERIALS AND METHODS

Collection of samples

Bronchial mucus, saliva, and blood were obtained from children with asthma. The saliva was obtained first by expectoration, followed by expectoration of sputum to minimize contamination of the saliva with sputum. After collection of these, a 4 to 5 ml. sample of blood was obtained by venipuncture. Sputum samples obtained from the Chest Service at Fitzsimons General Hospital (courtesy of Colonel Warren G. Morse) from patients with arrested tuberculosis served as the "nonasthma" control samples. All specimens were stored at –20° C.

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The first part of this work was supported by Research Grant E-2504, United States Public Health Service. The experiments were repeated and supported by General Research Support Grant FR-5216.

Received for publication April 14, 1964.

γ_{1A}- and γ_2-globulins

A γ_2-globulin fraction was purified from human Cohn fraction II (Pentex, Inc.) by starch block electrophoresis in barbital buffer of pH 8.6 and ionic strength 0.1.[7] Eluates from the slowly migrating globulin region were pooled and concentrated by pervaporation. The γ_{1A}-globulin was purified from fresh human serum by the method of Vaerman, Heremans, and Vaerman.[8] The protein concentrations were determined by nesslerization. The γ_{1A}-globulin preparation contained 192 γ N per milliliter and the γ_2-globulin contained 640 γ N per milliliter. The purity of these fractions was determined by double diffusion in agar and by immunoelectrophoresis against anti–whole human serum, antihuman γ_2-globulin and antihuman γ_{1A}-globulin. Both of these preparations gave a single precipitin band with antihuman serum by immunoelectrophoresis (see following section). One precipitin band of identical specificity was obtained when each fraction was directed against its specific antiserum and anti–whole human serum in agar diffusion.

Immunodiffusion and immunoelectrophoresis

The method of Ouchterlony[9] was employed for double diffusion studies. The precipitin bands were stained with Amido-Schwarz. Immunoelectrophoresis was carried out by the method of Scheidegger[10] on microscope slides. The precipitin lines were stained with azocarmine. Concentrations of γ_{1A}- and γ_2-globulins in samples were estimated by the method of Gell[11] with standard preparations of γ_{1A}- and γ_2-globulins. The goat antisera used in these tests were obtained from Hyland Laboratories. They were goat antisera against whole human serum and those specific to human γ_2-globulin (No. 10) and to γ_{1A}-globulin (No. 206).

RESULTS

Serum proteins in sputum

Serum proteins in sputum from individuals with asthma and from the patients without asthma were analyzed by immunoelectrophoresis against antihuman serum. Thirty-six sputum specimens from asthma patients were classified according to the condition of the patient at the time of collection of the sample, as shown in Table I. Thirteen specimens were obtained during an attack of asthma or during status asthmaticus from patients without any symptoms of infection. Sixteen cases (six of upper respiratory infection, five of pneumonia, four of bronchitis, and one of sinusitis) included respiratory infections with or without the symptoms of asthma. The presence of infection was determined by the presence of fever over 100° F., injected throat, and swelling and tenderness of the regional lymph nodes and by x-ray when so indicated. The patients without asthma had diagnosed arrested tuberculosis and had no acute infection in the upper respiratory tract. The results of the immunoelectrophoretic analyses indicated that albumin and β_2-globulin were present in most of the specimens, irrespective of the presence of asthma symptoms. Only one to two precipitin bands were observed in the α- to β_1-globulin region in three out of the thirteen specimens obtained from patients during an asthma attack or during status asthmaticus without infection, whereas all specimens obtained from the patients

with infection gave several precipitin bands in the α and/or β_1 region. As many as fifteen precipitin bands appeared in a specimen obtained from a patient with nasopharyngitis, bronchitis, and mild asthma.

In view of previous findings[1] that γ_{1A}-globulin is the major serum protein in saliva, the possibility was considered that the β_2-globulin detected in most sputum specimens might be γ_{1A} (β_{2A})-globulin. In order to investigate this possibility, Osserman's technique[12] was applied. The specimens showing only albumin and β_2 precipitin bands were separated by electrophoresis in the center of the slide. One side trough was filled with antihuman serum and another with purified γ_{1A}-globulin. The precipitin band in the β_2-globulin region had identical specificity to γ_{1A}-globulin–anti-γ_{1A}-globulin as indicated by a dipping of this band (Fig. 1). The presence of γ_{1A}-globulin in sputa was confirmed by double diffusion in agar. Twenty-six out of twenty-eight samples tested gave a definite precipitin band with anti-γ_{1A}-globulin.

A significant difference between the asthma and nonasthma groups was observed in the γ_2-globulin region (Table I). This protein was detected in nine out of thirteen sputa from patients having asthma (without infection), but not in sputa from the same patients in the convalescent state (Fig. 2). All specimens collected during infection also gave a γ_2-globulin precipitin band. Only two out of eighty-three sputa from subjects with arrested tuberculosis showed a γ_2-globulin precipitin band. However, an absence of the precipitin band in immunoelectrophoresis does not necessarily indicate an absence of this protein in the specimens. When twenty-seven specimens which gave no γ_2-globulin band in immunoelectrophoresis were applied to Ouchterlony plates against a goat antiserum specific to 7S γ-globulin, all twenty-seven showed definite precipitin bands. It seems that the difference between sputa in asthma and convalescence, with respect to γ_2-globulin, is quantitative rather than qualitative. The data shown in Table I suggest that the presence of α- and/or β_1-globulin is associated with acute infection in the respiratory tract rather than with asthma.

Table I. *Immunoelectrophoretic analyses of sputum from patients with and without asthma*

Symptoms	No. of patients	Albumin		α		β_1		β_{2A} (γ_{1A})		γ_2	
		No.	%	No.	%	No.	%	%	No.	No.	%
Asthma											
Asthma	13	13	100	3	23.1	2	15.4	13	100	9	69.2
Asthma + infection	9	9	100	9	100	7	77.8	9	100	9	100
Infection	7	7	100	7	100	3	42.9	7	100	7	100
Convalescent	7	7	100	0	0	0	0	4*	57.1	0	0
Total	36	36	100	19	52.8	12	33.3	33	91.2	25	69.4
Arrested tuberculosis											
No symptoms	83	72	86.7	2	2.4	0	0	68	81.9	2	2.4

*The other three sputa showed the presence of γ_{1A}-globulin upon agar diffusion.

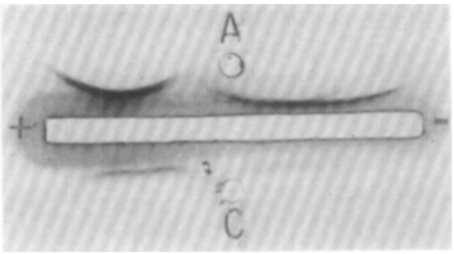

Fig. 1

Immunoelectrophoresis of sputum. The upper trough was filled with γ_{1A}-globulin and the lower trough with antihuman serum. A migrated sputum sample indicates the presence of γ_{1A}-globulin in sputum.

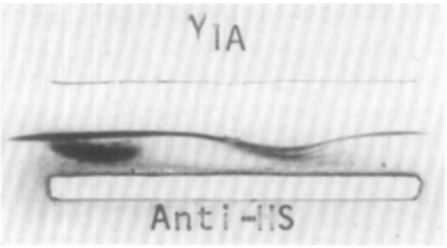

Fig. 2

Immunoelectrophoresis of sputum. *A*, Sputum from a patient with asthma. *C*, Sputum from the same patient during convalescence. The center trough is filled with antihuman serum.

Relative concentration of γ_{1A}- and γ_2-globulins

The results obtained by immunoelectrophoresis suggest that the γ_{1A}/γ_2-globulin ratio in sputum is higher than that in serum. In order to compare the concentrations of these proteins in sputum, saliva, and serum, these three specimens were obtained from the same patients with asthma at the same time (Table II). In some patients, the three specimens were obtained when there were no subjective symptoms, during an asthma attack and during status asthmaticus, in order to investigate possible changes in the protein concentrations associated with the symptoms of asthma. The concentrations of globulins in these specimens were estimated by the method of Gell[11] in Ouchterlony plates. Twofold dilutions of both γ_{1A}- and γ_2-globulin preparations were made with saline and ranged from 6 to 128 γ N per milliliter. Each of the dilutions was placed in the center well of an Ouchterlony plate and the peripheral wells were filled with six dilutions (i.e., undiluted and 1.5, 2, 2.5, 3, and 4 times dilutions of antiserum specific to the respective protein). The densities of the precipitate lines forming a hexagon around the center well were different, depending on the concentration of antigen. Appropriate dilutions of the test samples were then placed in the center well with the same antiserum dilutions in the peripheral wells. The hexagons formed by the test samples were compared line by line with the standard hexagons. The standard hexagon that matched the hexagon of the test sample most exactly was taken as the concentration of γ_{1A}- or γ_2-globulin in the sample. Both γ_{1A}- and γ_2-globulins were detected in most saliva and sputum specimens. As shown in Table III, the γ_{1A}/γ_2-globulin ratio in sputum was generally much higher than that in serum. Although the ratio was highest in saliva, absolute concentrations of both proteins in sputum were much higher than those in saliva. It was also found that concentrations of γ_{1A}-globulin in

sputum obtained during attacks of asthma and status asthmaticus were two to sixteen times higher than the concentration in samples obtained during the convalescent state. The concentration of γ_2-globulin in sputum was four to eighty times more in asthma attacks than in the convalescent state. There was generally a decrease in the γ_{1A}/γ_2-globulin ratio during asthma attacks. This, of course, is to be expected because the increase of γ_2-globulin was usually higher than the increase of γ_{1A}-globulin during these attacks.

DISCUSSION

The data presented in this article clearly show that the major serum protein components in sputum of patients with asthma are albumin, γ_{1A}-, and γ_2-globulins. Albumin and γ_{1A}-globulin were detected in most asthma sputa even when

Table II. *Concentrations of γ_{1A}- and γ_2-globulins in sputum, saliva, and serum of patients with asthma*

		Sputum		*Saliva*		*Serum*	
Patient	*Symptoms*	*γ_{1A} (μg N/ml.)*	*γ_2 (μg N/ml.)*	*γ_{1A} (μg N/ml.)*	*γ_2 (μg N/ml.)*	*γ_{1A} (μg N/ml.)*	*γ_2 (μg N/ml.)*
1	Asthma	16	25	8	<6	160-320	2,500
	Status asthmaticus	32	250	64	50	320	2,500-5,000
	Convalescent	–*	–	<8	0	320	2,500
	Pneumonia	32	50	8-16	<6	320	1,200-2,500
	Convalescent	–*	–	<8	Trace	400	1,200-2,500
2	Status asthmaticus	16	50	<8	Trace	320	2,500-5,000
	Convalescent	8	<6	<8	Trace	320	2,500
3	Status + infection	32	128	8-16	<6	640	1,200-2,500
	Convalescent + infection	16	25	<8	<6	640	2,500-5,000
4	Status + infection	8-16	25	<8	Trace	128	1,200-2,500
	Convalescent	8-16	6	8	<6	128	1,200
5	Status + infection	32	500	16	25	640	5,000
	Convalescent	16	25-50	8	6	640-1,280	5,000
6	Status + infection	128	500	8	12	640	5,000
	Convalescent	8-16	<6	8	<6	640-1,280	2,500-5,000
7	Asthma	16-32	100	8-16	6	160	2,500-5,000
	Convalescent	32	25-50	8	Trace	320	2,500
8	Asthma	8-16	25-50	<8	<6	320	1,200-2,500
	Convalescent	–*	–	<8	<6	320	1,200-2,500
9	Asthma	8-16	6	<8	6	128	1,200-2,500
	Convalescent	–*	–	8-16	<6	128	1,200-2,500
	Asthma	16-32	25-50	–	–	128	1,200
10	Asthma	16	50	16	6	128	1,200
	Convalescent	32	12	Trace	Trace	128	1,200
11	Asthma + sinuitis	32	25	–*	–	64-128	2,500
12	Asthma	64	50	–*	–	320	2,500-5,000
13	Asthma	32	50	–*	–	320	2,500-5,000
14	Asthma	16	50-100	–*	–	160-320	2,500-5,000

*Specimen not obtained.

Table III. *Ratio of γ_{1A}/γ_2-globulins in sputum and saliva of patients with asthma*

Patient	*Symptoms*	*Sputum* γ_{1A}/γ_2	*Saliva* γ_{1A}/γ_2
1	Asthma	5.0-10.0	10.0-20.0
	Status asthmaticus	1.0-2.0	10.0-20.0
	Convalescent	–*	–†
	Pneumonia	2.5-5.0	10.0
	Convalescent	–*	–†
2	Status asthmaticus	2.5-5.0	–†
	Convalescent	10.0	–†
3	Status + infection	1.0	3.0
	Convalescent + infection	2.5-5.0	–†
4	Status + infection	6.0	–†
	Convalescent	10.0-20.0	12.5-25.0
5	Status + infection	2.0	5.0
	Convalescent	2.5-5.0	5.0-10.0
6	Status + infection	2.0	5.0
	Convalescent	5.0-10.0	5.0
7	Asthma	5.0-10.0	>40.0
	Convalescent	5.0-10.0	–†
8	Asthma	1.0-2.5	–†
	Convalescent	–*	–†
9	Asthma	24.0	13.0-26.0
	Convalescent	–*	13.0-26.0
	Asthma	6.0	–†
10	Asthma	3.0	26.0
	Convalescent	26.0	–†
11	Asthma + sinuitis	5.0-10.0	–†
12	Asthma	10.0-20.0	–†
13	Asthma	5.0-10.0	–†
14	Asthma	2.5-5.0	–†

*Specimen not obtained.

†Ratio not obtained because of low concentrations of γ-globulins.

the patients were without subjective symptoms and in most specimens from arrested tuberculosis patients (Table I). As γ_{1A}-globulin is the major serum protein in saliva,[1, 13] the possibility arises that the protein in sputum may be present because of contamination of sputum with saliva. However, this possibility is unlikely, because the concentration of γ_{1A}-globulin in sputum is much higher than in saliva (Table II). Our findings differ from the observations of Mendes and colleagues[6] that only 69 per cent of asthma sputa contained albumin and 46 per cent contained γ_{1A}-globulin. The discrepancies may be due to the differences in method and the antisera used for detecting serum proteins. After we had obtained the present data. Keimowitz[14] reported the detection of albumin and γ_{1A}-globulin in the tracheobronchial washings of twelve normal individuals without infection.

Presence of γ_{1A}-globulin in sputa of convalescent patients and in the specimens from arrested tuberculosis patients suggests that γ_{1A}-globulin may be

secreted through the mucous membrane under physiologic conditions. This idea is in accord with the recent observations by Chodirker and Tomasi[2] that γ_{1A}-globulin represents a major fraction of γ-globulin of tears, bile, saliva, colostrum, and fluids of the small intestine.

It was also found that γ_2-globulin is secreted into bronchial mucus. A trace amount of this protein was detected in some specimens which were obtained during the convalescent state. The results are in accord with the findings of Keimowitz,[14] who detected γ_2-globulin in eleven out of twelve specimens of the tracheobronchial washings from normal individuals. The presence of γ_2-globulin in most specimens of saliva was contradictory to our previous findings. This may be due to contamination of saliva with bronchial mucus and/or nasal secretion, because the saliva specimens were obtained by expectoration in the present experiment.

It is evident that the γ_{1A}/γ_2-globulin ratio of sputum in the convalescent state is much higher than the ratio in serum. These findings, as well as those reported by Tomasi and Ziegelbaum,[13] support the view that γ_{1A}-globulin is selectively transferred to the external secretions. When patients have asthma attacks, secretion of albumin, γ_{1A}-globulins, and γ_2-globulins into bronchial mucus increases. Anderson and associates[15] reported that normal secretion on the surface of nasal epithelium showed serum albumin as the sole serum protein and that γ-globulin appeared in the nasal secretion following virus infection. It is evident from our experiment that the secretion of γ_2-globulin into bronchial mucus is not characteristic of infection alone. It seems that allergic inflammation, which increases capillary permeability, induces increased secretions of this protein into sputum. It is also possible that the "selective" mechanism for secretion of γ_{1A}-globulin is disturbed by allergic inflammation. A decrease of the γ_{1A}/γ_2-globulin ratio in sputum was observed when a patient had pneumonia without asthma symptoms (Table I), suggesting that the decrease is not characteristic of asthma.

High γ_{1A}/γ_2-globulin ratio in sputum may be of clinical importance in view of increasing evidence that reagin is associated with γ_{1A}-globulin.[3-5] Perlmutter and colleagues[16] have reported that blocking antibody is associated with γ_2-globulin. If reagin is a γ_{1A}-protein, our results suggest that reagin is easily secreted through the bronchial mucous membrane, whereas blocking antibody is secreted to a lesser extent. Studies on the distribution of γ_{1A}- and γ_2-globulin in lung tissues are under way.

Finally, our experiment suggests the possibility that the secretion of α- and β_1-globulins in sputum may be characteristic of infection. Even when asthma patients had severe attacks, these proteins were not detected in their sputum. It seems, therefore, that the detection of many precipitin bands in the α- to β_1-globulin region in asthma sputa suggests a combination of asthma and infection.

SUMMARY

Immunoelectrophoretic and agar diffusion analyses of thirty-six asthma sputa and eighty-three nonasthma sputa were performed. Almost all specimens contained albumin and γ_{1A}-globulin as major serum protein components.

Gamma-2 globulin was also detected in most cases. The ratio of γ_{1A}/γ_2-globulin in sputum during convalescence was found to be much higher than this ratio in serum. The γ_{1A}-globulin, γ_2-globulin, and albumin in asthma sputum was increased compared to that in sputum from individuals without symptoms. A decrease in the γ_{1A}/γ_2-globulin ratio in sputum was noted as a result of asthma attacks and/or infection in the upper respiratory tract. The significance of a high γ_{1A}/γ_2-globulin ratio in sputum was discussed.

We are indebted to Dr. Yoshio Tsuchiya for collection of specimens. We also wish to express our gratitude to Dr. David W. Talmage for his advice and criticism concerning the preparation of our manuscript.

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