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Herpesvirus-Induced "Early" Glycoprotein: Characterization and Possible Role in Immune Cytolysis

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Glycoprotein GVP-11 (molecular weight, 71,500), induced by bovine herpesvirus type 1, was detected on the external surface of infected cells. It could be categorized as an "early" or "β" class protein since it was synthesized early in the infectious process and its expression was not dependent upon prior viral DNA replication in the infected cells. Monoclonal antibodies directed against GVP-11 immunoprecipitated that glycoprotein and some low-molecular-weight polypeptides from infected cells labeled with either [^{35}S]methionine or [^{3}H]glucosamine. Immunoprecipitation of extracts from cells surface labeled with ^{125}I yielded an additional 138,000-molecular-weight polypeptide. Tunicamycin- or bromovinyl deoxyuridine-treated infected cells yielded polypeptides that were smaller in size than corresponding glycoproteins in untreated cells. Tunicamycin-sensitive glycosylation appeared to be necessary for the expression of the glycoproteins on the surface of the infected cells. The monoclonal antibodies directed against GVP-11 and serum from an immune cow could participate in antibody- and complement-mediated immunocytolysis of infected cells, and this immunocytolysis could be enhanced by arresting cells in the early phase of viral gene expression by treatment with inhibitors of viral DNA synthesis.

During infection, the expression of herpesvirus genes is controlled in a manner such that virus-induced proteins can be categorized as "immediate early" (α), "early" (β), or "late" (γ) depending upon the temporal order of their synthesis and the degree to which their synthesis is dependent upon the successful progression of certain physiological processes in the infected cell (13, 18, 25). Thus only immediate early genes are transcribed in the absence of prior protein synthesis, and in the absence of viral DNA synthesis, infected cells contain relatively low concentrations of late gene products (5, 13, 18, 20, 25).

Herpes simplex viruses specify a number of glycoproteins (2, 3, 8, 9, 21, 24) which are located on the virion envelope as well as on the surface of infected cells. These glycoproteins have been termed "social" proteins (14) since they mediate interactions between virions and host cells (24, 26, 27), between infected cells (11, 19), and between various components of the immune system and virions or infected cells (15, 23, 26, 27).

Among virus-induced glycoproteins, any early glycoproteins that are expressed on the surface of infected cells would be of particular interest for a number of reasons. (i) During viral replication after a primary infection, early glycoproteins would probably be the first viral gene products recognized by the afferent arm of the host's immune system. (ii) During subsequent infections or episodes of reactivation, early glycoproteins would again be the first viral targets available to the efferent arm of the immune system. (iii) These proteins may be the only viral targets available to the immune system in individuals who have been treated with antiviral agents that block viral DNA synthesis. Since at least some of these agents are virostatic and do not destroy infected cells (unpublished observation), it is important that these infected cells be recognized and destroyed by the immune system. Otherwise these drugs would merely delay, rather than prevent, replication. (iv) Early glycoproteins may be the only targets expressed in the absence of viral DNA synthesis in latently infected cells.

Recently (13) we identified an early glycoprotein in cells infected with bovine herpesvirus type 1 (BHV-1). In this communication we describe the characterization of this glycoprotein. Our results indicate that although this glycopro-

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tein can act as a target for immune cytolysis, its ability to function as such may be hindered by the expression of late viral gene products.

MATERIALS AND METHODS

Virus and cells. Strain P8-2 of BHV-1 was cultured in Madin Darby bovine kidney (MDBK) cells. Details of cell growth, virus purification, infection, and labeling of cells have been described previously (1, 13).

Immunization of mice and preparation of monoclonal antibodies. Female BALB/c mice obtained from Jackson Laboratories were injected intraperitoneally with 0.1 ml of purified BHV-1, ultrasonically emulsified with an equal volume of Freunds complete adjuvant. The mice were injected again 2 weeks later with virus emulsified in Freunds incomplete adjuvant, followed by subcutaneous injections of purified virus in Freunds incomplete adjuvant administered at 15-day intervals. Four days before the fusion, mice were injected via the tail vein with 0.1 ml of purified virus in phosphate-buffered saline.

Spleen cells were fused with NS-1 cells, and hybrid cells were selected as described by Kennett et al. (10). Hybridomas were screened for their ability to produce antibodies against BHV-1 proteins by indirect immunofluorescence, using as test cells an equal mixture of BHV-1-infected and uninfected cells acetone fixed onto printed slides (Reboz Surgical Instrument Co., Washington, D.C.). The secondary antibody was fluorescein-conjugated goat anti-mouse (H and L chain) immunoglobulin (Cappel Laboratories, Cochranville, Pa.). Identification of cultures secreting antibody to herpesviruses was later confirmed by immunoprecipitation. Positive clones were recloned at least once and injected into pristane (2, 6, 10, 14-tetramethylpentadecane)-treated BALB/c mice for production of ascites containing specific antibody.

Preparation of antigen for immunoprecipitation. Radioactively labeled cells were resuspended in RIPA buffer (0.05 M Tris-hydrochloride [pH 7.0], 0.15 M NaCl, 1% sodium deoxycholate, 1% Triton X-100) and sonicated on ice for 3 s at a setting of 4 on a Sonifier cell disrupter (Ultrasonics Inc., Plainsview, N.Y.). The cell debris was removed by centrifugation at 80,000 × *g* for 1 h, and the supernatant was used immediately for immunoprecipitation. Inclusion of 0.1 mM phenylmethylsulfonyl fluoride in RIPA buffer did not alter the pattern of immunoprecipitation.

Immunoprecipitation. Ten microliters of ascites fluid was added to BHV-1 antigen in 200 μl of RIPA buffer. After standing overnight at 4°C, immunoglobulin G fraction of rabbit anti-mouse immunoglobulin (Cappel Laboratories) was added, and incubation was continued for 4 h more. Ten milligrams of protein A Sepharose CL-4B beads (Pharmacia Fine Chemicals, Inc., Sweden) in 100 μl of RIPA buffer was then added. After 45 min, the Sepharose beads were washed four times with RIPA buffer containing 0.1% sodium dodecyl sulfate, resuspended in 50 μl of sample buffer (0.06 M Tris-hydrochloride [pH 6.8], 0.15 M β-mercaptoethanol, 0.00125% bromophenol blue, 1.25% sodium dodecyl sulfate, 12.5% glycerol), and heated in boiling water for 4 min.

Analysis of proteins by polyacrylamide gel electrophoresis. Samples were electrophoresed in the presence of sodium dodecyl sulfate through polyacrylamide gels (12) using a 15-cm vertical electrophoresis apparatus (Richter Scientific, Vancouver, British Columbia). Gels containing ^{125}I- or ^{35}S-labeled samples were autoradiographed on 3M film. ^{3}H- and ^{14}C-containing gels were soaked in En^{3}Hance (New England Nuclear Corp., Lachine, Quebec) and fluorographed on preflashed film.

Iodination of cell surface proteins. A technique modified from that of Glorioso and Smith (6) was used. Infected or mock-infected cells were harvested by mild trypsinization, which has previously been shown not to alter surface antigen expression or immune lysis (1), and washed once with Hanks balanced salt solution (HBSS)–2% fetal bovine serum (FBS) and once with HBSS containing KI at a concentration of 10^{-5} M. The cells were then counted in a hemacytometer after staining with trypan blue, and only cultures with greater than 95% viable cells were used. The cells (2×10^6) were resuspended in 0.49 ml of HBSS–10^{-5} M KI and mixed with 25 μl of a 2% solution of β-D-glucose, 100 μl of Enzymobeads (Bio-Rad Laboratories, Mississauga, Ontario), and 0.5 mCi of $Na^{125}I$ (New England Nuclear Corp.). After standing at room temperature for 30 min, 10 ml of HBSS–10^{-4} M KI was added. The cells were then pelleted and washed three times with HBSS–10^{-4} M KI. The cells retained their viability throughout the procedure. Soluble antigen was then prepared from the cells as outlined above.

Antibody- and complement-mediated cytolysis. BHV-1-infected or mock-infected MDBK cells were labeled overnight with 100 μCi of $Na_2{}^{51}CrO_4$ (New England Nuclear Corp.). The cells were then harvested and washed twice with Veronal buffer (0.15 M NaCl, 3 mM barbitol, 1.8 mM sodium barbitol, 0.5 mM magnesium chloride, 0.15 mM calcium chloride) containing 2% FBS, and the concentration of viable cells was determined by counting trypan blue-unstained cells in a hemacytometer. The target cells (2×10^4) in 100 μl of Veronal buffer–2% FBS were dispensed into disposable tubes (12 by 75 mm). Serum was added to the tubes which were then incubated at 37°C for 30 min. A 100-μl amount of rabbit complement (Cappel Laboratories) diluted 1/10 in Veronal buffer–2% FBS was added to each tube, and incubation was continued for an additional 90 min. A 1-ml amount of Veronal buffer–2% FBS was added to each tube, cells were pelleted by centrifugation for 10 min at 500 × *g*, 1 ml of supernatant was removed, and the amount of ^{51}Cr was determined in a Searle model 1185 γ counter. All tests were performed in triplicate, and each test contained samples lysed with 3% Triton X-100 to estimate total releasable radioactivity. The percent specific ^{51}Cr release was used as an indicator of cell death. This index was calculated as: 100 × [(counts per minute of ^{51}Cr released from cells with serum plus complement) − (counts per minute released with complement alone)]/[(counts per minute released from cells with Triton X-100) − (counts per minute released with complement alone)].

RESULTS

Early glycoprotein GVP-11. We have reported earlier (13) that BHV-1 induces at least one glycoprotein, GVP-11, that can be categorized as an early protein. To confirm this observation,

two approaches were used. First, cultures of MDBK cells infected at a multiplicity of infection of 10 PFU per cell with BHV-1 were labeled for 2-h intervals with [^{35}S]methionine or [^{3}H]glucosamine. Cells harvested at the end of the 2-h labeling periods were analyzed by polyacrylamide gel electrophoresis. Figure 1 shows that expression of BHV-1-induced polypeptides and glycoproteins was temporally controlled. Among the glycoproteins, GVP-11 was labeled in the first 2 h after infection, whereas GVP-9, the most heavily labeled glycoprotein in infected cells, was not labeled until later in the infection process.

The second approach was based on the observation that in the absence of viral DNA synthesis only early polypeptides are synthesized. Figure 2 shows that in infected cells treated with cytosine arabinoside (araC), a nonspecific inhibitor of DNA synthesis (lanes E and F), or phosphonoformate (PFA), an inhibitor that preferentially blocks herpesvirus DNA synthesis (lanes C and D), more GVP-11 was radioactively labeled than in untreated cells (lane B). In contrast, inhibition of DNA synthesis reduced the radioactivity associated with GVP-9, a late glycoprotein (13). Bromovinyl deoxyuridine (BVdU), an inhibitor which effectively prevents the production of infectious BHV-1 (G. A. Weinmaster, V. Misra, R. McGuire, L. A. Babiuk, and E. Declercq, Virology, in press) but does not block viral DNA synthesis (results not shown), did not alter the relative amounts of GVP-11 and GVP-9. However, the glycopolypeptides made in BVdU-treated cells appeared to be more electrophoretically mobile than the corresponding glycoproteins in untreated cells.

Monoclonal antibodies to GVP-11. To further characterize GVP-11, we made hybridomas that secreted antibody against BHV-1 proteins. Monoclonal antibodies against BHV-1 proteins were detected by immunofluorescence and immunoprecipitation. One such hybridoma cell line produced antibodies, of the immunoglobulin G1 class, which immunoprecipitated GVP-11 from antigen extracts of BHV-1-infected cells labeled with [^{35}S]methionine (Fig. 3).

GVP-11 expressed on the surface of infected cells. GVP-11 is a glycoprotein since it is labeled with [^{3}H]glucosamine and is associated with the

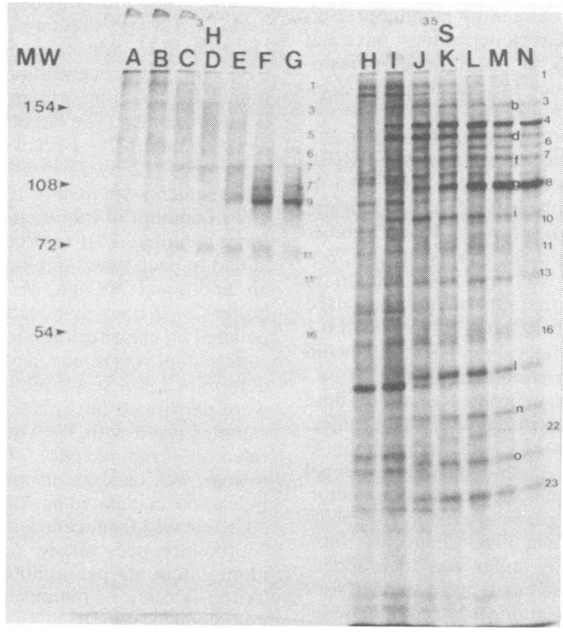

FIG. 1. Time course of BHV-1 glycoprotein synthesis. BHV-1-infected cells were labeled with [^{3}H]glucosamine (lanes A to G) or with [^{35}S]methionine (lanes H to N) at 0 to 2 (lane B), 2 to 4 (lane C), 4 to 6 (lane D), 6 to 8 (lane E), 10 to 12 (lane F), and 12 to 14 (lane G) h after infection. Mock-infected cells were labeled during the 0- to 2-h interval (lanes A and H). At the end of the labeling period, the cells were harvested and analyzed by electrophoresis on 7.5% polyacrylamide gels. Structural polypeptides are numbered, while lowercase letters indicate nonstructural polypeptides (13) and numbers (molecular weight × 1,000) with arrows indicate the position of molecular weight markers electrophoresed in the same gel.

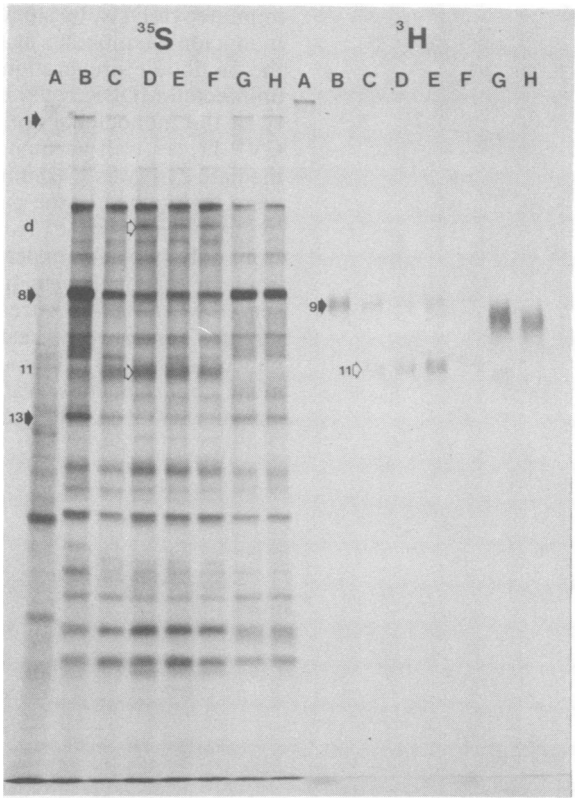

FIG. 2. Effect of PFA, araC, or BVdU on proteins and glycoproteins synthesized in BHV-1-infected cells. Mock-infected cells (lane A), BHV-1-infected cells (lane B), or infected cells treated with 100 μg of PFA per ml (lane C), 500 μg of PFA per ml (lane D), 50 μg of araC per ml (lane E), 100 μg of araC per ml (lane F), 5 μg of BVdU per ml (lane G), or 20 μg of BVdU per ml (lane H) were labeled with either [^{3}H]glucosamine or [^{35}S]methionine. In each case, the drugs were added immediately after virus adsorption. At 20 h after infection the cells were harvested and analyzed on a 7.5% polyacrylamide gel.

BHV-1 virions (13). It would, however, be immunologically of little interest if it was not expressed on the surface of infected cells. To establish that GVP-11 was a surface protein, the external proteins of infected cells and infected cells treated with PFA, araC, or BVdU were radioactively labeled with ^{125}I. Antigen extracts prepared from these cells were then analyzed by polyacrylamide gel electrophoresis either directly or after immunoprecipitation with the monoclonal antibodies directed against GVP-11. The results (Fig. 4) show that GVP-11, along with the other BHV-1-induced glycopolypeptides, was expressed on the surface of infected cells and of infected cells treated with the inhibitors. In BVdU-treated cells, however, the glycoproteins were of a lower molecular weight than in untreated cells. These results also indicate that; in the absence of viral DNA synthesis, more GVP-11 was made inside infected cells (Fig. 2), but the amount of this glycoprotein on the surface of these cells was not any greater than that on untreated cells. In addition to immunoprecipitating GVP-11, the monoclonal antibodies also precipitated 138,000-dalton and 53,000-dalton polypeptides. The immunoprecipitation, however, was specific since no GVP-9 was precipitated.

Partial blockage of the glycosylation of GVP-11 by tunicamycin. To further characterize GVP-11 we examined the effect of tunicamycin, an antibiotic that inhibits the addition of carbohydrate side chains attached by N-terminal glycosydic linkages (22), on the glycosylation and surface expression of GVP-11. Figure 5 shows that the glycosylation of GVP-11, as well as other glycoproteins, was only partially inhibited by tunicamycin and that monoclonal antibody directed against GVP-11 immunoprecipitated a [^{3}H]glucosamine-labeled polypeptide that was lower in molecular weight than the glycopolypeptide precipitated from uninfected cells. Although GVP-11 was glycosylated in tunicamycin-treated cells, albeit partially, tunicamycin-sensitive gly-

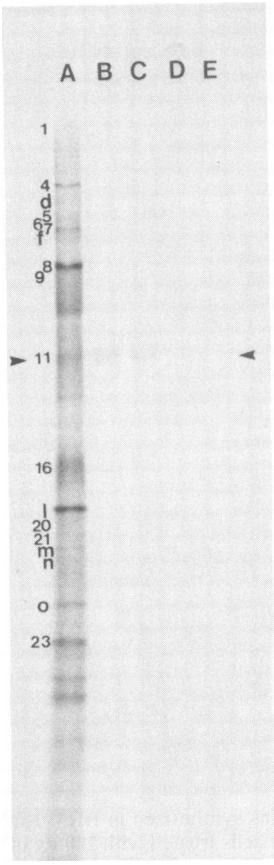

FIG. 3. Immunoprecipitation of [^{35}S]methionine-labeled BHV-1-infected-cell proteins with monoclonal antibodies. Extract from infected whole cells (lane A) was immunoprecipitated with monoclonal antibodies directed against GVP-11, clone 28B (lane B), and clone 28C (lane C) or was immunoprecipitated with monoclonal antibodies against VP-23, clone 27A (lane D). Mock-infected cell extract was immunoprecipitated with monoclonal antibodies directed against GVP-11 (lane E).

cosylation was necessary for expression of the glycoproteins on the surface of infected cells since GVP-11 could not be surface iodinated in tunicamycin-treated cells (Fig. 6) and these cells could not be killed by antibody- and complement-mediated cytolysis (Table 1).

Ability of monoclonal antibodies against GVP-11 and complement to mediate immunocytolysis. The monoclonal antibodies directed against GVP-11 could mediate immune cytolysis of BHV-1-infected cells in the presence of rabbit complement, whereas monoclonal antibodies directed against VP-23, a nucleocapsid protein, were unable to do so (Table 1). Serum from a cow infected with BHV-1 and reactivated with dexamethasone (16) was also able to mediate immunocytolysis, but to a lesser extent than the monoclonal antibodies against GVP-11. None of the antibody preparations were able to lyse uninfected MDBK cells. Interestingly, the ability of the monoclonal antibody directed against GVP-11, as well as immune bovine serum, to mediate cytolysis was enhanced if infected cells were maintained in the early phase of infection by treatment with PFA. Although the results from only two such experiments are depicted in Table 1, this enhancement was consistently observed whenever PFA-treated cells were used as targets. Tunicamycin-treated cells, on the other hand, were not lysed by antibodies and complement, confirming observations (Fig. 5 and 6) that although GVP-11 was partially glycosylated in

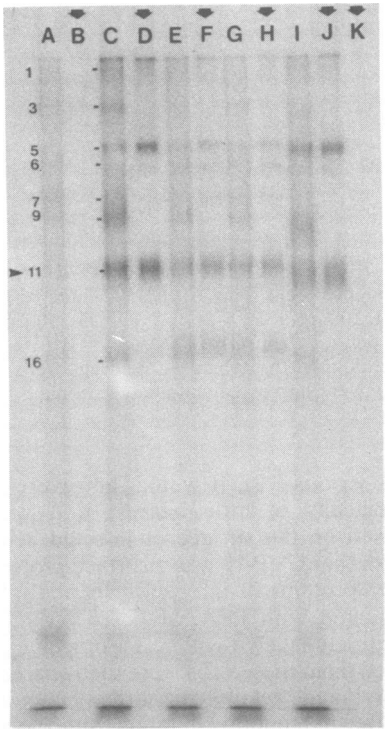

FIG. 4. Cell surface proteins labeled with ^{125}I-labeled Enzymobeads (Bio-Rad) from mock-infected (lanes A and B), BHV-1-infected cells (lanes C, D, and K), and BHV-1-infected cells treated with 75 μg of araC per ml (lanes E and F), with 150 μg of PFA per ml (lanes G and H), or with 7.5 μg of BVdU per ml (lanes I and J). Whole-cell extracts (lanes A, C, E, G, and I). Cell extracts immunoprecipitated with monoclonal antibodies directed against GVP-11 (lanes B, D, F, H, and J). Cell extracts from BHV-1-infected cells immunoprecipitated with monoclonal antibodies directed against VP-23 (lane K).

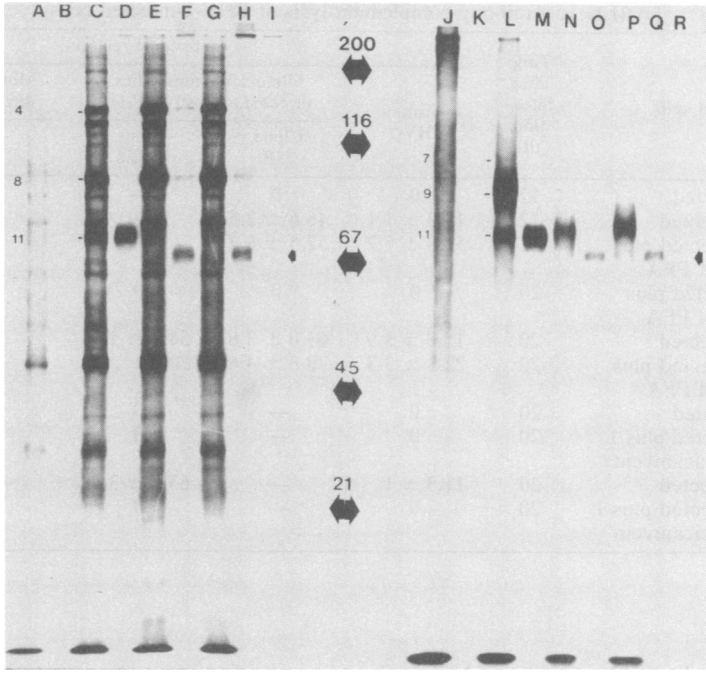

FIG. 5. Proteins and glycoproteins in mock-infected and BHV-1-infected cells treated with tunicamycin. Mock-infected cells (lanes A, B, J, and K) or BHV-1-infected cells were labeled with [^{35}S]methionine (lanes A to I) or [^{3}H]glucosamine (lanes J to R). BHV-1-infected cells were treated with either 1 μg of tunicamycin (lanes E, F, N, and O) or 2.5 μg of tunicamycin (lanes G, H. P, and Q). Cell extracts were prepared and analyzed by polyacrylamide gel electrophoresis either directly (lanes A, C, E, G, J, L, N, and P) or after immunoprecipitation with monoclonal antibodies directed against GVP-11 (lanes B, D, F, H, K, M, O, and Q) or with monoclonal antibodies directed against VP-23 (lanes I and R).

the presence of tunicamycin, the antibiotic blocked expression of the glycoprotein on the surface of infected cells.

DISCUSSION

The glycoprotein GVP-11, induced by BHV-1, could be categorized as an early or β protein because it was synthesized early in the infectious process and because its expression was not dependent on prior viral DNA replication in the infected cell. Inhibition of DNA synthesis led to increased intracellular synthesis of this glycoprotein in BHV-1-infected cells, although this was not reflected by increased expression of GVP-11 on the surface of cells. Such early antigens have been observed on Epstein-Barr virus-infected cells (5, 20), and unpublished observations (Balachandran and Rawls, personal communication) suggest that at least two of the glycoproteins specified by herpes simplex virus type 2 may be expressed earlier in infection than others.

In this communication we have used monoclonal antibodies to further characterize GVP-11. Monoclonal antibodies directed against this glycoprotein immunoprecipitated GVP-11 and small amounts of a glycosylated polypeptide of lower molecular weight from extracts of cells labeled after infection with either [^{3}H]glucosamine or [^{35}S]methionine. However, when extracts of cells surface labeled with ^{125}I were used, the monoclonal antibodies also precipitated a larger (138,000-molecular-weight) polypeptide which possessed the same electrophoretic mobility as the virus-induced glycoprotein, GVP-5. Since the 138,000-dalton polypeptide was not precipitated from [^{35}S]methionine- or [^{3}H]glucosamine-labeled cell extracts, it may be a virus-induced non-glycosylated polypeptide that has a low methionine content. Alternatively, the 138,000-dalton polypeptide may be a host-specified surface protein that is intimately associated with GVP-11 in the infected cell and hence is immunoprecipitated with it. Host-specified polypeptides would not be extensively labeled in infected cells to which radioactive amino acids were added after the cessation of host cell gene expression (13) and therefore would not appear in autoradiograms of the immunoprecipitates. We are at present trying to establish the nature of the 138,000-dalton polypeptide.

Tunicamycin, an antibiotic that inhibits the

TABLE 1. Antibody-complement lysis of BHV-1-infected cells

Expt	Target cells	Time after infection (h)	% Specific release			
			Anti-BHV-1	Monoclonal antibodies directed against GVP-11		Monoclonal antibodies directed against VP-23
				Clone 28B	Clone 1-5E8	Clone 27A
1	Mock infected	12	0	0	—[a]	0
	BHV-1 infected	12	13.3 ± 1.1	46.6 ± 2.5	—	0
	BHV-1 infected plus 100 μg of PFA	12	51.8 ± 5.7	57.5 ± 6.2	—	0
2	Mock infected plus 100 μg of PFA	20	0	0	0	0
	BHV-1 infected	20	15.8 ± 3.9	63.0 ± 3.6	54.3 ± 2.9	3.9 ± 0.8
	BHV-1 infected plus 100 μg of PFA	20	22.3 ± 3.3	78.0 ± 4.6	80.0 ± 2.1	0
3	Mock infected	20	0	—	0	—
	Mock infected plus 1 μg of tunicamycin	20	0	—	0	—
	BHV-1 infected	20	11.3 ± 1	—	63 ± 3.4	—
	BHV-1 infected plus 1 μg of tunicamycin	20	0	—	0	—

[a] —, Not done.

synthesis and attachment to proteins of N-linked carbohydrate side chains (22) only partially inhibited the glycosylation of GVP-11 and other BHV-1-induced glycoproteins. The tunicamycin-sensitive glycosylation was, however, necessary for expression of GVP-11 on the surface of infected cells since the glycoproteins were not iodinated on tunicamycin-treated cells which were also not susceptible to lysis by antibody and complement. The observation that tunicamycin only partially inhibited the glycosylation of BHV-1-induced glycoproteins did not come as a surprise as Norrild and Pedersen (Abstr. Int. Workshop on Herpesviruses, p. 108, 1981) have shown that tunicamycin only partially inhibits the glycosylation of at least some of the herpes simplex virus-induced glycoproteins. However, unexpectedly, we found that treatment of infected cells with BVdU, a thymidine analog which prevents the synthesis of infectious BHV-1, also led to the synthesis of glycosylated polypeptides which possessed a lower molecular weight than those of untreated cells. Since BVdU did not affect the electrophoretic mobility of any of the non-glycosylated polypeptides, it must have interfered only with post-translational glycosylation. Unlike tunicamycin, BVdU-sensitive glycosylation did not prevent the expression of glycoproteins on the surface of infected cells since these glycoproteins could be labeled with ^{125}I and these cells were susceptible to immunocytolysis. These results suggest that GVP-11 may have two kinds of carbohydrate side chains. One kind, which is attached by a tunicamycin-sensitive step, is necessary for

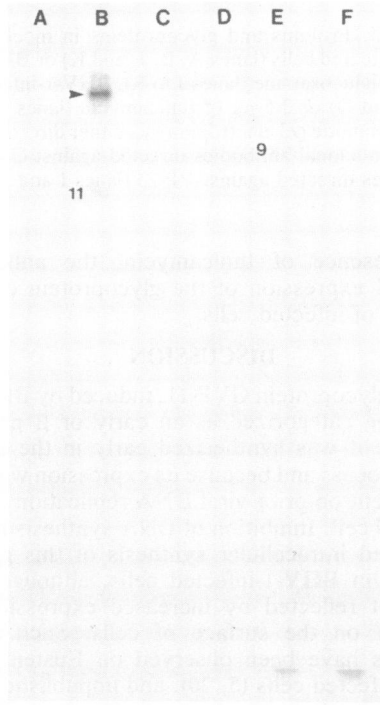

FIG. 6. Cell surface proteins labeled with ^{125}I-labeled Enzymobeads (Bio-Rad) from mock-infected cells (lanes A and D), BHV-1-infected cells (lanes B and E), or BHV-1-infected cells treated with 1.0 μg of tunicamycin (lanes C and F). Cell extracts were either electrophoresed through 7.5% polyacrylamide gels directly (lanes D, E, and F) or after immunoprecipitation with monoclonal antibodies directed against GVP-11 (lanes A, B, and C).

expression of the glycoprotein on the surface of infected cells. The other side chains, inhibited by BVdU, may be necessary for the production of infectious virus. BVdU is phosphorylated by herpesvirus-induced thymidine kinases (4; Weinmaster et al., in press) and is thought to interfere with virus replication by being incorporated into viral DNA (E. DeClercq, J. Descamps, and H. S. Allandeen, Abstr. Int. Workshop on Herpesviruses, p. 180, 1981). Our results suggest that it may also interfere with virus-induced glycoprotein synthesis or processing. This alternate mechanism of BVdU action is at present being investigated.

The monoclonal antibodies directed against GVP-11, as well as specific bovine antiserum, could participate in antibody- and complement-mediated immune cytolysis of BHV-1-infected cells as well as of infected cells in which only the early glycoproteins were expressed. This indicates that GVP-11 is probably accessible to the immune system and can participate as a target for immunocytolysis. The results also indicate that although antiherpetic agents such as PFA are inhibitory in vitro (results not shown), in conjunction with the immune system in vivo, they may destroy virus-infected cells. Interestingly, infected cells arrested in the early phase by PFA were more sensitive to antibody- and complement-mediated cytolysis than untreated cells. PFA-treated cells did not express more GVP-11 on their surface; this enhanced sensitivity to immunocytolysis in the absence of viral DNA synthesis and consequently in the absence of late glycoproteins and other late functions suggests that early glycoprotein-targeted immunocytolysis may in fact be suppressed or camouflaged by late functions. We are presently conducting experiments with other viral DNA synthesis inhibitors to determine whether they have similar effects. This should hopefully help us determine the reason for increased sensitivity to immune cytolysis. An alternative explanation for the increased sensitivity of PFA-treated cells to antibody and complement lysis would be that these cells are less able to repair the membrane damage caused by complement.

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LITERATURE CITED

1. **Babiuk, L. A., R. C. Wardley, and B. T. Rouse.** 1975. Defense mechanisms against bovine herpesvirus: relationship of virus-host cell events to susceptibility to antibody-complement cell lysis. Infect. Immun. **12**:958–963.
2. **Balachandran, N., D. Harnish, R. A. Killington, S. Bacchetti, and W. E. Rawls.** 1981. Monoclonal antibodies to two glycoproteins of herpes simplex virus type 2. J. Virol. **39**:438–446.
3. **Baucke, R. B., and P. G. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. V. Identification of an Fc-binding glycoprotein. J. Virol. **32**:779–789.
4. **Declercq, E., J. Descamps, G. Desomer, P. J. Barr, A. S. Jones, and R. T. Walker.** 1979. *E*-5-(2-Bromovinyl)-2′-deoxyuridine: a potent and selective antiherpes agent. Proc. Natl. Acad. Sci. U.S.A. **76**:2947–2951.
5. **Ernberg, I., G. Klein, F. M. Kourilsky, and D. Silvestre.** 1974. Differentiation between early and late membrane antigen on human lymphoblastoid cell-lines infected with Epstein-Barr virus. I. Immunofluorescence. J. Natl. Cancer Inst. **53**:61–65.
6. **Glorioso, J. C., and J. W. Smith.** 1977. Immune interactions with cells infected with herpes simplex virus: antibodies to radioiodinated surface antigens. J. Immunol. **118**:114–121.
7. **Glorioso, J. C., L. A. Wilson, T. W. Fenger, and J. W. Smith.** 1978. Complement mediated cytolysis of HSV-1 and HSV-2 infected cells: plasma membrane antigens reactive with type-specific and cross-reactive antibody. J. Gen. Virol. **40**:443–454.
8. **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.
9. **Honess, R. W., and B. Roizman.** 1975. Proteins specified by herpes simplex virus. XIII. Glycosylation of viral polypeptides. J. Virol. **16**:1308–1326.
10. **Kennett, R. H., K. A. Denis, A. S. Tung, and N. R. Klinman.** 1978. Hybrid plasmacytoma production: fusion with adult spleen cells, monoclonal spleen fragments, neonatal spleen cells and human spleen cells. Curr. Top. Microbiol. Immunol. **81**:77–91.
11. **Knowles, R. W., and S. Person.** 1976. Effects of 2-deoxyglucose, glucosamine, and mannose on cell fusion and the glycoproteins of herpes simplex virus. J. Virol. **18**:644–651.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
13. **Misra, V., R. M. Blumenthal, and L. A. Babiuk.** 1981. Proteins specified by bovine herpesvirus 1 (infectious bovine rhinotracheitis virus). J. Virol. **40**:367–378.
14. **Norrild, B.** 1980. Immunochemistry of herpes simplex virus glycoproteins. Curr. Top. Microbiol. Immunol. **90**:67–106.
15. **Norrild, B., S. L. Shore, T. L. Cromeans, and A. J. Nahmias.** 1980. Participation of three major glycoprotein antigens of herpes simplex virus type 1 early in the infectious cycle as determined by antibody-dependent cell-mediated cytotoxicity. Infect. Immun. **28**:38–44.
16. **Pastoret, P. P., L. A. Babiuk, V. Misra, and P. Griebel.** 1980. Reactivation of temperature-sensitive and non-temperature-sensitive infectious bovine rhinotracheitis vaccine virus with dexamethasone. Infect. Immun. **29**:483–488.
17. **Pereira, L., T. Klassen, and J. R. Baringer.** 1980. Type-common and type-specific monoclonal antibody to herpes simplex virus type 1. Infect. Immun. **29**:724–732.
18. **Roizman, B., M. Kozak, R. W. Honess, and G. Hayward.** 1974. Regulation of herpesvirus macromolecular synthesis: evidence for multilevel regulation of HSV-1 RNA and protein synthesis. Cold Spring Harbor Symp. Quant. Biol. **39**:687–701.
19. **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.
20. **Sairenji, T., Y. Hinuma, T. Seikazawa, and M. Yoshida.** 1977. Appearance of early and late components of Epstein-Barr virus-associated membrane antigen in Daudi cells superinfected with P3HR-1 virus. J. Gen. Virol. **38**:111-120.

21. **Sarmiento, M., M. Haffey, and P. G. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. III. Role of glycoprotein VP7(B_2) in virion infectivity. J. Virol. **29**:1149–1158.
22. **Schwarz, R. T., and R. Datema.** 1980. Inhibitors of protein glycosylation. Trends Biochem. Sci. **5**:65–67.
23. **Shore, S. L., T. L. Cromeans, and B. Norrild.** 1979. Early damage of herpes infected cells by antibody-dependent cellular cytotoxicity: relative roles of virus-specified cell surface antigens and input virus. J. Immunol. **123**:2239–2244.
24. **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.
25. **Stinski, M. F.** 1978. Sequence of protein synthesis in cells infected by human cytomegalovirus: early and late virus-induced polypeptides. J. Virol. **26**:686–701.
26. **Vestergaard, B. F., and B. Norrild.** 1979. Cross immunoelectrophoretic analysis and virus neutralizing activity of five monospecific sera against five different herpes simplex virus glycoproteins. Int. Agency Res. Cancer Sci. Publ. **24**:225–234.
27. **Yoshino, K., N. Isono, A. Tada, and M. Urayama.** 1979. Studies on the neutralization of herpes simplex virus. X. Demonstration of complement-requiring neutralizing (CRN) and slow-reacting CRN (S-CRN) antibodies in IgG. Microbiol. Immunol. **23**:975–985.