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Glycoproteins of Herpes Simplex Virus Type 2 As Defined by Monoclonal Antibodies

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We used monoclonal antibodies reacting with glycoproteins specified by herpes simplex virus type 2 (HSV-2) to characterize the individual antigens in terms of structure, processing, and kinetics of synthesis in BHK or Vero infected cells. Our results provided a direct demonstration of the structural identity of the gA and gB proteins of HSV-2 as well as confirmation of the existence of type-specific and type-common domains within the gD molecule. They also show that, with the exception of gC, processing of the viral glycoproteins differs to some extent in Vero and BHK infected cells, possibly as a result of different efficiency of glycosylation or different processing of underglycosylated and unglycosylated products in the two cell types. Finally, we showed that individual HSV-2 glycoproteins are synthesized at greatly different times during the infectious cycle, possibly in response to their different roles in virus replication and assembly.

Infection of mammalian cells with herpes simplex virus (HSV) results in the synthesis of over 50 virus-specified polypeptides (9, 21). Among these polypeptides are glycosylated species which become incorporated into the cell membranes and ultimately constitute part of the virion envelope (27). Herpesvirus glycoproteins represent the major target for the host immune responses and are likely involved in the processes of virus attachment, penetration, and maturation as well as in virus-induced cell fusion (27). The major glycoproteins of HSV type 1 (HSV-1) have been designated gC, gAB, gE, and gD; species with similar characteristics produced by HSV type 2 (HSV-2) infections have been given corresponding names (15, 17, 27).

To date, the majority of the studies on these viral products have made use of antisera raised in animals by a variety of procedures and reacting primarily, but not exclusively, with antigenic determinants specified by each glycoprotein (16). Although these sera have been excellent tools in the characterization of the viral glycoproteins, they are difficult to produce, usually have low potency, and are subject to batch variations. The recent development of the hybridoma technology (11) has, however, provided a method for obtaining unlimited supplies of well-defined and monospecific antibodies to desired antigens. This approach has been used by Pereira et al. (18–20) to raise monoclonal antibodies to HSV-1 and HSV-2 glycoproteins gAB, gC, and gD. Similarly, we have produced a number of monoclonal hybridomas secreting antibodies to HSV-2 antigens (10) and have reported the characterization of two HSV-2 glycoproteins as defined by the monospecific antibodies (1). Both of these glycoproteins exhibited electrophoretic patterns similar to that of HSV-1 gE described by Baucke and Spear (2), but they were shown to be structurally and antigenically distinct from each other. We also showed that each antibody reacted with molecules which had different electrophoretic mobilities but which shared the same peptide backbone and which were thus considered different forms of the same glycoprotein. We tentatively proposed that the antigen recognized by 17αA2 antibodies might be the HSV-2 equivalent of HSV-1 gE. The antigen reacting with 17βC2 antibodies could not be related to any of the known HSV glycoproteins and was therefore designated gF. Recent experiments carried out with our and other antibodies in the laboratory of P. G. Spear (personal communication) indicate, however, that the 17βC2 antibodies cross-react with a monoclonal antibody raised against glycoprotein gE (17), whereas the 17αA2 antibodies recognize gF, in contrast to our previous assignment (1). The revised designations will therefore be used throughout this report.

In this paper, we describe further characteristics of the HSV-2 gE and gF antigens as well as the characteristics of HSV-2 gAB, gC, and gD glycoproteins as defined by additional monoclonal antibodies we have isolated. Our data are in agreement with a previous report that gA and gB are two forms of the same protein (5). In addi-

tion, we present comparative data on the processing of all of the HSV-2 glycoproteins in two different cell types, BHK and Vero.

MATERIALS AND METHODS

Cells and virus. Baby hamster kidney (BHK) and Vero cells were grown in modified Dulbecco medium (GIBCO Laboratories) or Eagle minimal essential medium, respectively, with 5% heat-inactivated calf serum. The origins of the 333 strain of HSV-2 and the KOS strain of HSV-1 have been described previously (24).

Monoclonal antibodies. Hybridomas secreting HSV-specific antibodies were produced by fusing myeloma cells, Sp2/0.Ag14, with spleen cells from BALB/c mice immunized with Formalin-inactivated lysates of virus-infected L cells (10). Antibodies secreted by monoclonal hybridomas were isotyped from culture supernatant fluid using rabbit anti-mouse immunoglobulin subclasses obtained from Bionetics Laboratories (Toronto). Hybridoma clones were also injected intraperitoneally into pristane-primed BALB/c mice (12), and the resulting ascitic fluids were collected, analyzed, and used as a source of antibodies.

Radiolabeling and radioimmunoprecipitation. Detailed procedures have been described elsewhere (1). Briefly, monolayers of cells were mock infected or infected with HSV-2 at a multiplicity of infection of 20 PFU per cell and incubated in Hanks balanced salt solution. Except where otherwise stated, all experiments were carried out in BHK cells. Cells were labeled either between 3 and 20 h after infection or for 2-h pulses at different times after infection in Hanks balanced salt solution containing 20 μCi of L-[^{35}S]methionine per ml (specific activity, 900 to 1,100 Ci/mmol; New England Nuclear Corp.), 5% dialyzed fetal bovine serum, and 1/10 the normal concentration of methionine. In pulse-chase experiments, cells were labeled for 10 min with 100 μCi of L-[^{35}S]methionine per ml at 5 h after infection and chased for 5 h in medium containing 100 times the normal concentration of unlabeled methionine and 50 μg of cycloheximide per ml. Glycoproteins were also labeled by incubating cells with 100 μCi of D-[6-^{3}H]glucosamine hydrochloride per ml (specific activity, 19 Ci/mmol; New England Nuclear Corp.) for 3 to 18 h after infection in medium with 1/10 the normal concentration of glucose. Immunoprecipitations were carried out as described previously (1) by mixing RIPA-solubilized cell extracts with antibody and protein A–Sepharose CL-4B beads (Pharmacia Fine Chemicals, Inc.). The precipitates were washed, dissociated, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (9% acrylamide cross-linked with 0.24% *N,N'*-diallyltartardiamide). Molecular weight markers (Bio-Rad Laboratories) were electrophoresed in a parallel channel.

Cross-reactivity with reference sera. The identity of the glycoproteins immunoprecipitated by the monoclonal antibodies was confirmed by cross-adsorption with reference sera against the known major glycoproteins (gAB, gC, and gD). Briefly, [^{35}S]methionine-labeled infected-cell extracts were immunoprecipitated for 2 h at 4°C with a given monoclonal antibody, as described above. After centrifugation, the supernatant was again reacted with the same antibody, and the procedure was repeated three additional times. The supernatant collected after the last immunoprecipitation, as well as unadsorbed antigen, was then reacted with reference serum. All of the immunoprecipitates were analyzed on 9% polyacrylamide–0.24% *N,N'*-diallyltartardiamide gels as described above. The reference sera used were the gAB2, the gC2, and the gX2 antisera provided by R. J. Courtney and the anti-gD ENV-C serum provided by G. H. Cohen.

Tryptic peptide analysis. Labeled polypeptides were immunoprecipitated and visualized by autoradiography of stained gels. The regions of the gel containing the labeled species were excised, and the tryptic peptide analysis was carried out as previously described (1).

Immunofluorescence. Monoclonal antibodies were tested for their reactivity with HSV-infected cells (acetone fixed or unfixed) by an indirect immunofluorescence test using fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (IgG) (Cappel Laboratories). Vero cells growing on cover slips were infected with HSV-2 or HSV-1 at a multiplicity of infection of 0.1 PFU per cell and fixed for 10 min with cold acetone at 18 h after infection. Antibodies to antigens on the surface of infected cells were identified by a modification of a previously described technique (25). Briefly, monolayers of infected cells were monodispersed with trypsin, washed, and resuspended at a concentration of 10^5 cells per ml. After 30 min of incubation at 37°C with different dilutions of antibody, the cells were washed three times, incubated with conjugated antibody, washed again, and spread on microscopic slides which were mounted in buffered glycerol-saline and examined by fluorescence microscopy.

Complement-dependent antibody-mediated cytolysis. Antibodies to surface antigens were also assayed by adding guinea pig complement to ^{51}Cr-labeled infected BHK cells (New England Nuclear Corp.). Methods described by McClung et al. (13) were followed except that the test was carried out in microtiter plates with volumes of 0.05 ml of cells (10^4 viable cells), 0.05 ml of antibody, and 0.1 ml of 10 U of complement. Titers were expressed as the reciprocal of the highest dilution showing more than 10% specific ^{51}Cr release.

Neutralizing antibodies. The ability of the monoclonal antibodies to neutralize virus infectivity was tested by mixing 100 PFU of virus and various dilutions of ascitic fluid. The mixtures were incubated for 1 h at room temperature and assayed for surviving virus in microtiter plates containing Vero cells (22).

RESULTS

Properties of antibodies secreted by monoclonal hybridomas. The products secreted by 41 hybridomas making antibodies specific for HSV (1) were screened for reactivity with antigens on the surface of HSV-2-infected unfixed cells by indirect immunofluorescence. Twelve hybridomas producing such surface-reacting antibodies were identified, and their characteristics are listed in Table 1. Type-common antigenic determinants were recognized by antibodies secreted by two monoclones (18βB3 and 20αD4). Antibodies from 18βB3 reacted with HSV-2 and HSV-1

TABLE 1. Properties of monoclonal antibodies to HSV

Monoclone	Antibody isotype secreted	Serotype specificity[a]	Complement-dependent cytolysis[b]	Neutralization of HSV-2	Antigen specificity
18αA5	IgG1	2	1:30	<1:5	
17αD4	IgG2b	2	≤1:10	<1:5	
GH2-γ-C6	IgG1	2	≥1:5,260	<1:5	gC
13αC5	IgG1	2	1:1,000	<1:5	
20αD4	IgG1	2 + 1	≥1:5,260	<1:5	gAB
17αA2	IgG2a	2	1:160	<1:5	
17αC1	IgG1	2	≥1:5,260	<1:5	
17αD2	IgM	2	≤1:10	<1:5	gF
11A5	IgG1	2	ND[c]	ND	
17βA3	IgG1	2	1:1,000	<1:5	
18βB3	IgG1	2 + 1	1:4,000	1:25	gD
17βC2	IgG2a	2	1:40	<1:5	gE

[a] Tested by indirect immunofluorescence on acetone-fixed HSV-infected Vero cells or by radioimmunoprecipitation of [^{35}S]methionine-labeled (3 to 20 h) HSV-infected cells.
[b] Antibody dilution showing more than 10% specific lysis.
[c] ND, Not determined.

antigens with equal efficiency by both the immunofluorescence and immunoprecipitation assays; on the other hand, antibodies secreted by 20αD4 reacted with both HSV-2 and HSV-1 antigens by immunofluorescence but immunoprecipitated only HSV-2 antigens. None of the antibodies from the other monoclones reacted with HSV-1 antigens by either test, suggesting that these antibodies recognized type-specific domains of HSV-2 antigens. Antibodies from 9 of 11 monoclones tested were able to lyse HSV-2-infected cells in the presence of complement. Although binding activity to the surface of infected cells was demonstrated for antibodies from all of the monoclones, neutralization could only be demonstrated by microassays using antibodies from monoclone 18βB3. The identity of the glycoproteins to which individual antibodies are directed (see Table 1) was determined by immunoprecipitation of infected-cell lysates as described below.

Characteristics of HSV-2 glycoproteins gAB, gD, and gC. Autoradiograms of the [^{35}S]methionine-labeled polypeptides which were immunoprecipitated from BHK infected cells by antibodies from hybridoma 20αD4 revealed a complex pattern of four large polypeptides of molecular weights 125,000 (125K), 116K, 110K, and 98K to 100K and of five faster-migrating species with apparent molecular weights of 67K, 53K, 47K, 38K, and 30K (Fig. 1, track 1). All of these molecules, with the exception of 38K and 30K, were labeled also with [^{3}H]glucosamine (track 2). Pulse-chase experiments revealed the [^{35}S]methionine label to be associated initially with a diffuse band of molecular weight 98K to 116K (track 3) and at later times with the 125K species (track 4). This suggested the existence of a precursor-product relationship among at least the large polypeptides, a hypothesis confirmed by tryptic peptide mapping of most of the species immunoprecipitated by the 20αD4 antibodies. As shown in Fig. 2, panels A to C, the 125K, 116K, 110K, and ~100K molecules were all composed of identical methionine-containing peptides. The 67K, 53K, and 47K species, on the other hand, contained only subsets of these peptides (Fig. 3). The smallest polypeptides, 38K and 30K, were not analyzed.

The immunoprecipitation pattern obtained with the antibodies from 20αD4 resembled that produced by an antiserum raised against glycoprotein gAB of HSV-2 (5). To verify the identity of the antigen recognized by 20αD4, cross-adsorption tests were therefore carried out with the anti-gAB reference serum, as described above. As shown in Fig. 1, tracks 5 and 11, for Vero infected cells, both sera precipitated similar polypeptides. In addition, removal of the polypeptides by successive immunoprecipitations with the monoclonal antibodies from 20αD4 (tracks 6 to 9) depleted the extracts also of molecules precipitable by the reference serum (track 10). Finally, tryptic peptide analysis of all of the molecules precipitated by the anti-gAB serum (see Fig. 2D for the 125K and 110K species) confirmed that they are identical to the molecules recognized by the monoclonal anti-

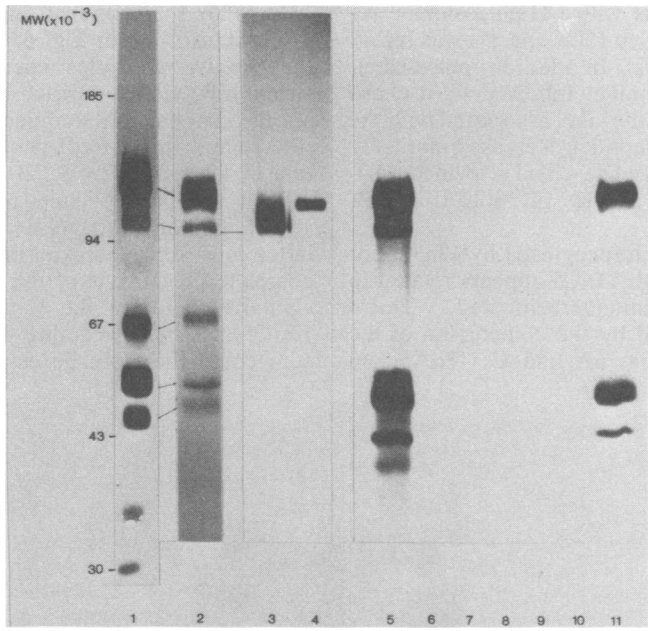

FIG. 1. Electrophoretic analysis of antigens immunoprecipitated from HSV-2-infected cells with antibodies secreted by hybridoma 20αD4 (tracks 1 to 4) and test of cross-adsorption between 20αD4 and anti-gAB rabbit serum (tracks 5 to 11). Infected BHK cells were labeled with [^{35}S]methionine (track 1) or [^{3}H]glucosamine (track 2) at 3 to 20 h after infection or were labeled with [^{35}S]methionine for 10 min at 5 h after infection (track 3) and chased for 5 h in nonradioactive medium (track 4). Infected Vero cells labeled with [^{35}S]methionine at 3 to 20 h after infection were immunoprecipitated five times consecutively with 20αD4 antibodies (tracks 5 to 9); the supernatant from the fifth reaction was then immunoprecipitated with anti-gAB serum (track 10). The products of direct immunoprecipitation of infected Vero cells with the anti-gAB serum are shown in track 11.

bodies (Fig. 2A to C). All of the above data thus established that the 20αD4 antibodies are directed against glycoprotein gAB of HSV-2.

Immunoprecipitation of cell extracts with antibodies from hybridoma 17βA3 (or 18βB3) yielded two polypeptides, 56K and 52K, which were labeled with either [^{35}S]methionine or [^{3}H]glucosamine (Fig. 4A, tracks 1 and 2). Upon longer exposures of the autoradiograms, a third methionine-containing polypeptide, which did not contain glucosamine and which had a molecular weight of 48K, could also be detected. The 52K polypeptide was the species prevalently labeled with [^{35}S]methionine in a 10-min pulse; upon chase, however, the label became associated mainly with the 56K species (tracks 3 and 4). As shown in Fig. 4B, the 56K and 52K molecules had identical peptide contents; the 48K species, on the other hand, lacked some peptides (Fig. 4C). This suggested that the 56K polypeptide is derived from the 52K polypeptide by the addition of carbohydrate side chains, whereas the 48K species could be a breakdown product. Immunoprecipitation patterns similar to the one described above have been reported for HSV-2 gD (7); cross-adsorption with the reference ENV-C serum (7) and peptide mapping of the antigens recognized by it or by 17βA3 confirmed the specificity of the monoclonal antibodies towards this glycoprotein (data not shown).

Immunoprecipitation of extracts of [^{35}S]methionine-labeled infected BHK cells with antibodies secreted by three hybridomas (18αA5, 17αD4, and GH2-γ-C6) yielded two major polypeptides of molecular weights of about 130K and 110K to 116K (Fig. 5A, track 1); of these, only the 130K species appeared to be labeled with [^{3}H]glucosamine (track 2). Labeling of cells with [^{35}S]methionine for a 10-min pulse followed by a 5-h chase indicated that the label, initially incorporated only into the 110K molecule, was subsequently partially chased into the 130K species (tracks 3 and 4).

An immunoprecipitation pattern rather similar to that described for 18αA5 antibodies was obtained with antibodies secreted by hybridoma 13αC5 (Fig. 5B). In this case, however, immunoprecipitation of [^{35}S]methionine-labeled infected BHK cells yielded most commonly only one diffuse polypeptide of molecular weight ranging from 100K to 130K (Fig. 5B, track 1); in some of the experiments, these molecules could, however, be resolved into two distinct species.

Labeling of the cells with [^{3}H]glucosamine revealed that the 100K to 130K species was highly glycosylated (track 2). In addition, pulse-labeling with [^{35}S]methionine, followed by a chase for 5 h, showed that the label associated with the 100K to 130K polypeptides decreased markedly in intensity during the chase and remained associated only with a species of 130K (Fig. 5B, tracks 3 and 4).

Neither the antigen recognized by 18αA5 nor the one reacting with 13αC5 appears related to the gAB complex immunoprecipitated by 20αD4 serum, as evidenced by the comparison of the peptide maps of Fig. 6A and C (18αA5 and 13αC5) to the maps of Fig. 2 (20αD4). The results illustrated in Fig. 6, however, indicated that the two molecules reacting with 18αA5 are structurally similar to each other (panel A) and are also similar to the antigen precipitated by a reference serum raised against the gC glycoprotein of HSV-2 (6) (panel B). They also appear related to the molecule precipitated by the 13αC5 antibodies (panel C), except that in this latter case, repeated experiments failed to yield complete proteolysis of the antigen. Structural similarity between the species recognized by 13αC5 and those reacting with 18αA5 and gC sera could therefore be established only for a

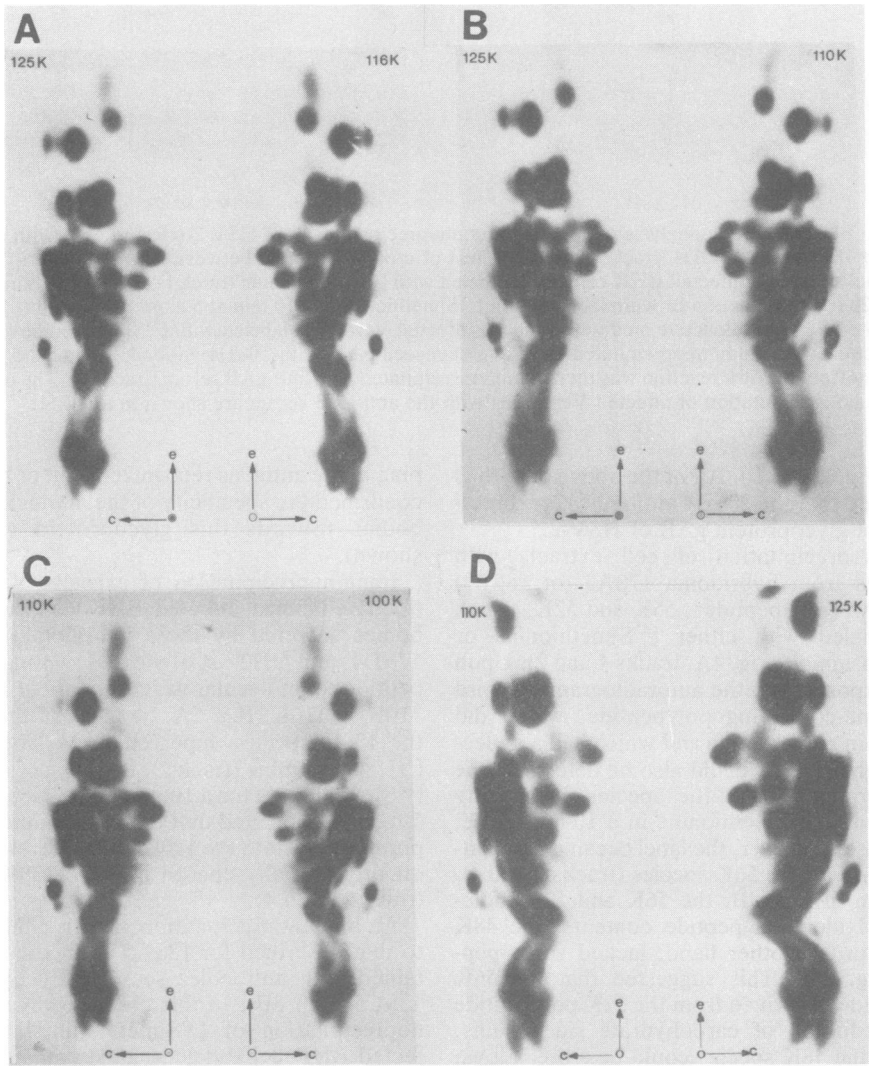

FIG. 2. Comparison of peptides obtained by tryptic digestion of the four high-molecular-weight species (125K, 116K, 110K, and 100K) precipitated by 20αD4 antibodies from [^{35}S]methionine-labeled BHK cells infected with HSV-2 (panels A to C). Tryptic digests of two of the molecules (110K and 125K) reacting with the anti-gAB reference serum are illustrated for comparison in panel D.

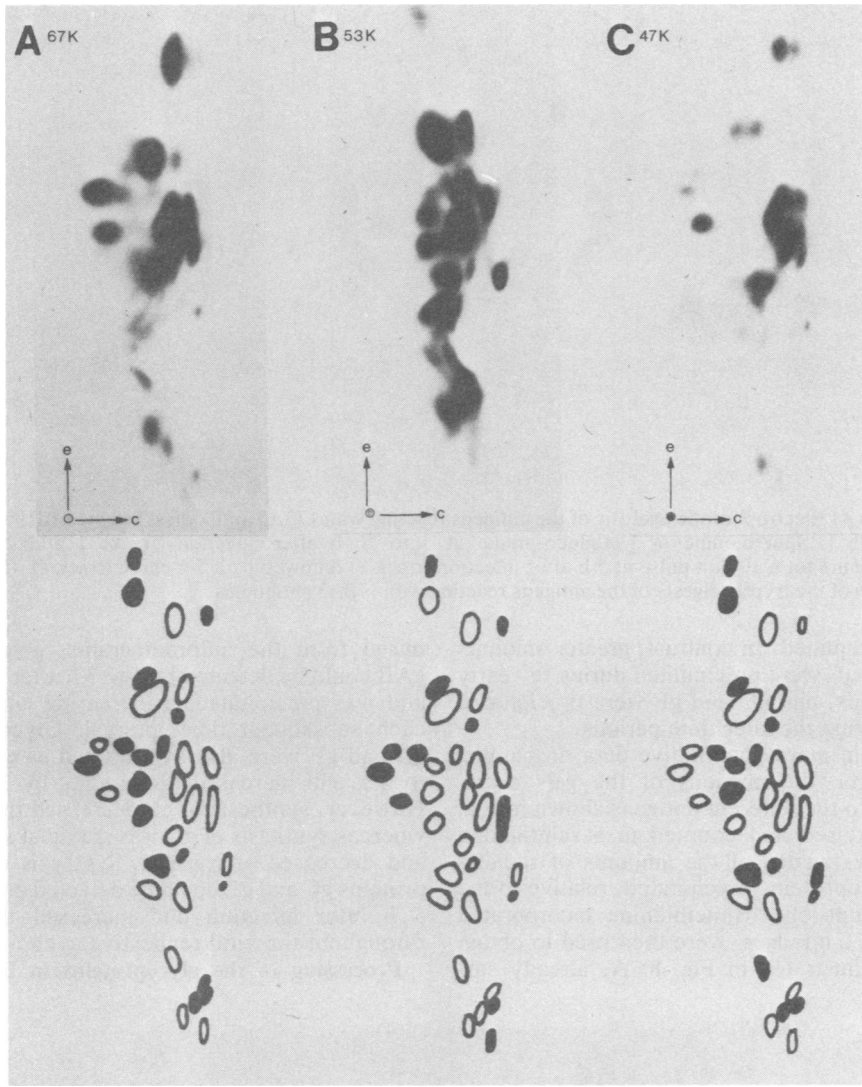

FIG. 3. Tryptic peptide analysis of three of the lower-molecular-weight species (67K, 53K, and 47K) immunoprecipitated by 20αD4 antibodies. In the schematic drawings below each panel, the white spots indicate peptides present in the above chromatograms as well as in the chromatograms of the high-molecular-weight species shown in Fig. 2. The black spots refer to peptides not represented in the lower-molecular-weight molecules.

portion of the molecule. The data presented in Fig. 5 and 6 were interpreted as indicating that both 18αA5 and 13αC5 sera react with the glycoprotein gC of HSV-2.

Kinetics of synthesis of glycoproteins. Antibodies secreted by the different monoclones described above were used to establish the kinetics of synthesis of HSV-2 glycoproteins. BHK cells were infected at a multiplicity of infection of 20 PFU per cell, labeled for 2 h with [^{35}S]methionine at different times after infection, and then reacted with the antibodies. Aliquots of the resulting immunoprecipitates were used to quantitate the radioactivity bound to the antibody, whereas the remaining portions were solubilized and analyzed by gel electrophoresis. For each time interval studied, the total amount of [^{35}S]methionine incorporated into trichloroacetic acid-precipitable material was also determined. Autoradiographs of the electropherograms (Fig. 7) indicated that the amounts of antigens precipitated at different times after infection were not the same for the different glycoproteins. Thus, glycoprotein gAB appeared early in the infectious cycle and was precipitated in substantial amounts for all time

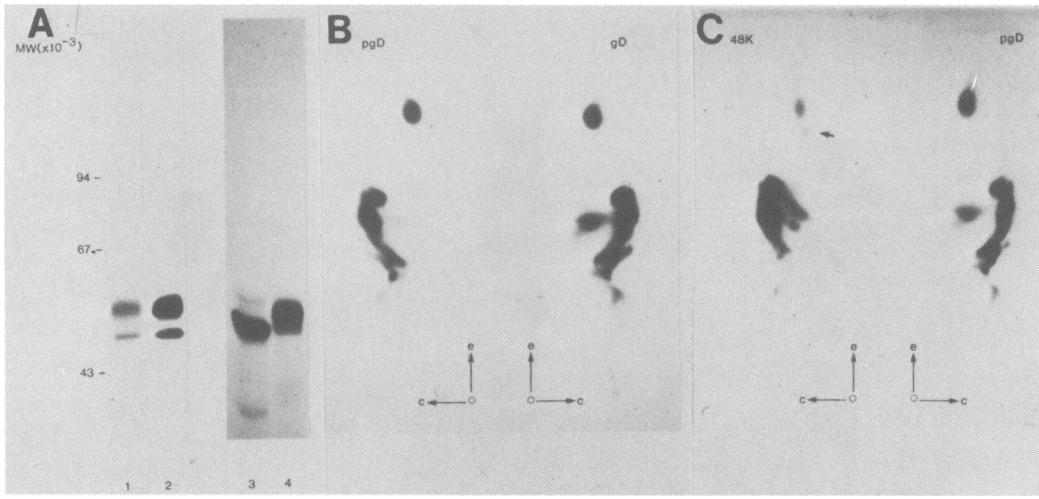

FIG. 4. (A) Electrophoretic analysis of the antigens reacting with 17βA3 antibodies. Infected BHK cells were labeled with [^{35}S]methionine or [^{3}H]glucosamine at 3 to 20 h after infection (tracks 1 and 2) or with [^{35}S]methionine for a 10-min pulse at 5 h after infection (track 3) followed by a 5-h chase (track 4). (B) and (C) Comparison of the tryptic digests of the antigens reacting with 17βA3 antibodies.

periods examined. In contrast, greater amounts of gD and gE were precipitated during the early time periods, and gC and gF were precipitated mostly during the later time periods.

To obtain more quantitative data, in parallel experiments, the portions of the gels corresponding to the different antigens shown in Fig. 7 were excised and counted in a scintillation counter. Estimates of the amounts of radiolabeled glycoproteins precipitated, relative to the total amount of [^{35}S]methionine incorporated during the 2-h pulses, were then used to obtain the data illustrated in Fig. 8. As already surmised from the autoradiograms, glycoprotein gAB could be detected by 1 to 3 h after infection and was precipitated in increasing amounts at each subsequent time interval. Glycoproteins gD and gF were also synthesized as early as 1 to 2 h and increased in amounts by 3 to 5 h. However, synthesis of gF decreased thereafter, whereas synthesis of gD was maximal at 5 to 7 h and decreased afterwards. Synthesis of glycoproteins gC and gE could be detected only at 3 to 5 h after infection and increased thereafter throughout the viral replicative cycle.

Processing of the glycoproteins in BHK and

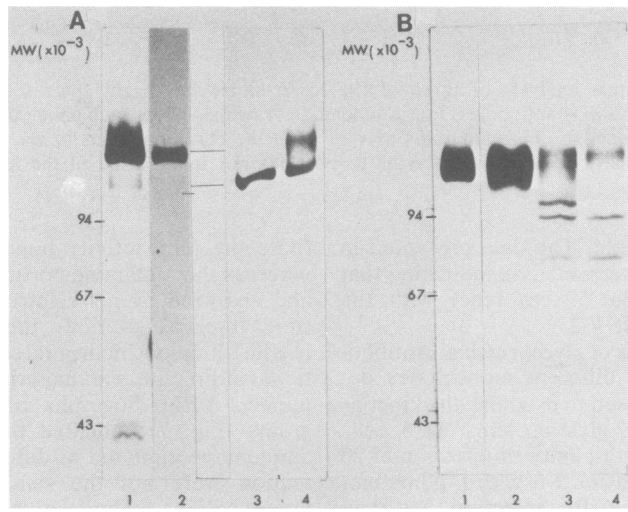

FIG. 5. Electrophoretic analysis of the antigens reacting with 18αA5 antibodies (A) of 13αC5 antibodies (B). Infected BHK cells were labeled in tracks 1 to 4 as described in Fig. 1 and 4.

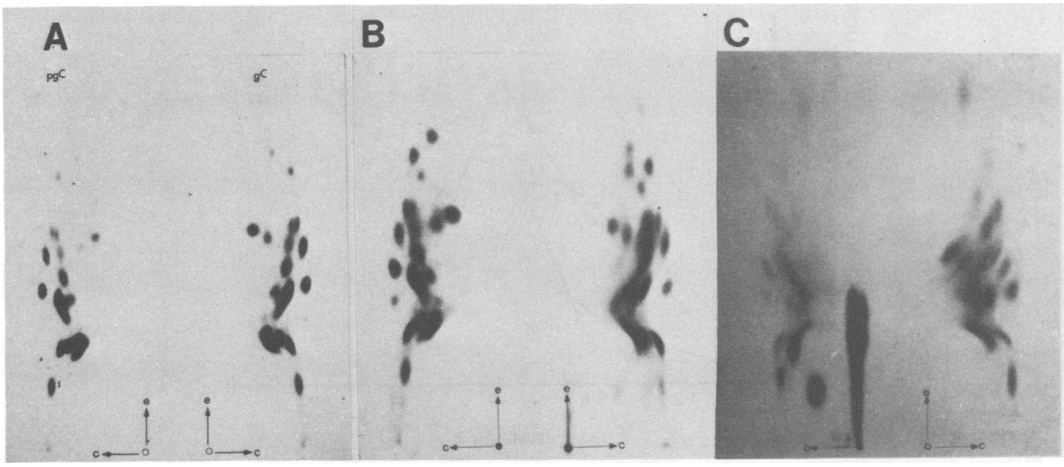

FIG. 6. Tryptic peptide maps of the precursor (pgC) and product (gC) molecules recognized by 18αA5 antibodies (A) and comparison of tryptic digests of the molecules reacting with 18αA5 and anti-gC sera (B) and 13αC5 and 18αA5 sera (C).

Vero cells. We have previously reported that antibodies from hybridomas 17αA2 and 17βC2 precipitated two HSV-2 glycoproteins, presently designated gF and gE, respectively, from infected Vero cells (1). When attempts were made to confirm our observations in BHK cells, substantial differences were observed in the migration characteristics of the immunoprecipitated polypeptides. Therefore, the characteristics of all of the HSV-2 glycoproteins recognized by our monoclonal antibodies were examined by parallel immunoprecipitation of extracts of infected Vero or BHK cells. The cells were labeled with [^{35}S]methionine between 3 and 20 h after infection, and equal amounts of protein from each extract were precipitated with equal amounts of the appropriate monoclonal antibodies, followed by electrophoresis of the reaction products.

No significant differences in the characteristics of the polypeptides precipitated by monoclonal antibodies to gC were observed (data not shown). On the other hand, clear differences could be seen in the pattern of the gAB polypeptides precipitated from the two cell types (Fig. 9, tracks 1 and 2). The group of major polypeptides with molecular weights from 100K to 125K in

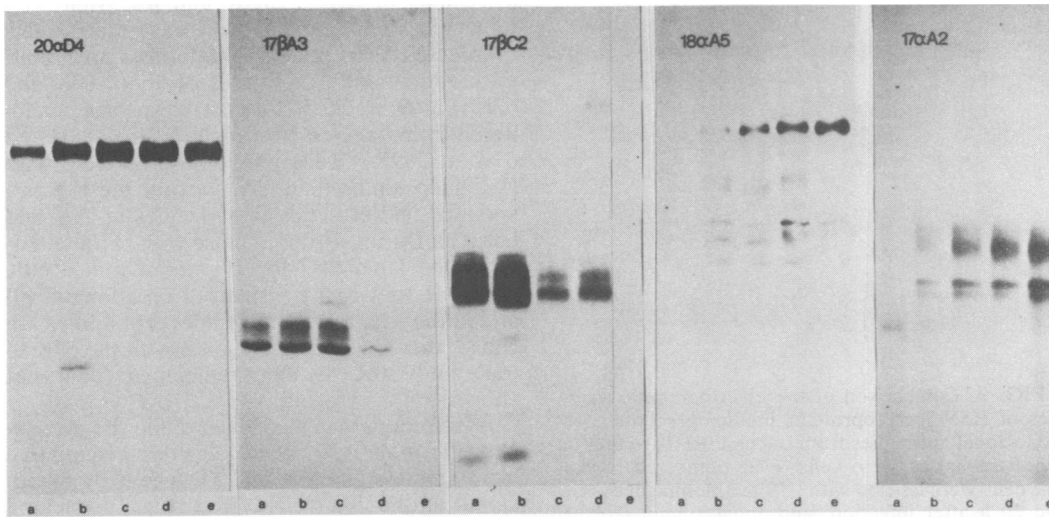

FIG. 7. Time course of synthesis of individual HSV-2 glycoproteins as determined by electrophoretic analysis of the antigens immunoprecipitated by monospecific antibodies: 20αD4 (gAB), 17βA3 (gD), 17βC2 (gE), 18αA5 (gC), and 17αA2 (gF). BHK cells were labeled with [^{35}S]methionine for 2 h and reacted with antibodies at 1 to 3 h (track a), 3 to 5 h (track b), 5 to 7 h (track c), 7 to 9 h (track d), and 9 to 11 h (track e) after infection.

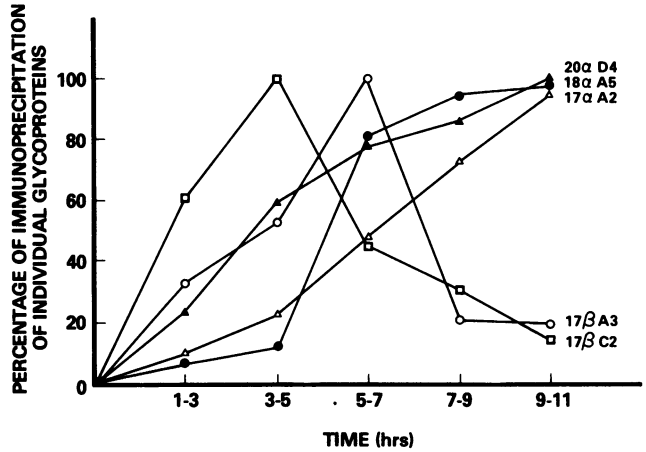

FIG. 8. Time course of synthesis of HSV-2 glycoproteins immunoprecipitated by monospecific antibodies. Infected BHK cells were labeled and reacted with antibodies as in Fig. 7. After electrophoresis of the reaction products, the immunoprecipitated polypeptides were excised from the gels and counted in a liquid scintillation counter. The amounts of radiolabeled glycoproteins precipitated relative to the total amount of [^{35}S]methionine incorporated into trichloroacetic acid-precipitable material during each 2-h pulse were then calculated and are expressed as percentages of the values corresponding to maximum synthesis of each glycoprotein.

BHK cells was represented in Vero cells by molecules of more diverse molecular weights ranging from about 94K to 125K. In addition, the 67K, 38K, and 30K species seen in BHK cells were absent in Vero cells and were substituted by molecules with approximate molecular weights of 50K and 40K. Differences were also observed for glycoprotein gD (Fig. 9, tracks 3 and 4). The 56K species precipitated from BHK cells was virtually absent in precipitates from Vero cells, and the relative distribution of the label was clearly cell dependent.

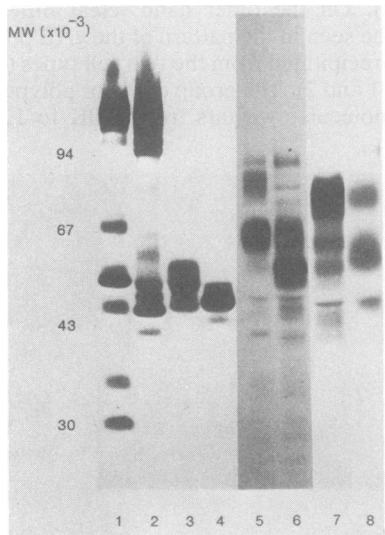

FIG. 9. Comparison of the electrophoretic mobilities of HSV-2 glycoproteins immunoprecipitated by monoclonal antibodies from infected BHK (odd-numbered tracks) or Vero cells (even-numbered tracks). The cells were labeled with [^{35}S]methionine between 3 and 20 h after infection, and equal amounts of cell proteins were immunoprecipitated with antibodies from the following hybridomas: 20αD4 (gAB), tracks 1 and 2; 17βA3 (gD), tracks 3 and 4; 17αA2 (gF), tracks 5 and 6; and 17βC2 (gE), tracks 7 and 8.

The glycoprotein precipitated by antibodies from hybridoma 17αA2 was also precipitated by antibodies from hybridomas 17αC1, 17αD2, and 11A5 (see Table 1). In extracts of infected BHK cells, these antibodies reacted primarily with molecules of 67K to 69K and less well with molecules of 80K to 86K, whereas from extracts of infected Vero cells, the antibodies precipitated predominantly molecules of about 60K and 67K (Fig. 9, tracks 5 and 6). Two species precipitated from infected BHK cells (80K to 88K and 67K to 69K) were efficiently labeled with [^{3}H]glucosamine (data not shown); these corresponded in Vero cells to molecules of 79K and 66K (1). During a 10-min pulse with [^{35}S]methionine, the label was found primarily in a 60K molecule (not easily visualized on a long label) and in the 67K to 69K molecules, but upon a 5-h chase, the label was associated with the 67K to 69K and 80K to 99K molecules (data not shown).

A difference was also observed in the electrophoretic mobility of the molecules precipitated from extracts of BHK and Vero cells by monoclonal antibodies from 17βC2 (Fig. 9, tracks 7 and 8). The slower-migrating molecule (80K) was more intensely labeled in BHK cells than in Vero cells, and the molecule migrating at about 56K to 60K, precipitated from Vero cells, was

represented as two species in BHK cells. All three of the major polypeptides precipitated from BHK cells by antibodies from hybridoma 17βC2 were labeled with [^{3}H]glucosamine, whereas in Vero cells, only the 56K to 60K species incorporated this label (1). Upon pulse-labeling of BHK cells with [^{35}S]methionine for 10 min at 5 h after infection, the radioactivity was associated with molecules of about 50K, 62K, and 68K; after a 5-h chase, it was associated primarily with 62K to 64K and 88K to 91K species (data not shown).

DISCUSSION

The major glycoproteins specified by HSV are all structural components of the virion envelope and play a role in all aspects of virus-cell interactions (27). A characterization of this important group of viral antigens is thus likely to further our understanding of viral infection and to indicate possible means of preventing it.

To date, characterization of many virally coded antigens, including the glycosylated ones, has been hampered by the genetic complexity of the virus and by the lack of monospecific sera able to react only with molecules of unique antigenic makeup. These difficulties have been overcome by the development of the hybridoma technology (11), which provides well-defined and monospecific antibodies ideally suited as reference sera for the identification and characterization of antigens and the study of their synthesis, processing and transport. In the present study, we have therefore undertaken a reexamination of all of the glycoproteins known to be coded by HSV-2 (15, 17, 27), using monoclonal antibodies which we have raised against each one of them.

Earlier studies on HSV-1 glycoproteins have indicated that, unlike the others, glycoprotein gA is not incorporated into virions (26). Subsequently, it has been shown that this glycoprotein is antigenically and possibly structurally similar to glycoprotein gB and, as such, might represent one form of the gAB polypeptide (5). A similar hypothesis has been put forward for the gA and gB molecules of HSV-2 (6). In our study we have made use of a monoclonal antibody against the gAB complex (20αD4) as well as of an anti-gAB serum raised in rabbits against the purified gAB molecules of HSV-2 (6). The two antisera were shown to immunoprecipitate molecules with similar electrophoretic mobility from extracts of infected cells; in addition, they cross-reacted fully so that removal of the molecules recognized by one antibody also eliminated the species reacting with the second antibody. Finally, peptide mapping of all of the major species recognized by the two sera indicated that within the gAB complex all molecules share the same protein backbone but differ as to the extent of glycosylation. Thus, these data constitute the first direct demonstration of the identity of the gA and gB proteins and confirm the hypothesis of Eberle and Courtney (6).

The gAB molecules recognized by 20αD4 antibodies in two cell lines, Vero or BHK, differed to some extent, indicating that processing of these viral antigens is in part cell-type specific. Similar observations have been reported by Pereira et al. (18) for the gAB molecules in Vero and HEp-2 cells and by Para et al. (17) for the gE molecules in different cell lines. The main reasons for the observed differences are likely related to different efficiencies of glycosylation in different cell types as well as to different processing of underglycosylated or unglycosylated products.

Similar but more pronounced differences were also observed for glycoprotein gD in Vero and BHK cells. In this case, the total amount of synthesis as well as the number of precursor molecules synthesized appeared reduced in Vero cells as compared with BHK cells. In our studies we have used two monoclonal antibodies against gD, 17βA3 and 18βB3; both of these antibodies were able to react with the precursor and the fully glycosylated form of glycoprotein gD, but they differed from one another in that 17βA3 antibodies recognized only the gD molecules specified by HSV-2, whereas 18βB3 serum reacted also with glycoprotein gD of HSV-1. The existence of type-specific and type-common domains within the gD molecule has been reported by others (7, 8); our data confirm this observation and in addition suggest the potential usefulness of monoclonal sera reacting with different epitopes for the immunological characterization of antigens and for the typing of viral isolates (19).

In contrast to glycoproteins gAB and gD, which have been shown to carry both specific and common determinants (3, 6) (Table 1), glycoprotein gC appears to be exclusively type specific (6). This might indicate that the gC of HSV-1 and the gC of HSV-2 are entirely different molecules, a hypothesis supported by the fact that the coding sequences for this glycoprotein do not map in the same region in the genome of the two viruses (23). That all four of our monoclonal antibodies to gC are type specific is also in agreement with (although does not prove) this hypothesis. An interesting feature of the monoclonal antibodies reacting with gC is that they precipitated antigens with different electrophoretic mobilities. These molecules appear to share methionine-containing peptides, but preliminary evidence suggests that they are only partially related from an immunological point of view. A similar behavior has been reported for

rabbit antisera to gC and gX of HSV-2 by Eberle and Courtney (6). The structural identity of the gX molecule was not established in their study, but it was shown that this molecule was immunologically related, though not identical, to the gC antigen. On the basis of these and our data, it seems therefore likely that all four sera (18αA5, 13αC5, gC, and gX) recognize glycoprotein gC of HSV-2 but possibly react with different forms of this antigen.

Both HSV-1 and HSV-2 code for a glycoprotein, gE, with Fc receptor binding capacity (9, 17). We have previously reported on the characterization of two HSV-2 glycosylated antigens synthesized in Vero infected cells and reacting with antibodies from hybridoma 17αA2 or 17βC2 (1). In the absence of direct measures of the ability to bind Fc receptors or of comparison with reference sera, we had tentatively designated the antigen recognized by 17αA2 as HSV-2 gE and the one reacting with 17βC2 as gF (1). Recent data by P. G. Spear (personal communication) have, however, provided direct evidence that the 17βC2 antibodies are reacting with HSV-2 gE, whereas 17αA2 antibodies react with gF. As mentioned above, we have therefore used this revised designation in the present report. In addition to 17αA2, we have identified three other antibodies (17αCl, 17αD2, and 11αA5) which exhibit the same specificity towards gF. All of these antibodies are type specific (which may suggest the absence of type-common domains in the gF molecule), and none appears to have neutralizing activity.

With the possible exception of gF, which has not been characterized in this respect, all known HSV glycoproteins become structural components of the virions. Studies on the kinetics of synthesis of the HSV-1 glycoproteins have however indicated that they are synthesized at different times during the infectious cycle (2, 4, 14), suggesting that they might fulfill distinct roles in virus replication and assembly. In the present study, we present similar evidence for the synthesis of the HSV-2 glycoproteins in BHK infected cells. Glycoproteins gE and gD can be detected as early as 1 to 2 h after infection and are synthesized at maximal rates at 3 to 5 and 5 to 7 h, respectively; on this basis, they could therefore be classified as early or β antigens. Similarly, glycoprotein gAB is present at 1 to 3 h after infection, but it continues to be synthesized throughout the cycle, with maximal synthesis at 9 to 11 h or later. On the other hand, gC and gF are not present in detectable amounts before 3 to 5 h after infection and attain maximum rates of synthesis only during or after DNA synthesis, a behavior exhibited by late or γ antigens. Qualitatively similar differences in the course of synthesis of gE and gF were also observed in Vero infected cells, where gE reached maximum synthesis at 5 to 7 h and gF at 9 to 11 h or later; it is not clear at present whether the different time course obtained in the two cell systems is dependent upon the observed differences in the processing of glycoproteins in different cells or due to different courses of infection between the two experiments.

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