IMMUNOCHEMICAL FEATURES OF SUBUNITS OF CHOLERA ENTEROTOXIN AND THERMOLABILE *E. coli* ENTEROTOXINS OF DIFFERING ORIGIN

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Many diseases accompanied by the development of diarrhea are caused by different species of bacteria, which produce cholera-like enterotoxins. These protein substances, responsible for the main manifest syndrome of the disease, namely the development of diarrhea, are similar in their antigenic properties, molecular structure, and mechanism of action [3, 5, 6, 9]. They consist of functionally different subunits, one of which is responsible for specific binding with receptors of intestinal epithelial cells, the other for the appearance of intracellular changes. None of these subunits, taken separately, is toxic for intact cells. The construction of an immunoprophylactic preparation on the basis of one subunit of a given enterotoxin can thus result in the creation of antitoxic immunity to the whole group of cholera-like toxins. However, cholera-like enterotoxins of different species are not identical with each other but exhibit definite differences [4, 5, 10].

The aim of this investigation was to undertake a comparative immunochemical analysis of subunits of cholera enterotoxin and thermolabile enterotoxins of *E. coli* of different origin.

EXPERIMENTAL METHOD

As producers of cholerogen (CT) we used strain 569B of *Vibrio cholerae* of the Inaba serotype, to obtain thermolabile *E. coli* enterotoxin of human origin (HLT) strain H74-114 of *E. coli*, and to obtain thermolabile *E. coli* enterotoxin a strain of *E. coli* (EWD_{299}) isolated

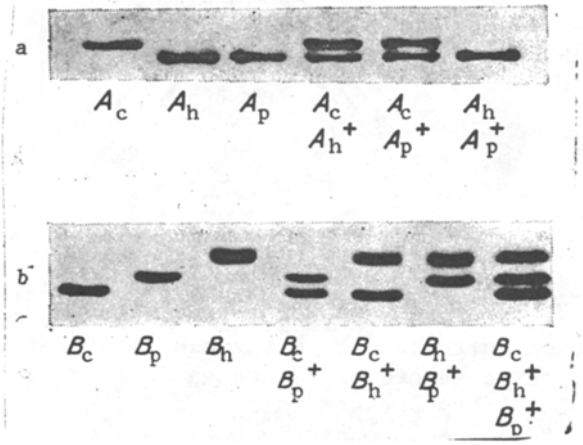

Fig. 1. PAG disk-electrophoresis with SDS of A (a) and B (b) subunits of cholera enterotoxin, of thermolabile enterotoxin of *E. coli* isolated from man, and from thermolabile enterotoxin of *E. coli* isolated from pigs (A_c, B_c, A_h, B_h, A_p, B_p respectively).

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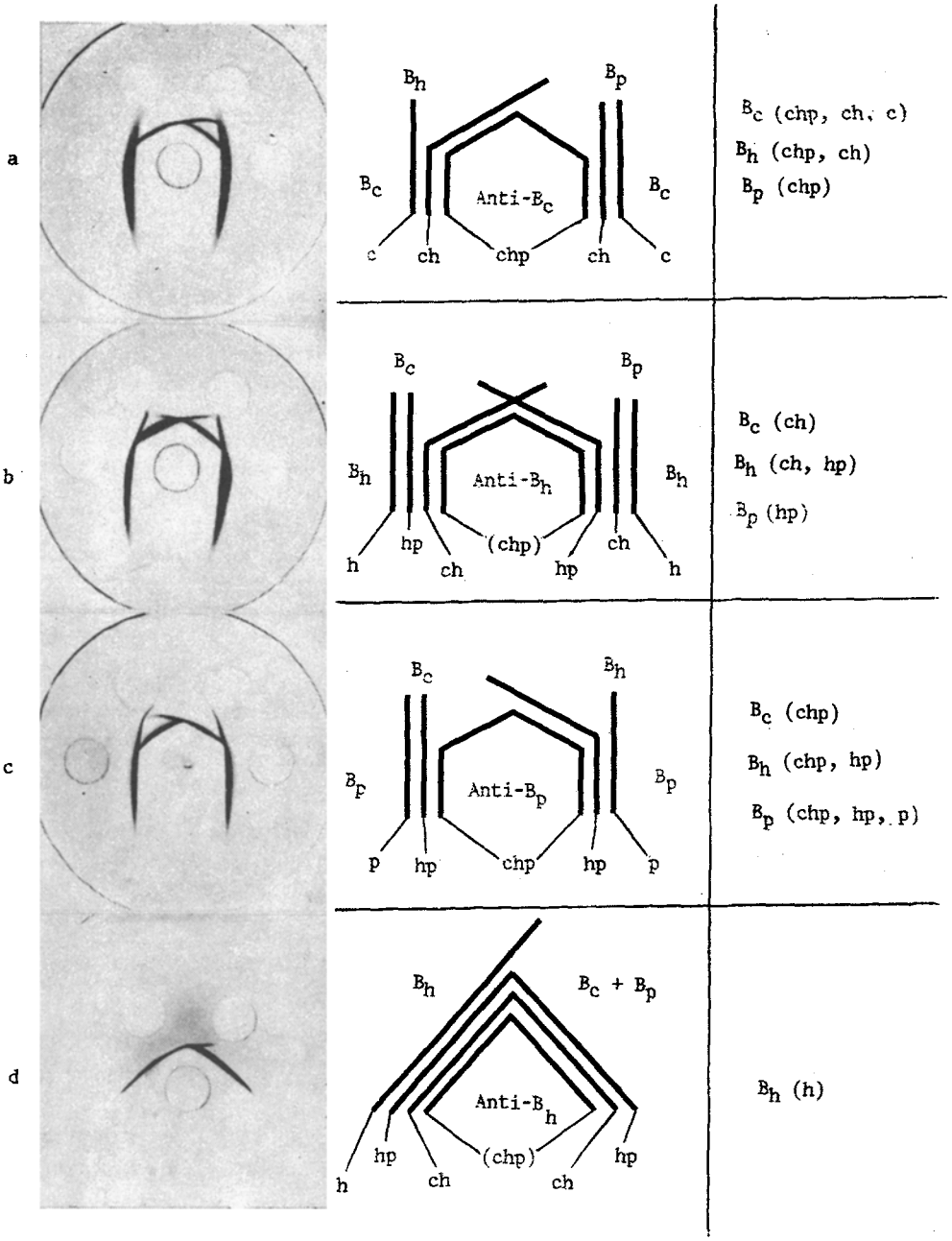

Fig. 2. Immunochemical analysis of B subunits of cholera enterotoxin and of thermolabile enterotoxins of *E. coli* isolated from man and pigs (B_c, B_h, and B_p respectively). Anti-B_c, anti-B_h, and anti-B_p) antisera to B_c, B_h, and B_p respectively. Lines on scheme of reaction correspond to antigenic determinants (AgD); B(chp) present in B subunits of all three enterotoxins and taking part in precipitate formation; B(ch) present in B_c and B_h; B(hp) present in B_h and B_p; B(c) present only in B_c; B(h) present only in B_h; B(p) present only in B_p. Here and in Fig. 3: I) reaction, II) scheme of reaction, III) AgD discovered.

from pigs PLT). The microorganisms were cultured and the toxins purified by methods described previously [1, 2, 11]. Subunits A and B of cholera enterotoxin and of thermolabile *E. coli* enterotoxins of human and animal origin (A_c and B_c, A_h and B_h, A_p and B_p, respectively) were obtained from whole toxins by fractionation by gel-filtration on a column with Sephadex G-75 under denaturing conditions at low pH and in the presence of urea, by a modified method [7]. The modification consisted of substitution of renaturation by gel-filtration on a column with Sephadex G-25 for renaturation by gradient dialysis. In this way losses of protein during renaturation through the pores of the dialysis membrane and also partial aggregation of the

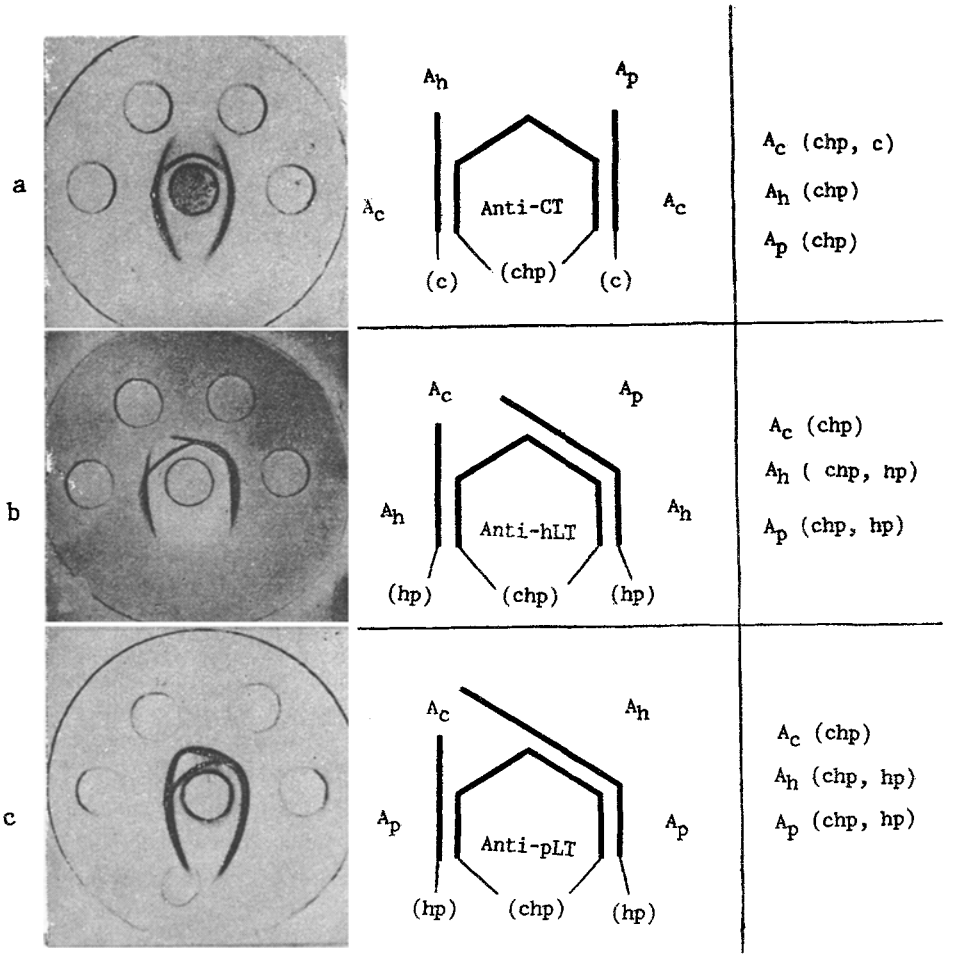

Fig. 3. Immunochemical analysis of A subunits of cholera enterotoxin and of thermolabile enterotoxins of *E. coli* isolated from man and pigs (A_c, A_h, and A_p respectively). Anti-CT) antiserum to cholera toxin; anti-hLT) antiserum to thermolabile enterotoxin of *E. coli* of human origin; anti-pLT) antiserum to thermolabile enterotoxins of *E. coli* of animal origin. Lines on scheme of reaction correspond to AgD: A(chp) present in A subunits of all three enterotoxins and taking part in precipitate formation, A(hp) present in A_h and A_p; A(c) present only in A_c.

denatured protein were eliminated. Monospecific antitoxic sera to whole toxins and to their B subunits were obtained by immunizing rabbits with purified preparations in accordance with the scheme described in [14]. In a comparative study of the immunochemical properties of the enterotoxin subunits, the double immunodiffusion test in agar gel was used [12]. The electrophoretic investigation of the enterotoxin preparations and their subunits was carried out in polyacrylamide gel (PAG) in the presence of sodium dodecylsulfate (SDS) [8].

EXPERIMENTAL RESULTS

To detect structural, functional, and immunochemical differences between the cholera-like enterotoxins and their subunits, they had to be obtained in the form of individual components. Preparations of subunits A and B were obtained by gel-filtration under denaturing conditions at low pH values followed by renaturation of the isolated proteins. According to the results of PAG electrophoresis with SDS, the A subunits of the toxins were proteins with mol. wt. of about 29,000 daltons and possessed somewhat different electrophoretic mobility (Fig. 1a). Allowing for the value of their electrophoretic mobility, these subunits could be arranged in the following order: $A_c < A_h \simeq A_p$. The B subunits of the three toxins were proteins with a molecular weight of between 11,000 and 12,000 daltons and differed in their electrophoretic mobility, which can be represented as $B_c > B_p > B_h$ (Fig. 1b).

According to data in the literature, previously, preparations of whole toxins were subjected to comparative immunochemical analysis [10, 13], and no similar investigations of individual subunits were undertaken. We carried out an analysis in order to discover related and individual antigenic determinants of each protein subunit.

To begin with B subunits of the three toxins were tested with serum to the B_c subunit, containing antibodies to all antigenic determinants (AgD) of this protein (Fig. 2a). By performing the gel-diffusion test in this way it was possible to discover AgD common to all three subunits — B(chp), AgD common to B_c and B_h — B(ch), and AgD corresponding only to B_c — B(c). Immunochemical analysis of the subunits of the three enterotoxins tested with serum to B_h (Fig. 2b) revealed additionally AgD in the B_h and B_p subunits common for these two proteins — B(hp). Performing the test with antiserum to B_p (Fig. 2c) revealed the presence of individual AgD in the protein B_p subunit — B(p) and confirms the data obtained in the two previous experiments. Individual B(h) AgD were found in the B_h subunit in the next version of the experiment (Fig. 2d). Similar results were obtained by the use of antisera to whole toxins (the data are not shown). AgD common to the B_c and B_p subunits were not found in these experiments.

The results thus show that ligand B subunits of the three related toxins exhibit immunological kinship on account of common AgD [B(chp)], characteristic of all three types of subunits tested, and on account of group AgD [B(ch) and B(hp)], characteristic of the B subunits of the three enterotoxins taken two at a time, and not found in the third toxin. Moreover, a larger number of types of AgD, determining general immunologic kinship, was observed in B_h than in B_c and B_p.

Immunochemical analysis of the A subunits of the three enterotoxins was carried out in the same way. Antisera to whole toxins were used as the source of antibodies. The analysis showed that each of the subunits has common AgD [A(chp) (Fig. 3, a, b, c)], and that the A_c subunit possesses individual AgD [A(c) (Fig. 3a)], characteristic of it alone, whereas the A_h and A_p subunits lacked individual AgD and had grouped AgD [A(hp)], characteristic of it alone (Fig. 3b, c); this means that the A subunits of the two thermolabile enterotoxins were immunologically identical.

Thus nine types of AgD were found in the subunits of cholera enterotoxin and the thermolabile enterotoxins of *E. coli* isolated from the different sources, and they can be grouped as follows. B subunits: 1) common AgD for the B subunits of all three enterotoxins — B(chp); 2) group AgD common to the B subunits of the three toxins taken two at a time — B(ch) and B(hp); 3) individual AgD — B(c), B(h), and B(p). A subunits: 1) common AgD for the A subunits of all three enterotoxins — A(chp); 2) group AgD common for the A subunits of the two enterotoxins hLT and pLT — A(hp); 3) individual AgD — A(c).

Accordingly the antigenic structure of the subunits of the three related enterotoxins can be presented in the form:

CT — B(chp), (ch), (c); A(chp), (c).
hLT — B(chp), (ch), (hp), (h); A(chp), (hp).
pLT — B(chp), (hp), (p); A(chp), (hp).

It can be postulated that the group of AgD common for the three enterotoxins is located at sites on the molecules of the A and B subunits that are evolutionarily more resistant, and that this is linked with the manifestation of their functional activity. It is therefore important that these AgD be included in immunoprophylactic preparations and also for the construction of immunodiagnostic test systems on their basis.

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TIME COURSE OF EXPERIMENTAL ANTIBODY FORMATION TO HERPES SIMPLEX VIRUS NUCLEOCAPSID AND ENVELOPE PROTEINS

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Investigation of the immune response in man to various forms of herpetic infection have shown [1, 3, 8] that the primary infection is characterized by a low level of antibodies to viral glycoproteins associated with a high level of antibodies to low-molecular-weight internal nucleocapsid proteins. However, the data published by the authors cited were obtained by the study of sera obtained once only from patients during the acute course of the disease and in the convalescent period. These results did not reflect the dynamics of antibody formation to individual viral proteins in the course of the infection. Yet such information would be of great importance for the development of precise methods of laboratory serodiagnosis of herpetic infection. The urgency of the development of tests of this kind is due to difficulties in the clinical diagnosis of herpetic encephalitis and of neonatal diseases.

The aim of this investigation was to study the dynamics of antibody formation to individual viral proteins during primary herpetic infection in experimental rabbits, using enzyme-linked immunosorbent assay (ELISA) and immunoblotting methods.

EXPERIMENTAL METHOD

Type I herpes simplex virus (HSV-I) of the VR-3 strain was cultured on a transplantable cell line. Virus-specific cytoplasmic envelope complexes (EC) and viral nucleocapsid (NC) were isolated from infected cells by methods described previously [6, 10]. The homogeneity of the antigens thus obtained was determined by polyacrylamide gel (PAG) electrophoresis and the microdiffusion in agar test, using hyperimmune rabbit serum obtained to the envelope glycoprotein of HSV-I.

Sera from rabbits (8 animals) infected with HSV-I, applied to the clarified cornea, were tested 7, 10, 14, 21, 28, and 75 days after infection.

The direct version of ELISA was carried out by the method in [12], using a volume of 100 µl. Immunosorbents (preparations of EC and NC) were used in a concentration of 1.25 µg/100 µl as protein.

Electrophoresis in 9.5% PAG was carried out by the method in [7]. The immunoblotting test followed the method in [11] with minor modifications. The sera were tested in a dilution of 1:10. An antirabbit peroxidase conjugate, prepared in the Laboratory of Serologic Micromethods, was used in a dilution of 1:2,000.

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