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Herpes Simplex Virus Type-Selective Enzyme-Linked Immunosorbent Assay with *Helix pomatia* Lectin-Purified Antigens

B. SVENNERHOLM,* S. OLOFSSON, S. JEANSSON, A. VAHLNE, AND E. LYCKE

Department of Virology, Institute of Medical Microbiology, University of Göteborg, Göteborg, Sweden

Received 23 May 1983/Accepted 24 October 1983

Helix pomatia lectin-purified antigens with specific reactivity to herpes simplex virus type 1 (HSV-1) and HSV-2 antibodies in human sera were used in an enzyme-linked immunosorbent assay. The type specificity of the antigens was assessed by double immunodiffusion precipitation in gel against rabbit HSV-1 and HSV-2 hyperimmune sera, and by enzyme-linked immunosorbent assay with human reference sera containing antibodies to either type of HSV. Fifty-two sera from patients with documented infection with either HSV-1 or HSV-2 were assayed for HSV type-specific immunoglobulin G antibodies. The reactivity of the sera against lectin-purified antigens correlated completely with the results of virus typing. We conclude that HSV type-specific immunoglobulin G antibodies can easily be measured by enzyme-linked immunosorbent assay with the use of *Helix pomatia* lectin-purified HSV-1 and HSV-2 antigens.

Due to the strong serological cross-reactivity between herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), the main problems associated with type-discriminating serodiagnosis of HSV infections derive from difficulties in obtaining viral antigens with a sufficient degree of type specificity.

One of the HSV-1-specified glycoproteins, designated gC, and its HSV-2 specified counterpart demonstrate mainly type-specific reactivities and should be good candidates for antigens in HSV type-specific immunoassays (9, 15). The conventional immunochemical procedures for isolation of individual HSV glycoproteins are cumbersome and often require use of denaturing conditions or access to monospecific antibodies (for a review, see reference 9). We have shown that gC, in contrast to the other HSV glycoproteins, contains an oligosaccharide with affinity for the *N*-acetylgalactosamine-specific lectin of *Helix pomatia* (HPA) (10). By using this lectin, it is possible to obtain large amounts of the type-specific glycoproteins. In the present paper, HSV-1 as well as HSV-2 type-specific glycoproteins were used in an enzyme-linked immunosorbent assay (ELISA) for demonstration of type-specific antibodies in human sera.

MATERIALS AND METHODS

Preparation of membrane and solubilization of membrane-associated antigens. BHK-21 cells of clone 13 were grown as previously described (5, 6). The HSV-1 F strain was obtained from B. Roizman, University of Chicago.

Crude membranes from HSV-1-infected BHK-21 cells and uninfected cells were prepared according to the method described by Olofsson et al. (10). Cells were mixed with 0.025 M Tris-hydrochloride (pH 8.0), homogenized with a Dounce homogenizer, and centrifuged at 1,500 × *g* for 15 min at 4°C. The membranes remaining in the supernatant phase were pelleted by centrifugation at 160,000 × *g* for 1 h, washed by suspending in 0.10 M glycine-sodium hydroxide buffer (pH 8.8), and centrifuged at 160,000 × *g* for 1 h. These washed membranes were used for preparation of two types of ELISA antigens.

(i) Type-common antigen. Washed membranes (4 ml) were suspended in 32 ml of cold 0.10 M glycine-sodium hydroxide buffer (pH 8.8), and 1.1 ml of 10% (wt/vol) solution of sodium deoxycholate (E. Merck, Darmstadt, Federal Republic of Germany) was added under stirring. The mixture was homogenized with 40 strokes of a Dounce homogenizer, incubated for 10 min on ice, and centrifuged at 100,000 × *g* for 1 h. The supernatant solution constituted the HSV antigen, and the negative control antigens were derived in the same manner from uninfected cells. This solubilized material was used in a solid phase assay with strong intertypic reactivity (7).

(ii) Type-specific antigens. For the affinity chromatography (10), the washed membranes were suspended in 32 ml of glycine-NaOH buffer (pH 8.8) with 1% Triton X-100 (wt/vol) and centrifuged at 100,000 × *g* for 1 h. The supernatant fluid (49 mg of protein) was applied on columns (diameter, 16 mm) containing 8 ml of HPA coupled to Sepharose 6 MB (Pharmacia Fine Chemicals, Uppsala, Sweden). The gel was equilibrated with 0.02 M Tris-buffered saline and 0.5% Triton X-100 and was washed at 4 ml/h with the same buffer. The biospecifically bound substances were eluted with 0.01 M *N*-acetylgalactosamine, and have previously been shown to contain the HSV-1 type-specific antigen gC (10). This material (2.8 mg of protein at a concentration of 0.35 mg/ml) was used directly in the immunoassays as described below. For preparation of HSV-2-specific glycoprotein, the same procedure was carried out on HSV-2-infected cells (210 mg of protein added to the HPA column gave 1.1 mg of biospecifically purified protein at a concentration of 0.22 mg/ml). The HPA-binding HSV-2-specific antigen migrated as a single electrophoretic band of ca. 130,000 daltons (Olofsson et al., manuscript in preparation) and is supposed to be identical to the HSV-2-specific glycoprotein of similar molecular weight described by Ruyechan et al. (12).

Protein assay. Protein concentrations were determined by the Lowry method, modified for measurement in Triton-containing solutions (2).

Sera. Serum specimens were obtained from 52 patients having a history of recurrent herpes labialis or herpes genitalis. At the time of an acute episode, virus was isolated and serum was collected from each patient. The virus strains were typed by using two methods, immunoelectroosmophoresis against type-specific antisera (4) and ELISA using monoclonal antibodies (8). According to the virus typing results, 26 sera were regarded as HSV-1 and 26 sera were regarded as HSV-2. Reference sera were used throughout the study.

* Corresponding author.

Rabbit hyperimmune sera to HSV-1 and HSV-2 were produced as previously described (10). In short, rabbits were inoculated in the scarified skin of the trunk. One month after the primary infection, the rabbits were inoculated in the eye and developed keratitis. Two weeks after healing of the corneal lesions, the animals were bled, and sera were tested for precipitins to HSV antigens. Using "crude" HSV-1 and HSV-2 antigens, i.e., before the biospecific adsorption to HPA, these sera gave several cross-reacting precipitation lines about double diffusion in agarose gels.

ELISA. Determination of immunoglobulin G (IgG) antibodies in human sera was performed on polystyrene microtiter plates (A/S Nunc, Denmark). The plates were coated with the antigen preparations (diluted in 0.05 M Tris-hydrochloride buffer, pH 8.2) by incubation with 100 μl at 4°C overnight. After three washes in PBS-Tween 20 (0.05%), 100 μl of sera diluted in PBS-Tween 20 with 1% bovine serum albumin (twofold dilution steps of a 1/100 dilution) was added, and the plates were incubated at 24°C for 4 h. The washing procedure was repeated. One hundred microliters of alkaline phosphatase-conjugated heavy chain-specific swine antibodies against human IgG (Orion Diagnostica, Helsinki, Finland), diluted 1:150, was added to each well and further incubated overnight at 24°C. The plates were again washed as described above, and 100 μl of substrate (*p*-nitrophenylphosphate 1 mg/ml in carbonate buffer, pH 9.8) was added. The substrate reaction was stopped after 45 min by adding 25 μl of 3 M NaOH, and optical density was recorded at 405 nm in a Titertek Multiscan spectrophotometer. The two negative control sera, used as reference, were assayed in multiple wells for each batch of coated microtiter plates. For a total of 64 trials, the optical density was 0.084 ± 0.029 (mean ± standard deviation) for the type-common antigen, 0.136 ± 0.041 for the type-1-specific antigen, and 0.148 ± 0.042 for the type-2-specific antigen.

Titers were expressed as the reciprocal of the dilution giving an absorbance of 0.3 units greater than background values of negative control sera. Titers of <100 were regarded as negative. Titers of all sera tested were adjusted in relation to the panel of reference sera included in each microtiter plate to compensate for inter-experiment variations. The intra-assay variation was assessed by a replicate test of single serum dilution incubated for 4 h (n = 30). The variation coefficient (Cv) was 5.6% for the deoxycholate-solubilized antigen (mean adsorbance, 1.465), 6.2% for the HSV-1 type-specific antigen (mean adsorbance, 1.580), and 5.9% for the HSV-2 type-specific antigen (mean adsorbance, 1.396).

Immunoprecipitation in gel. Agarose (Indubiose A37, L'Industrie Biologique Francaise, S.A.) was dissolved in 0.025 M Tris-hydrochloride buffer (pH 8.0) containing 0.2% (wt/vol) Triton X-100, and gels of 1% (wt/vol) were used. Samples of 15 μl of antigens and antisera were added to the wells, and the immunodiffusion was performed at room temperature for 24 h. The precipitates were photographed after staining with Coomassie brilliant blue.

RESULTS

Type specificity of HPA-purified HSV antigens. HSV-1- and HSV-2-solubilized glycoprotein antigens that had been biospecifically eluted from HPA columns were tested in gel diffusion against rabbit HSV-1 and HSV-2 hyperimmune sera (Fig. 1). One precipitation line was obtained between respective serum and homotypic antigen. There were no deviations of these precipitation lines indicating heterotypic cross-reactivities. Neither were any precipitation lines between the sera and the heterotypic antigens observed.

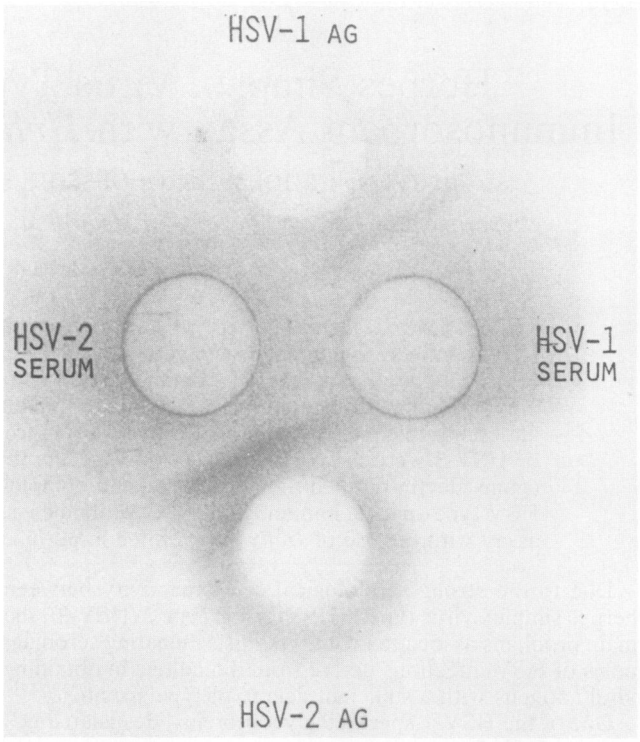

FIG. 1. Double immunodiffusion of HSV type-specific antigens and rabbit HSV-1 and HSV-2 hyperimmune sera in agarose gel. Immunodiffusion was performed at room temperature for 24 h. The precipitates were photographed after staining with Coomassie brilliant blue.

The type specificity of the lectin-purified HSV antigens was also tested with our two human reference sera. Microtiter plates were coated with different dilutions of respective antigen, and ELISA was performed with a constant dilution (1/100) of the sera. Again, both antigens displayed a high degree of type specificity within a wide concentration range (Fig. 2 and 3). For screening of sera (see below), an antigen dilution of 1/800 (corresponding to ca. 0.25 μg/ml) was used.

The strong type-selective properties of both antigens were further confirmed with an ELISA method of Vestergaard and Grauballe (16), showing only weak heterotypic reactions (B. F. Vestergaard, personal communication).

Screening of human sera for HSV type-specific antibodies. A panel of 26 sera from patients with verified HSV-1 and 26 sera from patients with verified HSV-2 infections was tested against (i) the deoxycholate-solubilized, type-common HSV-1 antigen, (ii) The HSV-1 type-specific lectin antigen, and (iii) the HSV-2 type-specific lectin antigen (Tables 1 and 2). All patients with HSV-1 infection demonstrated positive titers against the HSV-1-specific antigen. Eight of these sera contained antibodies also against the HSV-2 type-specific antigen. All sera from patients with verified HSV-2 infection demonstrated positive titers against the HSV-2-specific antigen. Of these patients, however, as many as 73% had antibodies reacting also against the HSV-1 antigen. In sera containing antibodies of both specificities, higher titers were generally obtained against the homotypic than against the heterotypic antigen. All sera from patients with HSV-1

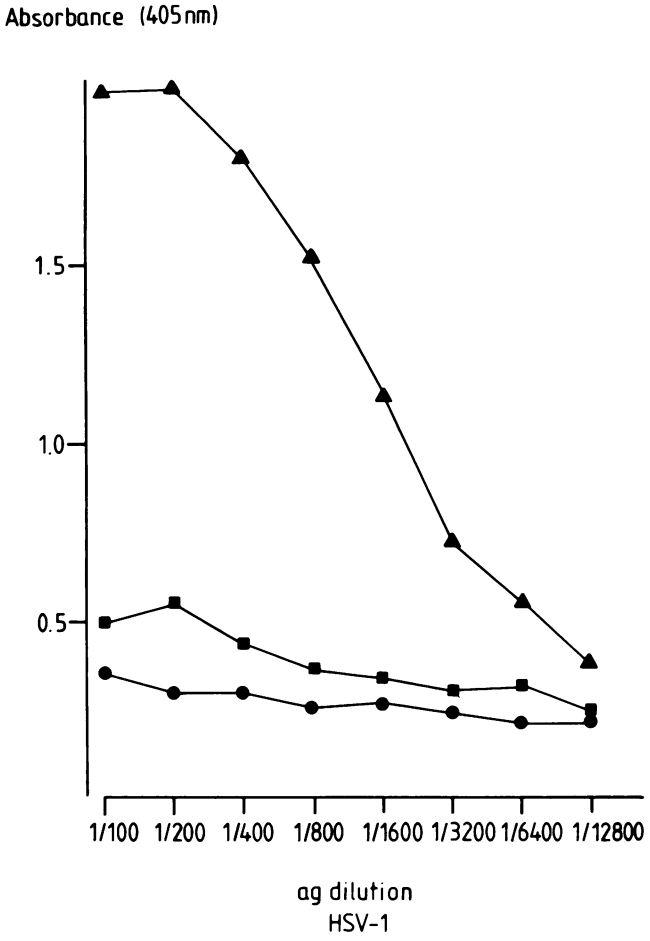

FIG. 2. Effect of the concentration of HSV-1 antigen on absorbance in ELISA. Symbols: ▲, patient serum with HSV-1 antibodies, diluted 1/100; ■, patient serum with HSV-2 antibodies, diluted 1/100; ●, patient serum without HSV antibodies, diluted 1/100.

infection reacted both with the lectin HSV-1 antigen and the type-common deoxycholate-solubilized antigen (Table 1). However, although all HSV-2-infected patients reacted with HSV-2-specific antigen, three sera in this panel had no antibodies against the type-common HSV-1 antigen (Table 2).

DISCUSSION

The need for a diagnostic technique differentiating HSV-1 and HSV-2 antibodies has been emphasized. A number of methods for serotyping have been applied, including microneutralization (11), neutralization kinetic tests (14), indirect hemagglutination inhibition (13), radioimmunoassay (1), and ELISA techniques (16) using type-specific antigens. However, these methods might be limited by such factors as (i) definition of the antigen used, (ii) time-consuming procedures for preparation of highly specific antigens, (iii) blocking of type-common antigenic sites with heterologous antibodies, and (iv) low sensitivity of the assay to detect type-specific determinants.

The method described here provides a simple quantitative technique for testing of human sera for type-specific HSV antibody. The type-specific antigen used was prepared rapidly by a non-immunological method, and no prior adsorption of sera to remove cross-reacting antibodies was necessary. The specificity of the antigens was clearly demonstrated by double diffusion against hyperimmune rabbit sera in gel and by ELISA with human reference sera. In addition, blocking of antigen with rabbit IgG of HSV-1 or HSV-2 specificity showed that none of the antigens contained dominating heterotypic determinants. Both antigens had small amounts of type-common determinants but were considerably enriched with regard to type specificity. (Bent F. Vestergaard, personal communication). However, it is evident from our data that the type-common determinants did not influence the type-specific reactions at the working dilution of the antigens.

When purified type-specific HSV antigens are used for sensitive serological assays, it has been argued that the antigenic variation observed among different HSV strains may induce altered antibody levels to individual antigens. This might lead to an inaccurate diagnosis when it is based on a serum reactivity with a single antigen (3). ELISA titers of sera to HPA-binding HSV-1 antigen and to the deoxycholate-solubilized antigens—containing all HSV glycoproteins—showed a very close correlation with respect to frequency of positivity and level of titers. In addition, the

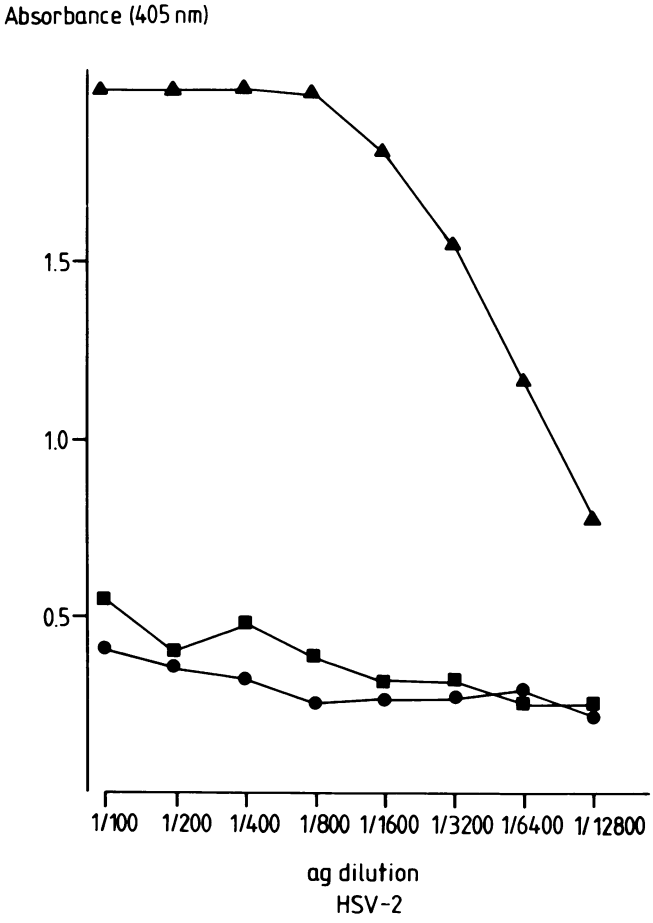

FIG. 3. Effect of the concentration of HSV-2 antigen on absorbance in ELISA. Symbols, ▲, patient serum with HSV-2 antibodies, diluted 1/100; ■, patient serum with HSV-1 antibodies, diluted 1/100; ○, patient serum without HSV antibodies, diluted 1/100.

TABLE 1. ELISA titers of sera from 26 patients with recurrent HSV-1 infection

Serum no.	ELISA titer against the following antigen[a]:		
	Type-common HSV-1	Lectin-1	Lectin-2
237	3,200	1,600	Neg[b]
105	3,200	1,600	Neg
108	6,400	3,200	Neg
124	3,200	1,600	Neg
129	1,600	1,600	Neg
130	800	800	Neg
131	1,600	800	Neg
132	1,600	800	Neg
133	6,400	3,200	100
134	1,600	800	Neg
135	3,200	3,200	400
136	3,200	3,200	Neg
137	1,600	800	100
31	1,600	3,200	Neg
88	800	1,600	100
89	3,200	3,200	200
165	800	800	400
234	800	1,600	Neg
235	1,600	400	Neg
236	12,800	3,200	Neg
243	6,400	12,800	Neg
253	12,800	3,200	Neg
256	6,400	3,200	Neg
258	3,200	1,600	1,600
260	3,200	6,400	400
247	6,400	6,400	Neg

[a] For descriptions of antigens, see the text.
[b] Neg, Negative titer (<100).

TABLE 2. ELISA titers of sera from 26 patients with recurrent HSV-2 infection

Serum no.	ELISA titer against the following antigen[a]		
	Type-common HSV-1	Lectin-1	Lectin-2
303	200	200	1,600
209	3,200	1,600	400
249	6,400	3,200	1,600
256	6,400	1,600	3,200
62	800	200	1,600
65	6,400	200	3,200
79	3,200	200	12,800
86	100	Neg	400
90	6,400	200	3,200
93	400	200	1,600
103	800	100	6,400
122	1,600	1,600	1,600
79	1,600	1,600	800
1689	Neg[b]	Neg	800
2404	200	100	800
11421	200	200	800
6994	Neg	Neg	100
7470	100	Neg	100
6861	400	400	1,600
10912	6,400	6,400	3,200
3297	Neg	Neg	200
3453	200	Neg	1,600
38229	200	Neg	800
13	1,600	1,600	6,400
17	800	800	3,200
558	1,600	1,600	6,400

[a] For descriptions of antigens, see the text.
[b] Neg, Negative titer (<100).

lectin-purified HSV-1 gC has been demonstrated to contain at least three different antigenic epitopes in studies using monoclonal antibodies (S. Jeansson et al., manuscript in preparation). Thus, it seems reasonable to assume that the type-specific antigen described is representative and will cover the antigenic variation among different HSV strains within a serotype.

In conclusion, the use of HPA-binding HSV-1 and HSV-2 glycoproteins provides an adequate and simple procedure for the type-specific serodiagnosis of HSV infections. The fact that no type-specific antibodies but only a commercially available lectin is needed for the preparation of antigens makes the technique suitable also for small or less well-equipped laboratories.

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