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IMMUNOCHEMICAL STUDIES OF THE ASTHMATIC SPUTUM

By

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Chemical studies of bronchial sputum by *Werner* (1953), *Springer et al.* (1954), *Baer* (1955), *Bukantz & Berns* (1958) demonstrated the presence of hexosamin, N-acetyl-hexosamin, uronic and sialic acid, fucose and mucopolysaccharides which exhibit blood group activity.

Immunological relationship between bronchial sputum and other human secretions and organ antigens was demonstrated by *Mendes et al.* (1961), suggesting chemical relations among some components from different sources.

The present report is concerned with the results obtained by gel double diffusion and immoelectrophoresis for the identification of protein components of bronchial sputum and their relationship with serum proteins.

MATERIAL AND METHODS

a) *Preparation of sputum.*

The bronchial sputum was obtained from patients with: 1) extrinsic asthma; 2) intrinsic asthma, and 3) mixed asthma. The criteria used for this classification was based upon clinical and laboratory data.

Sputum soon after collection, was vacuum dried, powdered and then redissolved in a sodium chloride solution at 0.9 %.

Sputum samples were generally used as 1 % solution.

With the technical assistance of Therezinha A. Salomão.

b) *Antisera.*

A) The following antisera against various protein fractions of human serum, kindly supplied by the Behringwerke (Marburg), were used in gel double diffusion:

- | | |
|---|--|
| 1 - Anti-prealbumin | 7 - Anti-beta ₂ M-globulin (rabbit) |
| 2 - Anti-albumin | 8 - Anti-beta ₂ M-globulin (horse) |
| 3 - Anti-alpha ₁ lipoprotein | 9 - Anti-beta ₂ A-globulin |
| 4 - Anti-alpha ₂ macroglobulin | 10 - Anti-transferrin |
| 5 - Anti-alpha ₂ lipoprotein | 11 - Anti-fibrinogen |
| 6 - Anti-beta ₁ lipoprotein | 12 - Anti-gammaglobulin |

B) Rabbit antisera against a pool of bronchial sputum from asthmatic patients of blood group A and AB were obtained by a technique described in a previous paper (10). These antisera were employed for the immunoelectrophoretic analysis. One of the antisera proved to be more active, at there were more precipitation lines.

C) Anti-total human serum was obtained by the immunization of rabbits with whole human serum. It was also used for immunoelectrophoresis.

Gel-Double-Diffusion.

Hartmann & Toilliez's (8) and *Jaculis & Heller's* (9) microtechniques with some minor modifications were used. Both the one per cent sputum and the different antisera were applied only once, and results noted after 48-72 hours. Readings were made with a magnifying lens after staining the slides with amido black 10 B.

Immunoelectrophoresis.

Grabar & Williams' (7) method was employed, modified by *Ferri & Cossermelli* (6).

The following substances were used as antigens:

- 1) sputum at 1 % solution, from patients of blood groups A and O;
- 2) selected human sera which previously had shown 3 precipitation bands with anti-sputum rabbit serum.

Human sera, which did not produce precipitation lines with the anti-sputum rabbit serum (nearly two thirds of the samples), were employed for absorption tests. These sera were designated as "normal".

TABLE I
Gel-double-diffusion tests with sputum from 13 asthmatic patients against specific antisera.*

Name	X.P.	G.H.	M.R.	E.S.	M.I.M.	F.S.	S.A.	I.B.	P.R.A.	A.L.	M.I.	A.S.	Y.S.
	Int.	Mix.	Int.	Mix.	Extr.	Mix.	Mix.	Extr.	Mix.	Mix.	Mix.	Mix.	Mix.
Pre-albumin	—	+	+	+	+	+	—	—	+	—	—	—	—
Albumin	+	—	—	+	—	+	+	+	+	+	—	+	+
Alpha ₁ -lipoprotein	—	—	—	—	—	—	—	—	—	—	—	—	—
Alpha ₂ -M-globulin	—	—	—	—	—	—	+	—	—	—	—	—	—
Alpha ₂ -lipoprotein	—	—	—	—	—	—	—	—	—	+	+	—	—
Beta lipoprotein	—	+	—	+	+	—	—	—	+	—	—	—	—
Beta ₂ M-globulin (rabbit)	+	+	+	—	+	—	—	—	—	—	—	—	—
Beta ₂ M-globulin (horse)	+	—	—	+	—	—	—	—	—	—	—	—	—
Beta ₂ A-globulin	—	—	—	+	+	+	—	+	—	—	—	—	—
Fibrinogen	+	+	+	—	+	—	—	—	—	—	—	—	—
Transferin	+	+	—	+	+	—	+	—	—	—	—	+	+
Gammaglobulin	—	+	—	+	+	+	+	—	+	—	—	—	+

* Kindly supplied by Behringwerke.

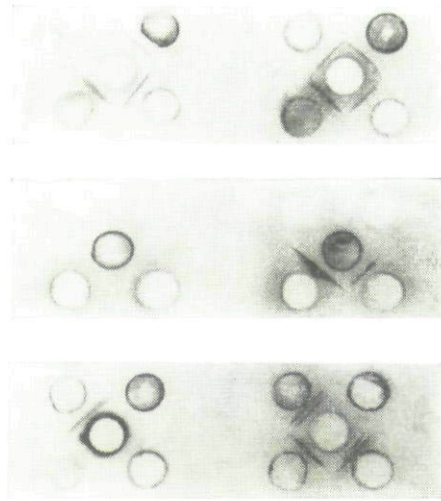


Fig. 1.
Gel-Double Diffusion

Outer wells: sputum fro different asthmatic patients

Center wells:

- a) left: anti-pre-albumin
right: anti-beta₂ M-globulin (rabbit)
- b) left: anti-alpha₁-lipoprotein
right: antigammaglobulin
- c) left: anti-beta₂ M-globulin (horse)
right: anti-transferrin

RESULTS

A) *Gel-Double-Diffusion.*

Results of gel-double-diffusion are condensed in table I: out of 13 sputum samples, 6 reacted against antiprealbumin; 9 against anti-albumin; none reacted against anti-alpha₁-lipoprotein; 1 against anti-alpha₂ M-globulin; 2 against anti-alpha₂-lipoprotein; 4 against anti-beta₁-lipoprotein; 4 against anti-beta₂ M-globulin (rabbit); 2 against anti-beta₂ M-globulin (horse); 6 against anti-beta₁ A-globulin; 4 against anti-fibrinogen; 7 against anti-transferrin, and 7 against anti-gammaglobulin.

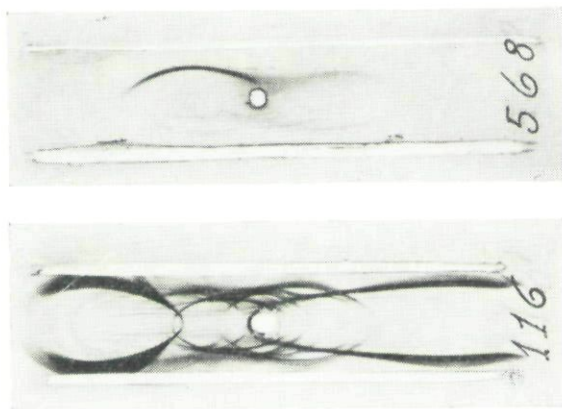


Fig. 2.

568—Electrophoresis of sputum from patient blood group A. Immunodiffusion against rabbit anti-human serum (above) and rabbit anti-sputum serum (below).

116—Electrophoresis of human serum. Immunodiffusion against the same rabbit anti-human serum used in number 568.

All antisera yielded one precipitation line, excepting anti- α_2 M-globulin, anti-transferrin and anti-albumin which in some slides presented 2 lines (Fig. 1).

B) *Immuno-electrophoresis.*

Immuno-electrophoretic analysis demonstrated the following:

1. The sputum from blood group A asthmatics contains various antigens different from serum proteins. By the diffusion of the sputum after electrophoresis, against antitotal human serum (prepared in rabbits), only two serum proteins were identified, corresponding, one to α_2 -globulin and the other probably to β_1 -siderophilin (Fig. II, 568).

2. The same sputum on reacting simultaneously with anti-sputum rabbit serum showed six lines of precipitation in the α_2 and β -globulin zones. Four of these precipitation lines corresponded to antigens specific to sputum, inasmuch as they were not detectable when the sputum reacted with rabbit anti-human serum (Fig. II, 116).

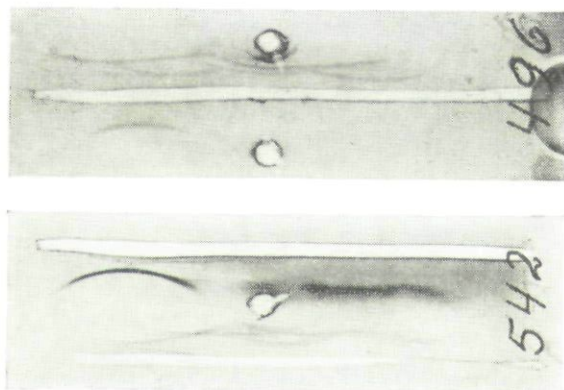


Fig. 3.

496—Electrophoresis of sputum from patient blood group O (above) and serum from asthmatic patient (below). Immunodiffusion against rabbit anti-sputum serum.

542—Electrophoresis of sputum from patient Blood group O (center). Immunodiffusion against rabbit anti-human serum (above) and rabbit anti-sputum serum (below).

3. On the other hand, the results of immunoelectrophoretic analysis of human serum in diffusion against anti-sputum rabbit serum, showed only 1 component in the α_1 zone, whereas the anti-sputum serum on reacting with sputum had revealed 5 to 6 components, confirming again that most of the sputum components here demonstrated were not serum proteins.

4. By previous absorption of the anti-sputum rabbit serum with "normal" human serum, three lines of precipitation were obtained with sputum of group A patients; thus, their different nature from the serum proteins was confirmed.

5. The anti-sputum rabbit serum on reacting with sputum from patients of blood group O revealed some of the components present in type A sputum, besides presenting some other components. In the albumin- α_1 globulin region appeared a precipitation line which could not be identified as serum-albumin (Fig. III, slide 496, upper part).

This anti-sputum serum proved to be more active than the pre-

vious one, as it revealed in type O sputum up to nine components different from serum components. On reacting against human serum, in the same immunoelectrophoretic operation, this anti-sputum serum showed only one precipitation line in the α_1 region (Fig. 496, lower part).

After absorption of this anti-sputum serum with 1/5 of its volume of "normal" human serum, the same components present in the sputum could still be detected by immunoelectrophoresis, showing, once again, that the antigens revealed are not serum-proteins, nor are they proteins immunologically related to them.

6. Type O sputum, on reacting simultaneously in immunoelectrophoresis against anti-human rabbit serum and anti-sputum serum, demonstrated 2 precipitation lines related to serum-proteins. One of the lines was located in the albumin zone and the other was the β_2 M-globulin or gammaglobulin zone (Fig. III, slide 542).

DISCUSSION

Antisera have been used recently to investigate a number of different pathologic conditions, for instance in the study of complement components (*Ellis & Gell*, 1958), proteins of the amniotic liquid (*Derrington & Soothil*, 1961, and ceruloplasmin in Wilson's disease (*Soothil et al.*, 1961).

Although secretions of various organs, such as urine, bile, etc., were exhaustively studied from the chemical point of view, generally for diagnostic purposes, the bronchial secretion has caused little curiosity. It is not known, whether the presence or absence of certain chemical components in the sputum might be useful in the diagnosis of different affections and particularly in the differential diagnosis of the three types of asthma.

In this paper, it has been demonstrated that there are sometimes evident difference, in the immunochemical constitution of various sputum samples of asthmatic patients, as far as the presence of various serum proteins is concerned. However, it is

possible that samples of sputum of the same patient, obtained at various times of illness, may produce different results.

The cases studied not only demonstrated the presence of several serum proteins in the sputum of asthmatic patients, but also proved, through immunoelectrophoresis, that sputum contains specific antigens, or, at least, possesses an immunochemical constitution different from that of the serum-proteins. These sputum substances which behaved immunologically different from serum-proteins, probably constitute specific sputum antigens. Further confirmation is provided by the finding that anti-serum against sputum (produced in rabbits) did not lose its capacity to react with sputum, even after absorption with "normal" human serum. By immunoelectrophoretic studies on human saliva *Ellison et al.* (1960) arrived at the same conclusion, and this fact strengthens our present observations.

The three main types of asthma — extrinsic, intrinsic and mixed — did not demonstrate any remarkable characteristics in relation to the presence or absence of certain serum proteins. Nevertheless, in 2 cases classified as intrinsic asthma, gammaglobulin was not present in the sputum, but, this fact, for the time being, should not be accepted as a rule inasmuch as in 9 cases of mixed asthma, neither was gammaglobulin not found in 3 samples of sputum.

The albumin fraction was not seen in 4 specimens of sputum obtained, respectively from 2 cases of mixed asthma, 1 from intrinsic asthma and 1 from extrinsic asthma.

Positive reactions were not found in diffusion of sputum against anti- α_1 lipoprotein.

Because of the lack of knowledge we still have about the immunochemistry of sputum, various lines of research are now being tackled which may help to elucidate its nature in various respiratory affections.

SUMMARY

1) By means of the gel-double diffusion technique using specific antisera against 12 different serum proteins, data

were obtained on the immunochemistry of sputum of asthmatic patients.

2) In 13 sputum samples of asthmatic patients (from 2 cases of intrinsic asthma, 2 cases of extrinsic asthma and 9 cases of mixed asthma) by means of the gel-double diffusion, we obtained 6 positive reactions for pre-albumin; 9 for albumin; 1 for α_2 M-globulin; 2 for α_2 -lipoprotein; 4 for β -lipoprotein; 4 for β_2 M-globulin (with anti- β_2 M-globulin, prepared in rabbit); 2 for β_2 M-globulin (with anti- β_2 M-globulin, prepared in horse); 6 for β_2 A-globulin; 4 for fibrinogen; 7 for transferrin (β_2 -siderophilin) and 7 for gammaglobulin. Some sputum samples contained neither albumin nor gammaglobulin.

3) By immunoelectrophoresis using sputum simultaneously against anti-sputum rabbit serum and rabbit serum against human serum, we could demonstrate in the bronchial sputum of asthmatic patients the presence of antigens, not immunologically related to serum protein.

4) The importance that immunochemical studies on sputum may have in the future is pointed out.

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