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IMMUNOLOGICAL STUDIES ON BRONCHIAL SECRETION

1. Antigenic Relationship Between Bronchial Secretion and Serum: Bronchial Secretion Constituents Detected in Human Sera

By

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Bronchial secretion contains specific antigens and several antigens in common with other secretions, organs, and serum (2, 4, 11, 13, 14).

Bronchial secretion can be eliminated by the expectoration mechanism and part of it is probably reabsorbed (18). The hypothesis that part of the bronchial secretion might be reabsorbed was first proposed by Gordonoff (3), and later by Messerklinger (15). With the enormous decrease in surface area of the airways from the distal airways to the trachea, and with the need for maintenance of a mucus layer of about 5 μ m in depth for proper functioning of clearance mechanisms, a very large amount of reabsorption must be taking place (9).

The purpose of the present paper was to test, by immunological methods, the hypothesis that bronchial secretion constituents are absorbed and that they can be detected in serum. Using the double gel-diffusion test, the reactions between rabbit anti-human sputum serum and human sera in different experiments and different diseases were analysed.

MATERIALS AND METHODS

Sera

Eighty-six sera from asthmatic patients, 25 sera from patients with other diseases, 17 sera from patients with tuberculosis and 27 sera from normal individuals were used. Sera were kept at -20°C .

Bronchial Secretion

Sputum from asthmatic patients, 0 Rh + and AB Rh + were obtained by bronchoaspiration. The material was dried and redissolved in distilled water. For immunization, solutions at a concentration of 20 g/100 ml in saline were used and kept frozen. For the double gel-diffusion, solutions at the concentrations of 0.5 to 1.0 g/100 ml were used.

Antisera

New Zealand rabbits weighing about 2 kg were given six intra-peritoneal injections of 1 ml of sputum solution emulsified in an equal volume of complete Freund's adjuvant at 4-day intervals. Fifteen days after the last intra-peritoneal injection, they were injected intravenously with 1 ml of 1 g/100 ml sputum in saline, three times a week. The animals were bled and the pooled sera inactivated at 56°C for 30 min, and kept frozen. Two kinds of antisera, raised against sputum from 0 Rh + and AB Rh + patients, were used.

Absorption

Anti-bronchial secretion sera were absorbed with 1/5 volume of a pool of normal human sera, for 12 hours at 4°C , followed by 1 hour at 37°C . Normal human sera, which did not produce more than 2 precipitation lines with the anti-sputum rabbit serum, were employed for absorption.

Double Gel-diffusion Test

The standard technique, as described previously (14), was employed.

The tests were made in duplicate. Readings were made after 24, 48, 72 and 120 hours. The following systems were used in the same slide: a) anti-sputum serum against human sera; b) anti-sputum serum against human sera and bronchial secretion; c) anti-sputum serum absorbed with human sera against human sera; d) anti-sputum serum absorbed with human sera against human sera and bronchial secretion.

The reactions were repeated 1 to 2 years later, using human sera from the same patients, and the results were compared, in the following circumstances: a) reaction between anti-sputum serum 0 Rh + and 19 human sera sampled at different times with 1-year intervals; b) reaction between anti-sputum serum AB Rh + and 29 human sera withdrawn twice, at 1- and 2-year intervals.

TABLE 1

Precipitation Reactions between Rabbit Anti-human Sputum Sera and Human Sera.

Rabbit anti-sputum sera	Human sera from patients with			
	Asthma	Tuberculosis	Other diseases	Normal controls
A) Anti-sputum sera (non-absorbed)				
<i>Positive reactions with:</i>				
1 to 3 bands	86	17	25	22
4 bands	2	9	1	1
5 bands	1	0	0	0
B) Anti-sputum sera absorbed with normal human sera				
<i>Positive reactions with:</i>				
1 to 2 bands	5	0	1	1
4 to 5 bands	0	0	0	0

RESULTS

The rabbit anti-bronchial secretion serum used in our experiments gave five precipitation bands with the homologous antigen. The number of bands was reproducible in replicate experiments.

Most reactions between anti-sputum serum and 150 human sera showed 1 to 3 precipitation bands; only 13 sera showed 4 precipitation bands; and one asthmatic serum showed 5 precipitation lines (Table 1).

Precipitation bands with reactions of identity were obtained when the anti-sputum sera reacted simultaneously with human sera and bronchial secretion.

Reactions of absolute identity between sputum and human sera were still obtained when the anti-sputum serum (absorbed with normal human sera) reacted at the same time with bronchial secretion and human sera from five asthmatic patients, one apparently normal individual and one patient from the group of other diseases (Fig. 1).

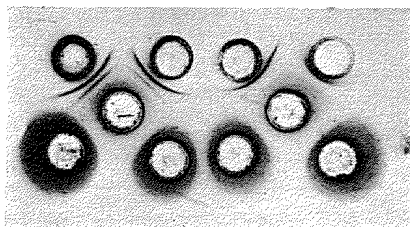


Fig. 1.

Reactions of identity between sputum and human sera, when the anti-sputum serum (absorbed with normal human sera) reacted at the same time with bronchial secretion and human sera.

The number of precipitation bands and their differences in number after 1 and 2 years are expressed in Table 2, as follows: After 1 year, in a group of 29 human sera the same number of precipitation bands were observed in 20 sera; one band of difference in 4 sera, and more than 1 band of difference in 5 sera. After 2 years, 22 sera showed the same number of precipitation lines; 5 sera, 1 line of difference; and 2 sera more than 1 line of difference. Another group of 19 sera showed, after 1 year, the same number of bands in 12 sera, one band of difference in 5 sera and more than 1 band in 2 sera.

DISCUSSION

The evaluation of the number of antigens in a given organ or secretion, such as bronchial secretion, presents many problems. The number of lines in agar indicates the minimum number of antigens, but not necessarily the total number of antigens in the secretion or serum (13).

It has usually been accepted that bronchial secretion contains, besides specific antigens, antigens also shared with other organs and secretions (13, 14). Double gel-diffusion and immunoelectrophoresis demonstrated the presence of spe-

TABLE 2

Number of Precipitation Bands with 1 to 2 Years' Interval.

Anti-bronchial secretion serum	Number of human sera	Interval between the reactions (years)	Precipitation bands		
			same number	one band of difference	more than one band of difference
AB Rh +	29	1	20	4	5
	29	2	22	5	2
O Rh +	19	1	12	5	2

cific components in the sputum. Serum antibronchial secretion absorbed with normal human sera still showed 2 or 3 precipitation bands, when reacting with sputum (4). It was demonstrated by several experiments that there are two antigens in bronchial secretion that are not common to organs or normal serum; and one of them seems to be common to intestinal mucus. When the anti-bronchial secretion serum yielded 5 precipitation bands reacting with sputum, it was suggested that 1 or 2 bands be considered specific, the remaining bands being considered common to other organs or secretions (13). From the basis of this observation one could speculate that the occurrence of 4 or 5 precipitation arcs between anti-sputum serum and some human sera could suggest the presence of specific antigenic determinants belonging to the bronchial secretion in the human sera studied. The identity reactions observed between sputum antigens and some human sera revealed by anti-sputum serum absorbed with normal human serum also seems to demonstrate the presence of specific bronchial secretion antigens in some sera. The differences in the number of precipitation arcs (2 or more) observed in the sera from some patients, when the double gel-diffusion was performed with intervals of 1 or 2 years, could be another argument favouring the hypothesis of the presence, in special circumstances, of bronchial secretion antigens in the sera. These findings could be explained by the reabsorption

of the sputum admitted by the physiological concept of the expectoration, according to which a certain part of bronchial secretion is absorbed (3, 5, 9, 15). Comprehensive and elucidating studies on this problem were also related by Kohler (10), Yeaguer (18) and Asmundsson & Kilburn (1). More recent evidence supports this possibility, such as numerous physiological studies on the permeability of the alveolar-capillary membrane (17). Also, Nettesheim & Williams (16), studying antigens deposited in the alveolar parenchyma, observed that a small proportion of the immunogen enters the blood stream and triggers an immune response. Specifically, the distal airways appear to clear to the larynx up the ciliary escalator, while the alveoli appear to be "cul-de-sacs" which are cleared by digestion and absorption (8). Deposition of radioiodinated albumin in the bronchi, through endoscopic technique, in patients without pulmonary lesions showed that human albumin can be absorbed at the bronchial mucosa without undergoing any change in its chemical structure (6).

The immunological evidence discussed above suggests that the bronchial secretion antigens or their determinants are demonstrated in some sera, supporting the physiological concept of expectoration, according to which part of the bronchial secretion may be absorbed.

SUMMARY

Rabbit anti-human bronchial secretion serum yielded, by double gel-diffusion, 5 precipitation bands with the homologous antigen. This anti-bronchial secretion serum reacting with 150 human sera (86 from patients with asthma, 17 from patients with tuberculosis, 25 from patients with other diseases and 22 normal sera) showed 1 to 3 precipitation bands in 136 sera, 4 precipitation bands in 13 sera and 5 precipitation lines in 1 serum. Precipitation bands with identity reactions were obtained when the anti-sputum sera reacted simultaneously with human sera and bronchial secretion. Identity reactions were also obtained when the anti-bronchial secretion serum

absorbed with normal human sera reacted with bronchial secretion and human sera from six patients and one normal individual. The number of precipitation bands in some sera was not the same after 1 or 2 years. Based on these immunological data, the physiological concept of expectoration, according to which part of the bronchial secretion may be absorbed, was discussed.

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