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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.

Laboratory of Serologic Micromethods and Laboratory of Genetics of DNA-Containing Viruses, Moscow Research Institute of Virus Preparations, Ministry of Health of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR O. G. Andzhaparidze.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 476-478, October, 1987. Original article submitted December 19, 1986.

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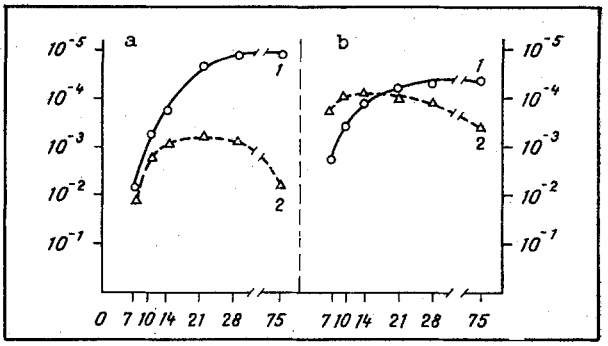

Fig. 1. Dynamics of antibody formation to EC (1) and NC (2) in ELISA. Abscissa, time after infection (in days); ordinate, antibody titers in ELISA: a) using native immunosorbents, b) using denatured immunosorbents.

EXPERIMENTAL RESULTS

The results of one typical ELISA experiment, obtained during the investigation of sera from one rabbit (Fig. 1a), enabled the time course of antibody synthesis to proteins of virus-specific structures (EC and NC) to be evaluated and compared. For instance, during a steady rise of antibody titers to EC proteins throughout the period of the investigation (2.5 months), antibody titers to viral NC proteins initially also rose, to reach a maximum by the 21st day, after which they fell.

The results of investigation of the sera by the immunoblotting method confirmed in principle the data of EIA (Fig. 2). In the course of the period of the infectious process studied an increase in the titer of antibodies to EC proteins was observed. The time course of the immune response was most clearly defined in the case of high-molecular-weight proteins with mol. wt. of 125, 110, 85, and 50 kilodaltons (kD) (Fig. 2: 3-8). These proteins are glycoproteins identified by the method in [2] (Fig. 2: 9) and correspond, it is considered, to herpetic glycoproteins gC, pgC, gE, and gD respectively [9]. The time course of antibody formation to a protein with mol. wt. of 100 kD (conjecturally glycoprotein gB) was less marked.

The main proteins of NC to which antibodies are formed are NC proteins with mol. wt. of between 39 and 45 kD, and also the major structural capsomeric proteins with mol. wt. of about 140 kD (Fig. 2: 11-16). The dynamics of antibody formation to NC proteins differs in character from that to EC proteins. For instance, in the initial stages of the infectious process (until the 21st day) an increase in the content of antibodies to low-molecular-weight NC proteins was observed. Later, the antibody titer fell. No marked time course of antibody formation was observed, however, to the protein with mol. wt. of 140 kD.

Despite qualitative agreement in the data on the time course of antibody formation to EC and NC, obtained by the ELISA and immunoblotting method, the marked quantitative disparity between antibody titers in ELISA and those obtained by immunoblotting will be noted. For example, titers of antibodies to EC and NC on the 21st day of the infection, according to the ELISA data differed tenfold (Fig. 1a), whereas according to the immunoblotting data, no such striking difference was observed (Fig. 2: 6, 14). One possible cause of the observed differences may be screening of the epitopes of the internal NC protein during interaction with antibodies against them, when intact subviral structures were used as immunosorbents in ELISA. To test this hypothesis, EC and NC preparations were treated with 2% sodium dodecyl sulfate (SDS) and, 5% 2-mercaptoethanol in order to create conditions for interaction between antibodies and screened antigenic determinants before the polystyrene plates were sensitized by them.

The ELISA data obtained by the use of denatured immunosorbents (EC and NC) are illustrated in Fig. 1b. The general character of the time course of antibody formation in sera tested to EC and NC remained unchanged. However, the quantitative ratio of the antibody titers to EC and NC in the early stages of the infectious process (until the 21st day) differed sharply from the ratio between the titers when intact immunosorbents were used (Fig. 1a, b). With intact immunosorbents the antibody titer to virus-specific envelope proteins was higher than the titers of antibodies to NC proteins throughout the period of study of the infectious process (Fig. 1a). When denatured antigens were used, there was a distinct increase in the

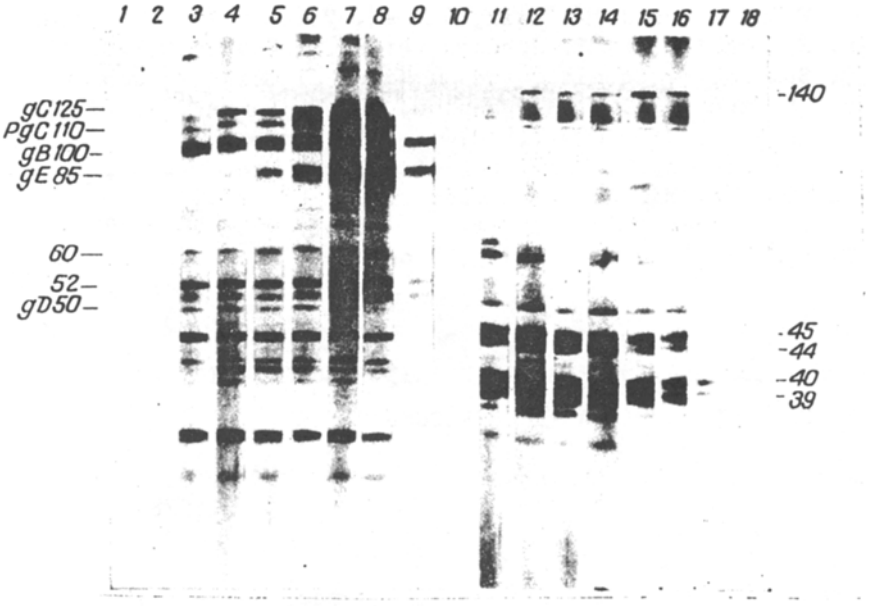

Fig. 2. Analysis of time course of antibody formation to EC (1-9) and NC (10-18) proteins by the immunoblotting method. 1, 18) preparations of EC and NC isolated from uninfected cells on analysis with immune serum; 2-17) EC and NC preparations from infected cells treated with sera taken before infection (2, 17) and 7 days (3, 11), 10 days (4, 12), 14 days (5, 13), 21 days (6, 14), 28 days (7, 15), and 75 days (8, 16) after infection. 9, 10) Glycoproteins revealed by the method in [2]. Numbers are molecular weights of proteins (kD). Letters correspond to nomenclature of HSV-I glycoproteins according to [9].

quantity of antibodies detectable in the early stages (before the 21st day), which reacted with epitopes of NC proteins, than to EC proteins (Fig. 1b).

The data showing predominant formation of antibodies to internal HSV proteins in the early stages of the infectious process thus confirmed data in the literature both on herpetic infection [3] and on several other viral infections [4, 5]. However, unlike data in the literature [3], the present investigation of antibody formation was undertaken over a period of time, with the use of highly purified homogeneous preparations of NC and EC and immunosorbents. In this way individual proteins to which specific antibodies were formed at different times of the experimental infection could be identified sufficiently unambiguously. Before the 21st day after infection antibodies were formed mainly to viral nucleocapsid proteins with mol. wt. of 39-45 kD, followed by a fall in the antibody level to them. From the 21st to the 75th day (period of observation) antibodies were formed mainly to glycoproteins with mol. wt. of 125, 100, 85, and 50 kD, corresponding, it was assumed to gC, gB, gE, and gD [9].

Comparison of the results of immunoblotting and ELISA obtained with the use of intact EC and NC (Figs. 1a and 2) suggests that some epitopes of NC proteins are inaccessible in ELISA for interaction with specific antibodies. When immunosorbents denatured by treatment with SDS and 2-mercaptoethanol were used the antibody titers to internal NC proteins on the 7th-14th days of infection became much higher than titers to EC proteins. This fact provides the basis for the construction of an ELISA test system for the diagnosis of primary and recurrent herpetic infection, using denatured EC and NC proteins as immunosorbents. The results showing the most distinct time course of antibody formation to glycoprotein gE and to the internal nucleocapsid protein with mol. wt. of 40 kD also provide a basis for further improvement of this ELISA test system.

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EFFECTIVE METHOD OF INCORPORATING PLASMID DNA INTO EUKARYOTIC CELLS USING LIPOSOMES TO PRODUCE VACCINIA VIRUS RECOMBINANTS

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KEY WORDS: recombinant vaccinia virus; liposomes; Sendai virus; AIDS virus.

Vaccinia virus has begun to be widely used recently as a vector for cloning foreign genes [8]. This is because of the high capacity of this vector [cloning of DNA fragments measuring 24 kilobase pairs (kb) has been reported [11]], and the broad spectrum of recipient cells sensitive to the virus. The recombinant virus, carrying genes of microorganisms pathogenic for man and animals, can also be used as a living subunit vaccine [15]. Production of recombinant vaccinia virus expressing HTLV-III-specific proteins is an important stage in the creation of a vaccine against AIDS. A fragment of the env gene of HTLV-III virus was therefore used for cloning.

To obtain recombinant vaccinia viruses, plasmid DNA containing the test gene in an environment of virus-specific sequences has to be incorporated into cells infected with this virus. Usually this can be done by the Ca-phosphate precipitation method [5]. Recently liposomes containing viral or plasmid DNA have begun to be used to transform eukaryotic cells [3, 14]. However, when using this method, investigators have encountered difficulties. Incorporation of DNA into cells with the aid of reverse-phase liposomes, consisting only of lipids, did not give the desired result because of the absence of any mechanism whereby genetic material can pass out of the liposomes into the cell cytoplasm [13]. At the same time, incorporation of DNA into liposomes into which envelope glycoproteins of Sendai virus had been incorporated for the purpose of membrane fusion, proved to be ineffective [4, 14].

An effective method of incorporating plasmid DNA into cells has recently been suggested, based on induction of fusion of the liposome membrane, into which gangliosides has been incorporated, with the plasma membrane of the cell with the aid of UV-inactivated Sendai virus. By means of this method a recombinant vaccinia virus carrying the env gene of HTLV-III virus has been obtained.

EXPERIMENTAL METHOD

Plasmid pGS20 [7] was used as the vector. Recombinant plasmid pGSenvL 1 was obtained by the scheme shown in Fig. 1. The EcoRI/BamHI-fragment of the env gene of HTLV-III virus was obtained after restriction of DNA of plasmid pBH10 [10]. The recipient of the recombinant plasmid was *E. coli* strain HB101 [(F^-, hsdS20(r_B^-, m_B^-), rec A13, lac Y1, rps L20(Str)]. The orientation of the cloned fragment in plasmid pGSenvL 1 was determined after restriction by

*Deceased.

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