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Herpes Simplex Virus Type 2 Glycoprotein gF and Type 1 Glycoprotein gC Have Related Antigenic Determinants

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The 104-S monoclonal antibody immunoprecipitated from herpes simplex virus type 2 (HSV-2)-infected cell extracts the 75,000-molecular-weight glycoprotein gF and its 65,000-molecular-weight precursor (pgF). The precursor pgF was sensitive to endoglycosidase H digestion, indicating the presence of high mannose-type oligosaccharides, whereas the stable gF product was sensitive to neuraminidase digestion, indicating the presence of sialic acid residues. The 104-S antibody also weakly precipiated the 130,000-molecular-weight herpes simplex virus type 1 (HSV-1) glycoprotein gC from both infected cell extracts and purified preparations obtained through the use of monoclonal antibody-containing immunoadsorbent columns. Immunofluorescence tests demonstrated that the 104-S antibody reacted with antigen present in cells infected with HSV-2 strain 333 and HSV-1 strain 14012 but not with antigen present in cells infected with HSV-1 strain MP, a strain deficient in HSV-1 gC production. These findings indicate that HSV-1 gC and HSV-2 gF have antigenic determinants that are related.

Herpes simplex virus (HSV) type 1 (HSV-1) specifies at least three to four glycoproteins (gA/gB, gD, and gE) that are biochemically and immunologically related to glycoproteins that are specified by HSV type 2 (HSV-2) (3, 5, 7, 9, 21, 28). In addition, there are viral glycoproteins (HSV-1 gC and HSV-2 gC) that have been reported to be unique to each HSV type. HSV-1 gC has been described as a type-specific antigen on the bases that antibodies to HSV-1 gC have been found to be predominantly, although not exclusively, type specific (22, 23, 26, 30, 33a) and that no detectable reactions have been reported to occur between HSV-1 gC and heterologous antisera (7, 33). Although an HSV-2 glycoprotein has been designated gC because its electrophoretic migration properties are similar to those of HSV-1 gC (7, 8, 28), the biochemical and immunological characteristics of the HSV-1 and HSV-2 proteins are quite distinct (7, 8), and the genes specifying these glycoproteins are not colinear (28). Recently, Balachandran et al. (1, 2) identified the previously undiscovered HSV-2 glycoprotein gF and have suggested that this glycoprotein may also be type specific, since they did not isolate any cross-reacting anti-HSV-2 gF monoclonal antibodies.

Recently, we described a battery of monoclonal antibodies to HSV-1 proteins (30), and we now have obtained a collection of monoclonal antibodies against HSV-2 proteins. In this report, we analyze a monoclonal antibody (designated 104-S) from this collection that surprisingly reacted with both the 75,000-molecular-weight HSV-2 glycoprotein gF and the 130,000-molecular-weight HSV-1 glycoprotein gC.

MATERIALS AND METHODS

Cells and virus. Vero, 10E2 (11), or HEp-2 cells were grown in Eagle minimal essential medium supplemented with 10% heat-inactivated fetal calf serum. The principal HSV strains used were 14012 (type 1) and 333 (type 2). Other type 1 (Miyama, MAL, MP, 17 *syn*, 17 *syn*$^+$ [19]) and type 2 (SAVAGE, MS, HG52 [19]) strains were also used. After viruses were adsorbed to cells at a multiplicity of infection of 10 to 20 for 1 h at 37°C, fresh Eagle minimal essential medium containing 5% heat-inactivated fetal calf serum was added, and the cultures were incubated at 37°C.

Preparation and characterization of monoclonal antibodies. Spleen cells from BALB/c mice inoculated with a clarified virus suspension from HSV-2 strain 333-infected mouse 10E2 cells were fused with NSI/1 cells (15) and cloned by limiting dilution, and antibody-producing cells were selected by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis analysis of radioimmunoprecipitates (see below) and propagated as ascites tumors. These procedures, which have been reported earlier in detail (30), were used to obtain ascites fluids containing 104-S and 82-S antibodies. The 104-S antibody is a nonneutralizing immunoglobulin G1 (IgG1). The 82-S antibody is a nonneutralizing IgG2b which reacts type specifically with HSV-2 glycoprotein gE. The specificity of this antibody was determined by comparing its reactivity with the H7E (27) and 17βC2 (1, 2, 14) anti-gE monoclonal antibodies. Virus neutralization by the antibodies was tested as described earlier (30). The immunoglobulin classes and subclasses of the antibod-

ies were determined by double diffusion with agar gel plates (Hyland Diagnostics, Costa Mesa, Calif.) and specific rabbit anti-mouse immunoglobulin (Miles Laboratories, Inc., Elkhart, Ind.) as previously described (30). The 19-S anti-HSV-1 gC and the 3-S anti-HSV-1 gA/gB monoclonal antibodies have been previously described (30). The cross-reacting H7E monoclonal antibody to HSV-1 gE (27) was a gift from J. Reactor, University of South Alabama College of Medicine, Mobile. The 17αA2 and 17βC2 monoclonal antibodies (1, 2, 14) were provided by N. Balachandran, McMaster University, Hamilton, Ontario, Canada.

Preparation of monoclonal antibody-Sepharose beads. 19-S antibody-containing ascites fluid (30) was dialyzed in 0.14 M sodium phosphate buffer (pH 8.0), passed through a 0.45-μm filter, and mixed with protein A-Sepharose CL-4B beads (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). The beads were washed four times with 0.14 M sodium phosphate buffer (pH 8.0), loaded into a polypropylene column (Bio-Rad Laboratories, Richmond, Calif.), washed again with 0.14 M sodium phosphate buffer (pH 8.0), and then washed with 0.1 M sodium phosphate buffer (pH 7.0). The antibody was eluted with 0.1 M sodium citrate buffer (pH 3.0) and dialyzed against 0.5 M NaCl–0.2 M sodium bicarbonate buffer (pH 8.5). The antibody was coupled to CNBr-activated Sepharose 4B beads (Pharmacia Fine Chemicals, Inc.) at 5 mg of protein per ml of gel, in accordance with the manufacturer's instructions. The antibody-coupled beads were washed three times with extraction buffer (0.1 M Tris-hydrochloride [pH 8.0], 10% glycerol, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 0.2 mM phenylmethylsulfonyl choride).

Purification of HSV-1 gC. Monolayers of HSV-1 strain 14012-infected Vero cells were washed with phosphate-buffered saline (PBS) and scraped, and the cells were collected by centrifugation (1,000 × *g*, 5 min) and stored at −70°C. The cell pellet was suspended in three volumes of extraction buffer, briefly sonicated, and clarified by centrifugation (40,000 × *g*, 1 h). HSV-1 gC was purified from the extract by a batch-wise adsorption procedure. Infected cell extracts and 19-S anti-HSV-1 gC monoclonal antibody-Sepharose 4B beads were mixed end-over-end on a rotator for 3 h at 4°C. The beads were pelleted (1,000 × *g*, 3 min) and washed three times with extraction buffer. The beads were then loaded into a polypropylene column (Bio-Rad Laboratories) and washed again with extraction buffer. HSV-1 gC was eluted from the beads with 3 M NaSCN–0.1% NP-40–0.01 M Tris-hyrdochloride (pH 8.0) and extensively dialyzed against either PBS (pH 7.2) or extraction buffer (pH 8.0). The purity of the preparation was verified by SDS-polyacrylamide gel electrophoresis analysis (see below).

Preparation of extracts containing radiolabeled viral proteins. Cells were labeled with 100 μCi of [^{35}S]methionine (800 to 1,200 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) per ml of Eagle minimal essential medium containing 5% dialyzed, heat-inactivated fetal calf serum and 10% the usual concentration of methionine between 5 and 20 h after infection. Shorter-term labeling experiments (see figure legends) were performed in methionine-free medium. Viral glycoproteins were labeled by adding D-[6-^{3}H]glucosamine (16 Ci/mmol; Amersham Corp.) to infected cells at a concentration of 50 μCi/ml in Eagle medium containing 5% dialyzed, heat-inactivated fetal calf serum and 20% the normal concentration of glucose between 5 and 20 h after infection. The cell monolayers were washed twice with Tris-buffered saline (pH 7.4) and then dissociated in extraction buffer. The extracts were clarified by centrifugation (60,000 × *g* for 1 h).

Glycosidase treatment. [^{35}S]methionine-labeled cell extracts were incubated at 25°C with endoglycosidase H (2.8×10^{-3} U/ml) (Miles Laboratories, Inc.) or with neuraminidase (8×10^{-2} U/ml) (type VIII, Sigma Chemical Co.) for 20 and 2 h, respectively. The extracts were then placed in an ice water bath and reacted with antibodies as described below.

SDS-polyacrylamide gel electrophoresis analysis of radioimmunoprecipitates. Radiolabeled cell extract or purified HSV-1 gC (0.5 ml) was mixed end-over-end on a rotator with antibody (5 μl) for 90 min at 4°C and then mixed again for 1 h with 0.1 ml of a 33% (vol/vol) suspension of either protein A-Sepharose CL-4B beads (Pharmacia Fine Chemicals, Inc.) or a complex of protein A-Sepharose CL-4B beads and rabbit anti-mouse IgG (Cappel Laboratories, Cochranville, Pa.). The beads were then washed with 0.5 M LiCl–0.1 M Tris-hydrochloride (pH 8.0)–0.1% Nonidet P-40, suspended in an equal volume of 2% SDS–20% glycerol–5% β-mercaptoethanol–0.125 M Tris-hydrochloride (pH 6.8)–0.004% bromphenol blue, and heated at 100°C for 5 min. The immunoprecipitated proteins and a [^{14}C]-methylated protein mixture catalog code (CFA.626; Amersham Corp.) serving as molecular-weight markers were separated by electrophoresis on either a 12.5% polyacrylamide gel or a 5 to 20% gel gradient in a discontinuous buffer system (16) as described earlier (35). Autoradiographs and fluorographs with En^{3}Hance (New England Nuclear Corp., Boston, Mass.) were prepared as previously described (12, 35). The absorbances at 595 nm of the bands in autoradiographs were measured with a scanning densitometer (Transidyne General Corp., Ann Arbor, Mich.).

Peptide mapping. [^{35}S]methionine-labeled proteins were immunoprecipitated as described above except that after washing, the protein A-Sepharose CL-4B beads were suspended in 0.0625 M Tris-hydrochloride (pH 6.7) containing various concentrations of trypsin-TPCK (Millipore Corp., Freehold, N.J.). The immunoprecipitated proteins were partially digested for 30 min at 37°C, and the digestion was stopped by the addition of an equal volume of 2% SDS–20% glycerol–5% β-mercaptoethanol–0.125 M Tris-hydrochloride (pH 6.8)–0.004% bromphenol blue followed by heating at 100°C for 5 min. The peptides were separated by electrophoresis on an SDS-containing 5 to 20% polyacrylamide gel gradient, and fluorographs were prepared as described above.

Enzyme-linked immunosorbent assay. Purified HSV-1 gC in 0.05 M sodium bicarbonate–carbonate coating buffer (pH 9.6) was adsorbed to wells (0.5 μg of protein per well) of U-bottomed polystyrene microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) overnight at 4°C. The wells were washed four times with PBS (pH 7.2) containing 0.05% Tween-20 (PBS-T) and protein-binding sites were then blocked by incubation with 5% bovine serum albumin (Miles Laboratories, Inc.) in PBS. Antibody diluted in 0.5%

bovine serum albumin in PBS was added (50 μl per well), and the plates were incubated for 1 h at 37°C. The wells were washed four times with PBS-T, and 50 μl of peroxidase-conjugated goat anti-mouse IgG heavy and light chains (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) diluted 1:400 in 0.5% bovine serum albumin in PBS-T was added to each well and incubated for 1 h at 37°C. The wells were washed four times with PBS-T, and 100 μl of a color-producing substrate solution [150 μg of 2,2 azino-di-(3-ethyl-benzylthiazoline-sulfonate) (Sigma Chemical Co.) per ml–0.05 M sodium citrate buffer (pH 4.0)–0.03% H_2O_2] was added to each well to detect bound peroxidase activity. The absorbance was measured at 414 nm with a Titertek Multiskan spectrophotometer (Flow Laboratories, Inc., McLean, Va.) after a 30-min incubation at room temperature in the dark.

Immunofluorescence. Antibody-containing ascites fluids were added to ethanol-fixed cells on cover slips and placed in a humidified chamber for 45 min at room temperature. After the cells were washed with PBS, they were reacted with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Kirkegaard and Perry Laboratories, Inc.), counterstained with Evans blue, and examined as previously described (30).

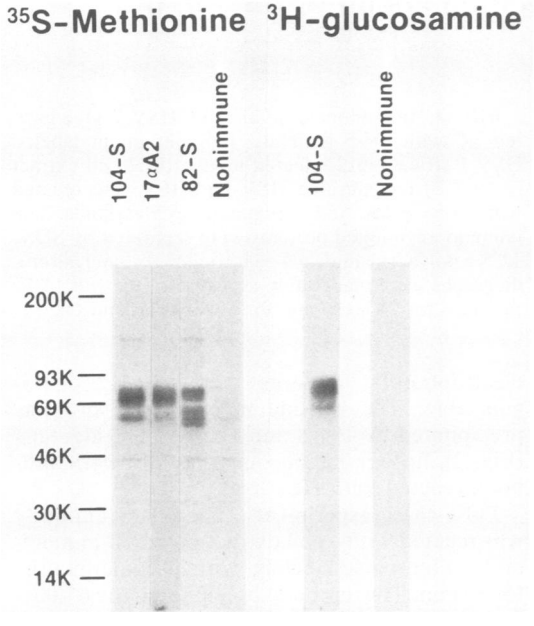

FIG. 1. HSV-2 protein immunoprecipitated by 104-S, 17αA2, and 82-S monoclonal antibodies. Mouse 10E2 cells were labeled with either [^{35}S]methionine or [^{3}H]glucosamine between 5 and 20 h after HSV-2 strain 333 infection, and cell extracts were prepared and reacted with 104-S, 82-S, 17αA2, and nonimmune ascites fluids. The immunoprecipitated proteins were separated by SDS–12.5% polyacrylamide gel electrophoresis, and an autoradiograph of the gel containing [^{35}S]methionine-labeled protein and a fluorograph of the gel containing [^{3}H]glucosamine-labeled protein were made.

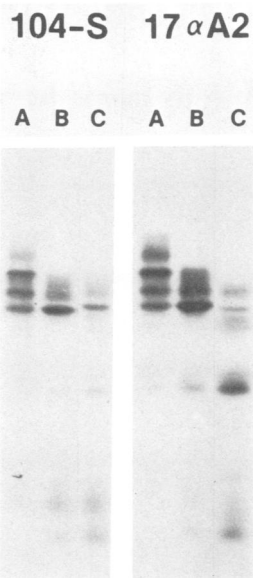

FIG. 2. Peptide map of the HSV-2 protein precipitated by 104-S and 17αA2. [^{35}S]methionine-labeled protein was treated for 30 min at 37°C with trypsin-TPCK at concentrations of 2.8 U (A), 5.6 U (B), and 22.3 U (C) per ml. The partially digested peptides were separated by electrophoresis on an SDS–5 to 20% polyacrylamide gel gradient, after which a fluorograph was made.

RESULTS

Immunoprecipitation of HSV-2 gF by 104-S monoclonal antibody. The nonneutralizing 104-S monoclonal antibody was first reacted with [^{35}S]methionine-labeled HSV-2 strain 333-infected cell extracts and then with protein A-Sepharose CL-4B–goat anti-mouse IgG complexes. These complexes were used instead of protein A-Sepharose CL-4B because 104-S IgG1 has a relatively weak affinity for protein A-Sepharose CL-4B. Electrophoresis analysis showed that the immunoprecipitated HSV-2 protein(s) formed a sharp band migrating at a molecular weight of 65,000 and a broad band migrating at a molecular weight of about 75,000 (Fig. 1). The protein(s) could be labeled with [^{3}H]glucosamine, indicating that it is a glycosylated protein. Proteins having the same mobility were precipitated from cells infected with HSV-2 strains MS, SAVAGE, and HG52 (data not shown). The 17αA2 monoclonal antibody against HSV-2 glycoprotein gF (1, 2) immunoprecipitated polypeptides having the same electrophoretic pattern. Although the molecular weights of the proteins precipitated by the 104-S monoclonal antibody the were similar to those of the proteins precipitated by the 82-S monoclonal antibody against HSV-2 glycoprotein gE, the

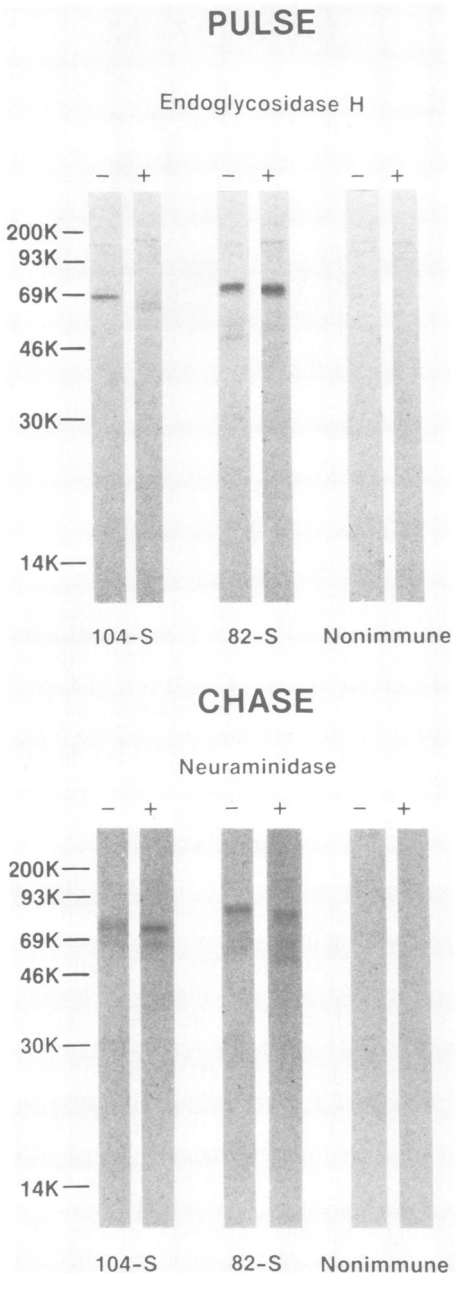

FIG. 3. Pulse-chase labeling of glycosidase-treated HSV-2-infected cells. At 5 h after HSV-2 strain 333 infection, cells were incubated for 1 h in methionine-free medium. The medium was then removed, and the cells were pulse-labeled for 30 min with [^{35}S]methionine in fresh methionine-free medium. Extracts were prepared immediately (top panel) or prepared after the cells were washed three times and incubated (chased) in complete nonradioactive medium for 4 h (bottom panel). Samples of extract were treated with endoglycosidase H for 24 h at room temperature and with neuraminidase for 30 min at room temperature. The extracts were reacted with 104-S, 82-S, and nonimmune ascites fluids. The immunoprecipitated proteins were separated by SDS–12.5% polyacrylamide gel electrophoresis, and an autoradiograph was made.

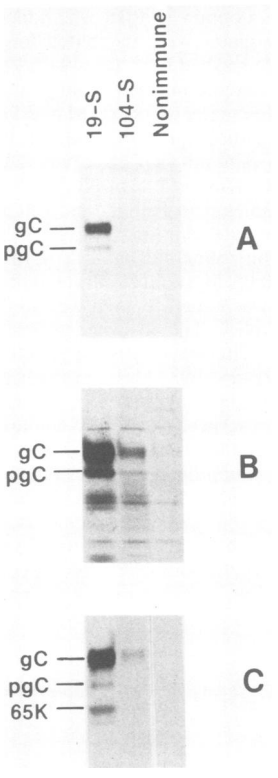

FIG. 4. Immunoprecipitation of HSV-1 glycoprotein gC with 104-S antibody. [^{35}S]methionine-labeled HSV-1 strain 14012-infected mouse 10E2 cell extract (A and B) and purified HSV-1 gC (C) were reacted with 19-S, 104-S, and nonimmune ascites fluids. The immunoprecipitated proteins were separated by SDS–12.5% polyacrylamide gel electrophoresis, and autoradiographs were made after exposure of the gels to X-ray films for 24 h (A) and for 3 weeks (B and C).

electrophoretic patterns were clearly distinguishable. The peptide maps of the proteins precipitated by 104-S and 17αA2 were identical (Fig. 2), further establishing that the 104-S antibody reacted with HSV-2 gF.

Pulse-chase experiments. The 104-S antibody was reacted with a cell extract prepared immediately after pulse-labeling with [^{35}S]methionine for 30 min. The antibody precipitated the 65,000-molecular-weight precursor to gF, pgF, which migrated as a single sharp band in polyacrylamide gels (Fig. 3). Protein migrating as a band doublet at a molecular weight of about 55,000 was very weakly precipitated from a pulse-labeled extract that was treated with endoglycosidase H, indicating the presence of high mannose-type oligosaccharides (32). In contrast, the

mobility of the 70,000-molecular-weight HSV-2 gE precursor, pgE, was not significantly affected by these conditions of endoglycosidase H treatment. From an extract prepared after a 4-h incubation in unlabeled medium the 104-S antibody predominantly precipitated the stable gF protein, which migrated as a broad band at a molecular weight of about 75,000. Minor diffuse bands were also seen at a molecular weight of about 60,000. The mobilities of the predominant proteins precipitated by the 104-S and 82-S antibodies were both reduced after neuraminidase treatment, indicating that they contain sialic acid.

104-S antibody-precipitated HSV-1 gC. Like the 19-S antibody to HSV-1 gC (30), the 104-S antibody precipitated from HSV-1-infected cell extracts glycoprotein gC (molecular weight, 130,000), its precursor pgC (molecular weight, 90,000), and often a 65,000-molecular-weight protein (Fig. 4), which may be a degradation product, since greater amounts of it were precipitated after extended incubation of the antigen (Fig. 5). However, the amount of radiolabeled protein precipitated by 104-S was only about 5 to 10% of that precipitated by 19-S, thus requiring prolonged exposure of the gels to X-ray film to clearly identify the precipitated polypeptides (Fig. 4). The 104-S antibody also weakly precipitated HSV-1 gC that was purified by an immunoadsorbent column containing 19-S antibody. The peptide maps of the proteins precipitated by the 104-S and 19-S antibodies were virtually identical (Fig. 6). These findings indicate that 104-S antibody reacted with HSV-1 gC, which means that HSV-2 gF and HSV-1 gC have antigenic determinants that are related. However, this relatedness may only be partial, since preadsorption of the 104-S antibody with HSV-1 gC attached to agarose beads for 18 h inhibited its capacity to precipitate [^{35}S]methionine-labeled HSV-2 gF by only 50% (data not shown).

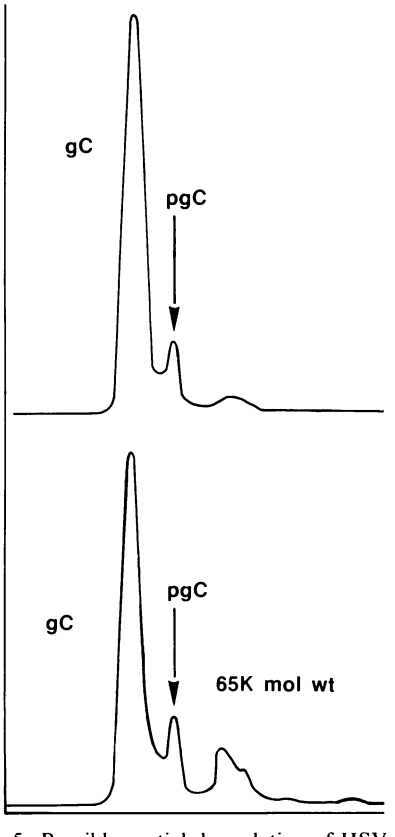

FIG. 5. Possible partial degradation of HSV-1 gC. [^{35}S]methionine-labeled HSV-1 strain 14012-infected mouse 10E2 cell extract was reacted with 19-S antibody immediately (top) or after incubation for 24 h at 4°C (bottom), resulting in the appearance of 65,000-molecular-weight (65K mol wt) protein bands. The immunoprecipitated proteins were separated by SDS–12.5% polyacrylamide gel electrophoresis. A densitometer tracing was made from an autoradiograph of the gel.

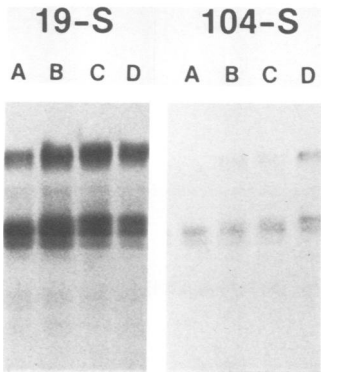

FIG. 6. Peptide map of the HSV-1 protein precipitated by 19-S and 104-S. [^{35}S]methionine-labeled protein was treated for 30 min at 37°C with trypsin-TPCK at concentrations of 22.2 U (A), 11.2 U (B), 5.6 U (C), and 2.8 U (D) per ml. The partially digested peptides were separated by electrophoresis on an SDS–5 to 20% polyacrylamide gel gradient, after which a fluorograph was prepared.

Immunofluorescence test. Antigen in cells infected with HSV-1 strain 14012 and HSV-2 strain 333 reacted with 104-S antibody, resulting in immunofluorescence staining of the cytoplasm (Fig. 7). However, this antibody did not react with cells infected with the MP strain of HSV-1, which is deficient in HSV-1 gC synthesis (13, 31). These immunofluorescence tests provided additional support that the 104-S antibody reacts with HSV-1 gC.

Enzyme-linked immunosorbent assay. Additional evidence for the reactivity of 104-S anti-

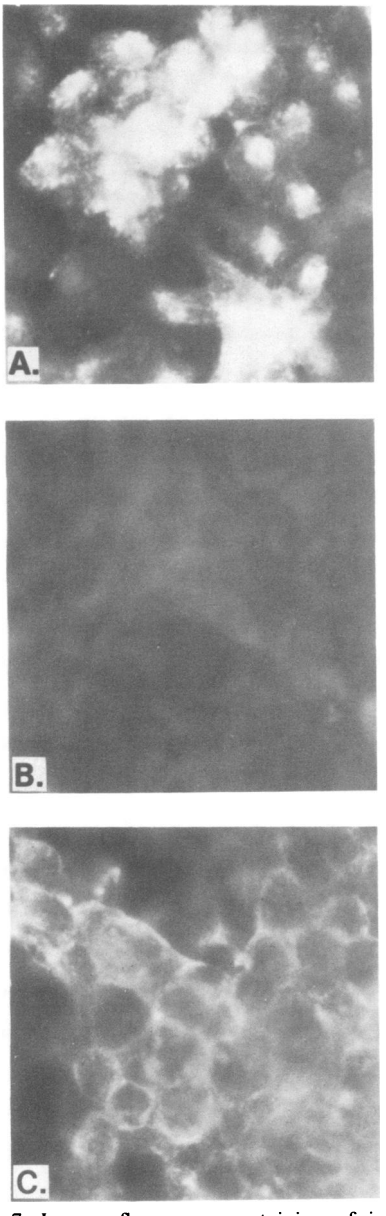

FIG. 7. Immunofluorescence staining of infected cells after treatment with monoclonal antibodies. Vero cells were ethanol fixed at 6 h after infection with HSV-2 strain 333 (A), HSV-1 strain MP (B), and HSV-1 strain 14102 (C) and reacted with 104-S.

body for HSV-1 gC was provided by enzyme-linked immunosorbent assay with microtiter plates that had wells containing adsorbed, purified HSV-1 gC. The titer of anti-HSV-1 gC antibodies in 104-S ascites fluid was somewhat lower than that in 19-S ascites fluid, whereas little or no antibody activity was detected in nonimmune ascites fluid and in those which contained monoclonal antibodies (3-S) to HSV-1 gA/gB (Fig. 8).

DISCUSSION

We have established that the 104-S monoclonal antibody reacts with HSV-2 gF, a glycoprotein which was recently identified by Balachandran et al. (1, 2). The lower-molecular-weight precursor of gF (pgF) contains high-mannose *N*-glycosidic linkages, as do the precursors of other HSV glycoproteins (4, 24, 25, 29, 34). The mature forms of HSV-2 gF and HSV-1 gB, gC, gD, and gE (6, 10) are sensitive to neuraminidase, indicating the presence of sialic acid.

The 104-S antibody also weakly precipitates HSV-1 gC, indicating that it reacts with a determinant on gC that is at least partically related to one on gF. This finding is unexpected, since it appears to be at variance with the proposal presented by several investigators that HSV-1 gC is antigenically type specific. Eberle and Courtney (7) made the proposition at least partly on the basis that their rabbit antiserum to HSV-2-infected cells did not precipitate HSV-1 gC. Additional evidence for this proposal is that Vestergaard and Norrild (33a) did not detect a reaction between their rabbit antiserum to HSV-1 gC (which was designated Ag 6 [20]) and HSV-2 antigen as analyzed by crossed and rocket immunoelectrophoresis. Possible reasons that the cross-reactive determinants on HSV-1 gC and HSV-2 gF were not detected in these and other studies are that they may be only partially related, relatively few in number, or weakly antigenic. If any of these reasons were correct, then the heterogeneous polyclonal antibodies used in these investigations could consist predominantly of type-specific antibodies which could mask the reactivity of a small population of weak cross-reacting antibodies. The use of monoclonal antibodies in this study allowed us to unambiguously show that HSV-1 gC is antigenically related to HSV-2 gF. In other studies, the application of monoclonal antibodies has also revealed antigenic relationships that were not clearly delineated with polyclonal antisera (see reference 17 for a review).

The fact that these proteins share determinants suggests that they have a common genetic origin and physiological function. The gene for HSV-1 gC is located between 0.620 and 0.640 map units on the HSV-1 genome (9a, 18, 28). Very recently, Para et al. (21a) found that the gene specifying HSV-2 gF maps in a DNA region that is colinear with this area of the HSV-1 genome. This is consistent with the finding of Marsden et al. (19) that a gene specifying a 63,000-molecular-weight glycoprotein, which may be HSV-2 gF, is located in this region of the HSV-2 genome.

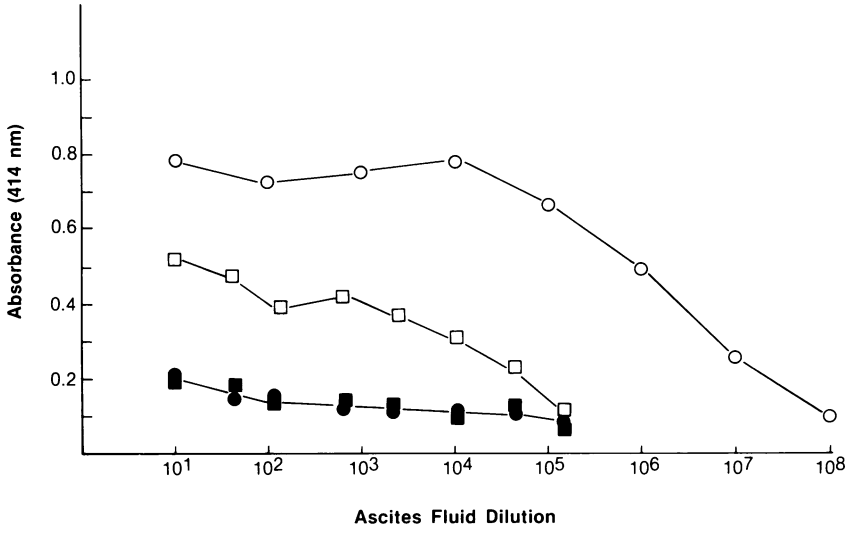

FIG. 8. Titration curves from enzyme-linked immunosorbent assay of monoclonal antibodies. The wells of a microtiter plate were coated with purified HSV-1 gC (0.5 μg per well) and incubated with ascites fluids containing 104-S (□), 19-S (○), and 3-S (●) antibodies and nonimmune ascites fluids (■) at the designated dilutions. The wells were then washed and incubated with peroxidase-conjugated antibodies to mouse immunoglobulin, followed by the addition of the color-producing substrate. The absorbance was then measured and plotted as a function of antibody dilution.

The physiological functions of HSV-1 gC and HSV-2 gF are unknown. HSV-1 gC is a component of the infected cell membrane and of the virion envelope (31). However, some syncitium-forming HSV-1 strains do not induce its synthesis (13, 31), indicating that at least under some conditions this glycoprotein is not necessary for virus propagation. The discovery of gF-deficient HSV-2 strains would suggest that these glycoproteins may play equivalent nonessential physiological roles.

Several workers (2, 7, 28) have identified an HSV-2 glycoprotein which they labeled HSV-2 gC. This designation implies that this protein is analogous to HSV-1 gC. However, the structural genes for these gCs are not colinear (28), and these glycoproteins have different immunological and biochemical properties (7, 8). These findings and those presented in this report raise the possibility that these gC glycoproteins may be completely unrelated, and therefore, the designation HSV-2 gC may be misleading.

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