Similarities and Differences in the Fc-Binding Glycoprotein (gE) of Herpes Simplex Virus Types 1 and 2 and Tentative Mapping of the Viral Gene for This Glycoprotein

MICHAEL F. PARA,^{1†} LYNN GOLDSTEIN,² AND PATRICIA G. SPEAR^{1*}

Department of Microbiology, University of Chicago, Chicago, Illinois 60637,¹ and Fred Hutchinson Cancer Research Center, Seattle, Washington 98104²

Received 18 June 1981/Accepted 3 September 1981

We performed affinity chromatography and immunoprecipitation experiments to determine whether cells infected with herpes simplex virus type 2 (HSV-2) expressed a glycoprotein that was functionally and antigenically related to the HSV-1 Fc-binding glycoprotein designated gE. We found that a protein from extracts of HSV-2-infected HEP-2 cells bound specifically to an Fc affinity column and that the electrophoretic mobility of this protein in sodium dodecyl sulfate-acrylamide gels was slightly less than the mobility of HSV-1 gE. Immunoprecipitation experiments performed with an antiserum prepared against HSV-1 gE revealed that (i) extracts from HSV-2-infected cells contained a glycoprotein that was antigenically related to HSV-1 gE; (ii) the electrophoretic mobility of the HSV-2 gE was indistinguishable from the mobility of the HSV-2 Fc-binding protein; (iii) the antiserum reacted with both newly synthesized transient forms and stable fully processed forms of both HSV-1 gE and HSV-2 gE; and (iv) the transient and stable forms of HSV-2 gE all had lower electrophoretic mobilities than their HSV-1 counterparts. Electrophoretic analyses of gE precipitated from extracts of HEP-2 cells infected with two sets of HSV-1 × HSV-2 intertypic recombinant viruses suggested that the gene for gE is located at the right end of the HSV genome (0.85 to 0.97 map units) in the unique portion of the S component.

A receptor with an affinity for the Fc region of immunoglobulin G (IgG) has been detected in a variety of cultured cell lines after their infection by herpes simplex virus type 1 (HSV-1) or HSV-2 (10, 18-20); this receptor is also present on the surfaces of HSV virions (12). Recently, we reported the isolation and characterization of an Fc-binding glycoprotein (designated gE) from extracts of HSV-1-infected cells (1) and from extracts of HSV-1 virions (12). Several lines of evidence have suggested that gE and the Fc receptors on intact infected cells and virions are identical; this evidence includes their common affinity for IgG Fc, their coincident appearance on cell surfaces after infection (1), and the ability of F(ab')₂ fragments from anti-gE antibodies to inhibit Fc receptor activity (13).

Because cells infected with HSV-2 as well as cells infected with HSV-1 express Fc-binding receptors, we searched for an HSV-2 glycoprotein with properties similar to those of HSV-1 gE. Results of affinity chromatography and immunoprecipitation experiments showed that HSV-2 induced the synthesis of an Fc-binding

glycoprotein and that this glycoprotein was structurally related to HSV-1 gE, but was not identical to it. Analyses of immunoprecipitates obtained from extracts of cells infected with HSV-1 × HSV-2 intertypic recombinants permitted the tentative mapping of the viral gene for gE or of a gene that determined the electrophoretic mobility and antigenicity of gE.

MATERIALS AND METHODS

Cells, viruses and reagents. The cell lines used were Vero and HEP-2 cells, which were obtained from Flow Laboratories, Rockville, Md. The cells were grown in Dulbecco modified Eagle minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum and were maintained after infection in medium 199 containing 1% inactivated calf serum (medium 199V). The Vero cells were used for viral titrations (15), and the HEP-2 cells were used for all other purposes. All of the viruses used in this study have been described previously; they included HSV-1 strains HFEM(tsN102-PAA⁺) (9), HFEM(syn) (1), mP (6), mP(50B) (17), and MacIntyre (2); HSV-2 strains G (4), 186 (14), and 333 (3); the C series of recombinants (9), which were obtained from mixed infections with HSV-1 strain HFEM(tsN102-PAA⁺) and HSV-2 strain 186; and recombinants of HSV-1 strain mP(50B) × HSV-2 strain G (17). The recombinant viruses and

[†] Present address: Department of Medicine, Ohio State University College of Medicine, Columbus, OH 43210.

parental strains were supplied by B. Roizman, University of Chicago, Chicago, Ill.; HSV-1 strain MacIntyre was obtained from J. McDougall, Fred Hutchinson Cancer Research Center, Seattle, Wash., who received it from J. G. Stevens, University of California at Los Angeles; and HSV-2 strain 333 was obtained from F. Rapp, Pennsylvania State University, Hershey. All virus strains were propagated in HEP-2 cells, as described in the accompanying paper (13). All reagents and chemicals used in this study were from the sources listed in the accompanying paper (13).

Conventional antiserum and hybridoma antibody. Anti-gE serum was prepared by immunizing a rabbit with purified HSV-1 strain HFEM(syn) gE, as described in the accompanying paper (13). In addition, a hybridoma cell line producing a monoclonal antibody designated A5 was isolated by the method of Köhler and Milstein (7). Spleen cells from BALB/c mice infected with HSV-1 strain MacIntyre were fused with BALB/c MOPC 21 NS1 myeloma cells (provided by C. Milstein, Molecular Research Council, Cambridge, England) by using procedures described elsewhere (11; L. Goldstein, P. G. Spear, and R. C. Nowinski, manuscript in preparation). Culture supernatants were screened for anti-HSV antibodies by an antibody-binding assay (11), using ^{125}I -labeled protein A from *Staphylococcus aureus* and HSV-1 strain MacIntyre or HSV-2 strain 333 antigens. The A5 hybridoma produced antibodies with apparently higher avidity for HSV-1 antigen than for HSV-2 antigen and was cloned by limiting dilution. An ascites fluid containing high concentrations of the A5 antibody was prepared after injecting pristane-primed BALB/c mice with the hybridoma cell line. Indirect immunofluorescence tests of the ascites fluid revealed that the A5 antibody bound to an antigen present in cells infected with several different HSV-1 strains, reacted less strongly with an antigen in HSV-2-infected cells, but did not react with uninfected cells. The A5 ascites fluid immunoprecipitated a glycoprotein from HEP-2 cells infected with HSV-1 strains MacIntyre and F and HSV-2 strain 333. The apparent molecular weight of the precipitated glycoprotein (the stable form detected in cells continuously labeled with [^{35}S]methionine or [^{14}C]glucosamine from 4 to 24 h after infection) was approximately 80,000 to 90,000 for all of the HSV strains tested, although strain-specific differences in size (or electrophoretic mobility) were evident.

Preparation of extracts from infected cells and immunoprecipitation. Monolayer cultures of HEP-2 cells were infected and incubated with medium containing [^{35}S]methionine, as described in the accompanying paper (13) and below. After the cells were washed with phosphate-buffered saline (PBS; 0.14 M NaCl, 3 mM KCl, 10 mM Na_2HPO_4 , 1.5 mM KH_2PO_4 , 0.5 mM MgCl_2 , 1 mM CaCl_2 , pH 7.2), they were detached by scraping with a rubber policeman and collected by centrifugation. To prepare cell extracts either for immunoprecipitation or for affinity chromatography, the cell pellets were suspended in extraction buffer (0.14 M NaCl, 3 mM KCl, 10 mM Na_2HPO_4 , 1.5 mM KH_2PO_4 , 0.1% sodium dodecyl sulfate, 0.5% sodium deoxycholate, 1% Nonidet P-40, pH 7.2) at concentrations of about 10^7 cells per ml, and the lysates were frozen at -20°C . Immediately before use, the lysates were thawed and centrifuged at 25,000 rpm for 2 h in an SW27.1 rotor to remove insoluble material. The

supernatants were used in immunoprecipitation experiments, as described in the accompanying paper (13).

Affinity chromatography. Fc affinity columns (CNBr-activated Sepharose 4B covalently coupled to bovine serum albumin [BSA] to which anti-BSA antibodies were bound) were prepared exactly as described by Baucke and Spear (1). Extracts of infected cells were prepared as described above and were applied to the columns or to control columns lacking the anti-BSA antibodies. After extensive washing of the columns with PBS containing 0.5% Nonidet P-40, gE was eluted selectively with PBS containing 0.5% Nonidet P-40 whose pH had been lowered to 5.5 by adding concentrated phosphoric acid. The results of other experiments (data not shown) demonstrated that the binding of gE to Fc affinity columns is highly dependent on the pH and that elution of the columns with pH 5.5 buffer instead of 3 M KSCN, as originally described (1), yields preparations of gE that are relatively free of anti-BSA antibodies and polypeptides that bind non-specifically to either BSA-Sepharose or IgG-BSA-Sepharose columns (manuscript in preparation). The eluted proteins were precipitated with trichloroacetic acid and analyzed by electrophoresis, as previously described (1).

Neutralization of viral infectivity. To test the effects of anti-gE on viral infectivity, we performed plaque reduction assays. For the experiments done with HSV-1 strains F and HFEM(syn) and HSV-2 strain G, we prepared mixtures which contained approximately 300 PFU of virus per ml and different amounts of untreated anti-gE or pre-immunization serum per milliliter of PBS containing 1% inactivated calf serum and 5 mM glucose. After the mixtures were incubated for 1 h at 37°C , 1-ml samples were plated in duplicate onto 25-cm 2 monolayer cultures of Vero cells to quantitate residual infectious units (15). For all of the other neutralization tests performed, the anti-gE was heat inactivated (56°C , 30 min) and adsorbed with HSV-2-infected cells (2 ml of a 1:2 dilution of anti-gE was incubated for 1 h at 37°C with a 125-cm 2 monolayer of HEP-2 cells that had been infected for 20 h with HSV-2 strain 186). The adsorbed serum was centrifuged for 2 h at 25,000 rpm in an SW27.1 rotor to remove virions. For the neutralization tests, 100- μl samples of diluted virus stocks containing approximately 300 PFU were mixed in duplicate with 25 μl of adsorbed serum and 15 μl of guinea pig complement. After the mixtures were incubated for 1 h at 37°C , each preparation was diluted to 1 ml with PBS containing 1% inactivated calf serum and 5 mM glucose and plated onto 25-cm 2 monolayer cultures of Vero cells.

RESULTS

Affinity of HSV-2 proteins for IgG-BSA-Sepharose. Extracts prepared from HEP-2 cells infected with HSV-1 strain HFEM(tsN102-PAA $^+$) or HSV-2 strain 186 were applied to IgG-BSA-Sepharose columns and to control BSA-Sepharose columns, as described above. These two virus strains were chosen because they were the parental strains used to generate a set of recombinant viruses (see below). Electrophoretic analysis (Fig. 1) revealed that after infection

with either virus strain, there was synthesis of one protein that bound specifically to IgG-BSA-Sepharose but not to BSA-Sepharose and could be eluted with pH 5.5 buffer. The HSV-1 strain HFEM(tsN102-PAA⁻)-induced column-binding polypeptide was indistinguishable in electrophoretic mobility from the polypeptide induced by HSV-1 strain HFEM(syn); in a previous study (1), Baucke and Spear showed that the HSV-1 strain HFEM(syn)-induced column-binding protein, which was designated gE, had an apparent molecular weight of about 80,000, was glycosy-

lated, was not able to bind to the affinity column if the Fc regions of the anti-BSA antibodies were destroyed by pepsin, and was not detectable in uninfected cells. Figure 1 shows that the HSV-2 strain 186-induced column-binding polypeptide had a lower electrophoretic mobility or a higher apparent molecular weight than HSV-1 gE. In other experiments (data not shown) we demonstrated that the polypeptide which bound to IgG-BSA-Sepharose from cells infected with this and other HSV-2 strains could be labeled with glucosamine, was presumably glycosylated, and consistently had a lower electrophoretic mobility than HSV-1 gE.

Identification of an HSV-2 protein antigenically related to HSV-1 gE. In the accompanying paper (13), Para et al. describe the properties of an antiserum that was prepared against HSV-1 gE and selectively precipitated transient and stable forms of gE from extracts of cells infected with different HSV-1 strains. This antiserum was tested for its capacity to react with polypeptides from extracts of HEP-2 cells infected with HSV-2 strain G. Extracts were prepared from HSV-2 strain G-infected cells and from HSV-1 strain HFEM(syn)-infected cells for comparison either immediately after a pulse with [³⁵S]methionine or after the pulse followed by 30 or 180 min of incubation in nonradioactive medium. Electrophoretic analyses of the immunoprecipitates obtained (Fig. 2) revealed that anti-gE reacted with polypeptides from HSV-2 strain G-infected HEP-2 cells, that the newly synthesized polypeptides appeared to be chased to more slowly migrating stable forms, as occurs with HSV-1 gE (1, 13), and that the transient and stable forms of HSV-1 gE exhibited greater electrophoretic mobility than the apparently equivalent transient and stable forms of HSV-2 gE. We also noted in this and other experiments that a larger fraction of the radiolabel that was detectable in immunoprecipitable newly synthesized polypeptides was recoverable in immunoprecipitable stable polypeptides for HSV-1 gE than for HSV-2 gE. This observation suggested that either HSV-2 gE turns over more rapidly than HSV-1 gE or anti-gE(HSV-1) cross-reacts better with the nascent forms of HSV-2 gE than with the fully processed forms.

In other experiments (data not shown), we compared immunoprecipitates obtained with anti-gE(HSV-1) from extracts of cells infected with several HSV-1 strains [strains HFEM, mP, 17(tsJ), and KOS(tsE6)] and several HSV-2 strains [strains G, GP-6, 186, and 186(PAA⁻)]. In all comparisons we observed that the transient and stable forms of HSV-1 gE had greater electrophoretic mobilities than their HSV-2 gE counterparts.

Effect of anti-gE(HSV-1) on HSV-2 infectivity.

FIG. 1. Viral proteins bound to and eluted from IgG-BSA-Sepharose columns (lanes IgG) and control BSA-Sepharose columns (lanes C). Extracts were prepared from HEP-2 cells that had been infected with HSV-1 strain HFEM(tsN102-PAA⁻) or HSV-2 strain 186 and incubated at 34°C from 3 to 28 h after infection with [³⁵S]methionine. After 0.5 ml of extract was applied to each column, the unbound proteins were washed through, and the bound proteins were eluted with pH 5.5 buffer as described in the text. The eluted proteins were then concentrated by precipitation with trichloroacetic acid and dissolved in sample buffer for the electrophoretic analysis. Samples (5 μ l) of the unfractionated extracts (lanes Ext) were also applied to this acrylamide gel for electrophoretic comparison. The molecular weight markers used in this and all other analyses were β -galactosidase (molecular weight, 135,000 [135K]), phosphorylase *b* (94,000), BSA (68,000), and ovalbumin (43,000).

FIG. 2. Electrophoretic analysis of immunoprecipitates obtained with anti-gE(HSV-1) from extracts of HEp-2 cells infected with HSV-1 strain HFEM(syn) or HSV-2 strain G. The infected cells were maintained at 37°C, pulse-labeled for 5 min with [³⁵S]methionine at 5.5 h after infection with the HSV-1 strain and 4 h after infection with the HSV-2 strain, and then harvested immediately or after incubation in nonradioactive medium for 30 or 180 min. A 1-ml amount of cell extract was mixed with 20 μ l of antiserum to obtain the immunoprecipitates, as described in the text.

Results presented in the accompanying paper (13) show that anti-gE(HSV-1) neutralizes HSV-1 strain HFEM(syn) infectivity in a complement-dependent reaction. We used this antiserum to perform neutralization tests with HSV-1 strain F and HSV-2 strain G in comparison with HSV-1 strain HFEM(syn). Figure 3 shows that unheated anti-gE(HSV-1) neutralized HSV-1 strain F almost as effectively as HSV-1 strain HFEM(syn) but had little if any neutralizing activity against HSV-2 strain G. Either HSV-2 virions lacked gE, which seems unlikely, or the cross-reactive determinants of HSV-2 gE were not accessible to or reactive with neutralizing antibodies.

Use of HSV-1 $\times$ HSV-2 recombinant viruses to map the gene for gE. Differences in the electrophoretic mobilities of HSV-1 and HSV-2 gE's and the poor neutralization of HSV-2 infectivity by anti-gE(HSV-1) provided two methods to distinguish HSV-1 gE from HSV-2 gE and offered the possibility of using HSV-1 $\times$ HSV-2 recombinant viruses to map the gene for gE. We used two sets of recombinant viruses for this purpose.

The first set was isolated by Morse et al. (9) from cells doubly infected with HSV-1 strain HFEM(tsN102-PAA⁻) and HSV-2 strain 186 and was designated the C series. These authors analyzed the DNAs of the recombinants to determine the provenance of the HSV-1 and HSV-2 DNA sequences in each, and their results are summarized in Fig. 4. To isolate gE by immuno-

FIG. 3. Effect of anti-gE(HSV-1) or preimmune serum on HSV infectivity. Different quantities of sera were added along with 300 PFU of virus per ml of reaction mixture, and 1-ml samples were plated onto Vero cells after 1 h at 37°C. The sera used were not heat inactivated and therefore contained sufficient endogenous complement to mediate the neutralization observed. Equivalent results were obtained by adding complement to heat-inactivated sera (13). Symbols: $\bullet$ and $\blacksquare$, anti-gE(HSV-1); $\circ$ and $\square$, pre-immune serum.

FIG. 4. Provenance of HSV-1 and HSV-2 DNA sequences present in the genomes of the recombinant viruses used in this study. The top line of each pair indicates HSV-1 DNA, and the bottom line indicates HSV-2 DNA. The recombinant viruses shown at the top were isolated and characterized by Morse et al. (9), and those at the bottom were isolated and characterized by Tognon et al. (17). This figure was adapted from figures of these authors. By convention, the region of the HSV genome from 0 to 0.82 map units is designated the L component, and the remainder is designated the S component.

precipitation, extracts were prepared from HEp-2 cells that had been infected with each of the parental strains or recombinant viruses and then pulse-labeled with [35 S]methionine. Figure 5 shows the electrophoretic analysis of the precipitated polypeptides, all of which exhibited mobilities characteristic of HSV-2 gE, except for the HSV-1 parental strain. The results of neutralization tests performed with two of the recombinant viruses and the two parental strains were consistent with the immunoprecipitation results in that in the presence of complement anti-gE(HSV-1) effectively inactivated only the HSV-1 parental strain (Table 1). These results and the information shown in Fig. 4 suggest that the gene for gE or a gene that influences both the mobility and antigenicity of gE maps to the

extreme left or right region of the HSV genome in its prototype arrangement.

The second set of recombinants used was isolated by Tognon et al. (17) from cultures that had been transfected with HSV-1 strain mP(50B) DNA and fragments of HSV-2 strain G DNA in order to rescue the cold-sensitive lesion of HSV-1 strain mP(50B). These workers determined the arrangement of HSV-1 and HSV-2 DNA sequences in the recombinant genomes, and their results are also summarized in Fig. 4.

To isolate the gE's of these viruses for comparison, we used two different preparations of antibodies. The first preparation was anti-gE(HSV-1) serum, and the second was mouse ascites fluid containing the hybridoma antibody A5, which reacted with the same HSV-2 protein as anti-gE(HSV-1), as shown by sequential immunoprecipitation experiments (Fig. 6). We selected this anti-gE hybridoma antibody because it preferentially precipitated the gE of HSV-2 parental strain G, whereas the anti-gE(HSV-1) serum preferentially precipitated HSV-1 strain mP(50B) gE. Figure 7 shows the immunoprecipitates obtained with the anti-gE(HSV-1) serum and the A5 hybridoma antibody from a single set

FIG. 5. Electrophoretic analysis of immunoprecipitates obtained with anti-gE(HSV-1) from extracts of HEp-2 cells infected with HSV-1 strain HFEM(tsN102-PAA⁺), HSV-2 strain 186, or the recombinant viruses indicated. The infected cells were maintained at 34°C, pulse-labeled for 5 min with [35 S]methionine at 5 h after infection, and then immediately harvested for preparation of the extracts. A 1-ml sample of extract was mixed with 10 μ l of anti-gE(HSV-1) to obtain the precipitates, which were all subjected to electrophoresis on the same gel slab; the HSV-1 strain HFEM(tsN102-PAA⁺) sample was exposed to X-ray film for a shorter period of time than the other samples.

TABLE 1. Neutralization of parental virus strains and recombinant viruses by anti-gE(HSV-1) in the presence of complement

Virus strain	% of control PFU ^a
HSV-1 HFEM(tsN102-PAA ^r)	15
HSV-2 186	96
Recombinant C5D	100
Recombinant C7D	100

^a See text for a description of the plaque reduction assay.

of extracts prepared from pulse-labeled infected HEP-2 cells. Whereas the anti-gE serum uniformly precipitated HSV-1 gE's [including that produced by HSV-1 strain mP(50B)] more efficiently than HSV-2 gE's, the A5 hybridoma antibody precipitated different relative proportions of the multiple HSV-1 gE polypeptides, exhibited less discrimination between HSV-1 and HSV-2 gE's by immunoprecipitation, and reacted very poorly with the gE of HSV-1 strain mP(50B). Not only did the gE of HSV-1 mutant strain mP(50B) appear to be antigenically different from the gE of HSV-1 parental strain mP, but its electrophoretic mobility was also slightly less (Fig. 7). We also found that anti-gE(HSV-1) serum failed to neutralize HSV-1 strain mP(50B) infectivity, even though it could precipitate gE made by the mutant and neutralized all of the other HSV-1 strains tested.

HEp-2 cells were infected with each of the recombinant viruses isolated by Tognon et al. (17) or with the parental strains and were then pulse-labeled before the preparation of extracts for immunoprecipitation with the two antibody preparations described above. Figure 8 shows that the electrophoretic mobilities and the relative amounts of gE precipitated from cells infected with each of the recombinant viruses were characteristic of HSV-2 parental strain G rather than HSV-1 strain mP(50B). The low-molecular-weight polypeptides detected in the immunoprecipitates were present in variable quantities from experiment to experiment and may have been degradation products of gE.

An examination of these results and the information presented in Fig. 4 suggested that the gene for gE is located in the unique sequences of the S segment between 0.85 and 0.97 map units. It should be noted that Tognon et al. (17) detected no restriction sites that were characteristic of HSV-2 DNA in the strain designated R50BG4, suggesting that recombination had occurred between the restriction sites used as markers or that this strain was a revertant rather than a recombinant. On the basis of our results, we favor the former alternative.

DISCUSSION

The results presented here indicate that cells infected with HSV-2 produce a glycoprotein that is antigenically and functionally related to the HSV-1 Fc-binding glycoprotein designated gE. Specifically, we found that an HSV-2-induced protein with a molecular weight of about 90,000 could be precipitated by anti-gE(HSV-1) and also could bind to IgG-BSA-Sepharose. However, the HSV-2 form of gE is clearly not identical to HSV-1 gE, because both newly synthesized and stable forms have higher apparent molecular weights and because antigenic differences were detected by neutralization assays and by efficiency-of-immunoprecipitation experiments.

The finding that anti-gE(HSV-1) neutralizes HSV-1 strains but not HSV-2 indicates that the type-common determinants detected by immunoprecipitation do not bind neutralizing antibodies or are not exposed on the surfaces of HSV-2 virions. Perhaps the shared determinants are inaccessible due to the interaction of gE with the lipid bilayer or with other proteins in the envelope. It should also be noted that the post-

FIG. 6. Electrophoretic analysis of immunoprecipitates obtained from extracts of HEP-2 cells infected with HSV-2 strain G. The infected cells were maintained at 37°C, pulse-labeled for 7 min with [³⁵S]methionine at 5.5 h after infection, and then immediately harvested for the preparation of extracts. Portions (200 µl from 10⁶ cells) of the extract were mixed with 0, 15, and 30 µl of the A5 ascites fluid to obtain the first set of precipitates (ppt.) (lanes a through c). Each supernatant was then mixed with 15 µl of anti-gE(HSV-1) to obtain the second set of precipitates (lanes d through f).

FIG. 7. Electrophoretic analysis of immunoprecipitates obtained with hybridoma antibody A5 and anti-gE(HSV-1) serum from extracts of HEp-2 cells infected with HSV-2 strains 333 and G and HSV-1 strains mP, mP(50B), and MacIntyre (Mac). The infected cells were maintained at 37°C [or 39°C for HSV-1 strain mP(50B)], pulse-labeled for 7 min with [35 S]methionine at 6 h after infection, and then immediately harvested for the preparation of extracts. A portion (0.2 ml) of each extract was mixed with 5 μ l of A5 ascites fluid or 10 μ l of anti-gE(HSV-1) to obtain the precipitates analyzed.

translational processing of HSV-2 gE appears to result in the loss or masking of some type-common determinants recognized by anti-gE(HSV-1) (Fig. 2), assuming that rates of gE turnover are similar in HSV-1- and HSV-2-infected cells. Thus, it is possible that the form of gE present in HSV-2 virions might react very poorly with anti-gE(HSV-1) even after solubilization.

Exploiting the different properties of HSV-1 and HSV-2 gE's, we characterized the immunoprecipitable products produced by members of two series of HSV-1 $\times$ HSV-2 recombinant viruses which had been isolated by others and characterized previously with respect to the provenance of the HSV-1 and HSV-2 DNA sequences (9, 17). Our results indicated that the structural gene for gE or another gene whose product influences both the mobility and the antigenicity of gE is located in the S component of the HSV genome between 0.85 and 0.97 map units. Marsden et al. (8) presented evidence that an HSV-2 glycoprotein with an apparent molecular weight of 92,000 mapped to the S component of HSV DNA approximately between map units 0.83 and 0.94; perhaps the 92,000-dalton component of these authors was HSV-2 gE. It is interesting that glycoprotein gD has also been mapped to the S component, whereas the other known HSV glycoproteins have been mapped in the L component of HSV DNA (5, 8, 16).

During the analysis of one set of recombinant viruses, we discovered that the gE produced by

FIG. 8. Electrophoretic analysis of immunoprecipitates obtained with hybridoma antibody A5 and anti-gE(HSV-1) from extracts of HEp-2 cells infected with HSV-1 strain mP(50B), HSV-2 strain G, or the recombinant viruses. The infected cells were maintained at 37°C [or 39°C for HSV-1 strain mP(50B)], pulse-labeled for 7 min with [35 S]methionine at 9 h after infection, and then immediately harvested for the preparation of extracts. A 0.5-ml portion of each extract was mixed with 15 μ l of A5 ascites fluid or 20 μ l of anti-gE(HSV-1) to obtain the precipitates analyzed.

HSV-1 mutant strain mP(50B) has properties that are different from the properties of the gE's produced by HSV-1 wild-type parental strain mP and other HSV-1 strains. Specifically, the electrophoretic mobility of HSV-1 strain mP(50B) gE is somewhat lower than that of HSV-1 strain mP gE, anti-gE(HSV-1) did not neutralize HSV-1 strain mP(50B) infectivity, and the hybridoma antibody A5 reacted very poorly with gE from this mutant compared with its reactivity with gE from the parental strain. The cold-sensitive mutation in this virus strain maps to the region of the genome which is substituted by HSV-2 DNA sequences in the recombinant viruses (17) (Fig. 4) and can be rescued by DNA sequences which are present in *Bam*HI fragment J of the HSV-1 genome (0.89 to 0.94 map units) (17). Therefore, it is possible that the 50B mutation is responsible for the altered physical and antigenic properties of gE and that these alterations play some role in determining the phenotype of mutant strain mP(50B). Tognon et al. (17) have shown that the 50B phenotype includes delayed production of plaques at 31°C compared with wild-type virus and accumulation of coreless capsids at nuclear pores late in infection; moreover, these authors showed that recombinants isolated after marker rescue exhibited a wild-type phenotype. They postulated that the accumulation of coreless capsids at the nuclear pores late in infection reflected the inability of the virus to preclude reentry of progeny viruses into the infected cell from which they were released. It remains to be determined whether and how these phenomena can be related to the altered properties of gE.

ACKNOWLEDGMENTS

Various aspects of this work were supported by grant MV13 from the American Cancer Society and by Public Health Service grants CA 19264 and CA 21776 from the National Institutes of Health. M.F.P. is a Fellow in Cancer Research supported by grant DRG-333 from the Damon Runyon-Walter Winchell Cancer Fund.

ADDENDUM

After this manuscript was submitted, we learned that R. G. Hope, J. Palfreyman, M. Suh, and H. S. Marsden (J. Gen. Virol., in press) had obtained similar results for the genomic location of the gene for gE. Both their results and our findings were presented at the International Workshop on Herpesviruses, 27–31 July 1981, Bologna, Italy.

LITERATURE CITED

- Baucke, R. B., and P. G. Spear. 1979. Membrane proteins specified by herpes simplex viruses. V. Identification of an Fc-binding glycoprotein. *J. Virol.* 32:779–789.
- Cook, M. L., and J. G. Stevens. 1973. Pathogenesis of herpetic neuritis and ganglionitis in mice: evidence for intra-axonal transport of infection. *Infect. Immun.* 7:272–288.
- Duff, R., and F. Rapp. 1971. Properties of hamster embryo fibroblasts transformed in vitro after exposure to ultraviolet-irradiated herpes simplex virus type 2. *J. Virol.* 8:469–477.
- Ejercito, P. M., E. D. Kieff, and B. Roizman. 1968. Characterization of herpes simplex virus strains differing in their effect on social behavior of infected cells. *J. Gen. Virol.* 3:357–364.
- Halliburton, I. W. 1980. Intertypic recombinants of herpes simplex viruses: a review. *J. Gen. Virol.* 48:1–23.
- Hoggan, M. D., and B. Roizman. 1959. The isolation and properties of a variant herpes simplex producing multinucleated giant cells in monolayer cultures in the presence of antibody. *Am. J. Hyg.* 70:208–219.
- Köhler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predetermined specificity. *Nature (London)* 256:495–497.
- Marsden, H. S., N. D. Stow, V. G. Preston, M. C. Timbury, and N. M. Wilkie. 1978. Physical mapping of herpes simplex virus-induced polypeptides. *J. Virol.* 28:624–642.
- Morse, L. S., T. G. Buchman, B. Roizman, and P. A. Schaffer. 1977. Anatomy of herpes simplex virus DNA. IX. Apparent exclusion of some parental DNA arrangements in the generation of intertypic (HSV-1 × HSV-2) recombinants. *J. Virol.* 24:231–248.
- Nakamura, Y., J. Costa, T. S. Traalka, C. L. Yee, and A. S. Rabson. 1978. Properties of the cell surface Fc-receptor induced by herpes simplex virus. *J. Immunol.* 121:1128–1131.
- Nowinski, R. C., M. E. Lostrom, M. R. Tam, M. R. Stone, and W. N. Burnette. 1979. The isolation of hybrid cell lines producing monoclonal antibodies against the p15(E) protein of murine leukemia viruses. *Virology* 93:111–126.
- Para, M. F., R. B. Baucke, and P. G. Spear. 1980. Immunoglobulin G(Fc)-binding receptors on virions of herpes simplex virus type 1 and transfer of these receptors to the cell surface by infection. *J. Virol.* 34:512–520.
- Para, M. F., R. B. Baucke, and P. G. Spear. 1982. Glycoprotein gE of herpes simplex virus type 1: effects of anti-gE on virion infectivity and on virus-induced Fc-binding receptors. *J. Virol.* 41:129–136.
- Rawls, W. E., D. Laurel, J. L. Melnick, J. M. Glicksman, and R. H. Kaufman. 1968. A search for viruses in smegma, premalignant and early malignant cervical tissues. The isolation of herpesviruses with distinct antigenic properties. *Am. J. Epidemiol.* 87:647–655.
- Roizman, B., and P. R. Roane, Jr. 1961. Studies on the determinant antigens of viable cells. I. A method, and its application in tissue culture studies, for enumeration of killed cells, based on the failure of virus multiplication following injury by cytotoxic antibody and complement. *J. Immunol.* 87:714–727.
- Ruyechan, W. T., L. S. Morse, D. M. Knipe, and B. Roizman. 1979. Molecular genetics of herpes simplex virus. II. Mapping of the major viral glycoproteins and of the genetic loci specifying the social behavior of infected cells. *J. Virol.* 29:677–697.
- Tognon, M., D. Furlong, A. J. Conley, and B. Roizman. 1981. Molecular genetics of herpes simplex virus. V. Characterization of a mutant defective in ability to form plaques at low temperatures and in a viral function which prevents accumulation of coreless capsids at nuclear pores late in infection. *J. Virol.* 40:870–880.
- Watkins, J. F. 1964. Adsorption of sensitized sheep erythrocytes to HeLa cells infected with herpes simplex virus. *Nature (London)* 202:1364–1365.
- Westmoreland, D., and J. F. Watkins. 1974. The IgG receptor induced by herpes simplex virus: studies using radioiodinated IgG. *J. Gen. Virol.* 24:167–178.
- Yasuda, J., and F. Milgram. 1968. Hemadsorption by herpes simplex-infected cell cultures. *Int. Arch. Allergy* 33:151–170.