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Antigenic Cross-Reactions among Herpes Simplex Virus Types 1 and 2, Epstein-Barr Virus, and Cytomegalovirus

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Polyvalent rabbit antisera against herpes simplex virus type 1 and 2 (HSV-1 and HSV-2), cytomegalovirus (CMV), and Epstein-Barr virus (EBV), monospecific antisera against affinity-purified HSV-2 glycoproteins gB and gG, and a panel of monoclonal antibodies against HSV and EBV proteins were used to analyze cross-reactive molecules in cells infected with the four herpesviruses. A combination of immunoprecipitation and Western blotting with these reagents was used to determine that all four viruses coded for a glycoprotein that cross-reacted with HSV-1 gB. CMV coded for proteins that cross-reacted with HSV-2 gC, gD, and gE. Both CMV and EBV coded for proteins that cross-reacted with HSV-2 gG. Antigenic counterparts to the p45 nucleocapsid protein of HSV-2 were present in HSV-1 and CMV, and counterparts of the major DNA-binding protein and the ribonucleotide reductase of HSV-1 were present in all the viruses. The EBV virion glycoprotein gp85 was immunoprecipitated by antisera to HSV-1, HSV-2, and CMV. Antisera to CMV and EBV neutralized the infectivity of both HSV-1 and HSV-2 at high concentrations. This suggests that cross-reactivity between these four human herpesviruses may have pathogenic as well as evolutionary significance.

Five herpesviruses, herpes simplex virus types 1 (HSV-1) and 2 (HSV-2), varicella-zoster virus (VZV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV), infect humans, and a major portion of the world population is infected in early childhood with HSV-1, VZV, CMV, and EBV (1, 18, 34, 39). The viruses exhibit complex interactions with their infected hosts, and primary infections usually result in the establishment of viral latency; reactivation and reinfection occur periodically (1, 18, 34, 39). Fifty or more virus-specified proteins can be identified in cells infected with herpesviruses (41), and in HSV at least 20 to 25 of them are virus structural components (41). The remainder are probably involved in the initiation and regulation of viral DNA transcription, translation, and synthesis (41).

The DNAs of HSV-1 and HSV-2 are about 50% homologous, and viral proteins show extensive antigenic cross-reactivity (5, 23, 45); cross-reactive determinants are present on both virion and nonstructural proteins (4, 5, 24, 45). The same protein may possess both type-specific and type-common determinants (5, 24, 45), and the genes coding for many of the cross-reactive proteins map colinearly in the genomes of the viruses (29, 35, 45). However, apart from the cross-reaction between HSV-1 and HSV-2, little information is currently available about antigenic cross-reaction between the other human herpesviruses. Early workers reported cross-reaction between HSV-1 and VZV in complement fixation and immunofluorescence assays (22), and by use of hyperimmune VZV serum, Shiraki et al. were able to immunoprecipitate proteins with molecular weights of 115,000 and 53,000 (115K and 53K proteins) from HSV-infected cells (44). More recently, cross-reactivity between gB of HSV and a VZV glycoprotein has been reported (16). Comparisons of newly available DNA sequences reveal homology between VZV and several HSV-1 glycoproteins (gB, gE, gG, and gH) and nonglycoproteins (11–13, 15, 30, 31, 33, 38) and also between potential EBV gene products and the ribonucleotide reductase, exonuclease, DNA polymerase, major DNA-binding protein, and glycoprotein gB of HSV (2, 15, 31, 33, 37, 38). No information about cross-reaction between CMV and the other four viruses has been published.

Antigenic cross-reactivity among this group of ubiquitous viruses is potentially of considerable biologic relevance. We are therefore studying the relatedness of the five human herpesviruses in greater detail. We report here on antigenic cross-reaction between proteins of HSV, EBV, and CMV.

MATERIALS AND METHODS

Cells and viruses. Monolayers of baby hamster kidney cells (BHK-21), Vero cells, and human embryonic fibroblast cells (WI38) were grown in Dulbecco modified Eagle medium (Gibco Laboratories, Grand Island, N.Y.) supplemented with antibiotics and heat-inactivated fetal bovine serum. B lymphoblastoid cell lines were grown in RPMI 1640 medium (Gibco) and included Raji cells (7, 21), which carry the EBV genome but do not produce virus; P3HR1-Cl13 cells (7, 21), which are an EBV-producing cell line that is superinducible with phorbol esters; MCUV5 cells, a marmoset line that produces a transforming strain of EBV (the latter two lines were a gift of George Miller, Yale University); and BJAB cells (7, 21), which are EBV negative. HSV-1 (strain KOS) and HSV-2 (strain 333) were grown and titers determined by plaque assay in Vero cells (3, 4); CMV (strain Davis) was grown and titers determined by plaque assay in WI38 cells. EBV was concentrated by centrifugation from 7-day spent culture media of MCUV5 or P3HR1-Cl13 cells that had been induced with 30 ng of 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) per ml (7, 21).

Monoclonal antibodies. The production and characterization of mouse monoclonal antibodies to HSV-2 and EBV have been described previously (3–7). All antibodies were

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TABLE 1. Specificities of antibodies used for testing cross-reactivity[a]

Antibody	Specificity[b]	Reacting protein(s)
Monoclonal		
B3	HSV-1 and HSV-2	gB
20αD4	HSV-1 and HSV-2	gB
13αC5	HSV-2	gG
17αC1	HSV-2	gC
17βC2	HSV-2	gE
18βB3	HSV-1 and HSV-2	gD
A4	HSV-1 and HSV-2	Major DNA-binding proteins: ICP8 (type 1) and ICP 11/12 (type 2)
3.24	HSV-1 and HSV-2	p45 (nucleocapsid)
8.20	HSV-2	Ribonucleotide reductase
13αA5	HSV-1 and HSV-2	gp350/gp250
D.1.17, E4D6, D4.16, E5B6	EBV	gp85
E1D1	EBV	p105 (early membrane protein)
E8B3	EBV	
Monospecific rabbit		
Anti-gB2	Affinity-purified HSV-2 gB	gB of HSV-1 and HSV-2
Anti-gG2	Affinity-purified HSV-2 gG	gG of HSV-2
Polyvalent rabbit		
Anti-HSV-1	HSV-1-infected BHK-21 cells	
Anti-HSV-2	HSV-2-infected BHK-21 cells	
Anti-CMV	CMV-infected WI38 cells	
Anti-EBV	P3HR1-Cl13 virus	

[a] Antibodies were purified by protein A-Sepharose before use. Monoclonal antibodies were raised against HSV-2 and EBV (3–7).
[b] For monospecific and polyvalent rabbit antibodies, entries are determinants the antibodies were raised against.

purified from high-titered ascitic fluids by chromatography on protein A-Sepharose (Sigma Chemical Co., St. Louis, Mo.). The reacting specificities of the antibodies used are given in Table 1.

Monospecific antibodies. Monospecific antibodies were made in rabbits against proteins purified by affinity chromatography on columns of monoclonal antibodies coupled to cyanogen bromide-activated Sepharose 4B (Sigma). Briefly, BHK-21 cells were infected with HSV-2 at a multiplicity of 5 PFU/cell and harvested when the cytopathic effect was extensive. Unlabeled infected cells from five 150-cm^2 flasks were mixed with cells from a flask that was labeled with 100 μCi of [^{35}S]methionine (specific activity, 1,380 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). Cells were lysed with buffer (0.05 M Tris hydrochloride, pH 7.2, 0.15 M NaCl, 1% Triton X-100, 1% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride [PMSF], and 100 U of aprotinin per ml), sonicated for 1 min, and centrifuged at 100,000 × *g* for 1 h. The supernatants were passed over the affinity columns at least five times, and each column was washed extensively with washing buffer (0.05 M Tris hydrochloride, pH 7.5, 0.5 M NaCl, 1% Triton X-100, 1 mM PMSF, 100 U of aprotinin per ml) until the radioactivity in the flowthrough reached background levels. Bound antigen was eluted with 3 M potassium thiocyanate (KSCN in phosphate-buffered saline [PBS], pH 7.8, 1 mM PMSF), dialyzed extensively to remove thiocyanate, and concentrated by dialysis against polyethylene glycol (PEG 20000; Sigma). The purity of the concentrated antigen was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), silver staining, and autoradiography. Purity was also checked by confirming appropriate reactivity with monoclonal antibodies of different specificities in an enzyme-linked immunosorbent assay (ELISA). All rabbits were bled prior to immunization and tested for their reactivity with HSV-infected cells in an ELISA. Rabbits that showed no reactivity were then immunized intramuscularly with the affinity-purified antigens emulsified in Freund adjuvant. Immune sera thus obtained were purified by chromatography on protein A-Sepharose.

Polyclonal antibodies. Antibodies against virus and virus-infected cells were raised by immunizing rabbits with HSV-1- or HSV-2-infected BHK-21 cells, CMV-infected WI38 cells, or EBV (P3HR1-Cel13 virus). Cells were infected with HSV-1, HSV-2, and CMV at a multiplicity of 5 PFU/cell and harvested when the cytopathic effect was extensive. They were frozen and thawed twice, and HSV-1- and HSV-2-infected cells were exposed to UV light for 15 min prior to inoculation to inactivate the virus. EBV was obtained by centrifugation from 7-day spent culture media of TPA-induced P3HR1-Cl13 cells. Cells and viruses were emulsified with complete Freund adjuvant for the first two intramuscular injections, and subsequent inoculations were given in incomplete Freund adjuvant. Immunoglobulin G (IgG) was obtained from the hyperimmune polyvalent sera by chromatography on protein A-Sepharose; it was then absorbed extensively with uninfected control cells (BHK-21, WI38, and Raji). After absorption, the antibodies showed minimal or no reactivity with uninfected cells.

Radiolabeling procedures. Confluent monolayers of BHK-21 cells were infected with HSV-1 and HSV-2 at a multiplicity of 10 PFU/cell and allowed to absorb for 2 to 3 h. They were labeled between 3 and 20 h postinfection with 20 μCi of [^{35}S]methionine (specific activity, 1,380 Ci/mmol; Amersham) per ml or with 50 μCi of [^{3}H]glucosamine (specific activity, 30 Ci/mmol; ICN Radiochemicals, Irvine, Calif.) per ml in Hanks balanced salt solution (4–7, 21). WI38 cells were infected with CMV at a multiplicity of 5 PFU/cell and labeled with [^{35}S]methionine or [^{3}H]glucosamine between 72 and 96 h postinfection. EBV-producing cells were induced with 30 ng of TPA per ml and labeled between 2 and 3 days postinduction (7, 21). Cells and viruses were also labeled with ^{125}I (Amersham) by use of Iodogen (tetrachlorodiphenylglycouril; Pierce Chemical Co., Rockford, Ill.) (17).

For iodination, HSV-1- and HSV-2-infected and unlabeled cells were collected at 24 h postinfection and CMV-infected cells at 96 h postinfection. EBV-producing cells were induced with TPA, and at 3 days postinduction they were fractionated on a discontinuous gradient of Ficoll-Hypaque (46). Cells expressing viral antigens were collected from the 25 to 50% interface for iodination. All cells to be labeled were suspended in PBS at a concentration of 10^7/ml and labeled with 0.5 mCi of ^{125}I. Labeled cells were washed five times before lysis for immunoprecipitation.

Immunoprecipitation and SDS-PAGE. Immunoprecipitation was carried out as described previously (4, 5, 7, 21). Briefly, cells were solubilized with RIPA buffer containing 0.1 mM PMSF and 100 U of aprotinin per ml (4, 5, 7, 21) and mixed with antibody and protein A-agarose beads (Genzyme Corp., Boston, Mass.). The precipitates were washed, dissociated by boiling in sample buffer with or without 2-mercaptoethanol, and analyzed by SDS-PAGE in 9% acrylamide cross-linked with 0.28% *N,N'*-diallyltartardiamide (DATD). Nonreduced samples were analyzed in separate gels, and molecular weight markers (Sigma) were electrophoresed in parallel channels. Gels were stained, destained, infused with 2,5-diphenyloxazole, dried on filter paper, and placed in contact with Kodak XAR-5 film at −70°C for fluorography (8).

Western blotting. BHK-21 cells were infected with HSV-2 at a multiplicity of 10 PFU/cell and collected at 24 h postinfection without radioactive labeling. Cells were solubilized in RIPA buffer and immunoprecipitated with monoclonal antibodies reacting with different HSV-2 proteins and glycoproteins. The immunoprecipitated proteins were electrophoresed in 9% acrylamide (0.28% DATD) and then electrotransferred onto nitrocellulose membranes (0.45-μm pore size; Schleicher & Schuell, Inc., Keene, N.H.). The transferred sheets were treated for 3 h with blocking buffer (10 mM Tris hydrochloride, pH 7.2, 0.15 M NaCl, 5% skimmed milk, and 0.05% sodium azide) and reacted for 3 h with blocking buffer containing rabbit antibodies of different specificities adjusted to the same protein concentration (400 μg/ml). They were then washed five to six times with wash buffer (10 mM Tris hydrochloride, pH 7.2, 0.15 M NaCl, 0.3% Tween 20) for 10 min each, followed by a final overnight wash. The washed sheets were reacted with alkaline phosphatase-conjugated goat anti-rabbit antibodies (HyClone, Logan, Utah) for 3 h, and the bound anti-rabbit antibodies were detected by reacting with substrate (5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium; Sigma).

Cross-reactivity with reference antibodies. The identities of cross-reactive HSV proteins were confirmed by cross-absorption with reference antibodies. Briefly, [^{35}S]methionine- or ^{125}I-labeled extracts were immunoprecipitated for 2 h at 4°C with a given antibody of known specificity as described above. After centrifugation, the supernatant was again reacted with the same antibody, and the procedure was repeated three to four times. The supernatant collected after the last immunoprecipitation, as well as a sample of the original unabsorbed antigen, was then reacted with the cross-reactive antibodies. All of the immunoprecipitates were analyzed by SDS-PAGE as described above.

Neutralization. The ability of rabbit antibodies to neutralize the infectivity of HSV-1 and HSV-2 was tested by mixing 250 PFU of virus and various dilutions of protein A-purified rabbit antibodies. The antibodies were used at equal protein concentrations, and the virus-antibody mixtures were incubated for 1 h at 37°C before assay for surviving virus in microtiter plates of Vero cells (3). The antibody dilution which protected 50% of the infected cultures was taken as the neutralization endpoint.

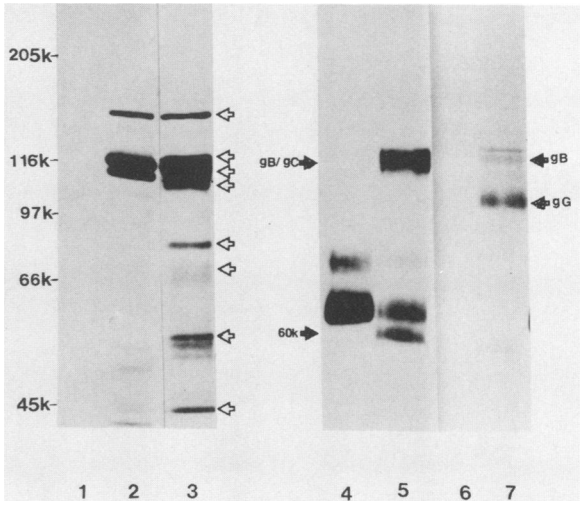

FIG. 1. SDS-PAGE analysis of HSV peptides immunoprecipitated by polyspecific rabbit anti-EBV antibodies. Control and infected cells were labeled with [^{35}S]methionine (lanes 1 to 3) or [^{3}H]glucosamine (lanes 4 to 7). Rabbit anti-EBV antibody with uninfected (lane 1), HSV-1-infected (lanes 2 and 5), and HSV-2-infected cells (lane 3 and 7); preimmune serum with HSV-1-infected (lane 4) and HSV-2-infected cells (lane 6). Arrowheads indicate HSV-specific peptides precipitated by rabbit anti-EBV antibody.

RESULTS

To determine antigenic cross-reactivity among HSV-1, HSV-2, EBV, and CMV, we used monoclonal antibodies of different specificities, monospecific rabbit antibodies against glycoproteins gB and gG of HSV-2, and polyvalent rabbit antibodies against virus and virus-infected cells (Table 1).

Reactivity of anti-EBV antibodies with HSV. To detect antigenic cross-reactivity between HSV and EBV, uninfected and HSV-1- and HSV-2-infected BHK-21 cells were labeled with [^{35}S]methionine or [^{3}H]glucosamine, solubilized in RIPA buffer, immunoprecipitated with rabbit anti-EBV antibodies, and analyzed by SDS-PAGE. Several HSV-specific [^{35}S]methionine-labeled peptides were immunoprecipitated by the anti-EBV antibodies; more peptides were precipitated from HSV-2-infected cells than from HSV-1-infected cells (Fig. 1, lanes 2 and 3). Peptides of 145K, 125K, and 110K were readily immunoprecipitated from cells infected with HSV-1. Longer exposure of gels revealed additional peptides of 105K, 70K, 60K, 58K, 53K, 45K, and 41K (data not shown). The anti-EBV antibody immunoprecipitated peptides of 145K, 125K, 110K, 108K, 85K, 72-66K, 60K, 58K, 53K, and 43K from cells infected with HSV-2. These data indicated the existence of proteins with antigenic determinants that were common to HSV-1, HSV-2, and EBV. Glycoproteins with mobilities similar to those of gC and gB and an additional 60K glycoprotein were immunoprecipitated from HSV-1-infected cells that had been labeled with [^{3}H]glucosamine. Two peptides of 67-75K and 61-64K that were also precipitated by the anti-EBV antibody from HSV-1-infected cells were immunoprecipitated by preimmune serum (Fig. 1, lane 4) and may be related to glycoprotein of HSV-1, which binds to the Fc region of normal rabbit and human antibodies (36). They were incon-

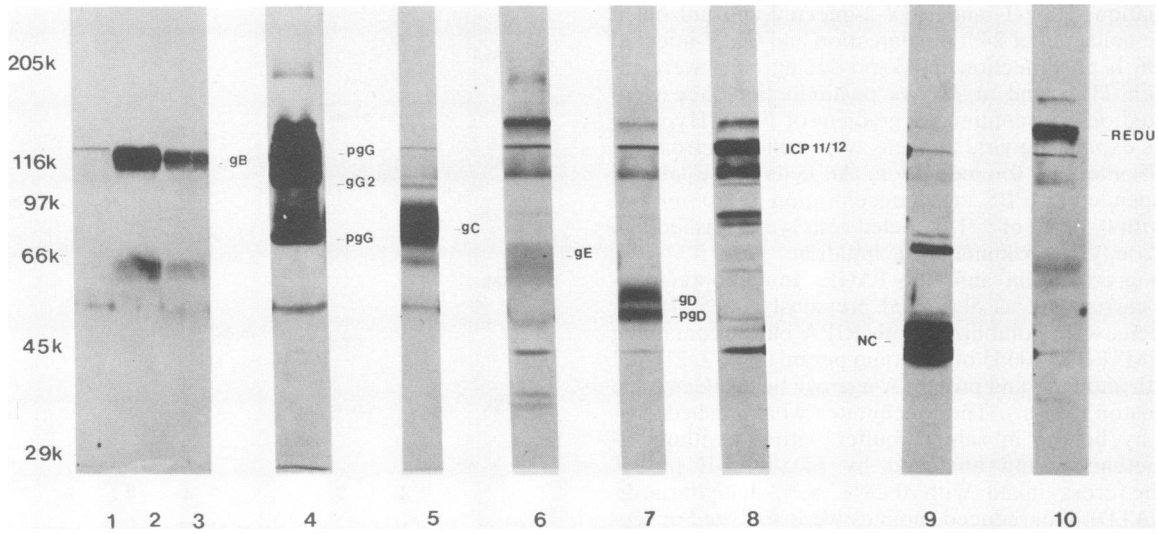

FIG. 2. Reactivity of polyspecific rabbit anti-HSV-2 antibody with different HSV-2 proteins that were immunoprecipitated and Western blotted. Unlabeled HSV-2-infected BHK-21 cells were immunoprecipitated with HSV-2 monoclonal antibodies of different specificities, electrophoresed, and transferred to nitrocellulose. The transferred proteins were reacted with rabbit antibodies and washed, and bound antibodies were detected with alkaline phosphatase-coupled anti-rabbit antibodies and substrate. Antibodies used for immunoprecipitation: lane 1, normal mouse serum (NMS); lane 2, B3 (gB); lane 3, 20αD4 (gB); lane 4, 13αC5 (gG); lane 5, 17αCl (gC); lane 6, 17βC2 (gE); lane 7, 18βB3 (gD); lane 8, A4 (ICP 11/12); lane 9, 8.20 (p45, nucleocapsid [NC] protein); lane 10, 13αA5 (ribonucleotide reductase [REDU]). Lanes were from a single transfer that was cut up for illustrative purposes. gG2, Glycoprotein gG of HSV-2; pgG, precursors of mature gG2; pgD, precursor of mature gD.

sistently immunoprecipitated by normal rabbit serum from HSV-1-infected cells only. Glycoproteins with mobilities similar to those of gB and gG of HSV-2 were immunoprecipitated from HSV-2-infected cells (Fig. 1, lane 7).

Immunoprecipitation of multiple proteins by polyvalent antisera would occur if each individual protein carried determinants recognized by antibodies in the sera (because of multiple reactivities within the sera or because of precursor-product relationships between the proteins) or if some or many of the proteins merely aggregated and coprecipitated as a consequence of only one or a few of the proteins being recognized. To distinguish between these possibilities and to analyze further the cross-reactivity of HSV and EBV, a combination of immunoprecipitation and Western blot experiments were done, as described in Materials and Methods. HSV-2-infected and unlabeled BHK-21 cells were immunoprecipitated with various HSV-2 monoclonal antibodies (Table 1), and the precipitated glycoproteins and nonglycoproteins were electrophoresed, transferred to nitrocellulose membranes, and reacted with rabbit antibodies against EBV and HSV-1. Rabbit anti-HSV-2, included as a positive control, reacted with all the proteins (Fig. 2). Normal rabbit serum did not react with any of the HSV-specific peptides (see Fig. 6), although it did react with proteins in samples immunoprecipitated with monoclonal antibodies against ICP 11/12 and the nucleocapsid. These reactive proteins did not have the same mobility as either ICP 11/12 or the nucleocapsid, and their identities are currently not known.

Rabbit anti-HSV-2 antibody reacted with the mature forms of the glycoproteins and also with their precursor and cleavage products (Fig. 2, lanes 2 to 7). The major DNA-binding protein of HSV is complexed to other HSV proteins, and monoclonal antibody usually immunoprecipitates this complex (47). The multiple peptides recognized by the anti-HSV-2 antibody (Fig. 2, lane 8) therefore probably represent the complex associated with ICP 11/12 of HSV-2. The monoclonal antibody against the nucleocapsid protein p45 immunoprecipitated the multiple forms of this protein and a related protein (80K), all of which were recognized by the rabbit anti-HSV-2 antibody (Fig. 2, lane 9). Rabbit anti-HSV-1 antibody reacted with HSV-2 gB, gE, gD, ICP 11/12, nucleocapsid (p45), and the ribonucleotide reductase, but not with HSV-2 glycoproteins gG and gC (Fig. 3). When rabbit anti-EBV antibody was tested with the different Western-blotted HSV-2 proteins, it reacted with glycoproteins gB and gG, the major DNA-binding protein (ICP 11/12), and the ribonucleotide reductase. These results indirectly demonstrated that EBV has counterparts to these HSV proteins. No reaction was seen with HSV-2 glycoproteins gC, gD, or gE; this may be due either to the absence of counterparts in EBV or to modification of determinants under the experimental conditions of Western blotting. This second possibility might also explain the failure of the anti-HSV-1 antibody to react with glycoprotein gC of HSV-2 (Fig. 3) despite the considerable DNA homology between the gC genes of these two viruses (14).

To help identify which EBV proteins were the counterparts of the HSV proteins, we immunoprecipitated HSV-infected cells with a number of EBV monoclonal antibodies (Table 1; E8B3, E1D1, D4.16, D1.17, and E4D6). One of the monoclonal antibodies (E8B3) which recognized an EBV-specific membrane protein (p105 [7]) reacted with HSV-1- and HSV-2-infected cells in a surface immunofluorescence assay and showed a ring-type membrane fluorescence (data not shown). The antibody immunoprecipitated glycopeptides of HSV-1 and HSV-2 (Fig. 4, lanes 2, 3, 7, and 8) which were identical in mobility to the peptides immunoprecipitated by a monoclonal antibody against HSV glycoprotein gB (Fig. 4, lanes 5 and 6). E8B3 also immunoprecipitated this peptide from a ^{125}I-labeled extract of HSV-1-infected cells (Fig. 4, Lane 10), indicating that the cross-reactive HSV peptide was probably located on the cell surface. Confirmation that the HSV peptide recognized by anti-EBV mono-

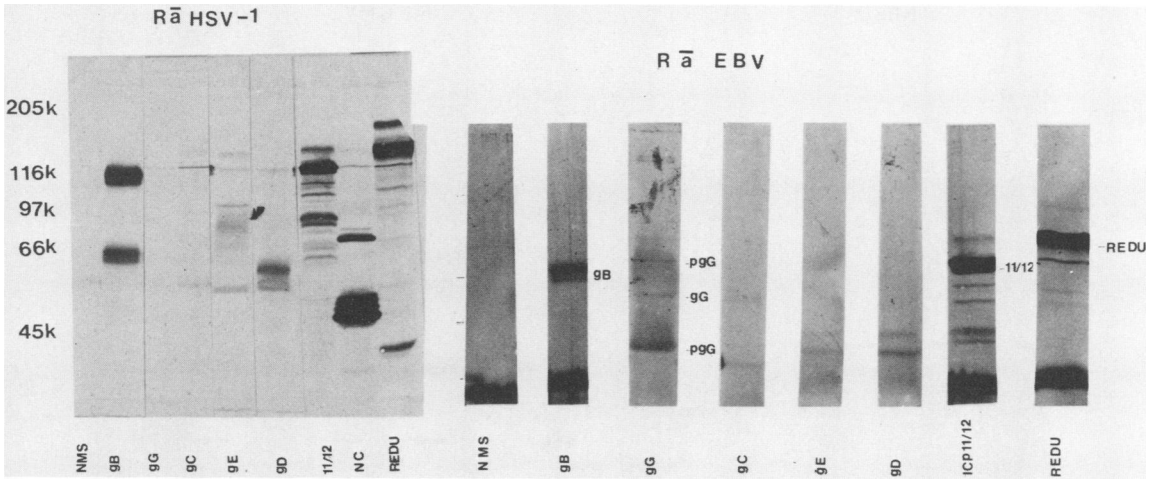

FIG. 3. Reactivity of polyspecific rabbit anti-HSV-1 (RaHSV-1) and rabbit anti-EBV (RaEBV) antibodies with different immunoprecipitated and Western-blotted HSV-2 proteins. Experimental details are the same as in legend to Fig. 2.

clonal antibody (E8B3) was glycoprotein gB was obtained by cross-absorption experiments. Complete removal of glycoprotein gB from ^{125}I-labeled extracts of HSV-1-infected cells by monospecific rabbit anti-gB antibody totally abolished the reactivity of E8B3 antibody (Fig. 4, lanes 11 to 15). These data also suggested that the p105 protein of EBV is an antigenic counterpart of HSV gB.

Reactivity of anti-CMV antibodies with HSV. Cross-reactivity between HSV and CMV was tested by immunoprecipitation of HSV-infected cells with rabbit anti-CMV antibodies. This antibody immunoprecipitated several [^{35}S]methionine-labeled peptides of 150K, 140K, 128K, 116K, 88K, 60K, and 58K from HSV-1 (Fig. 5, lane 5) and glycoproteins with mobilities similar to those of gB/gC and gD/gG (Fig. 5, lane 9). From HSV-2-infected cells it immunoprecipitated [^{35}S]methionine-labeled peptides of 150K, 145K, 140K, 128K, 116K, 110K, 108K, 85K, and 53-58K (Fig. 5, lane 6) and glycoproteins with mobilities similar to those of gB/gG and gD (Fig. 5, lane 10). To identify the cross-reactive proteins, the various HSV-2 proteins that were immunoprecipitated and Western blotted were reacted with anti-CMV antibodies (Fig. 6). The results showed that CMV has counterparts to HSV-2 glycoproteins gB, gG, gC, gD, and gE, to ICP 11/12, to the nucleocapsid, and to the ribonucleotide reductase. The reactivity with gD and gE was

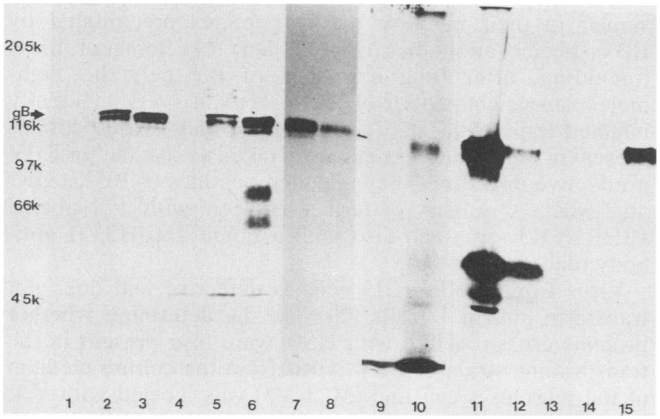

FIG. 4. SDS-PAGE analysis of HSV peptides immunoprecipitated by monoclonal antibody against an EBV membrane protein and by monoclonal and monospecific antibodies against HSV glycoprotein gB. Infected and uninfected cells were labeled with [^{35}S]methionine (lanes 1 to 6), [^{3}H]glucosamine (lanes 7 and 8), or ^{125}I (lanes 9 to 15). Monoclonal antibody E8B3 (against EBV p105) with uninfected (lanes 1 and 9), HSV-2-infected (lanes 2 and 8), and HSV-1-infected cells (lanes 3, 7, 10, 14, and 15); monoclonal antibody B3 (to HSV gB) with uninfected (lane 4), HSV-2-infected (lane 5), and HSV-1-infected cells (lane 6). Lanes 11 to 15, Cross-absorption between rabbit antibody to HSV-2 glycoprotein gB and anti-EBV monoclonal antibody E8B3. ^{125}I-labeled HSV-1-infected cells were immunoprecipitated five times consecutively with anti-gB antibody (lanes 11, 12, and 13, first, second, and fifth precipitation, respectively); the supernatant from the fifth reaction was then immunoprecipitated with E8B3 (lane 14) and compared with an immunoprecipitation of unabsorbed HSV-infected cells by the same antibody (lane 15).

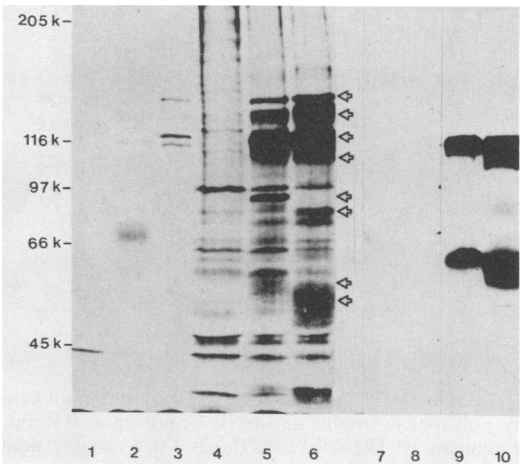

FIG. 5. SDS-PAGE analysis of HSV peptides immunoprecipitated by polyspecific rabbit anti-CMV antibodies. Uninfected and HSV-infected cells were labeled with [^{35}S]methionine (lanes 1 to 6) or [^{3}H]glucosamine (lanes 7 to 10). Preimmune serum with uninfected (lane 1), HSV-1-infected (lanes 2 and 7), and HSV-2-infected cells (lanes 3 and 8); rabbit anti-CMV antibody with uninfected (lane 4), HSV-1-infected (lanes 5 and 9), and HSV-2-infected cells (lanes 6 and 10). Arrows indicate the HSV peptides precipitated by rabbit anti-CMV antibody from [^{35}S]methionine-labeled cells.

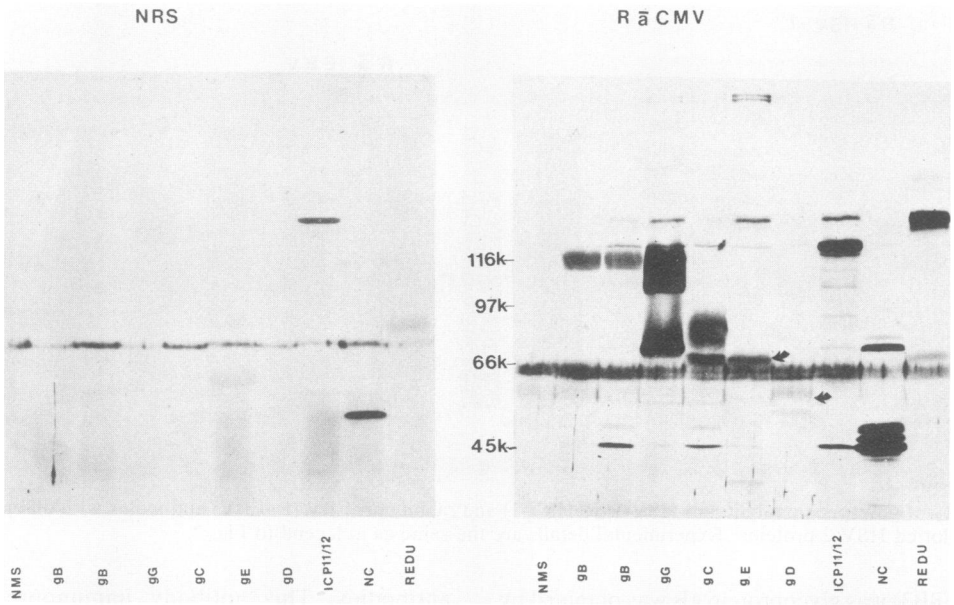

FIG. 6. Reactivity of normal rabbit serum (NRS) and polyspecific rabbit anti-CMV (RaCMV) antibodies with different immunoprecipitated and Western-blotted HSV-2 proteins. Details of the experiments are as given in the legend to Fig. 2.

very weak and may indicate only minimal sharing of antigenic determinants.

Reactivity of anti-HSV and anti-CMV antibodies with EBV. The experiments described above clearly demonstrated that anti-EBV and anti-CMV antibodies recognized a number of HSV-specific proteins. Reciprocal cross-reactivity was next examined by testing the reactivity of anti-HSV and anti-CMV antibodies with EBV. EBV-producing and nonproducing cells and virus from spent culture medium were extrinsically labeled with ^{125}I and used for immunoprecipitation. Positive controls included monoclonal antibodies and a rabbit antibody to EBV (Fig. 7). The monoclonal antibodies immunoprecipitated EBV-specific membrane proteins of 350-250K, 105K, and 85K from iodinated P3HR1-Cl13 cells (Fig. 7, lanes 1, 2, and 5). Anti-EBV antibody also immunoprecipitated these peptides, and no reaction was seen with normal rabbit serum (Fig. 7, lanes 3 and 4). Rabbit anti-HSV-1 antibody immunoprecipitated several ^{125}I-labeled peptides from EBV-producing cells which were similar in their mobility to the peptides precipitated by EBV-specific antibodies (Fig. 7, lane 6). Some of these (including, after long exposure of the gel, the high-molecular-weight peptide of 150K) were also seen in latently infected Raji cells (Fig. 7, lane 7), and only those peptides present in the producing cells were taken as specific for EBV productive infection. They included peptides of 105K, 85K, and 58K. A similar pattern was seen with ^{125}I-labeled P3HR1-Cl13 virus and also with a rabbit anti-HSV-1 antibody (data not shown).

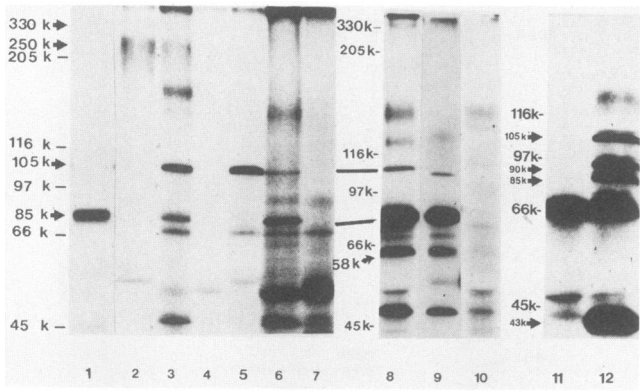

FIG. 7. SDS-PAGE analysis of EBV proteins immunoprecipitated by polyspecific rabbit anti-HSV-1 and anti-CMV antibodies. EBV-producing P3HR1-Cl13 cells (lanes 1 to 6 and 12), nonproducing Raji cells (lanes 7, 10, and 11), and virus from spent culture medium of MCUV5 cells (lanes 8 and 9) were labeled with ^{125}I. Lysates were immunoprecipitated with (lane 1) E1D1, monoclonal antibody against EBV glycoprotein gp85, (lane 2) D4.16, monoclonal antibody against EBV glycoprotein gp350/gp250; (lane 3) rabbit anti-EBV antibody; (lane 4) preimmune serum; (lane 5) E8B3, monoclonal antibody against an early EBV membrane protein, p105; (lanes 6 to 10) rabbit anti-HSV-1 antibody; (lanes 11 and 12) rabbit anti-CMV antibody. Samples were electrophoresed after reduction by 2-mercaptoethanol (lanes 1 to 8 and 10 to 12) or without reduction (lane 9). Arrows indicate EBV-specific peptides.

Virus from P3HR1-Cl13 cells is defective and does not transform human B cells (20, 34). To determine whether proteins cross-reactive with HSV were also present in the transforming strain of EBV, virus from the culture medium of the marmoset cell line (MCUV5) was labeled with ^{125}I, solubilized, and immunoprecipitated by anti-HSV-1 antibody. As shown in Fig. 7, lane 8, peptides of 105K, 85K, and 58K, similar to those in P3HR1-Cl13 cells, were precipitated from the transforming virus. In addition, a 330K peptide was precipitated by the anti-HSV-1 antibody. Similar results were also seen with MCUV5 cells (data not shown), and the mobility of the 105K, 85K, and 58K peptides under nonreducing conditions did not change (Fig. 7, lane 9). These results demonstrated that proteins that were cross-reactive with HSV were present on both the transforming and nontransforming strains of EBV.

Rabbit anti-CMV immunoprecipitated the 105K and 85K proteins from P3HR1-Cl13 virus (Fig. 7, lane 12), suggesting that they carry determinants that are shared among EBV, HSV, and CMV. Anti-CMV antibody also precipitated EBV-specific 90K and 43K peptides, indicating the presence of additional cross-reactive determinants.

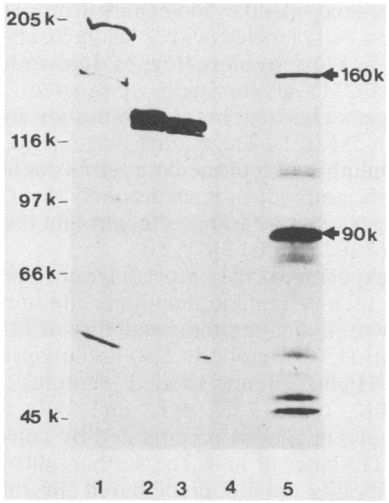

FIG. 8. SDS-PAGE analysis of EBV and HSV proteins immunoprecipitated by monoclonal antibody A4 against the major DNA-binding proteins of HSV-1 and HSV-2 (ICP8 [type 1] and ICP 11/12 [type 2]) from cells labeled with [^{35}S]methionine. Lane 1, Uninfected BHK-21 cells; lane 2, HSV-1-infected BHK-21 cells; lane 3, HSV-2-infected BHK-21 cells; lane 4, Raji cells; lane 5, TPA-induced P3HR1-Cl13 cells.

Cross-reactive EBV proteins were further analyzed directly with monoclonal antibodies made against HSV (Table 1). Antibody A4, which recognized the major DNA-binding protein of HSV-1 (ICP8) and HSV-2 (ICP 11/12) (Fig. 8, lanes 2 and 3), immunoprecipitated a prominent 90K peptide and a minor 160K peptide from [^{35}S]methionine-labeled TPA-induced virus-producing P3HR1-Cl13 cells (Fig. 8, lane 5); no reaction was seen with Raji cells (Fig. 8, lane 4). This result, together with the data shown in the Western blot experiments (Fig. 4), demonstrated that EBV has a counterpart to the HSV DNA-binding protein. Precipitation of two proteins (90K and 160K) may be due either to shared determinants or to protein-protein interactions, and further work is in progress to determine these relationships.

Another HSV monoclonal antibody (B3, Table 1) which reacted with glycoprotein gB of HSV-1 and HSV-2 reacted in an indirect immunofluorescence assay with the surface of EBV-producing cells (data not shown). Upon further testing by immunoprecipitation, antibody B3 precipitated peptides of 105K, 72K, and 58K from [^{35}S]methionine-labeled TPA-induced P3HR1-Cl13 cells but not from Raji cells (Fig. 9, lanes 1 and 2). This suggested that these peptides are related to HSV glycoprotein gB, and further experiments are in progress to determine the precursor-product, cleavage, or breakdown product relationships between them. Immunoprecipitation by the HSV gB monoclonal antibody suggested that the determinant recognized is conserved between HSV-1, HSV-2, and EBV. When we tested a rabbit monospecific antibody against glycoprotein gB of HSV-1 and HSV-2 (Fig. 9, lanes 4 and 5) with ^{125}I-labeled P3HR1-Cl13 virus, two prominent peptides of 105K and 72K were precipitated (Fig. 9, lane 7). A third, lower-molecular-weight peptide was also precipitated from virus-negative BJAB cells, indicating that it is not an EBV-specific protein (Fig. 9, lanes 6 and 7). The data were taken to indicate that the 105K and 72K peptides of EBV carry determinants that are counterparts of HSV gB. Since antibody E8B3, which recognized the 105K peptide of EBV (Fig. 7, lane 5), was also shown to react with glycoprotein gB of HSV (Fig. 2), it

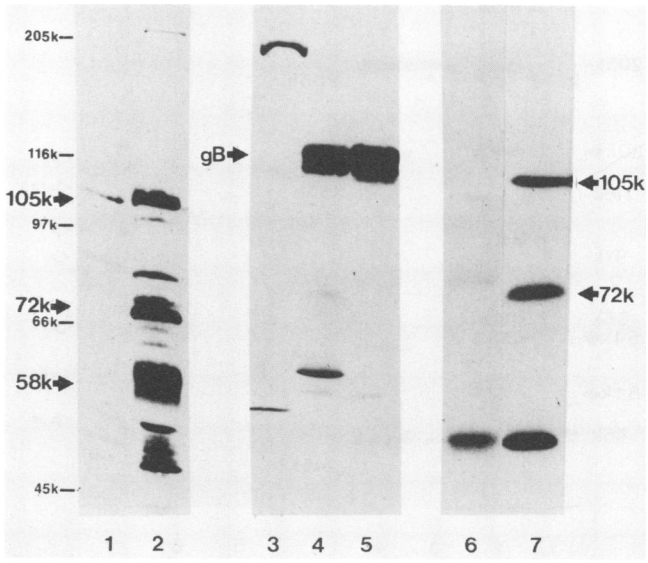

FIG. 9. SDS-PAGE analysis of EBV proteins immunoprecipitated by monoclonal and rabbit monospecific antibodies to glycoprotein gB of HSV-2. Cells and virus were labeled with [^{35}S]methionine (lanes 1 to 5) or ^{125}I (lanes 6 and 7). Monoclonal antibody B3 with Raji cells (lane 1) and TPA-induced P3HR1-Cl13 cells (lane 2); monospecific rabbit anti-HSV-2 gB with uninfected (lane 3), HSV-1-infected (lane 4), and HSV-2-infected (lane 5) BHK-21 cells, BJAB cells (lane 6), and P3HR1-Cl13 virus (lane 7).

seems likely that the virion-associated 105K peptide is an EBV counterpart of HSV gB.

Glycoprotein gG of HSV-2 maps in the unique short region of the HSV-2 genome and apparently possesses predominantly HSV-2-specific determinants (6, 25, 28, 42, 48). We raised a rabbit monospecific antibody against gG of HSV-2 which was purified with a monoclonal antibody (13αC5) affinity column. This monospecific rabbit antibody reacted only with glycoprotein gG of HSV-2 and did not react with

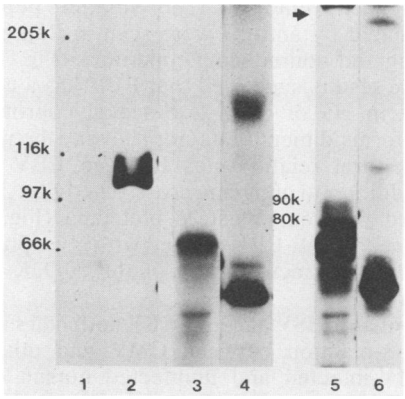

FIG. 10. SDS-PAGE analysis of EBV proteins immunoprecipitated by monospecific rabbit anti-HSV-2 glycoprotein gG. Cells and virus were labeled with [^{3}H]glucosamine (lanes 1 and 2) or ^{125}I (lanes 3 to 6). Anti-gG antibody with HSV-1-infected (lane 1) and HSV-2-infected (lane 2) BHK-21 cells, Raji cells (lanes 3 and 4), and MCUV5 virus (lanes 5 and 6). Samples were electrophoresed after reduction by 2-mercaptoethanol (lanes 1 to 3 and 5) or without reduction (lanes 4 and 6). The arrow indicates the polypeptide of >205K seen under nonreducing conditions.

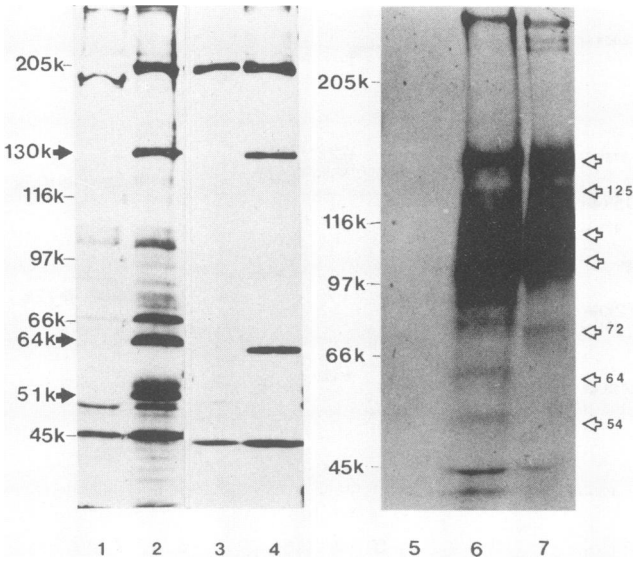

FIG. 11. SDS-PAGE analysis of CMV proteins immunoprecipitated from cells labeled with [^{35}S]methionine (lanes 1 to 4) or [^{3}H]glucosamine (lanes 5 to 7). Monospecific rabbit anti-HSV-2 glycoprotein gB with uninfected (lane 1) and CMV-infected WI38 cells (lane 2); polyspecific rabbit anti-EBV antibody with uninfected (lane 3) and CMV-infected WI38 cells (lane 4); preimmune serum with CMV-infected cells (lane 5); polyspecific rabbit anti-CMV antibodies with CMV-infected cells (lane 6); and polyspecific rabbit anti-HSV-1 antibodies with CMV-infected cells (lane 7). Open arrows, CMV-specific peptides precipitated by anti-HSV-1 antibody; solid arrows, CMV-specific peptides precipitated by anti-HSV-2 glycoprotein gB antibody.

HSV-1 (Fig. 10, lanes 1 and 2). The faintly labeled 64K peptide seen in HSV-1-infected cell extracts was also seen in experiments with preimmune sera (data not shown). The antibody to HSV-2 gG immunoprecipitated diffusely migrating peptides of 80K to 90K from ^{125}I-labeled virus obtained from the MCUV5 cell line (Fig. 10, lane 5). When electrophoresed under nonreducing conditions, the 80K to 90K peptide was replaced by a high-molecular-weight (>205K) peptide (Fig. 10, lane 6). The smaller peptides immunoprecipitated by anti-gG were seen in Raji cells under both reducing and nonreducing conditions (Fig. 10, lanes 3 and 4). These results suggested that EBV has a counterpart to glycoprotein gG of HSV-2. Since glycoprotein gG of HSV-2 also forms dimers (data not shown), the results also indicate structural relationships between EBV and HSV proteins in addition to antigenic cross-reactivity. The results are in accord with our Western blot data (Fig. 4) which showed that rabbit anti-EBV reacted with glycoprotein gG of HSV-2. Further characterization of the 80-90K protein of EBV is in progress.

Reactivity of anti-HSV and anti-EBV antibodies with CMV. To test cross-reaction between CMV and other herpesviruses, CMV-infected and uninfected human embryonic fibroblasts (WI38) were labeled with [^{35}S]methionine between 72 and 96 h postinfection, lysed, and used for immunoprecipitation. Rabbit anti-HSV-2 gB immunoprecipitated CMV-specific peptides of 130K, 64K, and 51K (Fig. 11, lanes 1 and 2), and rabbit anti-EBV antibodies also precipitated the 130K and 64K peptides (Fig. 11, lanes 3 and 4). These results demonstrated that CMV induces a protein that is a counterpart of HSV glycoprotein gB. This conclusion is strengthened by the fact that the rabbit anti-CMV antibody immunoprecipitated gB-like molecules from HSV-1- and HSV-2-infected cells labeled with [^{35}S]glucosamine and reacted with gB in Western blots (Fig. 6). It has recently been reported that a CMV glycoprotein, designated gA (150K to 160K), undergoes cleavage before maturation into proteins of 130K and 56-58K (21). The mature proteins were disulfide linked, and a number of intermediate forms could be precipitated by specific antibodies. It seems likely that CMV gA, in its various stages of maturation, is the protein recognized by the antibody raised against HSV gB.

After long exposure of the autoradiogram, the EBV antibody could be seen to immunoprecipitate additional peptides (data not shown), indicating the possibility of further cross-reactivity. Anti-HSV-1 antibody also immunoprecipitated a number of [^{3}H]glucosamine-labeled peptides (130-150K, 125K, 110-120K, 105K, 72K, 64K, and 54K) which were similar to the glycopeptides precipitated by anti-CMV antibodies (Fig. 11, lanes 6 and 7). Neither anti-HSV-1 nor anti-CMV antibodies immunoprecipitated any such peptides from control cells (data not shown). These data and the reactivity of CMV antibody with HSV and EBV (Fig. 5, 6, and 7) strongly suggest extensive cross-reaction between HSV, EBV, and CMV.

Neutralization of HSV-1 and HSV-2. The ability of antibody to cross-reactive determinants to neutralize virus was studied by assaying equal concentrations of purified IgG from antisera of different specificities for their ability to neutralize the infectivity of HSV-1 and HSV-2 in the absence of complement. Anti-HSV-1 and -HSV-2 antibodies neutralized both HSV-1 and HSV-2 even at low concentrations, while anti-EBV and anti-CMV antibodies neutralized at higher concentrations (Table 2). Interestingly, antibody to gB of HSV-2 was able to neutralize virus, indicating that glycoprotein gB plays an important role in virus infectivity; antibody to glycoprotein gG of HSV-2 failed to neutralize infectivity, providing a good control for the absence of artifactual neutralization by high concentrations of protein.

DISCUSSION

The five human herpesviruses differ from each other in DNA structure, host range, and in vitro rapidity of growth, characteristics which form the basis of their classification into three subfamilies (41). These differences suggest that they possess proteins of varying structures and functions. However, the viruses do also have common biologic features, such as morphology, mode of entry into host cells (fusion of virus envelope with the host membrane), nuclear

TABLE 2. Neutralization of HSV-1 and HSV-2[a]

Rabbit antibody against:	Neutralization[b] (50% neutralizing concn [μg])	
	HSV-1	HSV-2
HSV-1	+ (4.5)	+ (15)
HSV-2	+ (61)	+ (20)
CMV	+ (150)	+ (150)
EBV	+ (150)	+ (150)
gB of HSV-2	+ (45)	+ (15)
gG of HSV-2	—[c]	—

[a] Neutralization of HSV-1 and HSV-2 in the absence of complement was measured in a microneutralization assay; antibodies were purified by protein A chromatography, adjusted to contain equal concentrations of protein, and tested for neutralization of 250 PFU of virus.

[b] Purified antibodies which varied in concentration (45 to 3.2 mg/ml) were adjusted to 3 mg/ml. The concentrations of antibody which neutralized 50% of virus infectivity are given in parentheses.

[c] —, Rabbit antibody to gG and normal rabbit antibody both failed to neutralize infectivity at the highest concentration tested (300 μg).

TABLE 3. Summary of antigenic cross-reactions among HSV-1, HSV-2, CMV, and EBV

Protein	Antigenic counterpart[a]			
	HSV-1	HSV-2	EBV	CMV
HSV-1 gB	+	+	+	+
HSV-2 gB	+	+	+	+
HSV-1 gC	+	+	(+)	NT
HSV-2 gC	+	+	−	+
HSV-1 gD	+	+	−	NT
HSV-2 gD	+	+	−	+
HSV-1 gE	+	+	NT	NT
HSV-2 gE	+	+	−	+
HSV-1 gG	+	NT	NT	NT
HSV-2 gG	−	+	+	+
DNA-binding protein	+	+	+	+
Ribonucleotide reductase	+	+	+	+
p45 nucleocapsid	+	+	−	+
HSV-1 gH	+	NT	(+)	NT
HSV-1 U_s7	+	NT	(+)	NT
EBV gp85	+	+	+	+
EBV gp350	(+)	−	+	NT

[a] Antigenic counterparts as detected by immunoprecipitation with monoclonal, monospecific, and polyspecific antibodies and by the reaction of polyspecific antibodies with the Western-blotted HSV-2 proteins. Symbols: +, counterpart detected; −, counterpart not detected; (+), counterpart indicated only by sequence homology between genes coding for the specific proteins; NT, not tested.

location of viral DNA replication, and virus assembly and acquisition of an envelope from the inner nuclear membrane (41). Moreover, in a lytic cycle, viral DNA transcription and protein synthesis are similarly regulated in cascade fashion (41). This would imply that, in addition to unique proteins responsible for differential cell tropism and other characteristics, they probably possess some common structural and nonstructural regulatory proteins that are responsible for common functions. Ongoing analyses of viral DNA sequences reveal significant genetic homology (2, 11, 12, 15, 30, 31, 33), and the data presented in this paper (summarized in Table 3) demonstrate that a number of common antigenic determinants occur among the viral glycoproteins and nonglycoproteins of HSV-1, HSV-2, and EBV.

Earlier studies documented the antigenic relatedness of HSV-1 and HSV-2 and other animal herpesviruses and showed that the major DNA-binding protein of HSV-1 (ICP8) and HSV-2 (ICP 11/12) is one of the proteins which carries cross-reactive determinants (24, 27). Our results extend these observations, showing that both EBV and CMV also have antigenic counterparts to the major DNA-binding protein of HSV; they reinforce the conclusion (27) that such a highly conserved protein probably occupies a central role in herpesvirus DNA replication. The major DNA-binding protein of HSV has been shown to be associated in a protein-protein interaction with HSV DNA polymerase and alkaline nuclease, and it is believed that the three peptides interact as part of the HSV DNA replication complex (47). Precipitation of two EBV-specific proteins by the monoclonal antibody against the HSV DNA-binding protein suggests that a similar complex may exist in EBV. Quinn and McGeoch have identified the BALF2 sequence of EBV as coding for a protein equivalent to the HSV DNA-binding protein (38). The predicted size of the putative protein is 123K. Neither of the two proteins we immunoprecipitated were identical in size to this, but the differences may perhaps be a function of the gel system we used.

Sequence comparisons have shown that EBV and VZV code for proteins which have significant homology with the two proteins of 140K and 38K coded for by HSV-1 and HSV-2 which are associated with ribonucleotide reductase activity (2, 15). Reaction of rabbit anti-EBV antibody with the Western-blotted HSV-2 reductase molecule (Fig. 3) indicates that this sequence homology is also reflected in the conservation of antigenic determinants. Although no similar DNA homologies between HSV and CMV have yet been reported, the reaction of anti-CMV antibody with the HSV-2 reductase protein (Fig. 6) demonstrates that CMV also has a counterpart to the HSV reductase. Since reductase activity in the herpesviruses is believed to play an important role in viral DNA synthesis (15), the conservation of this molecule in the five human herpesviruses makes it a potential target for a common antiviral agent.

The sequence of glycoprotein gB of HSV-1 shows extensive homology with an open reading frame in the EBV genome, designated BALF4. The immunoprecipitation of HSV gB by rabbit anti-EBV antibody and its reaction with Western-blotted HSV gB demonstrate the antigenic relatedness between HSV gB and an EBV counterpart. HSV gB was also precipitated by a monoclonal antibody (E8B3) (7) to EBV p105, which suggests that p105 may be such an EBV counterpart. This conclusion is further strengthened by the ability of both monoclonal and monospecific antibodies to HSV gB to immunoprecipitate p105. However, unlike the RNA transcribed from the BALF4 open reading frame (2), synthesis of p105 is insensitive to phosphonoacetic acid (7). This raises the interesting possibility that p105 is an antigenic counterpart of gB that is distinct from the gene product of BALF4. The immunoprecipitation of HSV gB-like molecules by anti-CMV antibody and reactivity of anti-CMV antibody with Western-blotted HSV gB indicate that CMV also has a gB counterpart. This conclusion is further strengthened by the reciprocal precipitation of CMV-specific peptides of 130K, 64K, and 51K by monospecific antibody to HSV gB. CMV peptides of a similar mobility are also immunoprecipitated by anti-EBV antibody. The data presented here and elsewhere (16) thus indicate that gB is conserved in all the human herpesviruses. Glycoprotein gB of HSV-1 is believed to be essential to penetration of virus particles (43); conservation of a structurally similar protein among all of the human herpesviruses suggests that it may be playing a similar pivotal role in each.

Glycoprotein gG of HSV-1 is coded by the U_s4 gene in the unique short (U_s) segment of the genome, and the gene has been sequenced (32). Although gG of HSV-2 maps in the same region of the HSV-2 U_s segment (28, 42), the relationship between the two proteins is not yet clear. Available evidence suggests that they differ in a number of respects. First, the molecular weights of the mature forms of these glycoproteins vary. Thus, HSV-1 gG is a 59K protein (40), while proteins of ca. 94K, 108K, and 120K have been reported for HSV-2 gG (6, 28, 42). The in vitro translation product of HSV-1 gG is about 44K (40), and the unglycosylated precursor of HSV-2 gG is about 110K (6). These size differences in the primary products probably indicate that the genes coding for these proteins differ in the lengths of their reading frames. Second, the HSV-2 gG is synthesized from a partially glycosylated precursor of 120K by a proteolytic cleavage event via a 72K intermediate (6), while HSV-1 gG is synthesized from its precursor molecule by the stepwise addition of sugars (40). Third, seroepidemiologic studies indicate that gG of HSV-2 carries predominantly type-specific antigenic determinants (25, 48). Although the

rabbit anti-HSV-2 gG did not react with any HSV-1 proteins, it immunoprecipitated EBV-specific peptides. Moreover, both anti-EBV and anti-CMV antibodies reacted with the Western-blotted HSV-2 gG, indicating the presence of antigenic counterparts to HSV-2 gG in EBV and CMV. These data suggest that human herpesviruses may possess some antigenic determinants common to all members and in addition may contain other determinants that are shared by some, but not all, of the viruses. This notion is strengthened by the reactivity of some EBV-specific peptides with anti-CMV antibody but not with anti-HSV antibody (Fig. 7) and by the sequence homology of glycoprotein gC of HSV-1 with EBV sequences (BLLF1 and BDLF3) in regions not homologous with HSV-2 gC (unpublished observations made with a program of Lipman and Pearson [26]).

Immunoprecipitation of an EBV-specific peptide of 85K by both anti-HSV and anti-CMV antibodies indicates that the 85K protein contains conserved determinants. The HSV and CMV protein analogs that are responsible for this cross-reactivity have not yet been characterized. We are currently raising monospecific antibodies against the affinity-purified EBV 85K protein for this purpose. The reactivity of anti-HSV antibodies with a number of CMV peptides and the reciprocal reactivity of CMV antibodies with HSV peptides also show that HSV and CMV have a number of common determinants. The reactivity of anti-CMV antibody with Western-blotted HSV-2 protein identified some of the cross-reactive molecules. Major portions of the CMV genome have recently been sequenced, and sequence comparisons reveal homologies with a number of HSV and EBV glycoproteins and nonglycoproteins (T. Kouzarides, personal communication) which are supportive of our findings.

HSV-1 also codes for a glycoprotein, designated gH, which is believed to play a role in the egress of virus from infected cells (9). The gene coding for gH has been sequenced and shows homology with both VZV and EBV genes (31). Although the VZV and EBV protein counterparts to gH have not yet been characterized, such sequence homologies support our findings of additional antigenic cross-reactivity between these viruses. Glycoproteins gB, gC, gD, and gE of HSV are antigenically distinct proteins (49), and the sequences of known HSV-1 glycoproteins that are currently available do not show any significant homologies to each other (unpublished observations). However, the newly identified putative gene product of U_s7 of HSV-1 is homologous with HSV-2 gD and not with HSV-1 gD. In addition, the BLLF1 and BDLF3 sequences of EBV also have some degree of homology with each other (unpublished observations). This suggests the possibility that both gene duplication and gene diversion have resulted in the conservation of certain essential sequences in different proteins of these viruses.

Infection with each of the five herpesviruses is accompanied by seroconversion and, potentially, with life-long persistence of virus (1, 18, 34, 39). The frequency of clinically apparent reactivation in immunosuppressed patients suggests that the immune system plays an important role in the control of recurrent disease (1, 18, 34, 39). The effect of an immune response to one herpesvirus on infection with any one of the others is known only for the closely related HSV-1 and HSV-2, which induce reciprocal partial cross-protection. Primary infection with HSV-2 is less severe in people previously infected with HSV-1 (39), and even more impressively, mice immunized with HSV-1 genes introduced in the vaccinia virus genome have been protected against acute and latent infection with both HSV-1 and HSV-2 (10). There is thus precedent to suppose that hyperimmunization with cross-reactive antigens may prevent primary infections with more than one virus. The data presented in this paper show that HSV, EBV, and CMV have a number of common antigenic determinants, some of which are present in the virion envelope and in the infected cell membrane. Neutralization of HSV-1 and HSV-2 in the absence of complement by rabbit anti-CMV and anti-EBV antibodies demonstrates that a response to the membrane-associated antigens of one of the herpesviruses can result in generation of a response to other members of the group. Further identification and characterization of cross-reactive antigens of the five human herpesviruses and analysis of the in vivo immune response to them will determine the feasibility of vaccination with common immunogens to modify diseases induced not merely by one, but by several members of the group.

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