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Herpes Simplex Virus Glycoproteins: Participation of Individual Herpes Simplex Virus Type 1 Glycoprotein Antigens in Immunocytolysis and Their Correlation with Previously Identified Glycopolypeptides

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Tissue culture cells infected with herpes simplex type 1 virus express virus-specified glycoprotein antigens on the plasma membrane. Three of these have been previously identified and have been designated as Ag-11, Ag-8, and Ag-6. In the present study, immunoglobulins to each of the antigens were shown to be capable of mediating immunocytolysis in the presence of either complement (antibody-dependent complement-mediated cytotoxicity) or peripheral blood mononuclear cells (antibody-dependent cell-mediated cytotoxicity [ADCC]). Two herpes simplex virus type 1 strains, VR-3 and F, reacted similarly in the ADCC test in the presence of immunoglobulins to Ag-11, Ag-8, and Ag-6 in both infected Chang liver cells and HEp-2 cells. Anti-Ag-6, however, produced a lower ADCC reaction in HEp-2 cells than in Chang liver cells, suggesting differences in the Ag-6 surface expression in, or release from, these cells. Chang liver and HEp-2 cells infected with the MP mutant strain of herpes simplex virus type 1 showed reduced ADCC in the presence of anti-Ag-11 and anti-Ag-8, but no reactivity at all with anti-Ag-6. Crossed immunoelectrophoretic analysis showed that MP-infected cell extracts contain Ag-11 and Ag-8, but lack Ag-6. Polypeptide analysis of herpes simplex virus type 1 strains F, VR-3, and MP showed that Ag-11 consists of the glycoproteins gA and gB, that Ag-8 consists of gD, and that Ag-6 consists of gC. In conclusion, the present study demonstrates that either one of the glycoproteins (gC, gD, and a mixture of gA and gB) can function as a target for immunocytolysis and that the antibody preparation to gC (Ag-6) does not cross-react with any of the other glycoproteins.

Herpes simplex virus type 1 (HSV-1) induces the incorporation of viral proteins into the membranes of infected cells (6, 21). Insertion of the viral proteins in the plasma membrane imparts a new antigenic specificity to the infected cells, as demonstrated by various immunological assays. These have included lysis of cells with antibody and complement (antibody-dependent complement-mediated cytotoxicity [AbC]) and lysis by antibody in the presence of peripheral blood leukocytes (K-lymphocytes, polymorphonuclear leukocytes, or monocytes), a process termed antibody-dependent cell-mediated cytotoxicity (ADCC) (8, 14, 15, 19). With the aid of the crossed immunoelectrophoretic technique, we have demonstrated previously that HSV-1-infected cells contain three major membrane-bound glycosylated antigens, designated as Ag-11, Ag-8, and Ag-6 (10, 24). When injected into rabbits, each of these antigens produced antibody which reacted only with the respective antigen when tested by the crossed immunoelectrophoretic technique, employing an intermediate gel (1, 25).

In the present paper we report that: (i) each of the glycoprotein antigens Ag-11, Ag-8, and Ag-6 reacts as a target for immunocytolysis when rabbit sera reacting to each of the three antigens are employed in either the complement-dependent or the cell-dependent cytolytic assay; (ii) Ag-11, Ag-8, and Ag-6 consist of the glycopolypeptides gA plus gB, gD, and gC, respectively; (iii) the antibody preparation to Ag-6 (gC) only reacts to gC, as demonstrated by the lack of reactivity to cells infected with HSV-1 strain MP which lacks gC.

MATERIALS AND METHODS

Virus strains and cells. HSV-1 strains F and 17 (2, 22) were propagated in HEp-2 cells; HSV-1 strain

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VR-3 was propagated in Chang liver (CL) cells in Eagle minimum essential medium (MEM) with 2% fetal calf serum (FCS) (maintenance medium). The mutant HSV-1 (MP) previously shown to lack one major glycopolypeptide, gC (5, 20), and used primarily to demonstrate the specificity of the reagents, was also propagated in HEp-2 cells.

Infection of cells. (i) For ADCC and AbC tests, HEp-2 or CL cells were infected with HSV-1 as follows. Monolayers in 25-cm^2 plastic flasks were infected at a multiplicity of infection of 1 to 2 PFU of VR-3 per cell, 5 to 10 PFU of F per cell, and 20 to 30 PFU of MP per cell. After 1 h of absorption at 37°C, the monolayer was washed twice in phosphate-buffered saline, and replenished with 10 ml of maintenance medium. (ii) For antigen production and polypeptide analysis, 150-cm^2 monolayers of rabbit kidney (RK), CL, or HEp-2 cells were exposed to either VR-3, F, MP, or 17 at 10 to 20 PFU/cell. After 1 h of adsorption, the monolayer cultures were overlaid with 50 ml of maintenance medium.

Labeling of cells with radioisotopes. The maintenance medium was replaced at 9 h postinfection by medium containing 1/10 the normal amount of leucine, isoleucine, and valine, but supplemented with 5 μCi of each of the amino acids [*U*-^{14}C]leucine, [*U*-^{14}C]isoleucine, and [*U*-^{14}C]valine per ml of maintenance medium (300 mCi/mmol for each amino acid; New England Nuclear Corp., Boston, Mass.). The cells were processed at 10 h postinfection. For labeling with glucosamine, the maintenance medium was replaced at 9 h postinfection with medium containing 1/10 the normal concentration of glucose, but supplemented with 1 μCi of D-[1-^{14}C]glucosamine per ml of medium (45 to 55 mCi/mmol; New England Nuclear Corp.). The cells were processed at 20 h postinfection.

Preparation of viral protein and polypeptides. (i) Triton X-100 solubilization. For Triton X-100 solubilization, HSV-1-infected cells were scraped off the flasks, washed once in Hanks balanced salt solution, and suspended in 4 volumes of 5% Triton X-100 in 0.020 M glycine–0.0076 M Tris buffer, pH 8.6. The suspension was disrupted by sonication and centrifuged as described elsewhere (10).

(ii) SDS-mercaptoethanol solubilization. For sodium dodecyl sulfate (SDS)-mercaptoethanol solubilization, monolayers of infected cells were washed once in Hanks balanced salt solution and disrupted in 2% (wt/vol) SDS–5% (vol/vol) 2-mercaptoethanol, as reported by Morse et al. (9).

Antibodies. Polyspecific antibodies were produced by inoculation of rabbits with HSV-1 (F)-infected rabbit cornea cells disrupted in distilled water (25). Antibodies to Ag-11, Ag-8, and Ag-6 were obtained after immunization of rabbits with individual immunoprecipitates, obtained from lysates of HSV-1 (VR-3)-infected RK cells, as described elsewhere (25). The globulin fractions of the sera were obtained by ammonium sulfate precipitation, followed by serial dialysis against water, 0.05 M acetate buffer (pH 5.0), and phosphate-buffered salt solution, pH 7.2 (3). All purified globulin fractions were absorbed with uninfected HEp-2 or CL cells as follows. Globulin aliquots (1 ml each) were absorbed three times for 1 h at 37°C with 5×10^7 cells. All serum globulins were heat inactivated at 56°C for 30 min before use in the cytotoxicity assays.

Crossed immunoelectrophoresis. Crossed immunoelectrophoresis, including the intermediate gel procedure, has been detailed elsewhere (10, 25). Briefly, 30 μl of Triton X-100-solubilized antigens from HSV-1 (F)- or HSV-1 (MP)-infected cells were electrophoresed in a 1% (wt/vol) agarose gel (Indubiose, Vitry-sur-Seine, France) in 0.18 M Tris–0.06 M barbital buffer, pH 8.6. First-dimension electrophoresis was performed at 5 V/cm for 1.5 h. For second-dimension electrophoresis, the agarose gel contained 15 μl of polyspecific rabbit immunoglobulin per cm^2 (30 to 40 mg of protein per ml). The intermediate gel contained either preimmune rabbit globulin or monospecific immunoglobulin at a concentration of 10 μl/cm^2. The second electrophoresis was performed at 2 V/cm for 18 h. According to the experiment, individual immunoprecipitates were either (i) examined only after staining, (ii) cut out for antibody preparation, or (iii) extracted and solubilized for polypeptide analysis (11).

SDS-polyacrylamide gel electrophoresis. HSV-1-infected cell extracts and solubilized immunoprecipitates were treated with 2% (wt/vol) SDS–5% (vol/vol) 2-mercaptoethanol and boiled for 1 min. Electrophoresis was performed in a 9.25% (wt/vol) acrylamide separation gel cross-linked with 0.24% (wt/vol) *N,N*-diallyltartardiamide (Bio-Rad Laboratories, Richmond, Calif.). The stacking gel was 3.0% (wt/vol) acrylamide–0.08% (wt/vol). *N,N*-diallyltartardiamide. Buffer conditions were as specified previously (9). Autoradiographs of the dried gels were obtained on Cronex X-ray film (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.).

AbC. HSV (VR-3)-infected CL cells were suspended with 0.25% trypsin–0.05% EDTA, washed three times in MEM containing 2% heat-inactivated FCS (I-FCS), and labeled in suspension at 18 h postinfection with 50 to 100 μCi of ^{51}Cr (sodium chromate, 200 to 500 Ci/g of chromium; New England Nuclear Corp.) for 1 h. After labeling, the cells were washed through a cushion of 4 ml of I-FCS and resuspended in MEM with 10% I-FCS. The AbC assay was performed in a total volume of 0.3 ml. One-tenth of the target cells (7×10^3) was incubated with 0.1 ml of various antibody dilutions and 0.1 ml of undiluted guinea pig complement. The complement source was a pool of fresh guinea pig sera. After incubation for 2 h at 37°C, 0.7 ml of MEM with 10% I-FCS was added. The ^{51}Cr release was measured in triplicate samples in a gamma counter, as previously described (19).

ADCC. The ADCC test, detailed elsewhere (8, 14, 19), was done as follows. CL or HEp-2 cells infected with VR-3, F, or MP for 18 h were trypsinized and labeled in suspension with 50 to 100 μCi of ^{51}Cr for 1 h. The target cells were washed as described for the AbC assay and resuspended in MEM with 10% I-FCS to a concentration of 5×10^4 cells per ml. The mononuclear effector cells were prepared from the peripheral blood of healthy HSV antibody-positive or -negative human donors by the Ficoll-Hypaque density centrifugation technique (4). The cells were washed three times in Hanks balanced salt solution and resuspended in MEM with 10% I-FCS at a concentration of 1.5×10^6 cells per ml. The assay was performed in a

1.0-ml volume with 0.4 ml of target cells, 0.4 ml of effector cells, and 0.2 ml of rabbit antibodies diluted as specified in the figure legends; the effector/target cell ratio was 30:1. After 6 h of incubation at 37°C, the specific ^{51}Cr release was measured in triplicate samples, as previously described (19).

Calculations. For AbC, percent specific ^{51}Cr release was calculated as the percent release in the presence of rabbit immunoglobulin and complement minus the percent release in the presence of preimmunoglobulin and complement. For ADCC, percent specific ^{51}Cr release was calculated as the percent release in the presence of immunoglobulin and mononuclear effector cells minus the percent release in the presence of pre-immunoglobulin and mononuclear effector cells. The release from target cells alone into the medium was between 9 and 11% for the 2-h incubation time used in the AbC measurements and between 7 and 14% for the 6-h incubation period used in the ADCC assay. In the latter assay, the release obtained after the addition of effector cells alone never exceeded 21%. The maximal cell-associated isotope release was 85%. With either the AbC or the ADCC assay, antibody titers were defined as the reciprocal of the dilution yielding half of the maximal specific release (23).

RESULTS

Surface expression of individual viral glycoprotein antigens as measured by immunocytolysis. Previous experiments with either AbC or ADCC assays utilized polyspecific antibodies to HSV-1 obtained by immunization of rabbits (14) or from human convalescent serum (19). The recent availability of antibodies to three HSV glycoprotein antigens (Ag-11, Ag-8, and Ag-6) has enabled us to measure the cytotoxicity due to the reaction of each of these antigens with the corresponding antibody (26, 27).

(i) Reactivity of individual antigens in the AbC reaction. HSV-1 (VR-3)-infected CL cells were labeled with ^{51}Cr at 18 h postinfection. The ^{51}Cr-labeled target cells were reacted with polyspecific immunoglobulin, as well as with anti-Ag-11, Ag-8, and Ag-6 rabbit immunoglobulins diluted from 1:30 to 1:1,000. All of the immunoglobulin preparations initiated immunocytolysis, giving a maximal percent specific release ranging from 30 to 65% (Fig. 1). This finding indicates that all three antigens are exposed at the surface of the cells and are able to bind antibody and complement. The complement was used in a final concentration of 1:3, as higher dilutions were found to give a lower specific ^{51}Cr release (data not shown).

(ii) Reactivity of individual antigens in the ADCC reaction. Previous studies with human sera have suggested that the ADCC assay is more sensitive than the AbC method for the detection of small amounts of antibodies (19, 23). To confirm participation of all three antigens in the ADCC reaction and to compare the difference in sensitivity between the two methods, we diluted the various immunoglobulins from 1:1,000 to 1:300,000 and reacted them with target HSV-1 (VR-3)-infected CL cells labeled with ^{51}Cr at 18 h postinfection (Fig. 2). The results show that all of the anti-HSV sera tested in these assays mediated cytolysis and that the ADCC assay was more than 100-fold more sensitive in detecting HSV antibodies than was the AbC method (Table 1). Therefore, in all subsequent experiments, only the ADCC reaction was used.

(iii) Possible influence of host cells on the surface expression of an individual anti-

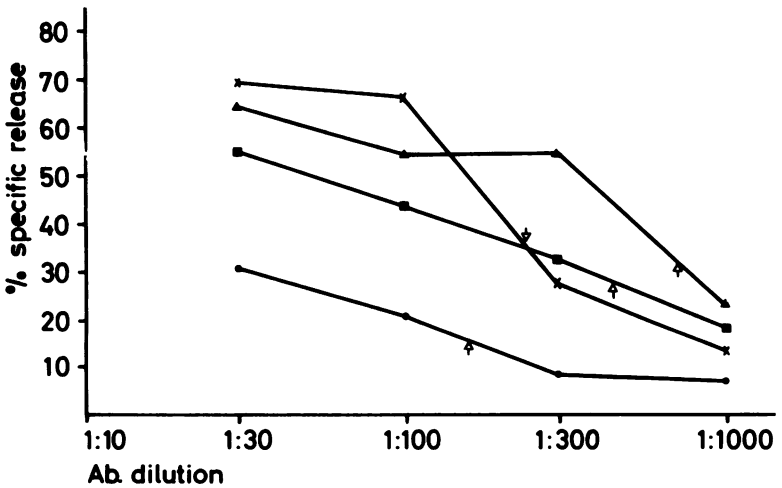

FIG. 1. *Titration of the immunoglobulin preparations in the AbC assay with HSV-1 (VR-3)-infected CL cells. Symbols: ×, polyspecific anti-HSV-1; ●, anti-Ag-11; ■, anti-Ag-8; ▲, anti-Ag-6. Arrows represent antibody titers of the various immunoglobulins.*

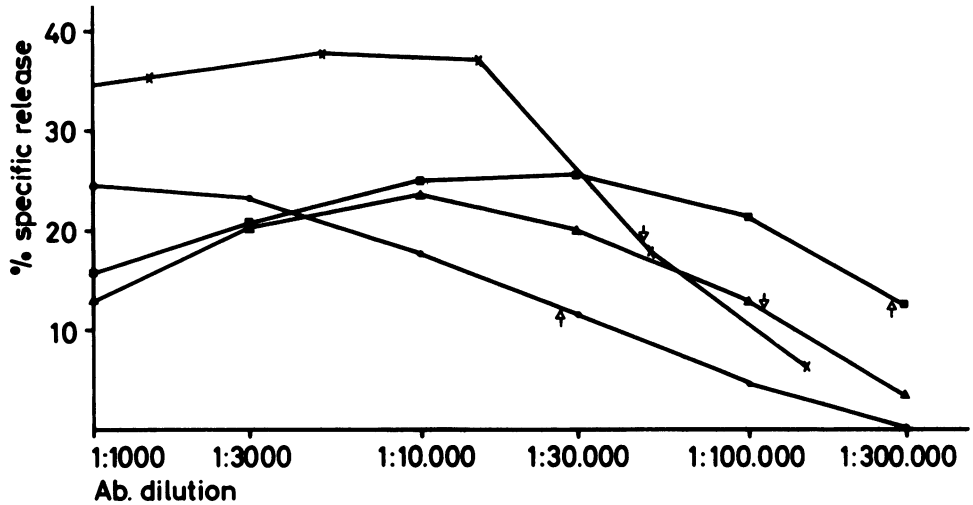

FIG. 2. *Titration of the immunoglobulin preparations in the ADCC assay with HSV-1 (VR-3)-infected CL cells. Symbols: ×, polyspecific anti-HSV-1; ●, Anti-Ag-11; ■, anti-Ag-8; ▲, anti-Ag-6. Arrows represent antibody titers of the various immunoglobulins.*

TABLE 1. *Comparison of antibody titers in the AbC and ADCC assays*

Immunoglobulin	Antibody titer		Ratio of ADCC/AbC titers
	AbC	ADCC	
Anti-HSV-1	240	47,000	195
Anti-Ag-11	160	27,000	168
Anti-Ag-8	440	270,000	613
Anti-Ag-6	700	110,000	157

gen. Experiments were done to ascertain whether the host cell might influence surface viral antigen expression. This was relevant as most of our previous studies with immunocytolytic assays were performed in CL cells infected with HSV-1 (VR-3) (8, 14, 19), whereas most of the information regarding HSV-1 polypeptides and antigens has been obtained in HEp-2 cells infected with other strains of HSV-1 (5, 11). The immunocytolysis obtained with HEp-2 cells infected with HSV-1 (VR-3) or (F) was compared with that measured with CL cells infected with the same strains. Table 2 gives the maximum percent specific release for each of the immunoglobulins. In both types of cells infected with either HSV-1 (VR-3) or (F), similar percent specific releases were obtained in the presence of the polyspecific globulin and the anti-Ag-11 and anti-Ag-8 immunoglobulins. In contrast, lower specific ^{51}Cr releases were obtained with anti-Ag-6 globulin when this was employed in the test with HEp-2 target cells, but not when employed with CL cells, infected with either strain F or VR-3.

Antigenic analysis of cells infected with HSV-1 (MP). Inasmuch as a mutant strain of HSV-1, (MP), fails to accumulate glycoprotein gC (20), we investigated antigen reactivity of cells infected with this strain in the ADCC test and by crossed immunoelectrophoresis. The ADCC test was done with both HEp-2 and CL infected cells. The results (Table 3) show that anti-Ag-6 failed to mediate the ADCC reaction with either type of infected cells. It is of interest that polyspecific anti-Ag-11 and anti-Ag-8 immunoglobulins also produced a lower specific ^{51}Cr release in MP-infected cells than in F- or VR-3-infected cells.

TABLE 2. *Comparison of different host cells and HSV-1 strains in ADCC mediated by polyspecific and monospecific immunoglobulins*

Immunoglobulin	Specific release (%)			
	HSV-1 (VR-3)-infected cells		HSV-1 (F)-infected cells	
	HEp-2	CL	HEp-2	CL
Anti-HSV-1	32.0	27.7	29.3	36.7
Anti-Ag-11	27.5	24.5	26.5	34.7
Anti-Ag-8	32.2	25.8	30.5	34.8
Anti-Ag-6	13.4	23.7	12.0	27.8

The lack of reactivity of the anti-Ag-6 immunoglobulin with MP-infected target cells prompted us to study HSV-1 (MP)-infected cells by the crossed immunoelectrophoresis technique to determine whether all three antigens (Ag-11, Ag-8, and Ag-6) are expressed. Analysis of Triton X-100-solubilized antigens by this technique showed that Ag-11 and Ag-8 were

identified by electrophoresis through intermediate gels containing antibodies to either Ag-11 or Ag-8 (data not shown). On the other hand, Ag-6 could not be demonstrated, since the anti-Ag-6 in the intermediate gel did not shift any of the precipitates to a lower position (Fig. 3A and B). It should be noted that an antigen that we designate as Ag-5 is present and that its electrophoretic mobility is similar to that of Ag-6 in HSV-1 (F)-infected cells (Fig. 3C and D). Ag-5 was slightly glycosylated by D-[1-^{14}C]glucosamine under labeling conditions used in the present study (data not shown).

Correlation between viral antigens and glycopolypeptides of HSV-1 (MP)-infected cells. The polypeptides present in the individual Triton X-100-solubilized antigens of HSV-1 (MP)-infected HEp-2 cells were identified with a ^{14}C-amino acid-labeled antigen preparation. Individual immunoprecipitates were cut from the agarose gel after crossed immunoelectrophoresis, as shown with arrows in Fig. 3A, solubilized in SDS and 2-mercaptoethanol, and analyzed on SDS-polyacrylamide gels (Fig. 4). The Ag-11 precipitate contained three polypeptides (Fig. 4, slot d), two of which correspond to the glycopolypeptides gA and gB (Fig. 4, slots b and c) found by other workers (5, 20). The Ag-8 precipitate showed one broad band corresponding to the glycopolypeptide gD_2 and a minor band corresponding to gD_1 (Fig. 4, slots b, c, and e). Only one band, corresponding to the nonglycosylated ICP-5, was demonstrated in Ag-5 (Fig. 4, slots a and f). As a control for Ag-6, an Ag-6 immunoprecipitate was cut from an HSV-1 (F) reference antigen preparation as shown in Fig. 4, slot g. Only one band corresponding to the glycopolypeptide gC could be detected. HSV-1 strains 17 and F have been used as reference strains interchangeably, as no difference with respect to either virus-specified polypeptides or antigens has been demonstrated (9; Norrild, unpublished data). The reference bands for D-[^{14}C]glucosamine-labeled HSV-1 (MP) polypeptides are shown in Fig. 4, slot c, which demonstrates the absence of the glycopolypeptide gC.

DISCUSSION

Viral antigens expressed on the surface of HSV-1-infected cells have been shown to initiate different immunocytolytic reactions in the presence of human, as well as polyspecific rabbit, antibodies to HSV-1 (15, 19). The present study has extended these observations by analyzing the ability of individual glycoprotein antigens (Ag-11, Ag-8, and Ag-6) to initiate immunocytolysis. Two different immunocytolytic mechanisms were used: AbC and ADCC. The antibody directed to the Ag-11, Ag-8, and Ag-6 preparations mediated immunocytolysis in both assays, thus demonstrating the surface expression of the three antigens late in the infectious cycle and the ability of each of the viral antigens to participate independently as a target for the cytolytic reactions (Fig. 1 and 2). Titration of the immunoglobulins by both methods showed that the sensitivity of the ADCC test for the detection of antibodies was more than 100-fold that of the AbC reaction (Table 1), leading us to use this method for later studies. The interaction between the membrane antigens and the antibodies to Ag-11, Ag-8, and Ag-6 is in agreement with previous findings demonstrating the neutralizing capacity of these antibodies (27) and also confirms earlier findings demonstrating the presence of identical viral antigenic determinants at the surface of the plasma membrane of HSV-1-infected cells and at the surface of the viral envelope (15, 16). The ability of individual viral glycoprotein antigens to participate in the AbC and ADCC cytolytic reactions was demonstrated with two wild-type HSV-1 strains, VR-3 and F. No difference was noted with the two types of HSV-1 when the same host cell line was used, but the expressions of Ag-11, Ag-8, and Ag-6 at the surface of infected cells appeared to be host cell dependent when tested in the ADCC assay (Table 2). Thus, the specific release obtained with HSV-1-infected HEp-2 or CL cells was similar for the polyspecific, anti-Ag-11 and anti-Ag-8 antibodies. The reactivity of anti-Ag-6 antibody was lower in HEp-2 cells than in CL cells infected with HSV-1 (VR-3) or (F). The reason for this difference is unknown at present. Three possibilities should be considered. First, the expression of Ag-6 at the surface of infected cells varies depending on the host cell used. Second, the plasma membrane of tissue culture cells varies in intrinsic properties, as recently reported by Schlehofer et al., who observed different permeabilities of HSV-1-infected cells to ^{51}Cr (18). Third, the possibility also exists that the OOviral antigen Ag-6 is preferentially shed from the surface of HEp-2 cells into the tissue culture medium, as previously suggested by several

TABLE 3. *ADCC mediated by polyspecific and monospecific immunoglobulins, using different host cells infected with the HSV-1 (MP) strain*

Immunoglobulin	Specific release (%)	
	HEp-2 cells	CL cells
Anti-HSV-1	14.6	7.7
Anti-Ag-11	13.9	3.5
Anti-Ag-8	19.4	7.1
Anti-Ag-6	−2.8	−2.6

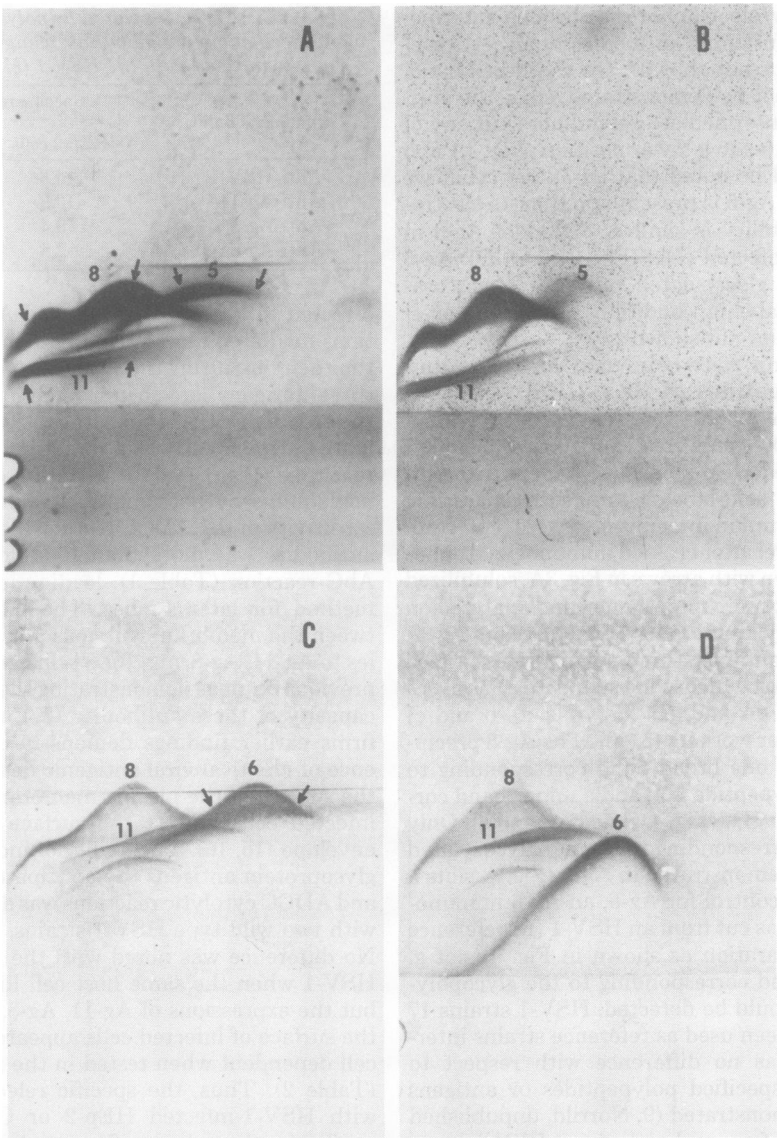

FIG. 3. *Crossed immunoelectrophoresis of Triton X-100-solubilized antigen preparations extracted from HSV-1 (MP)-infected HEp-2 cells (A and B) or HSV-1 (F)-infected cells (C and D). Antigen (30 μl) was applied for the first-dimension electrophoresis in all cases. The second-dimension electrophoresis was performed in anti-HSV-1 containing agarose (15 μl/cm²). The intermediate gels contained 15 μl of either normal rabbit globulin (A and C) or anti-Ag-6 (B and D) per cm². The arrows indicate how precipitates were cut when SDS-polyacrylamide gel electrophoresis was performed.*

workers (7, 13). A selective loss of Ag-6 would be observed as less reactivity of anti-Ag-6 serum to the surface of infected cells.

By lactoperoxidase-catalyzed iodination of intact infected cells, we have shown previously that Ag-6 of HSV-1 (F)-infected HEp-2 cells could be detected only late in the infectious cycle (10), whereas recent observations with CL cells infected with HSV-1 (VR-3) demonstrated Ag-6 at the cell surface at 8 h postinfection (Norrild, unpublished observations). Although a different virus was used, these findings also support host cell-dependent differences in the exposure of viral antigens at the surface of infected cells.

The second major observation described in the present paper allows a correlation of the antigenic specificities of Ag-11, Ag-8, and Ag-6

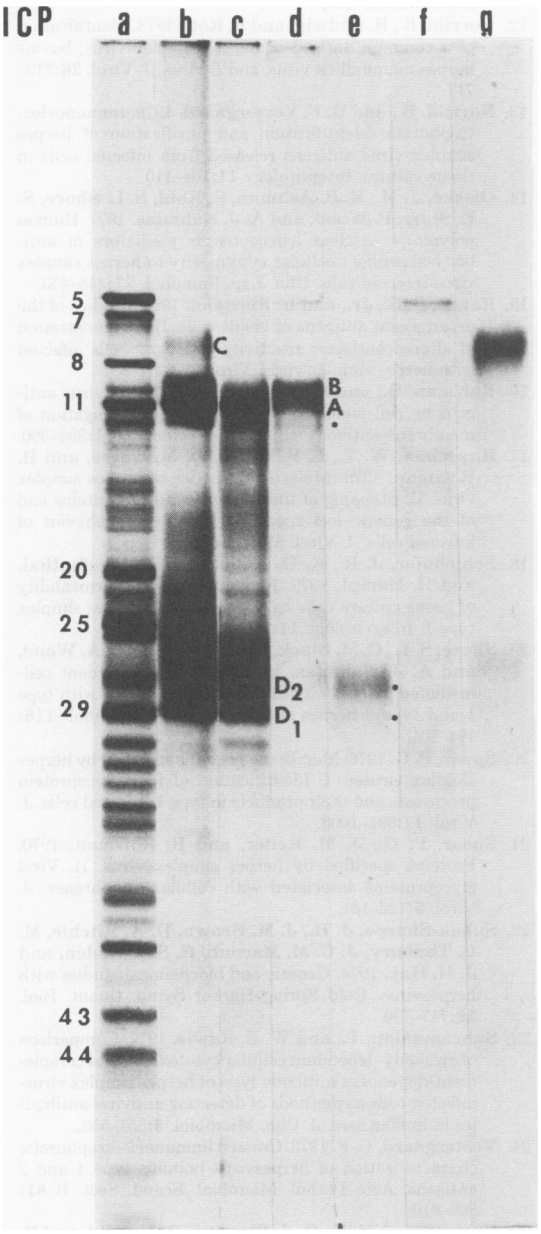

FIG. 4. *SDS-polyacrylamide gel electrophoresis in a 9.25% (wt/vol) separation gel cross-linked with 0.24% (wt/vol) N,N-diallyltartardiamide. (Slot a) HSV-1 (MP) ^{14}C-amino acid labeled and solubilized in disruption buffer; (slot b) HSV-1 (17) D-[1-^{14}C]-glucosamine labeled and solubilized in disruption buffer; (slot c) HSV-1 (MP) D-[1-^{14}C]glucosamine labeled and solubilized in disruption buffer; (slots d, e, and f) Ag-11, Ag-8, and Ag-5 of ^{14}C-amino acid-labeled HSV-1 (MP) solubilized from immunoprecipitates; (slot g) Ag-6 of ^{14}C-amino acid-labeled HSV-1 (F) solubilized from immunoprecipitates.*

to the glycoproteins described by other workers (17, 20). In addition, a new antigenic determinant not previously described was identified and designated as Ag-5. The polypeptides of antigens Ag-11, Ag-8, and Ag-5 were identified from ^{14}C-amino acid-labeled extracts of HSV-1 (MP)-infected cells. As Ag-5 in crossed immunoelectrophoresis showed an electrophoretic mobility similar to that of Ag-6 from extracts of HSV-1 (F)-infected cells (Fig. 3A and C), the polypeptides of Ag-6 were included as a reference (Fig. 4, slot g). The polypeptide analysis of Ag-11 identified three polypeptides (Fig. 4, slot d), of which the two with the highest molecular weight corresponded to glycoproteins gA and gB of HSV-1 demonstrated by other workers (Fig. 4, slots b, c, and d). This antigen has previously been demonstrated to be in common with HSV-1, HSV-2, B-virus, and bovine herpes mammillitis virus. Although the Ag-11 immunocomplex consists of different polypeptides when isolated from extracts of cells infected with these different viruses, one glycoprotein was in common with the Ag-11 of the four viruses, thus indicating that the major antigenic determinants were probably carried on this glycoprotein (12). The polypeptides of Ag-8 of HSV-1 (MP) showed several bands. A minor band corresponds to gD_1, and the broad band observed above gD_1 corresponds to gD_2 of Spear (20) (Fig. 4, slots c and e). Inasmuch as the different bands of ^{14}C-amino acid-labeled Ag-8 all correspond to various forms of gD, it might be concluded that the antigenic determinant sites are specified within gD. The polypeptide analysis of Ag-5 of HSV-1 (MP) demonstrated a significant difference from that of Ag-6 of HSV-1 (F) (Fig. 4, slots f and g). Thus, Ag-5 predominantly consists of ICP-5, which is identified as the major capsid polypeptide (5). The observation that Ag-5 is labeled by [^{14}C]glucosamine under certain conditions is most likely because glucosamine labeling was done in medium with 1/10 of the normal concentration of glucose. Reduced glucose allows labeling of non-glycosylated proteins, and a 1/10 concentration of glucose was near the critical value where additional proteins showed label (Spear, personal communication). As the present study also identified the major polypeptide of Ag-6 as gC (Fig. 4, slots b and g), the correlation of Ag-11, Ag-8, and Ag-6 with the major glycoproteins gA, gB, gC, and gD can be summarized as follows: Ag-11 consists of gA and gB, Ag-8 consists of gD, and Ag-6 consists of gC.

Regarding the specificity of the antibody preparations employed in the present study, we can conclude that the Ag-6 (gC) antibody reacts only to gC (26). This is based on the observation that

Ag-6 antibody did not react to HSV-1 (MP)-infected cells when employed in the ADCC reaction. As the test is very sensitive to the concentration of antibody, as discussed above, minor amounts of antibody directed to other viral antigenic specificities would be detected. The anti-Ag-11 and anti-Ag-8 antibodies cannot be tested for mono-specificity at present inasmuch as no mutants which lack either gA plus gB or gD are available.

In conclusion, the present paper demonstrates that gA, gB, gC, and gD carry different antigenic specificities and that either one of them can participate as a target for immunocytolytic reactions in the presence of either complement or mononuclear leukocytes from human peripheral blood.

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LITERATURE CITED

1. **Axelsen, N. H.** 1973. Intermediate gel in crossed and fused rocket immunoelectrophoresis. Scand. J. Immunol. **2**(Suppl. 1):71-77.
2. **Ejercito, P. M., E. D. Kieff, and B. Roizman.** 1968. Characterization of herpes simplex virus strains differing in their effects on social behavior of infected cells. J. Gen. Virol. **2**:357-364.
3. **Harboe, N., and A. Ingild.** 1973. Immunization, isolation of immunoglobulins, estimation of antibody titers. Scand. J. Immunol. **2**(Suppl. 1):161-164.
4. **Harris, R., and E. O. Ukaejiofo.** 1969. Rapid preparation of lymphocytes for tissue typing. Lancet **ii**:327.
5. **Heine, J. W., R. W. Honess, E. Cassai, and B. Roizman.** 1974. Proteins specified by herpes simplex virus. XII. The virion polypeptides of type 1 strains. J. Virol. **14**:640-651.
6. **Heine, J. W., P. G. Spear, and B. Roizman.** 1972. Proteins specified by herpes simplex virus. VI. Viral proteins in the plasma membrane. J. Virol. **9**:431-439.
7. **Kaplan, A. S., J. S. Erickson, and T. Ben-Porat.** 1975. Synthesis of proteins in cells infected with herpesvirus. X. Proteins excreted by cells infected with herpes simplex virus, types 1 and 2. Virology **64**:132-143.
8. **Kohl, S., S. E. Starr, J. M. Oleske, S. L. Shore, R. B. Ashman, and A. J. Nahmias.** 1977. Human monocyte-macrophage-mediated antibody-dependent cytotoxicity to herpes simplex virus-infected cells. J. Immunol. **118**:729-735.
9. **Morse, L. S., L. Pereira, B. Roizman, and P. A. Schaffer.** 1978. Anatomy of herpes simplex virus (HSV) DNA. X. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 × HSV-2 recombinants. J. Virol. **26**:389-410.
10. **Norrild, B., O. J. Bjerrum, H. Ludwig, and B. F. Vestergaard.** 1978. Analysis of herpes simplex virus type 1 antigens exposed on the surface of infected tissue culture cells. Virology **87**:307-316.
11. **Norrild, B., O. J. Bjerrum, and B. F. Vestergaard.** 1977. Polypeptide analysis of individual immunoprecipitates from crossed immunoelectrophoresis. Anal. Biochem. **81**:432-441.
12. **Norrild, B., H. Ludwig, and R. Rott.** 1978. Identification of a common antigen of herpes simplex virus, bovine herpes mammillitis virus, and B virus. J. Virol. **26**:712-717.
13. **Norrild, B., and B. F. Vestergaard.** 1979. Immunoelectrophoretic identification and purification of herpes simplex virus antigens released from infected cells in tissue culture. Intervirology **11**:104-110.
14. **Oleske, J. M., R. B. Ashman, S. Kohl, S. L. Shore, S. E. Starr, P. Wood, and A. J. Nahmias.** 1977. Human polymorphonuclear leucocytes as mediators of antibody-dependent cellular cytotoxicity to herpes simplex virus-infected cells. Clin. Exp. Immunol. **27**:446-453.
15. **Roane, P. R., Jr., and B. Roizman.** 1964. Studies of the determinant antigens of viable cells. II. Demonstration of altered antigenic reactivity of HEp-2 cells infected with herpes simplex virus. Virology **22**:1-8.
16. **Roizman, B., and P. G. Spear.** 1971. Herpesvirus antigens on cell membranes detected by centrifugation of membrane-antibody complexes. Science **171**:298-300.
17. **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.
18. **Schlehofer, J. R., K. O. Habermehl, W. Diefenthal, and H. Hampl.** 1979. Reduction of ^{51}Cr-permeability of tissue culture cells by infection with herpes simplex type 1. Intervirology **11**:158-166.
19. **Shore, S. L., C. M. Black, F. M. Melewicz, P. A. Wood, and A. J. Nahmias.** 1976. Antibody-dependent cell-mediated cytotoxicity to target cells infected with type 1 and type 2 herpes simplex virus. J. Immunol. **116**: 194-201.
20. **Spear, P. G.** 1976. Membrane proteins specified by herpes simplex viruses. I. Identification of four glycoprotein precursors and their products in type 1-infected cells. J. Virol. **17**:991-1008.
21. **Spear, P. G., J. M. Keller, and B. Roizman.** 1970. Proteins specified by herpes simplex virus. II. Viral glycoproteins associated with cellular membranes. J. Virol. **5**:123-131.
22. **Subak-Sharpe, J. H., J. M. Brown, D. A. Ritchie, M. C. Timbury, J. C. M. Macnab, H. S. Marsden, and J. H. Hay.** 1974. Genetic and biochemical studies with herpesvirus. Cold Spring Harbor Symp. Quant. Biol. **39**:717-730.
23. **Subramanian, T., and W. E. Rawls.** 1977. Comparison of antibody-dependent cellular cytotoxicity and complement-dependent antibody lysis of herpes simplex virus-infected cells as methods of detecting antiviral antibodies in human sera. J. Clin. Microbiol. **5**:551-558.
24. **Vestergaard, B. F.** 1973. Crossed immunoelectrophoretic characterization of herpesvirus hominis type 1 and 2 antigens. Acta Pathol. Microbiol. Scand. Sect. B **81**: 808-810.
25. **Vestergaard, B. F., O. J. Bjerrum, B. Norrild, and P. C. Grauballe.** 1977. Crossed immunoelectrophoretic studies of the solubility and immunogenicity of herpes simplex virus antigens. J. Virol. **24**:82-90.
26. **Vestergaard, B. F., and B. Norrild.** 1978. Crossed immunoelectrophoresis of a herpes simplex virus type 1-specific antigen: immunological and biochemical characterization. J. Infect. Dis. **138**:639-643.
27. **Vestergaard, B. F., and B. Norrild.** 1979. Crossed immunoelectrophoretic analysis and viral neutralizing activity of five monospecific antisera against five different herpes simplex virus glycoproteins. Boston Symposium 1977. IARC (Int. Agency Res. Cancer) Sci. Publ. **24**: 225-234.