COMMON ANTIGENS OF CHOLERA VIBRIOS AND MAN

T. N. Belova and D. D. Efimov

UDC 576.851.315.095.38.097.2

Antigens interacting with antibodies against human antigens were found in cholera vibrios. In preparations from human spleen an antigen reacting with antibodies against cholera vibrios was found. The common antigens of cholera vibrios and man are found by the passive hemagglutination and gel-diffusion tests. Cholera vibrios contain two types of antigens which are common with human antigens. The antigens of the first type resemble a substance of the Forssman type and are contained both in classical cholera vibrios and also in vibrios of the El-Tor biotype. Antigens of the second type differ from the Forssman antigen and are found in cholera vibrios of the Ogawa and Inaba serotypes but are not found in vibrios of the El-Tor biotype.

Antigens common with human antigens have been found in the agents of many infectious diseases: the bacilli of dysentery, paratyphoid fever, enteric infections, and plague, and in the viruses of smallpox and influenza [2, 4-7]. The existence of this common antigenic behavior of parasite and host is a manifestation of antigenic mimicry, and it may be due to adaptation of the parasite to the host and may determine the pathogenicity of the microorganism relative to that host [3, 7]. Antigens of the agent of infectious diseases which have common determinants with human antigens are interesting from the point of view of their participation in the susceptibility of the host to infection and the formation of postvaccinal immunity.

Common antigens of the cholera vibrio and man were studied in the investigation described below. This is a particularly urgent problem because in cholera the postinfectious and postvaccinal immunity is weak and of short duration [1].

EXPERIMENTAL METHOD

Antigens of cholera vibrios were obtained by extraction of cultures of cholera vibrios belonging to the Ogawa, Inaba, and Hikojima serotypes and cholera vibrios of the El-Tor biotype with physiological saline. The cultures of the vibrios were grown on solid nutrient medium and inactivated by the addition of merthiolate up to a concentration of 1:10,000 and of 40% formaldehyde solution at the rate of 10 ml per liter of vibrio suspension in a density of 200 to 400 billion cells/ml. Extracts of the vibrios were dialyzed in distilled water and dried by lyophilization.

Antigen preparations from human spleens were obtained by extraction of a dry acetone powder of spleen tissue at 100°C with water and by precipitation of the resulting extract with 5 volumes of alcohol. The precipitate was dried by washing with alcohol and ether. In this way preparations were obtained from the spleens obtained after accidental death from 6 previously healthy persons.

Antisera against the cholera vibrio antigens were obtained by immunization of rabbits with a mixture of equal volumes of bacterial suspension with a density of 200-400 billion cells/ml and Freund's complete adjuvant. The suspension was injected in volumes of 2 ml intraperitoneally and intramuscularly. After 21-25 days 2 ml of the vibrio suspension without Freund's adjuvant are injected intramuscularly, and blood was taken 7-10 days later.

I. I. Mechnikov Moscow Research Institute of Vaccines and Sera. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 9, pp. 62-64, September, 1972. Original article submitted January 2, 1972.

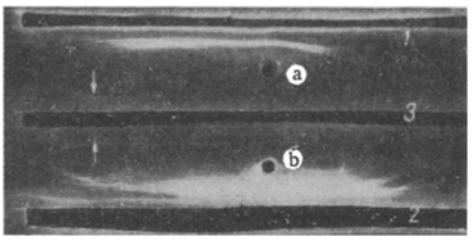

Fig. 1. Immunoelectrophoresis in gel of antigens from cholera vibrios: a) extract of cholera vibrios of the Inaba serotype; b) extract of cholera vibrios of the Ogawa serotype. Gutters for antisera filled with: 1) antiserum against cholera vibrios of Inaba serotype; 2) antiserum against cholera vibrios of Ogawa serotype; 3) antiserum against human leukocytes. Arrows denote precipitation arcs formed by interaction between antigens of cholera vibrios and antiserum against human leukocytes.

Antisera against human antigens were obtained by immunization of the rabbits with the following materials: a) human serum protein; b) normal human leukocytes isolated from citrated donor's blood by separation of the cells in 1% gelatin solution; c) human lymphoid tissue obtained by tonsillectomy. A mixture of equal volumes of the corresponding material and Freund's complete adjuvant was used for immunization. Again, 2 ml of the mixture was injected intraperitoneally and 2 ml intramuscularly. After 21-25 days 2 ml of the corresponding antigen was injected subcutaneously without Freund's adjuvant, and blood was taken 7-10 days later. Two series of immune horse globulin against human lymphocytes, prepared at the Moscow Research Institute of Microbiology and Epidemiology, Ministry of Health of the RSFSR, also were tested.

The passive hemagglutination test (PHT) was carried out with formalinized, tanninized sheep's erythrocytes, which were sensitized with 1% solution of lyophilized extract of cholera vibrios. The PHT was carried out in a microtitrator of the Takachi system.

For the agar precipitation test 3% solutions of lyophilized extracts of cholera vibrios and 10% solutions of the antigen preparations from human spleens were used.

Immunoelectrophoresis was carried out in 1% agar gel made up in veronal buffer, pH 8.6, ionic strength 0.015, with a potential gradient of 5 V/cm.

EXPERIMENTAL RESULTS

Rabbit antisera against human serum protein and against human lymphoid tissue gave high titers in the PHT (1:4096 and more) with all tested extracts of cholera vibrios. The same sera agglutinated unsensitized sheep's erythrocytes in a titer of 1:512. After absorption of the antisera with sheep's erythrocytes the titers in the PHT with extracts of cholera vibrios fell to zero or were sharply reduced to values of 1:2-1:8. Consequently, the PHT was due to antibodies interacting with sheep's erythrocytes. In the agar diffusion test solutions of lyophilized extracts of cholera vibrios and rabbit antisera against human serum proteins and lymphoid tissue formed a clear line, demonstrating interaction between the antigens of the cholera vibrios and antibodies against human antigens. After absorption of the antisera with sheep's erythrocytes no precipitation band was formed in the gel during diffusion against extracts of the cholera vibrios. Consequently, antisera against the human antigens contained antibodies reacting specifically both with sheep's erythrocytes and with cholera vibrios. These results indicate that common antigenic determinants exist in man, sheep, and cholera vibrios. Since antisera against cholera vibrios did not agglutinate sheeps's erythrocytes, it can be postulated that the substance in cholera vibrios which interacts with antibodies also reacting with sheep's erythrocytes, exists in the form of a hapten of the Forssman type.

Rabbit antisera against human leukocytes and both series of horse antilymphocytic globulin did not agglutinate unsensitized sheep's erythrocytes. After sensitization with extracts of cholera vibrios of serotypes Ogawa and Inaba these antisera agglutinated erythrocytes in titers of 1:1024 or more. The PHT titers were lower (1:2-1:8) with erythrocytes sensitized by extracts of cholera vibrios of the Hikojima serotype and and vibrios of the El-Tor biotype. During agar diffusion extracts of cholera vibrios of the Ogawa and Inaba serotypes formed a precipitation line with antiserum against human leukocytes. Immunoelectrophoretic analysis showed that antigens of cholera vibrios which react with antibodies against antigens of human leukocytes move in an electric field toward the anode (Fig. 1). Antigen from cholera vibrios of the Inaba serotype form a symmetrical arc, while antigen from vibrios of the Ogawa serotype form an asymmetrical, long arc of precipitation. This shows that antigen of the vibrio of the Ogawa serotype which reacts with antibodies against human antigens consists of molecules which differ from each other in their electrophoretic mobility.

By means of the agar diffusion test, an antigen forming a precipitation line with antiserum against cholera vibrios of the Ogawa serotype was found in a preparation of material from the human spleen.

These investigations showed that during immunization of rabbits and horses with human antigens antibodies which react with antigens of cholera vibrios are formed, and during immunization with cholera vibrios, antibodies interacting with human antigens are formed. Consequently, both in cholera vibrios and in man there are substances with common antigenic determinants. In cholera vibrios two types of these substances can be distinguished. Antigens of the first type have common determinants with human antigens and sheep erythrocytes, and they are evidently substances of the Forssman type. These substances are found in cholera vibrios of all serotypes and in vibrios of the El-Tor biotype. Antigens of the second type differ from the Forssman antigens, they are found in classical cholera vibrios of the Ogawa and Inaba serotypes, and they are absent in vibrios of the El-Tor biotype.

LITERATURE CITED

1. P. N. Burgasov, El-Tor cholera [in Russian], Moscow (1971), p. 77.
2. N. N. Zhukov-Verezhnikov and G. Guseva, Zh. Mikrobiol., No. 3, 14 (1964).
3. I. M. Lyampert, Abstracts of Proceedings of the 3rd All-Union Conference on Immunopathology [in Russian], Leningrad (1969), p. 16.
4. I. I. Podoplelov, G. M. Bochko, and V. I. Shipkov, Byull. Éksperim. Biol. i Med., No. 11, 61 (1971).
5. E. S. Stanislavskii, Zh. Mikrobiol., No. 10, 42 (1971).
6. V. P. Efroimson, Immunogenetics [in Russian], Moscow (1971), p. 258.
7. C. R. Jenkin, Advances in Immunology, Vol. 3 (1963), p. 351.