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Characterization of a Herpes Simplex Virus Type 2 75,000-Molecular-Weight Glycoprotein Antigenically Related to Herpes Simplex Virus Type 1 Glycoprotein C

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Evidence is presented that the herpes simplex virus type 2 glycoprotein previously designated gF is antigenically related to herpes simplex virus type 1 gC (gC-1). An antiserum prepared against type 1 virion envelope proteins immunoprecipitated gF of type 2 (gF-2), and competition experiments revealed that the anti-gC-1 component of the antiserum was responsible for the anti-gF-2 cross-reactivity. An antiserum prepared against fully denatured purified gF-2, however, and three anti-gF-2 monoclonal antibodies failed to precipitate any type 1 antigen, indicating that the extent of cross-reactivity between gC-1 and gF-2 may be limited. Several aspects of gF-2 synthesis and processing were investigated. Use of the enzymes endo-β-*N*-acetylglucosaminidase H and α-D-*N*-acetylgalactosaminyl oligosaccharidase revealed that the fully processed form of gF-2 (about 75,000 [75K] apparent molecular weight) had both complex-type N-linked and O-linked oligosaccharides, whereas newly synthesized forms (67K and 69K) had only high-mannose N-linked oligosaccharides. These last two forms were both reduced in size to 54K by treatment with endo-β-*N*-acetylglucosaminidase H and therefore appear to differ only in the number of N-linked chains. Neutralization tests and radioiodination experiments revealed that gF-2 is exposed on the surfaces of virions and that the 75K form of gF-2 is exposed on cell surfaces. The similarities and differences of gF-2 and gC-1 are discussed in light of recent mapping results which suggest collinearity of their respective genes.

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) specify several major glycoproteins. These glycoproteins are targets of neutralizing antibodies and play important roles in the adsorption and penetration of virus and also in virally induced cell fusion (43). HSV-1 and HSV-2 are closely related genetically, forming viable recombinants (15, 17, 27, 46). Therefore, structural and functional similarities between analogous HSV-1 and HSV-2 glycoproteins are to be expected, and attention is attracted by deviations in this pattern. Previous studies have shown that HSV-1 glycoproteins gB, gD, and gE have antigenically and functionally related counterparts in HSV-2 (7, 13, 28, 30, 32–34, 39). These related glycoproteins have been assigned the same alphabetic designation in accordance with conventions agreed upon by the participants in Cold Spring Harbor Workshops and of the International Congress on Human Herpesviruses (6).

A related HSV-2 counterpart for HSV-1 gC (gC-1), a glycoprotein nonessential for viral replication in cell culture (18, 25), has been more difficult to identify. Only recently have clues emerged as to the identity of this counterpart. We reported mapping results (31) which showed that the gene for the HSV-2 glycoprotein designated gF (1, 2) could be collinear with the gene for gC-1. In addition, Zweig et al. (49) recently reported that a monoclonal antibody to HSV-2 gF (gF-2) cross-reacted with gC-1. It should be noted that a different HSV-2 glycoprotein was previously assigned the name gC (37), based on the similarity of its molecular weight to that of gC-1. This HSV-2 glycoprotein is probably unrelated to gC-1, and therefore misnamed in that its gene is noncollinear with that for gC-1 (37) and no evidence for structural or antigenic relatedness has been reported.

We show by immunoprecipitation with a conventional antiserum that gF-2 and gC-1 are antigenically related. We also report that antigenic determinants unique to gF-2 are readily demonstrable, and we have further characterized gF-2 with respect to size, post-translational processing, and presence in virions and on cell surfaces. These findings, coupled with the ob-

servations described above, indicate that gF-2 and gC-1 are genetically and structurally related, although their genes have probably diverged considerably more than the genes for other major HSV glycoproteins. Implications with respect to the roles of these glycoproteins and problems of nomenclature are discussed.

MATERIALS AND METHODS

Cells and viruses. HEp-2 (human epidermoid carcinoma-2) cells obtained from Flow Laboratories (Rockville, Md.) were used in all experiments except for the neutralization assay in which Vero cells were used. The cells were grown as monolayer cultures in Dulbecco modified Eagle minimal essential medium supplemented with 10% fetal bovine serum. Viruses used were HSV-1 strains F (14), mP (19), MP (19), HFEM (obtained from A. Buchan, University of Birmingham, England), HFEM(syn) (3), KOS (obtained from M. Gibson and M. Levine, University of Michigan, Ann Arbor), and 14-012 (10) (obtained from F. Rapp, Hershey Medical Center, Hershey, Pa.) and HSV-2 strains G (14) and 333 (9) (also obtained from F. Rapp).

Hybridoma antibodies and antisera. Hybridoma cell lines secreting monoclonal antibodies directed against HSV-1 or HSV-2 glycoproteins were isolated by M. Para in this laboratory (M. F. Para, R. Sprague, A. G. Noble, K. M. Zezulak, M. L. Parish and P. G. Spear, manuscript in preparation). The monoclonal antibodies used (in the form of ascites fluids) were: III188, III596, and III211 directed against gF-2 (anti-gF-2); III114 and III255 directed against gD-1 and gD-2 (anti-gD-1/2); III347 directed against gE-2 (anti-gE-2); and II105 directed against gB-1 and gB-2 (anti-gB-1/2). Monoclonal antibody 157.4, specific for gC-1, was a gift from J. Glorioso and M. Levine (University of Michigan, Ann Arbor).

Rabbit antiserum no. 5 (antiserum R#5), prepared against HSV-1(F) virion envelope proteins solubilized by Nonidet P-40 as previously described (42), was characterized previously (41).

Antiserum no. 71 (antiserum R#71) was prepared by immunization of a New Zealand white rabbit with purified denatured gF-2. The antigen was isolated from extracts of HSV-2(333)-infected HEp-2 cells by immunoprecipitation with anti-gF-2 antibody (III188) followed by preparative electrophoresis of the immunoprecipitates on sodium dodecyl sulfate (SDS)-polyacrylamide gels. Antigen obtained from 8×10^7 infected cells was loaded per preparative gel (10 by 14 by 0.15 cm) and gF-2 was located in the gel after electrophoresis by detection of [^{35}S]methionine-labeled tracer added to the unlabeled extract before electrophoresis. Slices of gel were cut from top to bottom along the side of each gel, solubilized, and counted to determine the position of gF-2. After cutting out the gF-2 band, the remainder of each gel was exposed to X-ray film to ensure selective recovery of the gF-2 antigen. The rabbit, after having been bled for a preimmunization sample of serum, received six subcutaneous injections (at multiple sites on the back and neck) of gF-2, the first with Freund complete adjuvant and the rest with incomplete adjuvant. For the first four injections, adjuvant was emulsified with macerated gel containing gF-2. For the last two injections, gF-2 was extracted from the gel with 0.05 M NH_4HCO_3 containing 0.05% SDS, lyophilized (47), and dissolved in phosphate-buffered saline before emulsification with adjuvant. The amount of gF-2 injected was that obtained from 4×10^7 infected cells for the first three injections, 8×10^7 cells for the fourth injection, and 3.2×10^8 cells for the last two injections. The antiserum used was obtained from blood taken 12 days after the last injection.

Infection and labeling of the cells. HEp-2 cells were infected with virus at 10 PFU per cell. Virus was adsorbed for 2 h at 37°C in a small volume of phosphate-buffered saline (10 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 140 mM NaCl, 3 mM KCl, 0.5 mM $MgCl_2 \cdot 6H_2O$, 1 mM $CaCl_2$, pH 7.4) containing 1% inactivated calf serum and 0.1% glucose. The virus was removed and medium 199 containing 1% inactivated calf serum (199V) was added. For long-term labeling of cultures, medium was replaced 4 h after infection with 199V containing 10% of the usual concentration of methionine and the appropriate amount of [^{35}S]methionine (1,000 to 1,200 Ci/mmol; New England Nuclear Corp., Boston, Mass.). Cells were also labeled by the addition at 4 h after infection of [^{14}C]glucosamine (54 mCi/mmol; New England Nuclear) in 199V at 1 μCi/ml. For pulse-labeling, infected cells at 6 h after infection were washed three times with 199V lacking methionine, incubated for 5 min in this medium containing the appropriate amount of [^{35}S]methionine, and then harvested immediately or washed three times with 199V containing a 10-fold excess of unlabeled methionine; incubation was continued in this medium for the appropriate time before harvesting. Cell surface labeling with Na^{125}I (carrier-free, 17 Ci/mg; New England Nuclear) was performed by the procedure of Smith and Brown (40) except that labeling was performed at room temperature and afterward cells were washed with phosphate-buffered saline containing 10 mM NaI.

Immunoprecipitation and enzyme treatment. Cell extracts were prepared by washing infected cell monolayers three times with cold phosphate-buffered saline and adding extraction buffer containing 10 mM sodium phosphate buffer, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, ovalbumin (1 mg/ml), and aprotinin (100 U/ml; Mobay Chemical Corp., New York, N.Y.). One milliliter of extraction buffer was used per 25 cm^2 flask. Extracts were held on ice for 15 min and then centrifuged at 25,000 rpm in an SW27.1 rotor for 1 h to remove insoluble material. For immunoprecipitation, extracts were mixed with antibody and held on ice for 1 h. Sufficient quantities of Formalin-fixed *Staphylococcus aureus* (22) were added to obtain complete adsorption of antibody. After an additional 15 min on ice, the bacterial cells were pelleted in a Eppendorf microfuge. For immunoprecipitation experiments involving addition of a second antibody after removal of the first, a second portion of *S. aureus* was added and pelleted before addition of the second antibody to the supernatant fluid from the first reaction. Bacterial pellets were washed three times with 0.1 M Tris-hydrochloride buffer, pH 8–0.5 M LiCl–1% 2-mercaptoethanol. Pellets were solubilized for analysis on 8.5% SDS-polyacrylamide gels cross-linked with *N,N'*-diallyltartardiamide (18). Gels were fixed and stained and then impregnated with 2,5-diphenyloxazole by the method of Bonner and Laskey (4). Cronex medical X-ray film

was prefogged by the method of Laskey and Mills (23) and exposed to the gels at −70°C.

Immunoprecipitated glycoproteins were treated with endo-β-*N*-acetylglucosaminidase H (endo H; Miles Laboratories, Elkhart, Ind.) or α-D-*N*-acetylgalactosaminyl (GalNAc) oligosaccharidase (Bethesda Research Laboratories, Gaithersburg, Md.) as described by Johnson and Spear (21), except that for endo H treatment, immunoprecipitated glycoproteins were dialyzed against 0.1% SDS–50 mM NaCl, and for GalNAc oligosaccharidase treatment the buffer used was 50 mM potassium phosphate, pH 6.5.

Neutralization assays. We tested antibody-mediated neutralization of viral infectivity in the absence or presence of complement by a plaque reduction assay.

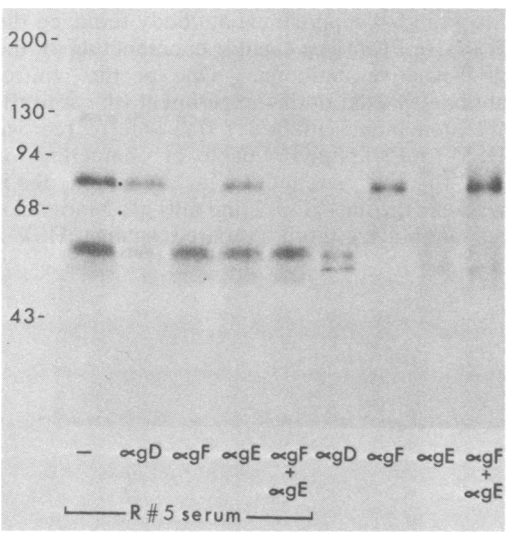

FIG. 1. Identification of HSV-2 glycoproteins immunoprecipitated by antiserum R#5 by use of monoclonal antibodies. Cell extracts used in this analysis were prepared from HEp-2 cells infected with HSV-2(333) at a multiplicity of 10 and labeled with 1 μCi of [^{14}C]glucosamine per ml from 4 to 27 h after infection. Antigens immunoprecipitated from the extract by antiserum R#5 after prior immunoprecipitation with no antibody (−), anti-gD-1/2 (III114) antibody (αgD), anti-gF-2 (III188) antibody (αgF), anti-gE-2 (III347) antibody (αgE) or both anti-gF-2 (III188) and anti-gE-2 (III347) antibodies (αgF + αgE) are shown on the left. The antigens that had been removed from the extracts by the first round of immunoprecipitation with the monoclonal antibodies are shown on the right (αgD, αgF, αgE, and αgF + αgE). For the first immunoprecipitation, 100 μl of extract was reacted with 1 μl of each monoclonal antibody and the immune complexes were collected for analysis. To supernatants of the extracts cleared of these immune complexes, 30 μl of antiserum was then added for the second immunoprecipitation. Precipitates were analyzed by electrophoresis on an 8.5% SDS-polyacrylamide gel here and in all other figures. Molecular weight markers used were myosin (200K), β-galactosidase (130K), phosphorylase b (94K), bovine serum albumin (68K), and ovalbumin (43K).

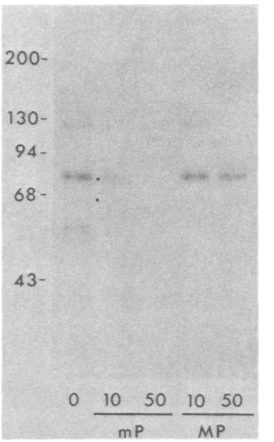

FIG. 2. HSV-2 glycoproteins immunoprecipitated by antiserum R#5 in the absence or presence of various amounts of unlabeled extract from HEp-2 cells infected with HSV-1(mP) or HSV-1(MP). HSV-2(333)-infected cells were labeled with [^{14}C]glucosamine as described in the legend to Fig. 1. Extract from the [^{14}C]glucosamine-labeled HSV-2-infected HEp-2 cells (50-μl samples) was mixed with 0, 10, or 50 μl of unlabeled extract as indicated and immunoprecipitated with 5 μl of antiserum R#5.

HSV-2(333) or HSV-2(G) virus at a concentration of approximately 400 PFU/ml was incubated at 37°C for 1 h with antiserum or monoclonal ascites fluid (that had been heated to 56°C for 30 min) at a final concentration of 1:50, 1:200, or 1:500, without or with guinea pig complement at a final concentration of 1:45. Samples (1 ml) were then added to Vero cell monolayers and adsorbed at 37°C for 2 h. After removal of the inoculum, medium 199V containing 0.1% gamma globulin was added. The cultures were incubated for 2 to 3 days at 37°C and then stained with Giemsa.

RESULTS

Cross-reactivity of gF-2 and gC-1. Two antisera and three monoclonal antibodies were used to probe for antigenic relatedness between gF-2 and gC-1.

An antiserum designated R#5, which was prepared against HSV-1(F) virion envelope glycoproteins (42), immunoprecipitated several HSV-2 glycoproteins (Fig. 1) as previously shown (41). Two of these glycoprotein bands (indicated by the dots in Fig. 1) were identified as different forms of gF-2 by their reactivity with an anti-gF-2 monoclonal antibody. Specifically, anti-gF-2 (III188) antibody (31) quantitatively precipitated these two glycopolypeptides so that neither remained available for precipitation by antiserum R#5 (Fig. 1). In similar fashion, the other glycoprotein bands detected in immunoprecipitates obtained with antiserum R#5 were identified as gD-2 (the 54,000 [54K]-molecular-weight glycopolypeptide seen in Fig. 1) and gB-2

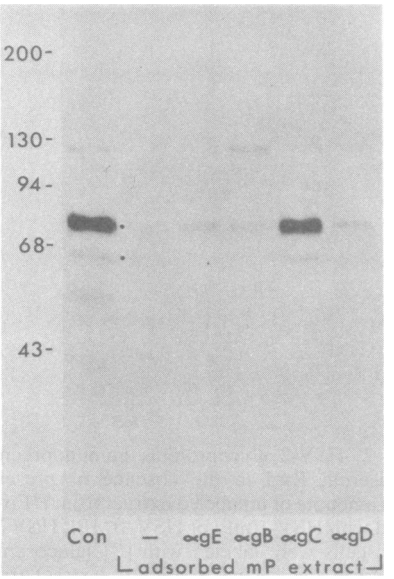

FIG. 3. HSV-2 glycoproteins immunoprecipitated by antiserum R#5 in the absence (Con) or presence (adsorbed mP extract) of unlabeled extract prepared from HSV-1(mP)-infected cells and previously immunoprecipitated with no antibody (−), anti-gE-2 (III347) antibody (αgE), anti-gB-1/2 (II105) antibody (αgB), anti-gC-1 (157.4) antibody (αgC), or anti-gD-1/2 (III114) antibody (αgD). HSV-2-infected cells were labeled as described in the legend to Fig. 1. The quantities used to obtain the immunoprecipitates shown were 50 μl of [^{14}C]glucosamine-labeled extract from HSV-2-infected cells, 100 μl of adsorbed unlabeled extract from HSV-1(mP)-infected cells, and 5 μl of antiserum R#5. The adsorption of each sample of HSV-1(mP) extract was done with 1 μl of monoclonal antibody as indicated except for adsorption with anti-gC-1 which was done with 4 μl of antibody.

(the 125K glycopolypeptide; see evidence presented below).

To determine the specificity of the antibodies in antiserum R#5 which cross-reacted with gF-2, competition experiments were performed with unlabeled extracts from cells infected with HSV-1(mP) or HSV-1(MP). Strain HSV-1(mP) produces all known HSV-1 glycoproteins, whereas the sibling mutant HSV-1(MP) (19) fails to produce gC-1 (18, 25). Antiserum R#5 was reacted with [^{14}C]glucosamine-labeled extract (from HSV-2(333)-infected cells) to which various amounts of competing unlabeled extract were added (Fig. 2). The extract from HSV-1(mP)-infected cells, but not from HSV-1(MP)-infected cells, competed for antibodies in antiserum R#5 that immunoprecipitated gF-2, as evidenced by decreases in the amount of [^{14}C]glucosamine-labeled gF-2 (identified by dots in Fig. 2) immunoprecipitated. Increased competition was observed with greater quantities of HSV-1(mP) extract added. Extracts from HSV-1(mP)- and HSV-1(MP)-infected cells competed equally well for antibodies in antiserum R#5 that precipitated other HSV-2 glycoproteins, providing internal controls for this experiment.

To verify that the competition for gF-2-reactive antibodies was due to gC-1 in the unlabeled HSV-1(mP) extract, an experiment similar to that described above was done except that samples of the HSV-1(mP) extract were first adsorbed with anti-gC-1 (157.4), anti-gB-1/2 (II105), anti-gD-1/2 (III114), or anti-gE-2 (III347) antibody to remove the respective antigens precipitated by these monoclonal antibodies. The results presented in Fig. 3 demonstrate that only the anti-gC-1 monoclonal antibody removed the HSV-1(mP) antigen capable of competing for the gF-2-reactive antibodies. One of the control antibodies used in this experiment (the anti-gB-1/2 monoclonal antibody) was able to remove HSV-1(mP) antigen capable of competing for gB-2-reactive antibodies in antiserum R#5, whereas the anti-gD-1/2 and anti-gE-2 monoclonal antibodies (both prepared against HSV-2

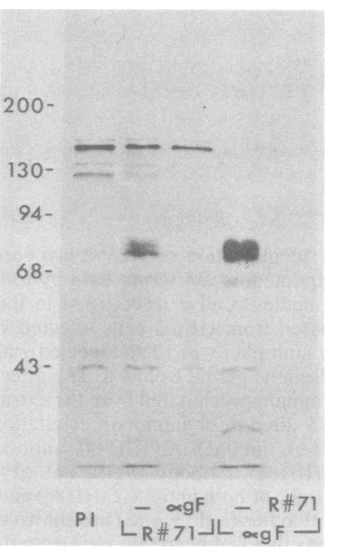

FIG. 4. Identification of polypeptides immunoprecipitated by antiserum R#71. Cell extracts used in this analysis were prepared from HEp-2 cells infected with HSV-2(333) at a multiplicity of 10 and labeled with 30 μCi of [^{35}S]methionine per ml from 4 to 31 h after infection. Cell extract was reacted with preimmune serum (PI), with immune serum (R#71) after no prior immunoprecipitation (−) or after prior immunoprecipitation with anti-gF-2 (III188) antibody (αgF), or with anti-gF-2 (III188) antibody (αgF) after no prior immunoprecipitation (−) or after prior immunoprecipitation with immune serum (R#71). Volumes used in the reactions were 100 μl of extract, 30 μl of serum, and 1 μl of ascites fluid.

antigens) were unable to remove HSV-1(mP) competing antigens. This was the anticipated result for the anti-gE-2 antibody, because not only does the antibody fail to react with HSV-1 antigens but precipitation of gE-2 by antiserum R#5 is not readily evident anyway. The anti-gD-1/2 antibody used, however, does cross-react with at least a fraction of gD-1, but apparently not sufficiently well under the conditions used here to remove HSV-1(mP) antigen capable of competing for the gD-2 reactive antibodies in antiserum R#5.

Taken together the results presented in Fig. 1 to 3 demonstrate that the anti-HSV-1 antiserum R#5 has antibodies reactive with gF-2 and that these antibodies are specific for gC-1.

The second antiserum used (R#71) was prepared against SDS-denatured gF-2 purified from extracts of HSV-2(333)-infected HEp-2 cells by immunoprecipitation with anti-gF-2 (III188) monoclonal antibody followed by preparative electrophoresis. This antiserum, but not preimmune serum, immunoprecipitated polypeptides of 63K and 75K from extracts of HSV-2(333)-infected cells (Fig. 4). Sequential immunoprecipitations performed with antiserum R#71 and anti-gF-2 (III188) antibody, using extracts prepared from HSV-2(333)-infected cells labeled with [^{35}S]methionine from 4 to 24 h after infection (Fig. 4) or from HSV-2(G)-infected cells pulse-labeled with [^{35}S]methionine for 5 min at 6 h after infection (data not shown), demonstrated the antigenic identity of the polypeptides recognized by both reagents.

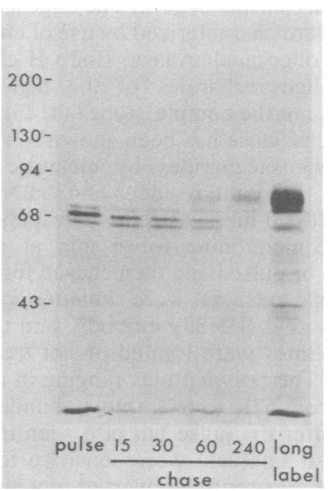

FIG. 5. Newly synthesized and processed forms of gF-2 immunoprecipitated with anti-gF-2 (III188) antibody. Cell extracts used in this analysis were from HEp-2 cells infected with HSV-2(333) at a multiplicity of 10. Infected cells were labeled for 5 min at 6 h after infection with 50 μCi of [^{35}S]methionine per ml and extracts were prepared immediately (pulse) or after chase periods of 15, 30, 60, or 240 min (chase). For comparison, an immunoprecipitate obtained from cells continuously labeled from 4 to 24 h after infection with 25 μCi of [^{35}S]methionine per ml (long label) was included. Immunoprecipitated products seen were obtained from reactions of 100 μl of extract with 1 μl of ascites fluid.

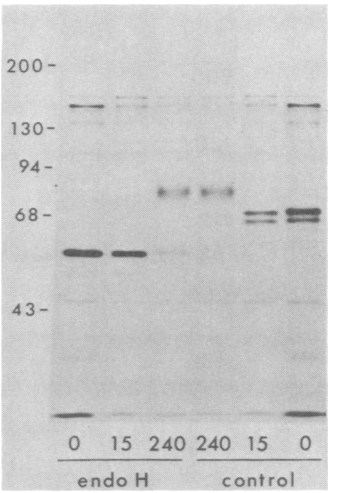

FIG. 6. Effect of endo H on newly synthesized and processed forms of gF-2 immunoprecipitated with anti-gF-2 (III188) antibody. Cell extracts used for this analysis were prepared as described in the legend to Fig. 5. Precipitated polypeptides were treated with endo H or not treated (control) as described in the text. The amounts of protein treated were one-fifth of the total products immunoprecipitated by 3 μl of ascites fluid with 300 μl of extract.

Immunoprecipitation experiments were done to determine whether the anti-gF-2 antiserum R#71 and three anti-gF-2 monoclonal antibodies cross-reacted with any HSV-1 antigen. Negative results were obtained in each instance. Specifically, antiserum R#71 failed to precipitate any polypeptide from HEp-2 cells infected with HSV-1 strain mP, MP, HFEM, F, 14-012, or KOS. Similarily, monoclonal antibody III188, previously shown to be specific for gF-2 (31), and antibodies III596 and III211, which precipitate the same polypeptides as III188 as determined by sequential precipitation experiments (data not shown), failed to immunoprecipitate any polypeptide from HEp-2 cells infected with HSV-1. Antibodies III188 and III596 were tested with HSV-1 strains mP, MP, HFEM, and KOS, and III211 was tested with HSV-1 strain HFEM(syn). Balachandran et al. (2) recently described four other anti-gF-2 monoclonal antibodies, all of which were shown to be HSV-2 specific.

Newly synthesized and fully processed forms of gF-2. Newly synthesized and fully processed forms of gF-2 made in HSV-2(333)-infected

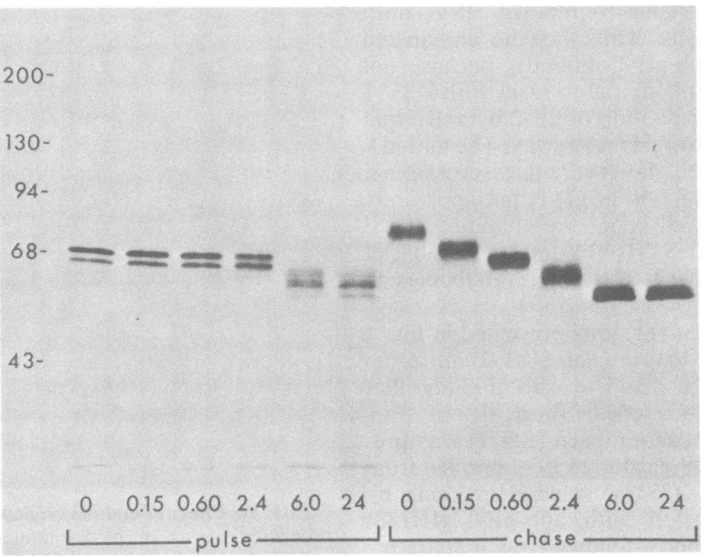

FIG. 7. Effect of GalNAc oligosaccharidase on newly synthesized and processed forms of gF-2 immunoprecipitated with anti-gF-2 (III188) antibody. Cell extracts used in this analysis were prepared as described in the legend to Fig. 5. The chase period was 240 min. Precipitated polypeptides were treated with GalNAc oligosaccharidase at concentrations of 0, 0.15, 0.6, 2.4, 6.0, or 24 mU/ml. The amounts of protein treated were 1/10 of the total products immunoprecipitated by 6 μl of ascites fluid with 600 μl of extract.

HEp-2 cells were characterized. Cells were exposed to [^{35}S]methionine for 5 min at 6 h after infection and then harvested immediately or incubated in nonradioactive medium containing a 10-fold excess of methionine for 15, 30, 60, or 240 min. Lysates were reacted with anti-gF-2 (III188) antibody, followed by electrophoresis of the immunoprecipitates on an SDS-polyacrylamide gel (Fig. 5). Polypeptides precipitated immediately after the pulse had molecular weights of 69K and 67K. After 30 min of chase, a 75K form appeared in the immunoprecipitates, and this form increased in amount up to 4 h, whereas the 69K and 67K forms disappeared. An immunoprecipitate obtained from extracts of HSV-2(333)-infected cells labeled with [^{35}S]methionine continuously from 4 to 24 h after infection is shown alongside the pulse-labeled forms in Fig. 5 for comparison. The protein band seen in all lanes of Fig. 5 (about 71K in molecular weight) was not always detected in precipitates obtained with the III188 antibody and is believed to be nonspecifically precipitated. Results similar to those shown in Fig. 5 were obtained in immunoprecipitations done with anti-gF-2 (III596) antibody (data not shown).

Balachandran et al. (1) characterized precursor and processed forms of gF-2 made by HSV-2(333) in Vero cells. They reported molecular weights of 60K for the precursor and 66K, 79K, and 31K for the processed forms.

Oligosaccharides of gF-2. The oligosaccharides of gF-2 were characterized by use of endo H and GalNAc oligosaccharidase. Endo H cleaves N-linked oligosaccharides of the high-mannose type, but not the complex type (44, 45). GalNAc oligosaccharidase has been shown to release O-linked oligosaccharides by cleavage between serine or threonine residues and GalNAc (20).

HSV-2(333)-infected cells were pulse-labeled with [^{35}S]methionine for 5 min at 6 h after infection or pulsed and then chased for 15 or 240 min. Cell extracts were immunoprecipitated with anti-gF-2 (III188) antibody, and the immunoprecipitates were treated or not treated with endo H. The polypeptides ranging in molecular weight from 67K to 69K (most abundant immediately after the pulse but also seen in the 240-min chase samples) were sensitive to endo H and were apparently converted to a single band of 54K (Fig. 6). Therefore, these polypeptides contain high-mannose N-linked oligosaccharides; moreover, the difference in electrophoretic mobility between the discrete 67K and 69K species is probably due to a difference in the number of N-linked chains attached, whereas the faster and more heterogenous electrophoretic mobility of the endo H-sensitive polypeptides precipitated from the 240-min chase sample could be due to trimming of the N-linked chains before the addition of terminal sugars. The mature 75K protein precipitated from the 240-min chase sample was insensitive to endo H, pre-

sumably because the high-mannose N-linked oligosaccharides had been converted to complex N-linked oligosaccharides.

In similar fashion, the newly synthesized and processed forms of gF-2 were treated with GalNAc oligosaccharidase. Extracts were immunoprecipitated with III188 antibody, and the immunoprecipitates were treated with various concentrations of this enzyme. Concentrations of GalNAc oligosaccharidase which had no effect on the 67K and 69K forms converted the 75K form to species of higher electrophoretic mobility, demonstrating the presence of O-linked oligosaccharides (Fig. 7). At higher enzyme concentrations, all forms of gF-2 were affected, probably due to contaminating glycosidic activities. The O-linked oligosaccharides in the mature 75K protein seem to account for the large difference in electrophoretic mobility between immature and mature forms of gF-2, as has been shown for several HSV-1 glycoproteins (21).

Expression of gF-2 on infected cell surfaces. It has been reported by Balachandran et al. (1) that their monoclonal antibody 17αA2, directed against gF-2, stained the surfaces of unfixed cells infected with HSV-2 in an immunofluorescence assay. We obtained similar results in a radioimmune assay in which unfixed HSV-2(333)-infected HEp-2 cells exposed to the monoclonal antibody III188 bound ^{125}I-labeled protein A from *S. aureus* (data not shown), thereby confirming the presence of gF-2 on infected cell surfaces.

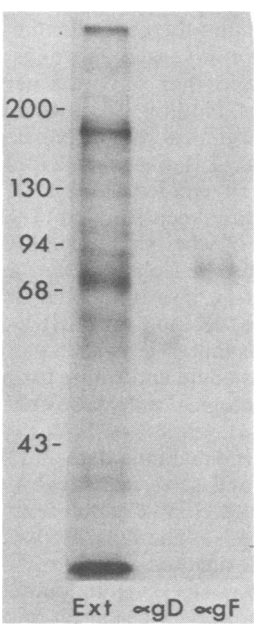

FIG. 8. Presence of gF-2 on the surfaces of infected cells. HSV-2(333)-infected HEp-2 cells were labeled at 16 h after infection with Na^{125}I as described in the text. A sample of the cell extract was electrophoresed (Ext) along with polypeptides immunoprecipitated by anti-gF-2 (III188) antibody (αgF) or anti-gD-1/2 (III255) antibody (αgD) as a control. Immunoprecipitated products seen were obtained from reactions of 100 μl of extract with 1 μl of ascites fluid. Cell extract seen is from 10 μl.

TABLE 1. Complement-dependent neutralization by anti-gF-2 antibodies

Reagent	Virus strain	Neutralization[a]
Monoclonal ascites fluid		
III211	HSV-2 (G)	1:500
III188	HSV-2 (G)	<1:50
III596	HSV-2 (G)	<1:50
R#71		
Pre-immune serum	HSV-2 (333)	<1:50
Immune serum	HSV-2 (333)	1:50

[a] Antibody dilution producing 50% plaque reduction of the indicated HSV-2 strain.

To identify the form of gF-2 exposed on cell surfaces, we labeled HSV-2(333)-infected HEp-2 cells at 16 h after infection with ^{125}I in a lactoperoxidase-catalyzed reaction. Electrophoretic analysis of the labeled cell lysate and of labeled polypeptides precipitated by anti-gF-2 (III188) or anti-gD-1/2 (III255) antibody is shown in Fig. 8. The fully processed 75K form of gF-2 is exposed on infected cell surfaces.

Neutralization by anti-gF-2 antibodies. Three monoclonal antibodies specific for gF-2 and the antiserum prepared against purified denatured gF-2 (R#71) were tested for neutralizing activity against HSV-1 or HSV-2 virions. Although none of these antibodies had neutralizing activity against HSV-1 virions (data not shown), monoclonal antibody III211 and antiserum R#71 were able to mediate complement-dependent neutralization of the HSV-2 strain against which the antibodies were prepared (Table 1). Complement-independent neutralization was not observed (data not shown). These neutralization results demonstrate the presence of gF-2 on virion surfaces.

DISCUSSION

Evidence presented here and elsewhere indicates that the HSV-2 glycoprotein previously designated gF is the HSV-2 counterpart of gC-1. In this paper we have shown that gC-1 and gF-2 are antigenically related. Specifically, the anti-gC-1 component of an antiserum prepared against HSV-1 membrane glycoproteins immunoprecipitates gF-2. Also, Zweig et al. (49)

have isolated a monoclonal antibody which immunoprecipitates both gC-1 and gF-2.

The shared antigenic determinants of gC-1 and gF-2 may be few in comparison with the type-specific determinants, however, based on the following observations. An antiserum raised by us (R#71) against SDS-denatured gF-2 and an antiserum raised by Eberle and Courtney against SDS-denatured gC-1 (11, 12) do not cross-react with antigens of the heterologous serotype. Also, most monoclonal antibodies directed against either gC-1 and gF-2 (1, 2, 31, 32, 39) appear to be type specific.

We have previously mapped the gene for gF-2 to a region of the HSV-2 genome (0.58 to 0.69 map units) that overlaps the gene encoding gC-1 in the HSV-1 genome. Recently we have refined the mapping of the gF-2 gene and located it in the region within map units 0.62 to 0.64 in the HSV-2 genome (Zezulak and Spear, manuscript in preparation). This region of the HSV-2 genome hybridizes to the *Sal*I fragment R of HSV-1 DNA, within which the structural gene for gC-1 has been mapped (0.63 to 0.64 map units) (16, 24). Consequently, it seems likely that the genes for gC-1 and gF-2 will prove to be both collinear on the HSV-1 and HSV-2 genomes and at least partially related in nucleotide sequence. Comparison of our results with those of Marsden et al. (26) suggest that the 63K HSV-2 glycoprotein they mapped to the region from 0.57 to 0.66 map units is actually gF-2.

Treatment of gF-2 with endo H and GalNAc oligosaccharidase showed that addition and processing of gF-2 oligosaccharides is similar to the processing of HSV-1 gC, as well as to all other HSV-1 glycoproteins examined (21, 29, 35, 36, 38, 48). The newly synthesized 67K and 69K polypeptides were sensitive to endo H, indicating the presence of high-mannose N-linked oligosaccharides. What is unusual about this glycoprotein compared to other HSV glycoproteins examined so far is that there are two forms of the newly synthesized gF-2 as resolved by electrophoresis, apparently differing only in the number of N-linked oligosaccharides attached. The mature form of gF-2 has a much lower electrophoretic mobility than the immature forms and contains both complex N-linked oligosaccharides and O-linked oligosaccharides. Removal of O-linked oligosaccharides by treatment with GalNAc oligosaccharidase increases its mobility dramatically. Thus O-linked oligosaccharides appear to be responsible for much of the difference in electrophoretic mobility between immature and mature forms of gF-2, just as was demonstrated for several HSV-1 glycoproteins (21).

We also showed that gF-2 is present on virion and infected cell surfaces. This is similar to results obtained for all other HSV-1 and HSV-2 glycoproteins examined (reviewed in reference 43).

Although gC-1 and gF-2 are antigenically related, structural differences between these two glycoproteins are also very evident. As mentioned above, each glycoprotein appears to have many type-specific antigenic determinants, and the two glycoproteins differ considerably in size as estimated by electrophoresis in SDS gels. The apparent molecular weights of the immature forms of gF-2 in HEp-2 cells are 67K and 69K and the mature form is 75K. For gC-1, the immature form (105K) and mature form (130K) are much larger in size. Wenske et al. (48) have reported that the precursor form of gC-1 has an apparent molecular weight of 75K after treatment with endo H. Johnson and Spear (21) reported similar results for newly synthesized gC-1. After endo H treatment of gF-2, we found its apparent molecular weight to be 54K, indicating that differences in sizes of all forms of the two glycoproteins may be accounted for by differences in sizes of the polypeptide chains.

Considering the similarities in size and greater antigenic cross-reactivity of other related HSV-1 and HSV-2 glycoproteins (7, 13, 28, 30, 32–34, 39), the striking differences between gC-1 and gF-2 suggest that the genes for these two glycoproteins have diverged more as HSV-1 and HSV-2 evolved than have the genes for other glycoproteins. Nothing is known about the function or physiological role of either gC-1 or gF-2 except that gC-1 is dispensable for replication of HSV-1 in cell culture. Viable gC-1$^-$ mutants have repeatedly been isolated (5, 8, 19), raising the question of whether viable gF-2$^-$ mutants of HSV-2 can also be isolated. One can predict that an investigation into functional differences between related HSV-1 and HSV-2 membrane glycoproteins that exhibit such marked structural differences could contribute to an understanding of the biological differences between HSV-1 and HSV-2.

Based on the mapping data and cross-reactivity of gC-1 and gF-2, we suggest that gF-2 be considered the HSV-2 counterpart of gC-1 and be renamed gC-2. The 130K HSV-2 glycoprotein previously designated gC-2 based on its similarity in size to gC-1 (37) is undoubtedly unrelated to gC-1. Therefore, we suggest this glycoprotein be renamed gF-2. These suggestions are in accordance with conventions agreed upon by participants in Cold Spring Harbor Workshops and of the International Conference on Human Herpesviruses (6).

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