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Detection of Type-Specific Antibody to Herpes Simplex Virus Type 1 by Radioimmunoassay with Herpes Simplex Virus Type 1 Glycoprotein C Purified with Monoclonal Antibody

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Herpes simplex virus type 1 (HSV-1) and HSV-2 specify at least four glycoproteins designated gA/gB, gC, gD, and gE. Previous studies have shown that gC produced by HSV-1 is antigenically distinct from the corresponding HSV-2 glycoprotein. With the exception of gC, the glycoproteins of both serotypes share antigenic sites. Standard serological assays fail to differentiate the antibody to the shared antigenic determinants from the type-specific antibody. In this paper, we describe a procedure for purifying gC from HSV-1-infected cell extracts with an immunoadsorbent prepared with an HC1 monoclonal antibody. When used in a solid-phase radioimmunoassay, gC proved to be a type-specific antigen for quantitation of antibody to HSV-1. Among individuals who had no antibody to HSV at the onset of infection, all of those with primary HSV-1 infection developed antibody to gC. Subjects with primary HSV-2 infection failed to develop antibody reactive with gC of HSV-1 ($P < 0.01$). Both immunoglobulin G and M antibodies against gC were detected in sera from subjects with either primary or recurrent HSV-1 infection. Higher antibody titers to gC were found in sera from individuals with recurrent infection than in sera from those with primary HSV-1 infection.

Glycoprotein C (gC) is one of at least four glycoproteins of herpes simplex virus type 1 (HSV-1) and HSV-2 present in the virion envelope and in infected-cell membranes (8, 12, 13). This glycoprotein is considered to be type specific for HSV-1 because antisera produced against gC fail to react with the corresponding HSV-2 glycoprotein (2, 19, 21). Recent studies with a monoclonal antibody to gC of HSV-1, designated HC1, demonstrated that the antibody reacted with HSV-1-infected cells, but not with HSV-2 infected cells, by immunofluorescence staining. HC1 antibody neutralized HSV-1 in vitro and in vivo but did not inhibit infection with HSV-2 (3, 14, 16). Serological analysis of HSV isolates with HC1 monoclonal antibody demonstrated that all of the 63 naturally occurring HSV-1 strains specified a glycoprotein with the antigen properties of HSV-1 gC (14).

Epidemiological and immunological studies of HSV-1 and HSV-2 infections have been difficult because standard serological tests such as complement fixation, neutralization, hemagglutination, and indirect immunofluorescence measure antibodies against a mixture of type-specific and type-common antigens. The reactivity of sera with type-common antigens obscures the detection of type-specific antibodies, making it difficult to determine whether an individual has had infection with HSV-1 or HSV-2 or both (10). Cross-adsorption of sera with the heterologous virus may not eliminate cross-reactive antibodies and substantially reduces the antibody titer (4, 7). Electrophoretically separated glycoproteins have been used in a radioimmunoassay (RIA) for antibody directed predominantly against type-specific antigen (4). With the exception of gC, however, HSV-1 and HSV-2 glycoproteins have been shown to contain cross-reacting determinants as well as type-specific sites (12, 15, 16, 17, 18, 20). Sera containing type-specific antibodies to gC have been typed by polyacrylamide gel electrophoresis of immune precipitates from HSV-1- and HSV-2-infected cell extracts (5, 18). The major drawback of this technique is that it is neither quantitative nor suitable for large-scale, rapid diagnostic testing.

We report the purification of gC with HC1 monoclonal antibody as an immunoadsorbent for HSV-1 gC and its use in a solid-phase RIA for type-specific antibodies to HSV-1.

MATERIALS AND METHODS

Preparation of HSV infected cell extracts. Human epidermoid carcinoma no. 2 (HEp-2) cells were grown

in Dulbecco minimal essential medium supplemented with 10% fetal calf serum. HEp-2 cells were inoculated with 5 to 10 PFU per cell of HSV-1 strain VR3 and maintained in medium with 2% fetal calf serum. Eight hours after inoculation, the medium was replaced with methionine-free medium supplemented with 10 μCi of L-[^{35}S]methionine (≥600 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) per ml. Extracts were prepared by harvesting the cells after 16 h and incubating 4×10^7 cells per 1.5 ml of 10 mM Tris buffer, pH 7.4 (0.5 M NaCl, 0.01 M N_3Na, 1% Triton-X 100, and 1% sodium deoxycholate), for 45 min on ice. Insoluble material was removed by centrifugation at $400 \times g$, followed by centrifugation at $100,000 \times g$.

Monoclonal antibody. The immunological properties of HC1 monoclonal antibody produced by fusing NS-1 myeloma cells with spleen cells of mice immunized with HSV-1 have been described previously (16). Immunoglobulin G (IgG) was precipitated from mouse ascites fluid with 40% saturated ammonium sulfate and used at a concentration of 5.7 mg of protein per ml.

Preparation of HC1 immunoadsorbent and purification of HSV-1 gC. Sepharose (0.5 g) (cyanogen bromide-activated Sepharose 4B; Pharmacia Fine Chemicals, Piscataway, N.J.) was washed with 2.5 ml of coupling buffer (0.2 M $NaHCO_3$ [pH 8.5] with 0.5 M NaCl and 0.01 M N_3Na) and combined with 20 mg of HC1 IgG that had been dialyzed against coupling buffer. The Sepharose and antibody were mixed overnight at 4°C, washed four times with coupling buffer, mixed overnight with glycine buffer (pH 8.5), and washed alternately with coupling buffer and 0.2 M acetate buffer (pH 4.0). The percent binding of HC1 IgG was ≥80%. HC1 immunoadsorbent was stored at 4°C in coupling buffer.

gC was purified by immunoaffinity chromatography in the following manner. An extract of HSV-1-infected HEp-2 cells (5.0 ml) was added to 400 μl of antibody-coated Sepharose that had been equilibrated with 10 mM Tris buffer for 6 h at 4°C. After mixing overnight, the HC1 immunoadsorbent was washed 10 times with extract buffer to remove unreacted antigen. gC was eluted with 800 μl of 3 M KSCN, 5 M lithium chloride, or 0.1 M glycine-hydrochloride for 2 h at 4°C. The purity of the eluted glycoprotein was determined by electrophoresis of radiolabeled eluted protein in 9.25% sodium dodecyl sulfate-polyacrylamide gels cross-linked with *N,N'*-diallyltartardiamine and autoradiography (19). The preparation contained approximately 0.01 μg of protein per ml. The eluate was also incubated with lentil lectin-Sepharose and eluted with 0.2 M α-methyl-D-mannoside in extract buffer for comparison with purification, using monoclonal antibody only.

RIA for antibody to HSV-1 gC. To determine the optimal antigen concentration for use in the RIA, the eluate containing purified gC and the eluting agent was diluted in phosphate-buffered saline (PBS), pH 7.2. Diluted antigen (0.025 ml) was added to polyvinyl U plates (Dynatech Laboratories, Inc., Alexandria, Va.) and allowed to dry. Before use, the plates were washed with PBS, incubated with PBS containing 20% fetal calf serum for 1 h at 37°C, and washed twice with PBS. With a microdilutor, duplicate serial fourfold dilutions of test sera were made in PBS containing 10% fetal calf serum and 0.05% Tween 20 (J. T. Baker Chemical Co., Phillipsburg, N.J.). The plates were incubated for 1 h at 37°C and washed three times with PBS–0.05% Tween 20, and ^{125}I-labeled goat anti-human IgG or IgM antiserum (Tago, Inc., Burlingame, Calif.) was added to each well (50,000 cpm/0.025 ml per well). The plates were incubated for 1 h at 37°C, washed five times with PBS–0.05% Tween 20, and dried. Each well was counted for 1 min in a gamma scintillation counter. The HSV-1 gC antibody titer of each serum was calculated by subtracting the average counts per minute in duplicate wells incubated with a known HSV-negative serum from the average counts per minute in the test serum wells at the same dilution. If this difference minus the mean counts per minute ± 2 standard deviations of eight wells containing diluent only was ≥100 cpm, specific binding was considered to be present. The antibody titer was expressed as the highest serum dilution showing specific binding to gC antigen.

Sera. Serum was obtained from 12 subjects with primary genital HSV infections within 1 week after the appearance of symptoms and then 20 to 30 days later, from 9 subjects with recurrent herpes labialis within 3 months after an episode, and from 10 subjects with no history of HSV infection. Subjects with primary genital HSV infection were also tested for the development of lymphocyte transformation to HSV-1 and HSV-2 antigens (22). Viral isolates were typed by restriction endonuclease analysis (1). All sera were tested for HSV antibody by microneutralization or complement fixation (22). For the neutralization assay, serial dilutions of serum were incubated with human foreskin fibroblasts and an HSV-1 or HSV-2 inoculum in replicates of six. After scoring for cytopathic effects, the 50% endpoint for the six replicates was calculated by the Karber method.

RESULTS

Purification of gC. The first series of experiments was carried out to determine whether gC could be purified from other HSV proteins and host cell proteins with an immunoadsorbent prepared with HC1 monoclonal antibody. Two polypeptides with the electrophoretic properties of gC and its precursor, pgC, were eluted from HC1 immunoadsorbent as demonstrated autoradiographic images obtained after electrophoresis of the eluate in sodium dodecyl sulfate-polyacrylamide gels (Fig. 1). Small amounts of faster-migrating radiolabeled polypeptides coeluting appear to share antigenic determinants with gC (L. Pereira, unpublished results). Gels stained with Coomassie brilliant blue were free of host cell proteins. The eluted polypeptides were adsorbed to a lectin column and had the lectin-binding properties of HSV-1 gC. In addition to potassium thiocyanate, lithium chloride and glycine-hydrochloride were effective in eluting purified gC and its precursor (data not shown). Elution with potassium thiocyanate consistently produced an antigen that could be diluted at least 1:100 for use in the RIA, and this method was chosen for the purification of HSV-1 gC to test patient sera.

Standardization of the RIA with purified gC. In the second series of experiments, specific bind-

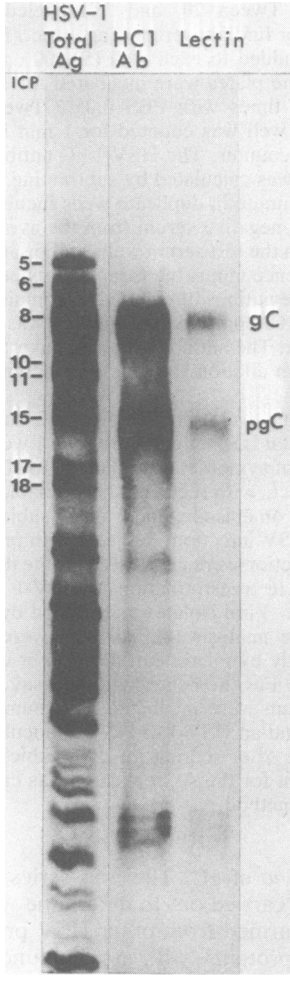

FIG. 1. Autoradiogram of electrophoretically separated polypeptides from extracts of HSV-1-infected cells and purified proteins eluted from HC1 monoclonal antibody immunoadsorbent. The extract of [^{35}S]methionine-labeled infected cells before application to the immunoadsorbent is shown (left lane) along with the proteins eluted with 3 M KSCN (middle lane); these proteins were applied to a lentil lectin column and eluted (right lane). The numbers to the left of the figure designate high-molecular-weight HSV-1-infected cell polypeptides (ICP) made late in infection (11).

ing was evaluated with gC antigen purified from HC1 immunoadsorbent and test sera from subjects known to have been HSV seronegative at the onset of primary HSV-1 infection (HSV-1 serum) or HSV-2 infection (HSV-2 serum) and from a subject who had no detectable HSV antibody by neutralization and complement fixation assays and no cellular immunity to HSV (HSV-negative serum). gC antigen was used at dilutions of 1:5, 1:10, 1:50, 1:100, and 1:200. Specific binding was demonstrated with the HSV-1 serum to a dilution of 1:4,096 when each of three different antigen preparations were used at a 1:100 dilution (Fig. 2A). The antigen titration curve demonstrating binding of the HSV-1 serum at each dilution of antigen tested is shown in Fig. 3. Binding was diminished when the antigen was used at a 1:5 or 1:10 dilution, as compared with a 1:50 or 1:100 dilution. No specific binding was found with the HSV-2 serum or with the HSV-negative serum with any of the HSV-1 gC antigen preparations. The antibody titer to HSV-1 gC in the HSV-1 serum was reproducible at 1:1,024 to 1:4,096 when tested in five different assays with the same antigen preparation (Fig. 2B), whereas no specific binding occurred at any dilution of the HSV-2 serum or the HSV-negative serum in any of the five assays.

Antibody titers to purified gC. Of the 12 initially seronegative subjects with primary genital HSV infections, only those with HSV-1 infections developed antibody to HSV-1 gC (Table 1). All six patients had detectable IgG antibody to HSV-1 gC during the convalescent phase of the infection. Five of the six subjects also developed IgM antibody to HSV-1 gC. Six subjects who had primary HSV-2 infection and no detectable HSV antibody at the onset of the infection did not develop IgG or IgM antibodies to HSV-1 gC. All of these patients showed seroconversion when the same sera were tested by neutralization or complement fixation (Table 1). One of these patients (subject 6) had a neutralizing antibody titer of 1:6, which is borderline, but developed lymphocyte transformation to HSV-1 antigen with an increase in stimulation index from <2.5 to 18.0. The frequency of seroconversion to HSV-1 gC antigen in patients with proved primary HSV-1 infections, as compared with those with primary HSV-2 infections, was significantly different ($P < 0.01$; chi-square test with Yates correction). Antibody to HSV-1 gC was present by RIA in one serum sample before neutralizing antibody was detectable (subject 2). This patient had a relatively high titer of antibody to HSV-1 gC 4 days after the onset of symptoms. The diagnosis of primary HSV infection in this patient was substantiated by the absence of neutralizing antibody and of lymphocyte transformation to HSV-1 and HSV-2 antigens at the initial evaluation. Although antibody titers to HSV-1 gC were low in three subjects tested during the convalescent phase of primary HSV infection, these sera showed detectable antibody, whereas the acute-phase sera remained negative in five separate assays. Two of these subjects were also tested early enough to document primary infection based on the acquisition of cellular immunity to HSV antigens. The demonstration of seroconversion to HSV-1 gC antigen suggests primary HSV-1 infection.

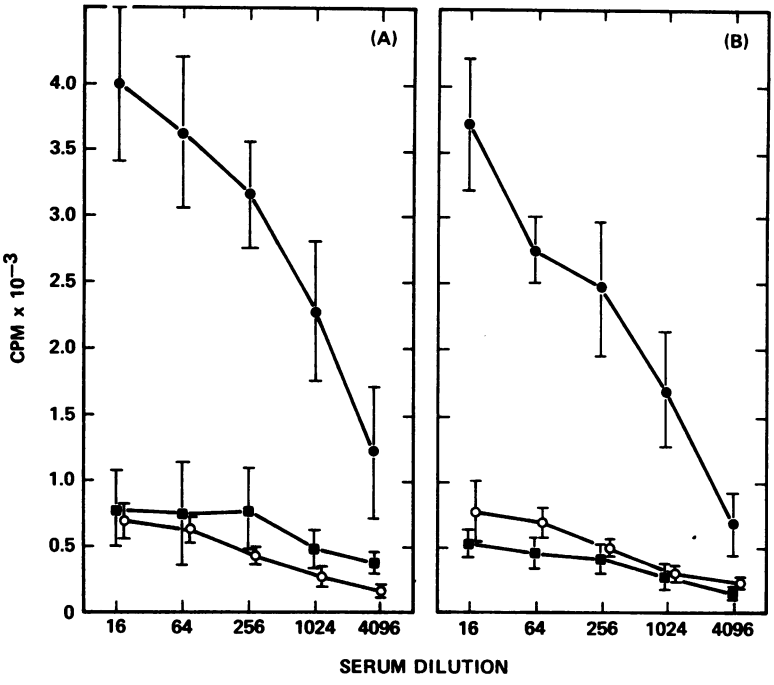

FIG. 2. Binding of serial dilutions of HSV-1 serum (●), HSV-2 serum (■), and HSV-negative serum (○) in a solid-phase RIA with HSV-1 gC as antigen. (A) Mean counts per minute ± standard error detected when the test sera were incubated with each of three HSV-1 gC antigen preparations; (B) mean counts per minute ± standard error detected when the test sera were incubated with the same HSV-1 gC antigen preparation in five separate assays.

All nine subjects with a recent recurrence of herpes labialis had IgG antibody to HSV-1 gC (Table 1). The mean antibody titer in these subjects was 1:851, compared with 1:230 in patients tested during the convalescent phase of primary HSV-1 infection ($P < 0.05$; Student's t test). Six of the nine subjects with recurrent herpes labialis also had IgM antibody to HSV-1 gC. The IgM titers ranged from 1:64 to 1:1,024 in patients with recurrent herpes labialis, compared with a range of 1:16 to 1:64 during convalescence from primary HSV-1 infection.

Ten normal subjects with no history of HSV infection who had no antibody to HSV by the complement fixation assay were tested for IgG and IgM antibodies to HSV-1 gC. Nine of these individuals had no detectable IgG antibody to this antigen. One subject whose HSV-1 gC titer was 1:64 also had cellular immunity to HSV antigen. None of these subjects had IgM antibody to HSV-1 gC.

DISCUSSION

Immunoaffinity chromatography with type-specific HC1 monoclonal antibody proved to be an efficient, reliable method for the purification of HSV-1 gC from infected cell extracts. Eisenberg et al. have recently reported the purification of gD from HSV-1 and HSV-2 with affinity columns constructed from cross-reacting HD1 monoclonal antibody (6). In both instances, polyacrylamide gel analysis demonstrated that the glycoprotein preparation was free of other viral and host cell proteins. Purified gD retained its native antigenic determinants as demonstrated by immunoprecipitation with polyvalent sera. In the present study, the reactivity of human sera with HSV-1 gC indicated that the major antigenic domains of the glycoprotein were not altered by the purification procedure.

The suitability of HSV-1 gC as an antigen for the detection of HSV-1 specific antibody was demonstrated by RIA with sera from subjects with primary and recurrent HSV-1 infections. All of the subjects with primary HSV-1 infections developed antibody to HSV-1 gC, whereas none of those with primary HSV-2 infections developed cross-reacting antibody to HSV-1 gC ($P < 0.01$; chi-square test). High titers of antibody to HSV-1 gC were found in sera from subjects with recent episodes of herpes labialis. The data suggest that gC induces a specific antibody response in the human host. Previous studies have shown that gC elicits type-specific neutralizing antibody (3, 16, 21). The development of type-specific antibody to HSV-1 gC

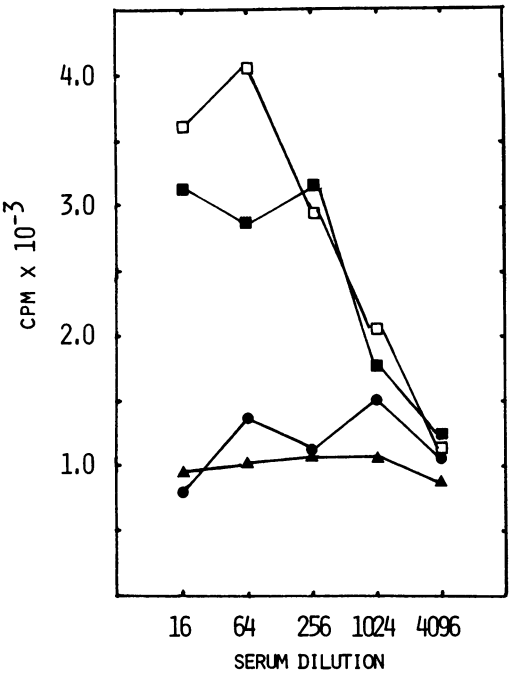

FIG. 3. Binding of serial dilutions of HSV-1 serum in a solid-phase RIA with HSV-1 gC as antigen. The antigen was used at dilutions of 1:5 (●), 1:10 (▲), 1:50 (■), and 1:100 (□). The figure indicates the mean counts per minute in duplicate wells when serial dilutions of the test serum were incubated with each dilution of the HSV-1 gC antigen.

antigen provides a useful method for differentiating primary HSV-1 from primary HSV-2 infection.

The solid-phase RIA with HSV-1 gC provided a rapid, quantitative method for measuring HSV-1 type-specific antibody in a large number of serum samples. With this method, IgG and IgM antibodies to HSV-1 gC could be detected in unfractionated serum. The data suggest that the presence of IgM antibody to HSV-1 gC could be used for the diagnosis of recent HSV-1 infection but that its presence does not distinguish primary from recurrent infections. Although the number of sera tested was small, antibody to HSV-1 gC was detected in sera from 14 individuals with epidemiologically distinct sources of HSV-1 infection. The data indicate that the gC specified by unrelated HSV-1 strains contains a common antigenic site and are consistent with serological analysis of HSV-1 strains with HC1 monoclonal antibody. The previous demonstration that HC1 antibody reacted with 63 HSV-1 isolates by immunofluorescence staining confirms the expression of gC and the HC1 antigenic domain on naturally occurring HSV-1 strains. The RIA for type-specific HSV-1 antibody has potential application for investigating whether past HSV-1 infection reduces susceptibility to HSV-2 infection or alters its severity. The analysis of humoral immunity to the individual HSV glycoproteins will be useful in studies of the pathogenesis of primary and recurrent HSV

TABLE 1. IgG and IgM antibody to HSV-1 gC in individuals with primary HSV-1 and HSV-2 infections and recurrent herpes labialis measured by RIA

HSV type isolated and subject no.	Primary HSV infection at indicated time (days)								Recurrent HSV infection at <3 mo		
	Titer of antibody to HSV-1 gC				Neutralizing antibody				Subject no.	Titer of antibody to HSV-1 gC[a]	
	IgG		IgM		HSV-1		HSV-2			IgG	IgM
	<7	20–30	<7 d	20–30	<7	20–30	<7	20–30			
HSV-1											
1	<1:16	1:256	<1:16	<1:16	<1:5	1:40	<1:5	1:40	13	1:4,096	<1:16
2	1:256	1:1024	1:16	1:16	<1:5	1:20	<1:5	1:10	14	1:256	1:64
3	<1:16	1:16	<1:16	1:16	<1:5	1:14	<1:5	1:7	15	1:16,384	1:1,024
4	<1:16	1:16	<1:16	1:64	<1:5	1:22	<1:5	1:14	16	1:1,024	<1:16
5	<1:16	1:16	<1:16	1:16	<1:8	1:16[a]			17	1:64	1:256
6	<1:16	1:64	<1:16	1:256	<1:8	1:64[a]			18	1:1024	1:256
HSV-2											
7	<1:16	<1:16	<1:16	<1:16	<1:5	1:6	<1:5	1:10	19	1:1,024	<1:16
8	<1:16	<1:16	<1:16	<1:16	<1:5	1:10	<1:5	1:11	20	1:256	1:64
9	<1:16	<1:16	<1:16	<1:16	<1:5	1:7	<1:5	1:16	21	1:4,096	1:1,024
10	<1:16	<1:16	<1:16	<1:16	<1:5	1:10	<1:5	1:16			
11	<1:16	<1:16	<1:16	<1:16	<1:8	1:64[a]					
12	<1:16	<1:16	<1:16	<1:16	<1:8	1:32[a]					

[a] Complement fixation titer.

infection; for example, Zweerink and Corey have related differences in the development of antibodies against virus-specific antigens to the severity of HSV-2 infection (23). Such studies will provide important background for the development of HSV subunit vaccines (9).

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