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RECOMBINANT env PROTEIN IN DIAGNOSIS OF AIDS

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The acquired immunodeficiency syndrome (AIDS) is a dangerous disease caused by a retrovirus which is spreading rapidly at present in many countries of the world. Test systems based on the ELISA technique are most commonly used for the diagnosis of AIDS. Production of suitable diagnostic kits requires a considerable number of virus antigens, the preparation of which requires the use of large quantities of expensive media and sera to culture the virus, and also special measures of protection of personnel working with the virus. The use of antigens obtained by genetic engineering methods in these test systems is therefore interesting. With test systems of this kind it is possible to detect antibodies to single virus antigens, and they may therefore, be useful to determine the stage of the disease and to predict its outcome [6, 8].

The writers previously described a method of obtaining AIDS virus antigens coded by the env gene in *Escherichia coli* cells [1, 2]. In this paper we describe the results of testing blood sera from patients with AIDS and AIDS-related complex and also of sera from groups at increased risk of developing AIDS, by the use of a diagnostic kit based on recombinant protein.

EXPERIMENTAL METHOD

The lysate of a culture of AIDS virus of antigen-producing strain JM 107/pUCenv1 [2] was obtained by the method of Emtage et al. [4] with certain modifications. The cell residue obtained from 2 liters of induced culture [2] was resuspended in 15 ml of cold buffer containing 25% sucrose, 50 mM Tris-HCl (pH 8.0), 20 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride (PMSF), after which 5 ml of a solution of lysozyme (initial concentration 10 mg/ml) was added and the mixture was incubated for 5 min at 0°C. Next 2.5 ml of 0.5 M EDTA solution was added and the sample was incubated for a further 5 min at 0°C. The suspension was then treated with 25 ml of lytic buffer (0.1% Triton X-100, 50 mM Tris-HCl, pH 8.0, 50 mM EDTA), and the lysate was treated with ultrasound until disappearance of viscosity, and then centrifuged at 10,000g for 45 min at 4°C. The supernatant was used in the subsequent work.

Blood sera were tested by ELISA, using the complex sandwich technique with competition [9]. For this purpose, 96-well polystyrene panels (Costar, Great Britain) were sensitized

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TABLE 1. Results of Comparative Testing of Blood Sera by a Test System Based on Recombinant env Protein and a Commercial Test System (Organon-Teknika)

Test system	Total number of sera	Number of positive sera	Number of false-positive sera	Number of negative sera
Based on recombinant protein	150	59	1	90
Organon-Teknika	150	66	0	84

with immunoglobulins isolated by the affinity chromatography method on Protein A-Sepharose (Pharmacia, Sweden) from blood serum of AIDS patients. Sensitization was carried out in physiological saline for 1 h at 37°C, followed by incubation for 12 h at 20°C. The panels were then washed four times with physiological saline containing 0.1% Triton X-100 (PST). Next, 100 µl of lysate of induced *E. coli* JM 107/pUCenv1 cells, diluted tenfold with PST, was introduced into each well. After incubation for 12-16 h at 20°C the wells were washed five times with PST, a mixture (90 µl) of anti-AIDS-IgG conjugated with peroxidase and 10 µl of the test serum was added, and the sample was incubated for 2 h at 37°C. The wells were then washed three times with PST and 100 µl of chromogenic substrate mixture for peroxidase (5 mM orthophenylenediamine in 50 mM sodium-citrate buffer, pH 4.5, with 0.005% H_2O_2) was added to each well. After 30 min (20°C, in darkness) the reaction was stopped by addition of 50 µl of 2N H_2SO_4. The results were read on a flowmeter (Flow Titertech, Great Britain) at 492 nm. Sera giving a decrease of optical density by 50% or more compared with control samples (a mixture of 90 µg of anti-AIDS conjugate with 10 µl of normal human serum) were considered to be positive.

Testing the sera by the immunoblotting method was carried out as described previously [7] with minor modifications. After electrophoresis in 12.5% polyacrylamide gel with sodium dodecylsulfate [5] the virus proteins transferred to a nitrocellulose filter by the electroblotting method, and treated with the test serum diluted 1:100. The filter was then incubated with goat antiserum against human immunoglobulins, conjugated with peroxidase (Bio-Rad, USA). Staining was carried out in a substrate solution containing 0.05%, 3,3'-diaminobenzidine and 0.003% H_2O_2.

The test blood sera were taken from the collection of the D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR.

EXPERIMENTAL RESULTS

According to recently published data, most persons who are seropositive for AIDS virus have antibodies to products of the env gene (gp160, gp120, gp41). As antigen for the test system we therefore used a recombinant protein containing a sequence coded by a fragment of the env gene with AIDS virus [2]. Table 1 gives the results of testing blood sera by the diagnostic kit we obtained. For control purposes the same sera were tested by a commercial test system marketed by the firm of Organon-Teknika (the Netherlands). It will be clear from Table 1 that the results of testing agreed in 94% of cases (142 of 150 samples analyzed). The experimental test system gave only one false positive result (serum No. 87 from a patient with hemophilia). Seven sera (Nos. 46, 51, 68, 92, 103, 125, 147), which were seropositive according to the Organon-Teknika test system gave a negative result in the experimental test system. These sera evidently contain only an exceedingly small quantity of antibodies to env gene products. To test this hypothesis, sera Nos. 46, 51, 68, 92, 103, 125, 147 were tested by the immunoblotting method (serum No. 23, positive in both tests, was used as the control). It will be clear from Fig. 1 that these sera were in fact without antibodies to gp120 and gp41, but contained considerable amounts of antibodies to products of other genes (p31, p24, p15). These blood sera were taken from patients with AIDS-related complex, in whom absence of antibodies to products of the env gene is often observed, although antibodies to products of gag and other genes are present [8]. The discovery of antibodies to envelope protein of the AIDS virus is usually an indication of an advanced stage of the disease and is a poor prognostic sign [3].

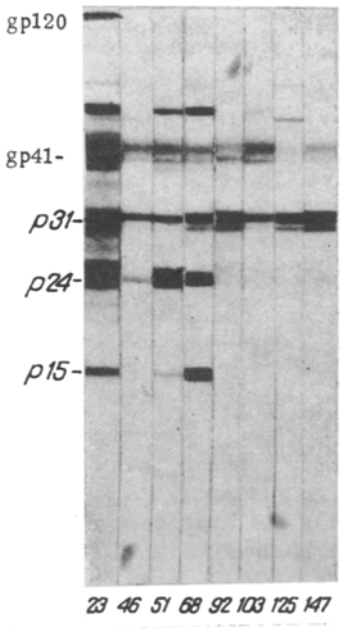

Fig. 1. Testing blood sera from patients with AIDS and AIDS-related complex by the immunoblotting method.

The diagnostic kit described in this paper can thus be used as a test to verify the presence of antibodies to products of the env gene of AIDS virus in order to predict the future development of the disease. In order to produce a test system by which all seropositive sera can be detected, we are currently conducting research in order to obtain a recombinant protein, carrying sequences coded by the gag gene of AIDS virus, in *E. coli* cells.

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