IMMUNOCHEMICAL STUDY OF HETEROGENEIC ANTIGENS SIMILAR FOR Yersinia pestis EV AND HUMAN ERYTHROCYTES

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It was shown that fractions containing heterogeneic antigens similar for Yersinia pestis EV and human erythrocytes can be obtained by the method of immunosorption of antigen with fixed antibodies on a basis of polyacrylamide gel.

KEY WORDS: heterogeneic antigens; immunosorption; polyacrylamide gel.

The presence of heterogeneic antigens similar to those from human and animal tissues has been established for many pathogenic species of microorganisms. Many workers associate their biological role with the virulence of microorganisms and with the development of autoimmune disorders [1, 3, 7]. Accordingly it is interesting to study heterogeneic antigens of vaccine strains of microorganisms.

In previous communications results showing the presence of heterogeneic antigens in a vaccine strain of Yersinia pestis EV were described [2-4]. The results of these investigations served as the basis for preparations of fractions of the agent of plague containing heterogeneic antigens similar to antigens of human erythrocytes.

EXPERIMENTAL METHOD

To obtain fractions of Y. pestis containing heterogeneic antigens the method of immunosorption of antigen with fixed antibodies on a basis of polyacrylamide gel [5] was used. The principle of the method is that of the total multiple-component complex of microbial antigens, those which possess common antigenic properties with human tissues used to obtain immune sera are fixed on the immunosorbent. Sera obtained by immunization of rabbits with human erythrocytes by Kosyakov's method [6] were used as the source of antibodies. The γ globulin fraction was isolated from the resulting immune sera by precipitation with ammonium sulfate at 50% saturation. Polyacrylamide gel, made up with reagent from Reanal (Hungary), was used as the carrier; its composition was as follows: 10 ml of γ globulin fraction, 0.6 g acrylamide, 0.2 g N,N-methylene-bis-acrylamide, 0.02 ml TEMED, and 5 mg ammonium persulfate. The gel was polymerized at 4°C for 1 h.

The polymerized gel was shredded with a glass rod and passed twice through a syringe with a No. 0840 needle. The shredded gel was washed with 10 volumes of an eluting solution of 3M sodium thiocyanate by centrifugation at 3000g for 5 min. The washed gel was mixed with an equal volume of Sephadex G-25 and the mixture was applied to a column 29 cm high and 1 cm in diameter.

The antigen consisted of 50 ml of a saline extract from Y. pestis cells with a protein concentration of 1 mg/ml. Perfusion of the antigen was carried out by means of an Ultragel (Sweden) apparatus. After the end of perfusion the column was carefully washed with Tris-HCl buffer, pH 7.5, to remove components not fixed to the immunosorbent. The result of washing was verified spectrophotometrically at $\lambda = 280$ nm. A 3M solution of sodium thiocyanate was used for elution. After elution the solution was dialyzed against Tris-HCl buffer in order to remove the CNS ions and then concentrated to a protein concentration of 1 mg/ml.

The concentration of polysaccharides in the fractions was determined by the anthrone method [9]. Dimethylaminobenzaldehyde was used for colorimetric determination of lipids [8].

The precipitation test in agar gel was carried out in the modification of Gusev and Tsvetkov. Antigens from human and animal (rat, mouse, guinea pig, suslik, and hamster) tissues were prepared by saline extraction.

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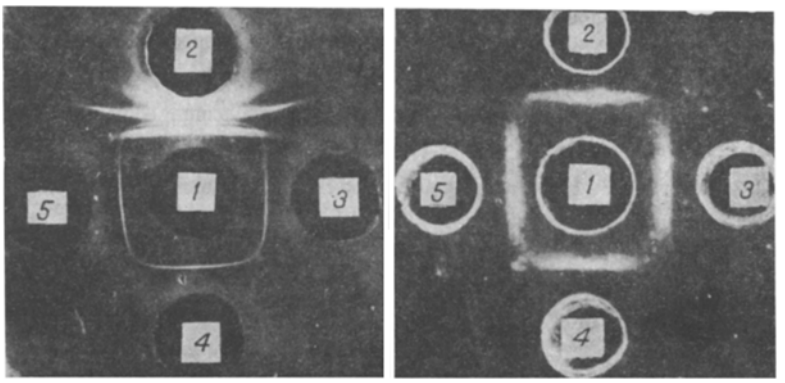

Fig. 1. Precipitation test on fractions of Y. pestis EV obtained by the use of immunosorbents with antierythrocytic sera. 1) In a) saline antigen from Y. pestis EV; 1) in b) fraction of Y. pestis EV obtained on immunosorbent with anti-O-serum; 2) plague antiserum; 3) antiserum against group O erythrocytes; 4) antiserum against group A erythrocytes; 5) antiserum against group B erythrocytes.

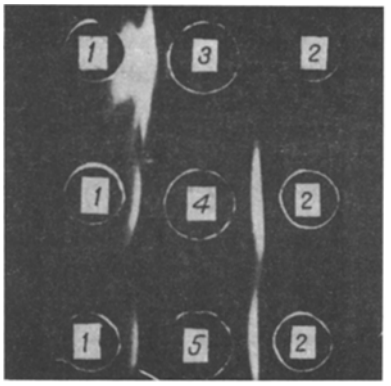

Fig. 2

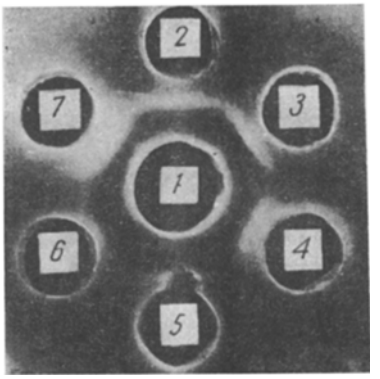

Fig. 3

Fig. 2. Comparative analysis of fractions of Y. pestis EV. 1) Plague antiserum; 2) antiserum against group O human erythrocytes; 3) saline antigen from Y. pestis EV; 4) fraction of Y. pestis EV obtained on immunosorbent with anti-O-serum; 5) fraction of Y. pestis EV obtained on immunosorbent with anti-A-serum.

Fig. 3. Precipitation reaction of plague antiserum with liver extracts from various animals. 1) Plague antiserum; 2, 3, 4, 5, 6, 7) extract of human, guinea pig, rat, mouse, suslik, and hamster liver, respectively.

EXPERIMENTAL RESULTS

The isolated fractions of Y. pestis EV contained 1 mg/ml protein, 0.08-0.1 mg/ml carbohydrates, and 0.002-0.003 mg/ml lipids.

Comparative analysis of the precipitation test on antigens of Y. pestis EV with immune sera against human erythrocytes before and after isolation from the immunosorbent is illustrated in Fig. 1. The Y. pestis fraction formed one precipitation band both with immune serum against Y. pestis EV and with immune sera against human erythrocytes of groups O, A, and B. The precipitation lines in this case were identical. Precipitation lines obtained with immune serum against group O erythrocytes and with isolated fractions of Y. pestis EV obtained on immunosorbents with immune sera against human group O and A erythrocytes also were identical (Fig. 2).

Antigenic similarity of Y. pestis and several human tissues and organs, as well as those of the rat, mouse, guinea pig, suslik, and hamster was discovered in a series of experiments. Immune serum against Y. pestis EV gave a positive precipitation reaction with human, guinea pig, rat, mouse, suslik, and hamster liver antigens, with human, guinea pig, mouse, and rat lungs, with human and rat spleen, with human heart, with hamster, mouse, and rat kidneys, and with rat brain tissue.

As Fig. 3 shows, precipitation lines obtained with plague antigen and with the liver of various animals were identical. Adsorption of the plague antiserum with human group A, B, and O erythrocytes had no significant effect on the results of the test. In this case nonidentical heterogeneic antigens were evidently present in the human organs and erythrocytes.

The results are evidence that fractions of Y. pestis EV containing heterogeneic antigens similar to antigens of human erythrocytes can be obtained by the use of an immunosorbent based on polyacrylamide gel. The fractions obtained are antigenically similar to human erythrocytes of any blood group.

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