HETEROGENEIC ANTIGENS OF Pasteurella pestis AND Vibrio cholerae SIMILAR TO HUMAN AND ANIMAL TISSUE ANTIGENS

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A study of some strains of Pasteurella pestis and Vibrio cholerae showed that they contain heterogeneic antigens similar to human and animal tissue antigens. Common antigens with those of human red cells and guinea pig liver and spleen were found in P. pestis, and common antigens with the epithelium of the mucous membrane of the human small intestine were found in V. Cholerae. In dried cholera vaccine prepared from V. cholerae of types Ogawa and Inaba no heterogeneic human type A, B, or O antigens and no Forssman antigen could be found. It is considered that the presence of cross-reacting heterogeneic antigens in microorganisms may increase their virulence to the host.

Heterogeneic antigens are found in many widely different species of living organisms. Investigations into cross-reacting heterogeneic antigens or mimicry antigens, i.e., those common to both host and infecting agent, are of great interest. Such antigens have been found among Gram-positive and Gram-negative bacteria [2, 5-7].

Heterogeneic antigens similar to human specific antigen were first found in Pasteurella pestis by Zhukov-Verezhnikov and Guseva in 1944 [2]. On the basis of their findings these workers put forward the original hypothesis that the presence of human antigen in pathogenic microorganisms helps to increase their virulence, for it makes it more difficult for the host to distinguish between "its own" and "the foreign" antigen, a phenomenon subsequently described in the literature as "antigenic mimicry" [3, 4].

The object of the investigation described below was to continue the study of the agents of particularly dangerous infections for the presence of heterogeneic antigens similar both to group-specific human antigens and to the organ antigens of man and animals.

EXPERIMENTAL METHOD

Experiments were carried out on 4 strains of Pasteurella pestis (including 2 vaccine strains) and 9 strains of Vibrio cholerae (including 1 vaccine strain).

To detect antigens similar to the organs and tissues of man and animals in microorganisms the precipitation test in agar gel and the reaction of adsorption of specific isoagglutinins by heterogeneic bacterial antigens were used [1].

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TABLE 1. Detection of Antigenic Similarity in Some Strains of P. pestis With Human and Animal Tissues (Results of Agar Diffusion Test)

Microorganism	Antiserum	Results
P. pestis Otten	a	1 line
	b	1 »
	c	—
P. pestis EV-5	a	2 lines
	b	1 »
	c	1
P. pestis EV	a	1 line
	b	1 »
	c	—
P. pestis 231	a	1 line
	b	1 »
	c	—

Legend: a) antiserum against human red cells; b) antiserum against guinea pig spleen; – no reaction.

For the gel-diffusion test, to rule out the possibility of denaturation of the antigens the bacterial cells were used untreated in the form of suspensions of living cells in physiological saline. Besides normal rabbit serum, the Hottinger's digest in which the nutrient agar for growing the bacteria was prepared was also used as a control. The test antisera and the normal rabbit serum formed no precipitation lines with Hottinger's digest.

The antisera used in the gel-diffusion tests were prepared by immunizing rabbits with the following antigens: human red cells, scrapings of epithelium of the mucous membrane and stroma of the residual part of the human small intestine, and extracts of guinea pig liver and spleen. A few special points about the technique of preparing intestinal antigens must be mentioned. Choice of the small intestine is explained by the fact that the pathological process in cholera arises in this part of the digestive tract, and it has the minimal content of incidental microflora. To remove the microflora a segment of intestine was thoroughly washed with physiological saline and placed in a solution containing oxytetracycline and streptomycin in doses of 50 units/ml of each. After the material had remained for 2 days in a refrigerator at 4°C control seedings were taken for sterility. Antigens were prepared from the sterile organ. The tissues were minced in a homogenizer and extracted with physiological saline in the ratio of 1:2 at 4°C. The resulting antigens were used to immunize rabbits (1 rabbit for each antigen) by the following scheme: the first injection was given subcutaneously as a mixture of antigens with Freund's adjuvant in a volume of 20 ml. Next, after 2 weeks, followed intramuscular injections of antigens without adjuvant at weekly intervals, in volumes of 5, 10, and 10 ml respectively. Blood was taken 7 days after the last injection. If necessary revaccination was performed. The antisera obtained had a titer of up to 1:20,000 in the agglutination test with homologous antigens.

Heterogeneic antigens of the human A, B, and O (H) isoantigen type were detected in dried cholera vaccine prepared from V. cholerea types Ogawa and Inaba, by the adsorption of specific isoagglutinins test. Monospecific forensic-medical anti-A and anti-B sera and anti-O(H) lectin, prepared from elderberries, were used in the experiments. The forensic-medical sera and lectin were diluted with physiological saline to a titer of 1:64 and added in a volume of 0.1 ml to a test tube with a residue of bacteria weighing approximately 50 mg. Adsorption was carried out at 37°C for 2 h. The tubes were then centrifuged and the supernatent tested with a 5% suspension of red cells of groups A, B, and O. For the detection of Forssman antigen, instead of the forensic medical sera a hemolytic serum was used and tested after adsorption with sheep's red cells.

EXPERIMENTAL RESULTS

The results obtained in the agar diffusion test with P. pestis Otten (vaccine strain), P. pestis EV-5 (vaccine strain), P. pestis EV (reference strain of the NIIEG line), P. pestis 231 (highly virulent strain), and with antisera against human red cells and guinea pig liver and spleen are given in Table 1. All strains used in the experiment formed 1 diffuse line with antisera against human red cells except P. pestis EV-5, which gave 2 distinct lines with antiserum against human red cells. When antiserum against human red cells and guinea pig liver and spleen extracts were used as antigens, each gave 1 diffuse line. Antisera against guinea pig liver and spleen extracts gave more than 10 distinct lines with homologous antigens and 1 clear line with extract from albino mouse spleen.

In the diffusion test with strains of El Tor vibrio and V. cholerae 1 or 2 diffuse lines were found in each case with antiserum against scrapings of epithelium from the mucous membrane of the human small intestine. Antiserum against stroma of the human small intestine gave no precipitation lines in the agar with the same strains of El Tor vibrio and V. cholerae.

The results of the experiments to test adsorption of the antisera showed that dried cholera vaccine obtained from V. cholerae types Ogawa and Inaba contains no antigens of the human A B O system and no Forssman antigens.

It can thus be concluded that common antigenic complexes exist between erythrocytes and human and animal tissues, on the one hand, and the agents of plague and cholera on the other hand. Characteristically, a few common antigens with V. cholerae were present only in the epithelium of the mucous membrane of the human small intestine. The presence of cross-reacting antigens in the agents of particularly dangerous infections which show a resemblance to the tissues and organs of the host may help to explain the high virulence of P. pestis and V. cholerae.

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