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# Monoclonal Antibodies to Two Glycoproteins of Herpes Simplex Virus Type 2

N. BALACHANDRAN, D. HARNISH, R. A. KILLINGTON,† S. BACCHETTI,* AND W. E. RAWLS

*Department of Pathology, McMaster University, Hamilton, Ontario, Canada L8N 3Z5*

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Monoclonal antibodies to herpes simplex virus type 2 were found to precipitate different numbers of radiolabeled polypeptides from lysates of virus-infected cells. Antibodies directed against two viral glycoproteins were characterized. Antibodies from hybridoma 17αA2 precipitated a 60,000-molecular-weight polypeptide which chased into a 66,000- and a 79,000-molecular-weight polypeptide. All three polypeptides labeled in the presence of [$^3$H]glucosamine and had similar tryptic digest maps. The 60,000-molecular-weight polypeptide also chased into a 31,000-molecular-weight species which did not label with [$^3$H]glucosamine. Antibodies from hybridoma 17βC2 precipitated a 50,000-molecular-weight polypeptide which chased into a 56,000- and an 80,000-molecular-weight polypeptide. These polypeptides also shared a similar tryptic digest map and labeled with [$^3$H]glucosamine. Both monoclonal antibodies were herpes simplex virus type 2 specific. The viral proteins precipitated by 17αA2 antibodies had characteristics similar to those reported for glycoprotein E, whereas the proteins precipitated by 17βC2 antibodies appeared to represent a glycoprotein not previously described. This glycoprotein should be tentatively designated glycoprotein F.

Over 50 polypeptides which appear to be specified by herpes simplex virus type 2 (HSV-2) have been identified by polyacrylamide gel electrophoresis of radiolabeled lysates of infected cells (17, 21). However, the genome of the virus ($10^8$ molecular weight) can theoretically code for considerably more than 50 polypeptides. This large number of polypeptides, the limitations of the accuracy with which they can be identified by migration characteristics in polyacrylamide gels, and the lack of information regarding precursor-product relationships between polypeptides greatly hamper studies of the origin and function of these molecules (8). Demonstration of unique identity and interrelationships between the different polypeptides found in virus-infected cells may be possible through the use of monoclonal antibodies which recognize single antigenic determinants. To this end, we have produced a number of hybridomas secreting antibodies to HSV-2-induced proteins (13). Immunoprecipitation of proteins from virus-infected cells by monoclonal antibodies was found to yield different numbers of polypeptides. We have examined the polypeptides precipitated by monoclonal antibodies secreted by two of the hybridomas and found that the antibodies are directed against type-specific determinants on two different viral glycoproteins.

† Present address: Department of Microbiology, University of Leeds, Leeds, United Kingdom.

## MATERIALS AND METHODS

**Cells and virus.** Vero cells were grown in minimal essential medium (MEM F-15; GIBCO Laboratories), supplemented with antibiotics and heat-inactivated calf serum (10%). The virus used was HSV-2 strain 333 (18).

**Production of hybridomas.** The method of production and culturing of hybridomas and the screening procedures for HSV-specific antibodies were given in detail elsewhere (13). Briefly, BALB/c mice were immunized with Formalin-inactivated lysates of virus-infected L cells, and spleen cells from them were fused with the myeloma cells Sp 2/0 Ag.14 (19). The hybrids were selected in Hy-HAT medium (13), and antibodies secreted were tested by $^{125}$I-protein A assay, indirect immunofluorescence, and enzyme-linked immunosorbent assay. Ascitic fluid with antibody was obtained by injecting hybrid clones intraperitoneally into pristane-treated mice. The nomenclature of the hybridomas used in the present report is as previously described (13), except for the omission of the letter F preceding each name.

**Radiolabeling of cells.** Monolayers of Vero cells were mock infected or infected with HSV-2 at a multiplicity of infection of 20 PFU per cell and incubated with Hanks balanced salt solution. For long-term labeling of infected cell peptides (1 to 16 h) or for short-time pulsing (2-h periods at different times postinfection), the medium contained 5% dialyzed fetal calf serum, 1/10 the normal amount of methionine, and 20 μCi of L-[$^{35}$S]methionine (specific activity, 900 to 1,100 Ci/mmol, New England Nuclear Corp.) per ml. For pulse-chase experiments, cells were labeled at 5 h postinfection for 30 min with 100 μCi of L-[$^{35}$S]methi-

onine per ml and chased for 5 h in medium containing 100× unlabeled methionine and 50 μg of cycloheximide per ml. For glycoprotein labeling, the cells were incubated with 100 μCi of D-[6-$^3$H]glucosamine hydrochloride (specific activity, 19.0 Ci/mmol, New England Nuclear Corp.) per ml for 3 to 16 h in medium with 1/10 the normal concentration of glucose.

**Immunoprecipitation and gel electrophoresis.** The cells were harvested by scraping, washed three times with cold phosphate-buffered saline, and solubilized with RIPA buffer (0.05 M Tris-hydrochloride, 0.15 M NaCl, 0.1% sodium dodecyl sulfate, 1% sodium deoxycholate, 1% Triton X-100, 100 U of Aproteinin per ml, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM Benzimidine-Hydrochloride and 50 μg of DNase per ml) for 5 min at 4°C. The lysate was sonicated and centrifuged at 100,000 × *g* for 1 h. One milliliter of the supernatant was mixed with 10 μl of antibody (ascitic fluid or 10× concentrated culture medium) and 100 μl of Protein-A Sepharose CL-4B beads (Pharmacia Fine Chemicals) (15 mg of beads in RIPA buffer) and kept at 4°C for 2 h with constant mixing. The beads were collected by centrifugation, washed three times with RIPA buffer and solubilized with 100 μl of sample buffer ((0.37 M Tris-hydrochloride pH 6.8, 10% glycerol, 5% 2-mercaptoethanol, 10% sodium dodecyl sulfate) (14). The samples were loaded onto 7.5 to 15% polyacrylamide gradient gels containing 0.8% Bis (Bio-Rad Laboratories) as cross-linker and 0.1% sodium dodecyl sulfate and run at constant voltage for 24 h. The gels were stained with Coomassie blue, destained, infused with 2,5-diphenyloxazole, dried on filter paper, and placed in contact with Kodak X-Omat film at −70°C for fluorography (2). Cross-adsorption between different sera was determined by sequential immunoprecipitation of lysates of infected cells for three times with one antibody, followed by immunoprecipitation of the third supernatant with a second antibody. All immunoprecipitates were then analyzed on sodium dodecyl sulfate gels as described above; in each case, molecular weight markers were electrophoresed in a parallel channel.

**Tryptic peptide analysis.** Tryptic digestion of [$^{35}$S]methionine-labeled polypeptides was performed by the method of Morrison and Lodish (15). HSV-infected Vero cell polypeptides were immunoprecipitated as described above and were resolved on 7.5 to 15% sodium dodecyl sulfate-polyacrylamide gradient gels. Labeled polypeptides were visualized by autoradiography of stained, dried gels, and the regions of the gel containing the labeled polypeptides were excised and placed in 8 ml of 0.2 M ammonium bicarbonate (pH 8.0) containing 50 μg of diphenylcarbamyl chloride-treated bovine trypsin (Sigma Chemical Co.). The gel slices were broken into smaller fragments and incubated at 37°C for 24 h with gentle shaking. Fresh ammonium bicarbonate-trypsin solution (5 ml) was added, and the digestion was continued for a further 24 h. The two digestions were pooled, and a third 24-h digestion in the presence of 50 μg of fresh trypsin was performed. Digested samples were lyophilized and [$^{35}$S]methionine tryptic peptides were analyzed by high-voltage electrophoresis in pyridine-acetic acid-water (1:10:100) (pH 3.5) and chromatography in *n*-butanol-pyridine-acetic acid-water (5:4:1:5) on silica gel, thin-layer sheets (20 by 20 cm, 0.2 mm; Polygram SIL N-HR) as described by Dobos and Rowe (5). Chromatograms were coated with 2,5-diphenyloxazole (20% [wt/vol] acetone), and peptides were visualized by autoradiography on Kodak X-ray film.

## RESULTS

**Polypeptides precipitated by monoclonal antibodies.** Vero cells which were mock infected or infected with HSV-2 and labeled with [$^{35}$S]methionine from 1 to 16 h postinfection were lysed with RIPA buffer. The lysates were reacted with antibodies from different hybridoma lines, and the immunoprecipitable polypeptides were separated on polyacrylamide gels as described above. None of the antibodies tested precipitated detectable polypeptides from lysates of mock-infected cells; neither did preimmune mouse serum react with lysates of mock-infected or infected cells (data not shown). Each antibody, however, precipitated one or more polypeptides from infected cells. Antibodies secreted from 10 different hybridoma lines were used to immunoprecipitate antigens from HSV-2-infected cells, and in all instances but one (17βA3) more than one polypeptide were resolved on gels (Fig. 1). The immunoprecipitation patterns, however, were different from one another, indicating that even in instances in which highly complex patterns were obtained, the antibodies had different specificities.

Several reasons could be invoked to explain the precipitation of multiple polypeptides by individual antibodies. First, the hybridoma cell line (and thus the antibodies) could be polyclonal in origin. Secondly, the antibodies may recognize an antigenic determinant on a single polypeptide which is complexed with other polypeptides in the lysates or alternatively may recognize a common antigenic determinant present on several polypeptides. Finally, the antibody may be directed to a single antigenic site on a polypeptide which, due to posttranslational modification, is present in several forms which differ in migration characteristics. Repeated monocloning of the hybridoma lines by limiting dilutions negated in most cases the polyclonal nature of the lines. To explore which of the remaining possibilities was responsible for the complex patterns, the polypeptides precipitated by antibodies obtained from two hybridoma lines, 17αA2 and 17βC2, were characterized in more detail.

As shown in Fig. 1, 17αA2 antibodies react specifically with three polypeptides with apparent molecular weights of 60,000, 66,000, and 79,000, whereas 17βC2 antibodies immunoprecipitate species with molecular weights of 50,000,

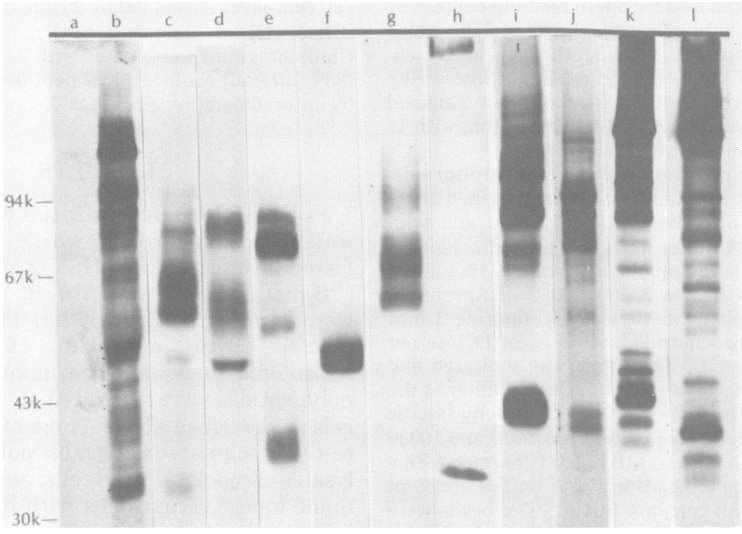


FIG. 1. *Autoradiograms of the electrophoretic analysis of immunoprecipitates obtained with antibodies secreted by different hybridoma lines or with immune mouse serum. Lysates of mock- or HSV-2 infected Vero cells, labeled with [$^{35}$S]methionine 1 to 18 h postinfection were immunoprecipitated with antibodies and electrophoretically separated on sodium dodecyl sulfate-polyacrylamide gels. Lane a, mock-infected cell lysate immunoprecipitated with immune mouse serum; lanes b to l, infected cell lysates immunoprecipitated with immune mouse serum (b) or antibodies from hybridomas 17αA2 (c); 17βC2 (d); 13αA3 (e); 17βA3 (f); 17αD2 (g); 15αD2 (h); 17βA4 (i); 13αA5 (j); 13αC5 (k); or 17αC5 (l).*

56,000, and 80,000. Both hybridoma lines when cloned gave rise to monoclonal lines secreting antibodies with specificities identical to those of the parental lines (Fig. 2). Immunodiffusion analysis, using rabbit antisera to mouse immunoglobulin G subclasses, indicated that the antibodies produced by both hybridoma lines were of the immunoglobulin G 2a type; however, internal labeling of the antibodies with [$^{35}$S]methionine revealed that they differed in the mobility of the light chain (Fig. 3), which indicates that the lines were derived by independent fusions. Cross-adsorption of infected cell lysates demonstrated that the antibodies from the two hybridoma lines recognized antigenic sites on different polypeptides (Fig. 4). Finally, as already reported (13) both antibodies reacted with HSV-2-infected, but not HSV-1-infected, cells.

**Kinetics of appearances of viral antigens.** To characterize the viral antigens recognized by the antibodies, cultures of infected cells were incubated in the presence of [$^{35}$S]methionine for 2-h intervals at 1, 3, 5, 7, and 9 h postinfection. The cells were then lysed, and the polypeptides were precipitated with antibodies from the two hybridoma lines. Using antibodies from 17αA2, the 60,000- and 66,000-molecular-weight species could be clearly detected only in cells labeled 3 to 5 h postinfection (Fig. 5, lane e). By 5 to 7 h postinfection, the amount of label associated with both polypeptides had increased, and an additional faster-migrating species with a molecular weight of 31,000 became apparent. At even later times (7 to 9 and especially 9 to 11 h postinfection), the major density of the label was associated with the 60,000-molecular-weight polypeptide; furthermore, with increasing times postinfection, a slight shift downwards in the mobility of the immunoprecipitated species was observed.

The results of a similar experiment with antibodies from 17βC2 are presented in Fig. 6. In this case, immunoprecipitable polypeptides of molecular weights 50,000 and 56,000 could be detected as early as 1 to 3 h postinfection, and maximal synthesis appeared to occur between 3 and 7 h postinfection; at these times a species of 80,000 molecular weight also became apparent. As noted for 17αA2, the polypeptides synthesized later postinfection migrated slightly faster than the polypeptides synthesized earlier in the replicative cycle (Fig. 6).

The relationships between the polypeptides precipitated by each antibody were also examined in pulse-chase experiments. Infected cells were labeled with [$^{35}$S]methionine for 30 min at 5 h postinfection: one set of cultures was harvested immediately, whereas a parallel set was incubated for an additional 5 h in the presence of 100× excess unlabeled methionine and 50 μg

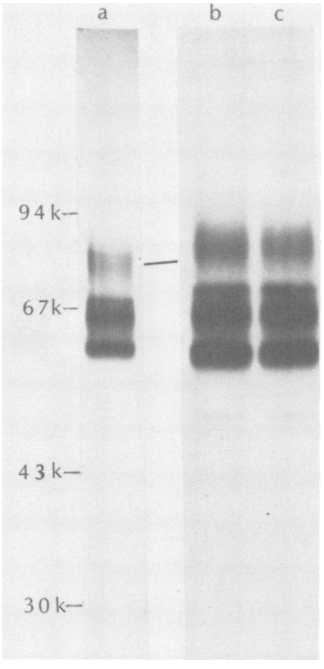


FIG. 2. *Comparison of the polypeptides immunoprecipitated with antibodies secreted from hybridoma 17αA2 and monoclonal lines derived from it. Lysates of HSV-2-infected Vero cells, labeled with [$^{35}$S]methionine (1 to 18 h postinfection) were reacted with antibodies from 17αA2 (lane a) and from monoclones 17αA2-1-A2 (lane b) and 17αA2-1-B2 (lane c).*

of cycloheximide per ml. At the end of the labeling period, antibodies from 17αA2 precipitated a 60,000-molecular-weight, methionine-containing polypeptide; during the 5-h incubation period, the radiolabel chased into the 66,000- and 79,000-molecular-weight species and to a lesser degree into the 31,000-molecular-weight species (Fig. 5c). Under similar experimental conditions, antibodies from 17βC2 precipitated a 50,000-molecular-weight polypeptide synthesized during the 30-min pulse; after 5 h of incubation, the radiolabel chased into diffuse, slower-migrating bands with apparent average molecular weights of 56,000 and 80,000 (Fig. 6c).

Antibodies from 17αA2 and 17βC2 reacted with the surface of HSV-2-infected, unfixed cells (data not shown), which suggested that the viral antigens recognized by them might be glycoproteins (10). This was confirmed by experiments (Fig. 5 and 6) in which the antibodies were reacted with lysates of infected cells labeled with [$^{3}$H]glucosamine between 3 and 16 h postinfection. In the case of 17αA2 antibodies (Fig. 5), the two major species precipitated had molecular weights of 66,000 and 79,000. A third polypeptide, less intensely labeled and with a molecular weight of 60,000, could also be detected. F17βC2 antibodies, on the other hand, precipitated a major diffuse species with molecular weights ranging from 56,000 to 60,000 and a minor 50,000-molecular-weight polypeptide.

**Tryptic peptide analysis.** The observations described above suggested that antibodies from 17αA2 and 17βC2 were directed against antigenic sites on different glycosylated polypeptides. In addition, in each case the antigenic sites appeared to be present on polypeptides with different migration characteristics derived by posttranslational modifications. To confirm these findings, the [$^{35}$S]methionine-labeled species from the polyacrylamide gels were digested with trypsin, and the tryptic digests were analyzed by two-dimensional peptide analysis as described above. Comparison of the major polypeptides with molecular weights of 60,000 and 56,000, precipitated by antibodies from 17αA2 and 17βC2, respectively, revealed unique tryptic digest maps for each species (Fig. 7).

The polypeptides migrating with molecular

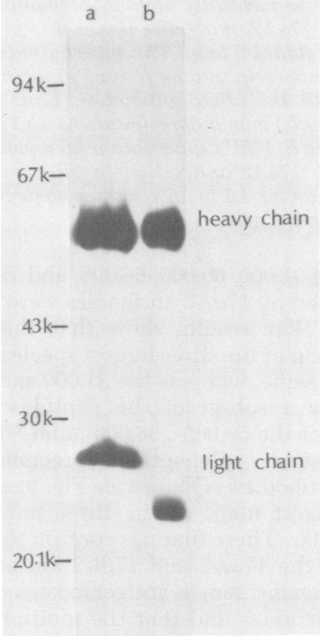


FIG. 3. *Autoradiograms of electrophoretically separated [$^{35}$S]methionine-labeled antibodies secreted by hybridomas 17αA2 and 17βC2. A total of 1 × 10$^{6}$ actively growing cells were labeled with [$^{35}$S]methionine (20 μCi/ml for 18 h); 1 ml of the culture fluid was then incubated with protein A Sepharose for 2 h, centrifuged, and suspended in sample buffer. Electrophoresis was carried out on 7.5 to 15% polyacrylamide gradient gels containing 0.1% sodium dodecyl sulfate. Lane a: 17αA2; lane b: 17βC2.*

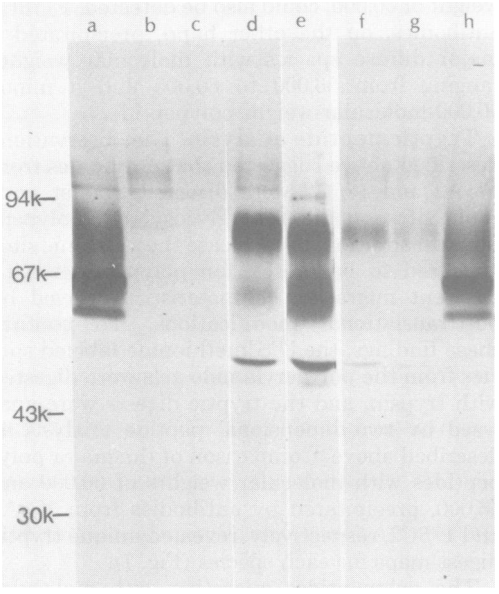


FIG. 4. *Test of cross-adsorption between 17αA2 and 17βC2 antibodies. Lysates of labeled infected cells were sequentially immunoprecipitated three times with 17αA2 antibodies (lanes a to c) or 17βC2 antibodies (lanes e to g). The supernatants from the last immunoprecipitations (c and g) were then adsorbed with the other antibodies. Lane d, 17αA2 supernatant (c) immunoprecipitated with 17βC2 antibodies; lane h, 17βC2 supernatant (g) immunoprecipitated with 17αA2 antibodies. In all cases, electrophoresis was on 7.5 to 15% sodium dodecyl sulfate-polyacrylamide gels.*

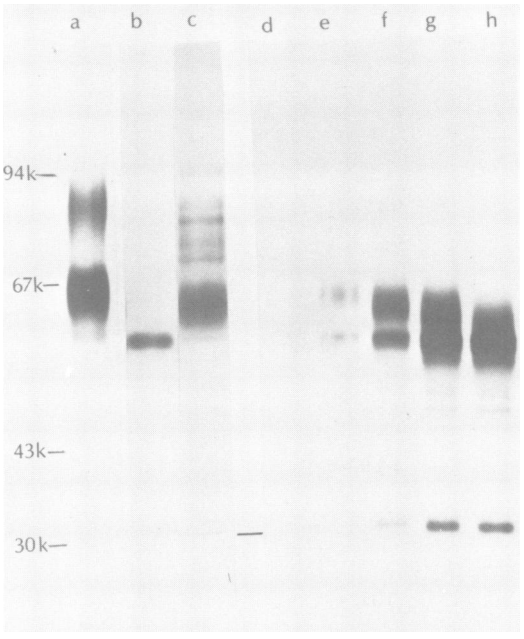


FIG. 5. *Characterization of antigens immunoprecipitated by 17αA2 antibodies from lysates of infected Vero cells labeled with: lane a, [$^3$H]glucosamine at 3 to 16 h postinfection; lane b, [$^{35}$S]methionine for 30 min at 5 h postinfection; lane c, as in lane b, followed by a 5-h chase in nonradioactive medium; lanes d to h, [$^{35}$S]methionine for 2 h at different times postinfection (d: 1 to 3 h; e: 3 to 5 h; f: 5 to 7 h; g: 7 to 9 h; h: 9 to 11 h). In all cases, the antigens were separated by electrophoresis on 7.5 to 15% sodium dodecyl sulfate-polyacrylamide gradient gels.*

weights of 31,000, 60,000, 66,000, and 79,000 and precipitated by 17αA2 antibodies were similarly analyzed. The results show that the peptide composition of the three largest species is essentially the same, whereas the 31,000 species contains only a subset of the peptides (Fig. 8). Analysis of the 50,000-, 56,000-, and 80,000-molecular-weight polypeptides precipitated by 17βC2 antibodies is shown in Fig. 9; again the tryptic digest maps of the three polypeptides were similar. These findings confirm the suggestion that the 17αA2 and 17βC2 antibodies are directed against single antigenic sites on different polypeptides and that the multiple species observed in sodium dodecyl sulfate-polyacrylamide gels resulted from posttranslational modification or cleavage.

**Cross-adsorption with antiserum to envelope glycoproteins (anti-ENV-C serum).** On the basis of their described properties and by comparison with data reported in the literature (1), 17αA2 and 17βC2 antibodies were tentatively identified as directed against viral glycoproteins gE and gD, respectively. Antibodies from 17βC2, however, immunoprecipitate an

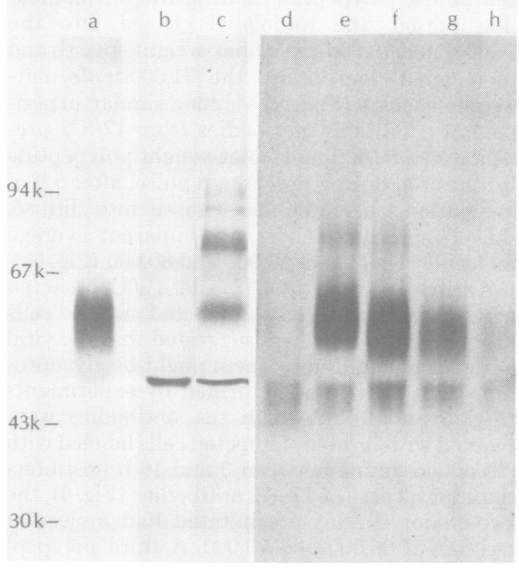


FIG. 6. *Characterization of antigens immunoprecipitated by 17βC2 antibodies. Lanes are as in Fig. 5.*

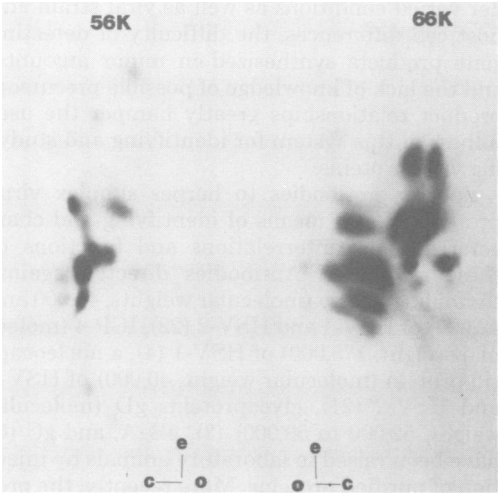


FIG. 7. *Two-dimensional tryptic peptide analysis of the 66,000-molecular-weight polypeptide precipitated by 17αA2 antibodies and the 56,000-molecular-weight polypeptide precipitated by 17βC2 antibodies. Polypeptides immunoprecipitated from infected [$^{35}$S]methionine-labeled Vero cells were separated by electrophoresis, eluted from the gel, and digested with trypsin. The tryptic digests were then subjected to electrophoresis (e) on a silica gel sheet in one dimension and chromatography (c) in the second dimension, as indicated at the origin (O).*

80,000-molecular-weight species not previously described in the gD group. To ascertain whether indeed 17βC2 antibodies reacted with gD, we compared immunoprecipitation patterns obtained with 17βC2 and anti ENV-C serum and determined the ability of 17βC2 antibodies to adsorb antigens precipitated by anti-ENV-C antibodies. ENV-C antiserum, kindly provided by G. H. Cohen, was raised against the envelope proteins of HSV-2 strain T2-Savage and is analogous to the anti-ENV-2 serum described in reference 7. Anti-ENV-C serum precipitated polypeptides with molecular weights of 51,000 and 56,000 (which have been designated pD and gD [7]) and also polypeptides with molecular weights of 48,000 and 110,000 to 130,000 (Fig. 10). Complete removal of the antigens reacting with 17βC2 antibodies did not reduce the quantity of the pD and gD antigens precipitated by ENV-C serum. Moreover, the antigens precipitated by the two antibodies have slightly different migration characteristics. Thus, 17βC2 antibodies do not appear to be directed against the gD glycoprotein group.

## DISCUSSION

Identification of the gene products of herpesvirus presents a special problem because of the genetic complexity of the viruses. Since herpes simplex virus type 2 inhibits the synthesis of proteins coded for by the host cell within a few hours postinfection (17), radiolabeled amino acid precursors appear to be incorporated primarily

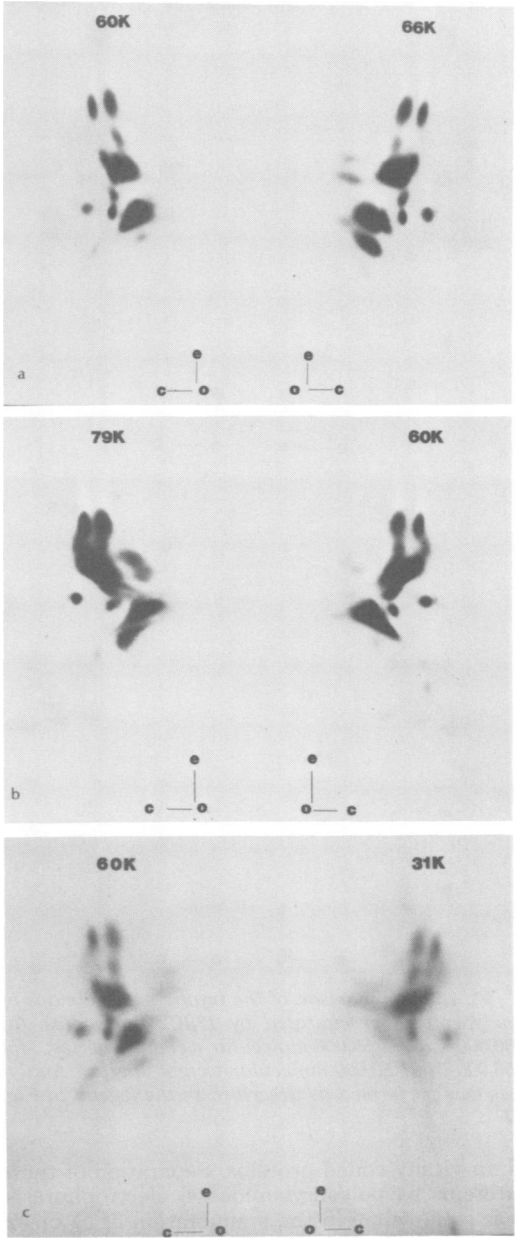


FIG. 8. *Comparison of the tryptic peptide maps of polypeptides immunoprecipitated with 17αA2 antibodies. (a) 60,000- and 66,000-molecular-weight species; (b) 79,000- and 60,000-molecular-weight species; (c) 31,000- and 60,000-molecular-weight species. Analysis was performed as described in the legend to Fig. 7.*

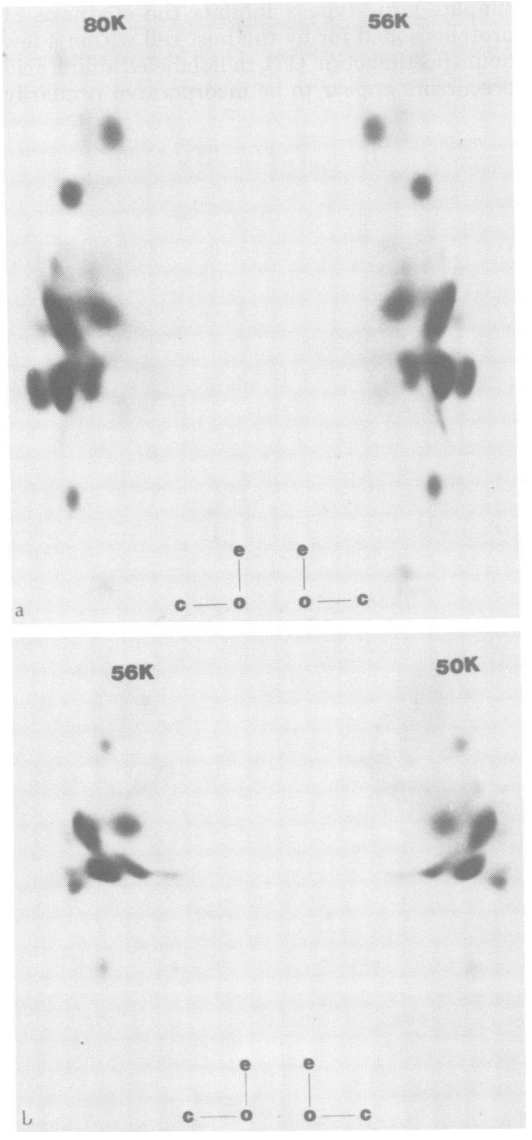


FIG. 9. *Comparison of the tryptic peptide maps of polypeptides precipitated by 17βC2 antibodies. (a) 80,000- and 56,000-molecular-weight species; (b) 56,000- and 50,000-molecular-weight species. Analysis was performed as described in the legend to Fig. 7.*

into virally coded proteins. Separation of these proteins by polyacrylamide gel electrophoresis has so far allowed the enumeration of 51 or 52 polypeptides of viral origin (17, 21) which have been identified by their order of migration (11) or by their apparent molecular weights (17). The large number of polypeptides detected, the variability in molecular weight estimates determined by polyacrylamide gel electrophoresis under varied conditions as well as viral strain and host cell differences, the difficulty of detecting gene products synthesized in minor amounts, and the lack of knowledge of possible precursor-product relationships greatly hamper the usefulness of this system for identifying and studying viral proteins.

Specific antibodies to herpes simplex virus proteins offer a means of identifying and characterizing the interrelations and functions of these molecules. Antibodies directed against thymidine kinase (molecular weights, 44,000 and 42,000) of HSV-1 and HSV-2 (22), ICP-4 (molecular weight, 175,000) of HSV-1 (4), a nucleocapsid protein (molecular weight, 40,000) of HSV-1 and HSV-2 (21), glycoproteins gD (molecular weight, 52,000 to 59,000) (3), gBgA, and gC (6) have been raised in laboratory animals by injection of purified proteins. More recently, the production of monoclonal antibodies to glycoproteins gC and gD of HSV-1 (16) and to the 40,000-molecular-weight nucleocapsid protein of HSV-1 (23) has been reported. The high degree of antigenic specificity of monoclonal antibodies and the ease of producing hybridomas secreting

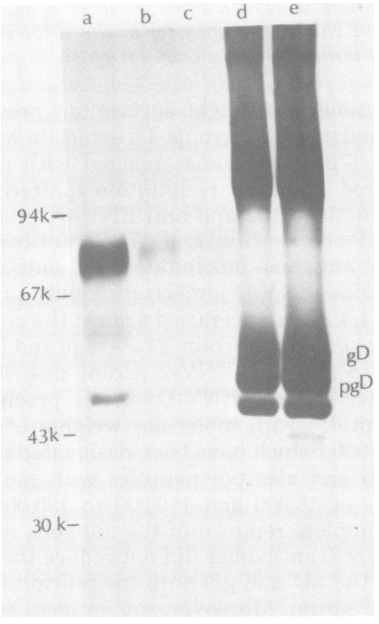


FIG. 10. *Comparison of the specificities of 17βC2 and ENV-C serum antibodies. Lysates of HSV-2-infected, [$^{35}$S]methionine-labeled Vero cells were sequentially precipitated three times with 17βC2 antibodies (lanes a to c). The supernatant from the last immunoprecipitation (lane c) was then reacted with ENV-C antiserum (lane d). Lane e shows the results obtained by immunoprecipitating the cell lysate with ENV-C antiserum alone.*

antibodies to HSV viral proteins (13) suggest that reagents produced by this approach may simplify analysis of the virus.

We found that monoclonal antibodies to HSV-2 antigens frequently precipitated more than a single polypeptide from lysates of infected cells. The patterns precipitated by monoclonal antibodies from any one hybridoma were, however, unique (Fig. 1), which suggests that polypeptides were not nonspecifically trapped in the immunoprecipitate. In our analysis of the polypeptides precipitated by antibodies from two hybridomas, all of the species detected appeared to be interrelated as precursors and products. Zweig et al. (23) have recently described monoclonal antibodies to a 40,000-molecular-weight nucleocapsid protein which also reacted with an 80,000-molecular-weight protein that was not a precursor or doublet of the 40,000-molecular-weight molecule. The authors concluded that the two proteins shared a common antigenic determinant, although they had distinctly different primary structures. Thus, the reasons for the precipitation of multiple polypeptides by monoclonal antibodies appears to vary in different cases.

Antibodies from hybridoma 17αA2 precipitated a partially glycosylated 60,000-molecular-weight precursor of the 66,000- and 79,000-molecular weight species. Molecules with similar migration characteristics and relationships have been described for HSV-1 and HSV-2 as different forms of gE. As reported by Baucke and Spear (1), a 64,000-molecular-weight species, pE, is processed to give $pE_1$ (molecular weight, 66,000) which in turn yields $gE_2$ (molecular weight, 80,000). These different forms derive from the same translation product and constitute a minor component of infected cell extracts. In pulse-chase experiments, label also chased into an immunoprecipitable 31,000-molecular-weight polypeptide. This previously undescribed molecule which is not glycosylated but shares peptides with the 60,000-molecular-weight species is likely a cleavage product. Although not conclusive, our data at present are in agreement with the designation of 17αA2 antibodies as directed against the gE glycoprotein group.

Antibodies from hybridoma 17βC2 precipitated a partially glycosylated 50,000-molecular-weight precursor of the 56,000- and 80,000-molecular-weight species. Molecules with similar but not identical characteristics have been designated pD and gD (7); however, no polypeptide of 80,000 has been described as belonging to the gD group. As reported above, this polypeptide, which migrates close to the 79,000-molecular-weight polypeptide precipitated by 17αA2 antibodies (probably anti-gE), has a peptide map similar to the 50,000- and 56,000-molecular-weight species and distinct from the 79,000-molecular-weight species. The absence of cross-adsorption between 17βC2 antibodies and anti-ENV-C serum, as well as the slightly different migration characteristics revealed by a direct comparison of antigens recognized by the two sera, indicate that 17βC2 antibodies are directed against a viral glycoprotein different from gD and from any of the glycoproteins so far described. We propose that this glycoprotein be tentatively designated gF.

Experiments reported elsewhere (13) indicated that antibodies from both hybridoma lines were specific for HSV-2, since no reactions were observed with HSV-1 antigens by enzyme-linked immunosorbent assay, immunofluorescence, or immunoprecipitation. Both antibodies stained the surface of unfixed cells infected with HSV-2 in an immunofluorescence assay; this was not unexpected since other workers have shown that HSV glycoproteins are expressed on the surface of virus-infected cells (10). However, the monoclonal antibodies did not lyse virus-infected cells in the presence of complement nor did they neutralize the virus (data not shown).

The utility of monoclonal antibodies in characterizing the viral proteins is further suggested by incidental observations made in the course of our study. For example, the kinetics of synthesis of two glycoproteins (Fig. 5 and 6) were found to be different. The precursor to one of them (17βC2, Fig. 6) was detectable by 3 h postinfection, and maximal synthesis occurred between 5 and 7 h postinfection. The synthesis of the other glycoprotein (17αA2, Fig. 5) occurred much later in the replicative cycle. Another observation referred to by Honess and Roizman (12) and by Spear (20) is the apparent decrease in efficiency of glycosylation late in the replicative cycle. The diffuse bands representing the glycosylated polypeptides precipitated by both monoclonal antibodies migrated faster when harvested late in the replicative cycle as compared with early harvest (Fig. 5 and 6). The migration of the precursor polypeptides, on the other hand, was not influenced by the phase of the replicative cycle. One explanation for this observation is that the host cell enzymes utilized in glycosylation are not replenished during the replicative cycle and become the rate-limiting step in glycosylation (12). The use of monoclonal antibodies will permit exploration of these interesting phenomena.

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