**Herpes Simplex Virus Replication and Protein Synthesis in a Human Blood-derived Cell Line**

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**SUMMARY**

Herpes simplex virus (HSV) types 1 and 2 were shown to replicate in a newly described human cell line (Meg) derived from the peripheral blood of a healthy volunteer. The cell line has both megakaryocyte-like and B cell-like properties. Upon infection with HSV-1 or -2, at a m.o.i. between 0.5 and 5, unlike B and T cells, the Meg cells were growth-arrested and this was accompanied by cytopathic effects and virus replication. The HSV proteins and glycoproteins B and D (gB and gD) made in the blood-derived Meg cells were compared to the corresponding proteins made in the non-blood-derived cell lines, Vero (African green monkey kidney cell) and HEp-2 (human epidermoid carcinoma cell). The maximum level of HSV protein synthesis occurred earlier in the Meg cells than in the Vero and HEp-2 cells. The electrophoretic pattern of HSV-1 and -2 proteins made in the Meg cell line was similar to the corresponding proteins made in the Vero and HEp-2 cell lines; however, some qualitative and quantitative differences were evident. There were no apparent differences detected in the migration pattern of gB made in all three cell lines while significant differences were observed with the gD species. However, upon hydrolysis with *Staphylococcus aureus* V-8 protease of the monoclonal antibody-purified gB and gD, distinct differences were observed in the electrophoretic pattern of the generated peptide fragments of both gB and gD made in the three cell lines. The results demonstrate that a human blood cell can support HSV replication and that species-specific post-translational modification of gB and gD occurs in HSV-infected Vero cells as compared to HSV-infected human cells.

**INTRODUCTION**

Various strains of herpes simplex virus (HSV) differentially affect the social behaviour of the HEp-2 cell line upon infection (Ejercito et al., 1968). This implies that strain-specific viral components inserted into the host cell membrane regulate differential cell to cell interactions (Keller et al., 1970). It has also been shown that a single HSV antiserum will interact very differently with a variety of HSV-infected cell types (Norrild, 1980). This may result from host cell-directed modifications of viral glycoproteins which ultimately become associated with the cell membrane. The major viral antigens expressed on the surface of both HSV virions (Olshevsky & Becker, 1972) and HSV-infected cells (Glorioso & Smith, 1977; Pauli & Ludwig, 1977) are the viral glycoproteins. Glycoproteins are, in fact, the most likely candidates for modulation of both cellular social behaviour (Lloyd, 1975) and for affecting antibody interactions since they can undergo host cell-directed modifications.

Several potential biological functions have already been ascribed to various HSV glycoproteins (Corey & Spear, 1986). The gE binds the Fc portion of immunoglobulin (Ig)G (Baucke & Spear, 1979; Para et al., 1982), gB appears to be required for viral replication and
possibly viral entry and cell fusion (DeLuca et al., 1982; Little et al., 1981; Manservigi et al., 1977; Sarmiento et al., 1979), gD may be involved in adsorption of the virion to the cell (Spear et al., 1978) and HSV glycoproteins are involved in the T cell-mediated lysis of HSV-infected cells (Carter et al., 1984).

The characterization of post-translational modifications of these glycoproteins in specific cell lines and the impact of these alterations on function may greatly further our understanding of the pathology of herpetic infections. Numerous studies have concentrated on the post-translational modifications of HSV glycoproteins (Braun et al., 1984; Cohen et al., 1980; Compton & Courtney, 1984; Eberle & Courtney, 1980; Johnson & Spear, 1983, 1984; Keller et al., 1970; Pereira et al., 1981; Spear & Roizman, 1970; Zezulak & Spear, 1984). Pereira et al. (1981) described differences in the molecular weights of HSV-1 and -2 gA/B (now pgB and gB) and gD synthesized in Vero cells (derived from African green monkey kidney) or HEp-2 cells (derived from human epidermoid carcinoma). Zezulak & Spear (1984) indicated that these observed differences in electrophoretic mobilities may be due, in part, to proteolysis of gB in Vero cells. However, they demonstrated differences in the electrophoretic mobilities of mature forms of gD and gF in these same cell lines. Cohen et al. (1980) have demonstrated that detectable levels of gD in the host cell appear 2 h post-infection (p.i.). The protein initially appears as a 50K core polypeptide followed by glycosylation to a 52K pgD. This species is then modified further by addition of carbohydrate and sialyl residues to a 59K gD product. These studies with infected KB cells (derived from human epidermoid carcinoma) demonstrated a maximal synthesis of gD at about 6 h p.i. which then remained nearly constant (to the last time point of 10 h p.i.). Johnson & Spear (1984), on the other hand, found that gD synthesis in infected HEp-2 and Vero cells reached a maximum peak of synthesis at about 4 to 6 h p.i. and then dropped dramatically over the next few hours with less than 50% of the synthetic levels observed at 10 h p.i. The differences in the apparent translational regulation of gD synthesis may be explained by differences in experimental procedures or that different cells were employed and that some host cell factors may be involved.

In the study of HSV glycoproteins, the choice of host cell is probably critical when determining structural and compositional properties in vitro that may relate to the conditions in vivo. The post-translational modification of HSV glycoproteins in host cells may be both species-specific and tissue-specific. Most of the recent studies described above have evaluated HSV glycoproteins synthesized in animal host cells or malignant human cells. Many studies with B-type, blood-derived, cell lines detected low levels of virus replication which were usually persistent infections with limited c.p.e. (Lehtinen, 1986; Leinbach & Summers, 1979; Rinaldo et al., 1979; Seigneurin et al., 1976). Similar results have also been obtained with T cells (Rinaldo et al., 1978, 1979). We describe in this report, for the first time, a normal human blood cell line (derived from peripheral blood of a healthy volunteer) which can support HSV-1 and -2 replication with the expected kinetics of viral replication, host cell growth inhibition and total cytopathic effects. The cell line has both megakaryocyte-like properties (Morgan & Brodsky, 1985) and B cell-like properties. The megakaryocyte (Meg) represents an excellent candidate blood cell capable of harbouring or replicating all or some of the herpesviruses. The pre-megakaryocyte and maturing megakaryocyte are found primarily in the bone marrow and the lungs. Upon initiation of differentiation/maturation the nucleus replicates and divides several times without cell division, reaching a polyploid state of 16 or 32 N. At maturation, the megakaryocyte sheds thousands of platelets from its cytoplasm into the plasma (Behnke & Pedersen, 1974). The developing megakaryocyte, therefore, has an inordinate capability for DNA replicative processes, an extremely attractive environment for virus replication, and upon its release of platelets into the circulation, a means to disseminate viruses throughout the body. We were the first to describe the observation of herpes-like viruses within the platelet (Soslau, 1983). Furthermore, other types of viral infection have been shown to reduce the platelet count greatly (Oski & Naiman, 1966; Zinkham et al., 1967) as well as a murine cytomegalovirus infection (Petursson et al., 1984) and a human varicella infection (Espinoza & Kuhn, 1974). This latter study also demonstrated virus particles in megakaryocytes at autopsy.

The studies presented in this report demonstrate that a newly derived human blood cell line,
with megakaryocyte-like and B cell-like properties, can fully support HSV-1 and -2 replication in a fashion similar to non-blood-derived cultured cell lines. There are, however, significant differences in the p.i. timing, when maximal levels of virus proteins are synthesized in the Meg cells as compared to HEp-2 and Vero cell lines. The viral gB and gD synthesized in the Meg cells are clearly more similar to those from the human HEp-2 cell line than those from the Vero cell line.

**METHODS**

**Cell culture methods.** All cell lines were maintained in culture medium RPMI 1640 from Gibco. The Vero and HEp-2 cell lines (both obtained from American Type Culture Collection, Rockville, Md., U.S.A.) were grown as monolayers in medium supplemented with 10% foetal calf serum and passaged with 0.01% trypsin-EDTA (Worthington) as they became confluent. The human blood cell line, Meg, was one of the 18 derived as previously described (Morgan & Brodsky, 1985) from the peripheral blood of a normal donor, FOS, and maintained in suspension culture in medium supplemented with 10% human serum. Cell counts were performed on either a haemocytometer or on a Coulter counter (model ZBI). Cell viability was monitored by trypan blue exclusion.

**Virus infection and plaque assay.** Stocks of HSV-1 (KOS) and HSV-2 (333) were routinely grown in human diploid cells (HEL, human embryo lung cells or Flora 5000, human diploid fibroblast cells). Cell-free supernatants were prepared when c.p.e. was extensive and virus stocks were stored at -70 °C. The Vero and HEp-2 cell lines were set up at 3 x 10^4 cells/well (in a 24-well plate; Nunc) the day prior to infection. Cell growth 1 day after passage was minimal due to some cell death and a lag phase induced by trypsinization. The Meg cells were pelleted from suspension, washed once with RPMI, counted and the appropriate number of cells suspended in RPMI supplemented with 10% foetal calf serum. The Vero and HEp-2 cells in monolayers were washed once and then 1 ml fresh medium was added. The virus was added to a 1 ml suspension of cells or to cells in a monolayer at a m.o.i. of 0.05 to 5 for HSV-1 and at a m.o.i. of 3 to 5 for HSV-2 as indicated in the figure legends. Cells were incubated with virus or without virus (mock-infected) for 1 h at 37 °C. The medium plus virus was then removed and the cells resuspended in the appropriate growth medium.

**Virus titres** were determined by plaque assay on primary rabbit kidney cells as previously described (Duff & Rapp, 1971).

**Microscopical methods.** Meg cell morphology and c.p.e. were monitored by both light and electron microscopy. We deposited 5 x 10^4 to 8 x 10^4 cells (0.15 ml aliquots) onto alcohol-washed glass microscope slides by using a cytocentrifuge (Shandon Southern Instruments Inc., Sewickley, Pa., U.S.A.) at 1000 r.p.m. for 5 min. Wright-Giemsa differential stain was used for morphological evaluation.

Cells obtained from control and HSV-infected cultures were washed twice with cold 0.2 M-phosphate buffer pH 7.4, pelleted and fixed in cold 1.25% glutaraldehyde for 30 min for electron microscopy. After a buffer wash, the pellet was post-fixed for 30 min in 1% osmium tetroxide, washed with buffer, and stained with 0.5% uranyl acetate for 30 min. Alcohol dehydration was followed by propylene oxide and Epon infiltration and the pellet was then embedded for 48 h. Samples were thin-sectioned, stained with uranyl acetate and lead citrate and viewed on a Hitachi HU-12A transmission electron microscope.

**DNA isolation.** Control and HSV-infected cells (approximately 4 x 10^6 cells) were cultured for 24 h in medium supplemented with 10 μCi [3H]thymidine/ml (New England Nuclear, 78 Ci/mmol) at various times p.i. The DNA was isolated by a method similar to that previously described (Maniatis et al., 1982). Briefly, cells were pelleted, washed and resuspended in 1 ml of a buffered proteinase K (0.5 mg) solution (10 mM-Tris-HCl, 10 mM-NaCl, 1 mM-EDTA, 0.5% SDS pH 8.0). The proteins were digested overnight at 37 °C after which the DNA was purified with phenol-chloroform, isoamyl alcohol extractions. The DNA was ethanol/sodium acetate-precipitated from the aqueous solution. The purified DNA was then analysed on a caesium chloride equilibrium gradient (initial refractive index 1.4000) run at 40000 r.p.m. for 72 h in a Beckman SW50.1 rotor. The gradients were collected in drops after puncturing of the bottom of the tube; portions of each fraction were counted in a liquid scintillation counter and samples were taken for refractive index measurements. DNA fractions were pooled, dialysed and ethanol-precipitated. Dot blot hybridization was performed as previously described (Bresser et al., 1983).

**Protein analysis.** Equal numbers of cells were labelled with 100 μCi [35S]methionine/ml (New England Nuclear, 1100 Ci/mmol) or 80 μCi [3H]mannose/ml (New England Nuclear, 25 Ci/mmol) for 24 h periods at specified times p.i. The labelling medium for 35S was 1 ml culture medium without methionine plus 10% foetal calf serum (no additional methionine was required as determined by titration studies; data not shown). The mannose labelling medium consisted of culture medium with 1/10 the normal glucose concentration plus 10% foetal calf serum (optimum labelling conditions were determined by titrating the glucose concentration, data not shown). The medium plus isotope was removed from the cells at the end of the labelling period and 150 μl of sample buffer (Laemmli, 1970) added. The cell samples were completely dissolved by heating at 100 °C for 3 min. To each sample was added 100 μl of a 20% deoxycholate solution in 0.01 M-phosphate buffer (Bio-Rad; Bio-Gel H) to remove DNA. The samples were heated at 60 °C for 10 min, centrifuged in a Phillips-Drucker table-top centrifuge at 2500
r.p.m. for 10 min and the total supernatant was analysed by 10-5% polyacrylamide gel electrophoresis as previously described (Laemmli, 1970).

When samples were immunoprecipitated prior to electrophoretic analysis equal numbers of the labelled cells were disrupted in 200 μl of dissociation buffer (20 mM-Tris-HCl, 50 mM-NaCl, 5 mM-EDTA, 0-5% Triton X-100, 0-5% deoxycholate, 100 μg-phenylmethylsulphonyl fluoride pH 7-8) at 37 °C for 15 min and the cellular debris pelleted in a Brinkman microcentrifuge for 4 min. The antibody (polyclonal anti-HSV-1 (Macintyre, VR3) or anti-HSV-2 (MS) from Accurate Chemical Corp., Westbury, N.Y., U.S.A.; monoclonal anti-gB (H233) or anti-gD (HD1) (Pereira et al., 1981) was added to the 200 μl sample (1:100 dilution) and incubated at 4 °C for 1 to 2 h followed by the addition of 40 to 70 μl of a 10% solution of Staphylococcus aureus Protein A (Sigma) incubated at 4 °C for 1 h. The Protein A to antibody ratio was maintained between 10:1 and 20:1. When samples were immunoprecipitated with monoclonal antibodies (MAbs) the reaction was followed by the addition of 5 μl of goat anti-mouse IgG-IgM for 1 h at 4 °C prior to the Protein A incubation. The Protein A-immune complex was pelleted through a 1 ml 1 M-sucrose pad (in dissociation buffer) and the pellet washed twice with dissociation buffer. Samples were then analysed by polyacrylamide gel electrophoresis as above. The MAb-immunoprecipitated gB and gD were, in specified cases, hydrolysed with S. aureus V-8 protease (Sigma) at 37 °C for 30 min prior to polyacrylamide gel electrophoresis.

In all cases the polyacrylamide gels were impregnated with PPO, dried and fluorographed as previously described (Bonner & Laskey, 1974).

RESULTS

Properties of the Meg cell line

The megakaryocyte-like properties of the Meg cell line have been described previously (Morgan & Brodsky, 1985). Recent studies have demonstrated that it has an IgG gene rearrangement and is Epstein–Barr nuclear antigen-positive, as is characteristic of B cells (data not shown). Unlike B cells, this Meg cell line does not produce any IgG. Also, the Meg cell cannot be induced by phorbol esters or HSV infection to produce any other Epstein–Barr virus markers. Finally, the electrophoretic gel pattern of phosphoproteins (32P-labelled) derived from Meg cells is dissimilar to patterns obtained with established B and T cells (data not shown). The exact identity of these blood-derived cells remains to be established. However, they represent an excellent model for the study of HSV replication in a human blood-derived cell line. This cell line may also represent an endogenous cell type capable of harbouring and disseminating other herpesviruses.

Characteristics of HSV replication in the Meg cell line

Studies were conducted with the Meg cell line to establish that this blood-derived cell line could be infected with HSV and support its replication in a fashion similar to other known permissive cells. Cells infected at m.o.i. ranging from 0-5 to 5 (all experiments employed 2 × 106 cells/ml) were completely growth inhibited p.i. At a m.o.i. of 0-05 or 0-1 cell growth was retarded for 48 h p.i. and normal growth then resumed with no appreciable loss of cell viability compared to mock-infected cells. It was not determined, at this time, whether these cells harboured latent virus or had a low level persistent viral infection. Cell viability, however, diminished steadily p.i. at the higher m.o.i. Cell morphology was monitored by light microscopy of Wright–Giemsa-stained cells at 24 h intervals p.i. The appearance of c.p.e. corresponded to the results observed in the cell viability and growth kinetics. Characteristic of HSV permissive cells, virtually all of the cells were observed to have some c.p.e. by 48 h p.i. (Fig. 1).

A series of experiments demonstrated that the inhibition of cell growth and the observed c.p.e. in the Meg cells infected with HSV were due to HSV replication and not due to a non-productive lytic infection. Fig. 2 demonstrates virus replication in the Meg cells with a 2 × 103- to 104-fold increase of HSV titre p.i. The complete synthesis of the virion de novo was further established by the demonstration of HSV DNA and protein synthesis. (Aspects of protein synthesis will be discussed in subsequent sections.) Approximately 50% of the DNA being synthesized in the HSV-1-infected Meg cells (labelled with [3H]thymidine at 6 to 30 h p.i.) was HSV DNA. The newly synthesized HSV-1 DNA migrated to an equilibrium buoyant density in a CsCl gradient equivalent to DNA with a G + C content of approximately 67-4%, which is similar to that previously reported for HSV DNA (Kieff et al., 1971) (data not shown). DNA was also purified from HSV-infected and mock-infected Meg cells and probed with a cloned HSV
HSV proteins in a blood-derived cell line

Fig. 1. Light microscopy of Meg cells infected with HSV-1 or HSV-2. Cells were deposited on slides and stained as described in Methods. (a) Mock-infected cells 2 days p.i., (b) HSV-1-infected cells (m.o.i. 5) 2 days p.i., (c) HSV-2-infected cells (m.o.i. 3) 2 days p.i. Bar markers represent 150 μm.

Fig. 2. Virus titres in the Meg cell line infected with HSV-1 (----) or HSV-2 (-----). The Meg cells at an initial concentration of 2 × 10^6 cells in 1 ml were infected at an m.o.i. of 5. After a 1 h incubation the unadsorbed virus was removed and the cells resuspended in 4 ml of culture medium with 0.5 ml samples removed at various times p.i. for assay. Infectious virus was quantified as p.f.u./ml of cell lysate as described in Methods.

DNA fragment (the EcoRI L fragment of HSV-1 kindly provided by Dr Richard Hyman, Hershey, Pa., U.S.A.; map units 0.456 to 0.496) by dot blot hybridization. The DNA from HSV-1-infected cells hybridized strongly with the HSV DNA probe while the DNA from the control cells was completely negative (data not shown).
Finally, electron microscopy demonstrated HSV particles at all stages of development present in the infected cells. Fig. 3(a) shows a typical nucleus containing virus particles. It should be noted that a significant number of particles appear to be empty capsids as they lack a DNA core. Fig. 3(b) and (c) demonstrates the apparent sequential maturation and movement of the virus from the nucleus, through the nuclear membrane, where it acquires its outer envelope, through the cytoplasm to the outer membrane. Typical c.p.e. can also be seen in Fig. 3(c).

**35S-labelled HSV proteins synthesized in Meg, Vero and HEP-2 cells**

Once the human Meg cell line was shown to be permissive to HSV it was important to demonstrate HSV protein synthesis and to determine whether the protein species were the same as those synthesized in other permissive cell lines. We employed the Vero and HEP-2 cell lines for these and all subsequent comparative studies. All three cell lines were infected under identical conditions at a m.o.i. of 5 with HSV-1 and a m.o.i. of 3 with HSV-2 as described in Methods. Fig. 4 demonstrates that the immunoprecipitated viral proteins synthesized in all three cell lines infected with HSV-1 had similar electrophoretic patterns. However, the times of appearance of the 35S-labelled HSV proteins p.i. were very dissimilar. Under our conditions of
HSV proteins in a blood-derived cell line

Fig. 4. Electrophoretic pattern of immunoprecipitated [³⁵S]methionine-labelled proteins from HEp-2 (H), Vero (V), and Meg (M) cells infected with HSV-1. Cells were incubated for 4 h (44) or 24 h with [³⁵S]methionine at various times p.i. The proteins from equal numbers of cells were immunoprecipitated with polyclonal antibodies to HSV-1. The immunoprecipitated proteins were electrophoresed on a 10-5% SDS-polyacrylamide gel and visualized by fluorography.

labelling and analysis the HSV proteins synthesized in the Meg cells reached maximum levels between 4 and 24 h p.i. whereas in the Vero cells maximum levels were observed between 24 and 48 h and in the human HEp-2 cells maximum virus protein synthesis appeared to remain elevated between 24 and 72 h. The same three cell lines were also infected with HSV-2 and the HSV protein synthesis was followed by [³⁵S]methionine labelling. As with HSV-1 the protein patterns for all three cell lines were similar. The time of appearance of maximum HSV-2 protein synthesis was again similar to that observed with HSV-1 except that the infected Vero cells produced maximum levels of HSV-2 proteins between 4 and 24 h p.i. as did the Meg cells (data not shown). HSV-1 and -2 viral proteins were synthesized at all p.i. time periods studied. However, at the X-ray exposure time used to resolve all the bands in the maximally or near maximally labelled lanes these proteins do not show up in some samples.

Comparison of HSV-1 and -2 proteins in three cell lines

The Meg, Vero and HEp-2 cell lines were infected with HSV-1 and -2 as described above. The HSV proteins were labelled with [³⁵S]methionine for a 24 h period during maximal synthesis as determined in the preceding section (24 to 48 h p.i. for HEp-2 and Vero cells and 4 to 28 h p.i. for Meg cells). The HSV-1 and -2 proteins were immunoprecipitated from equal numbers of cells with the corresponding polyclonal anti-HSV antibody. Fig. 5 demonstrates some distinct differences between HSV-1 and HSV-2 proteins synthesized within each cell line as has been shown before. There were also similarities and dissimilarities for each HSV type protein pattern from cell line to cell line. The most readily detected variations between cell lines in the protein
patterns appeared in the 25K to 50K range. The variations were both qualitative and quantitative. However, since the HSV proteins made in the infected cells were labelled at different times p.i. (to obtain maximum labelling) some of the observed differences in protein patterns may result from variations in the appearance of protein species at different times p.i. The protein patterns from the two human cell lines were more similar to each other than to the protein pattern derived from the Vero cells. These descriptive studies were extended to analyse the cross-reactivities of anti-HSV-1 and -2 antibodies to HSV proteins synthesized in each of the infected cell lines. As has been shown by others, there is considerable cross-reactivity with the two HSV types (data not shown).

\[3^H\]Mannose-labelled HSV proteins synthesized in three cell lines

The foregoing studies indicated that the HSV-1 and -2 virus proteins produced in the Meg cells were largely similar to HSV proteins produced in the Vero and HEp-2 cells. However,
qualitative and quantitative differences in the HSV proteins synthesized in the Meg cells exist as compared to proteins synthesized in the Vero and HEp-2 cell lines. Therefore, studies were initiated to analyse selectively the HSV glycoproteins synthesized in the Meg cell line as compared to the Vero and HEp-2 cell lines. The cells were labelled with [3H]mannose for 24 h periods at 4 to 48 h p.i. with HSV-1. The glycoprotein pattern observed with the Vero cells changed with each 24 h period of labelling p.i. These changes potentially indicate a step-wise alteration in the post-translational modifications of the carbohydrate moiety or degradative processing at different stages p.i. (Fig. 6). Similar changes were not observed with the HEp-2 and Meg cell lines. The pattern of maximal labelling in all three cell lines is very similar to the kinetics observed with [35S]methionine. Once again, the protein patterns from the two human cell lines were more similar to each other than to the protein pattern from Vero cells.

**Monoclonal antibody analysis of gB and gD**

The mannose studies indicated differences in the HSV glycoproteins synthesized in all three cell lines. Further resolution of these proteins could best be achieved by the selective precipitation with MAbs. Studies were, therefore, conducted with two specific glycoproteins to determine whether they were modified by post-translational processes differently in each of the three cell lines. The HSV glycoproteins B and D were immunoprecipitated from [3H]mannose labelled cells with MAbs as described in Methods. No gross differences could be detected in the gel pattern of gB (pgB/gB) derived from HSV-1- or HSV-2-infected cells (data not shown). The glycoprotein patterns of gD (pgD/gD) derived from all three HSV-1-infected cell lines, unlike gB, demonstrated qualitative and quantitative differences in each cell line (Fig. 7). Two discrete major glycosylated species appeared to exist in the HEp-2 and Meg cells (with mol. wt. 59K and 62K) while gD synthesized in the Vero cells appeared to contain a very heterogeneous mixture of glycosylated species. The higher molecular weight species was somewhat heavier than previously reported. The apparent molecular weight differences may result from slightly different analytical conditions.

**Proteolytic analysis of gB and gD**

While the altered gD patterns indicate cell-directed differences in the post-translational modification of this protein the observed similarity of the gB pattern does not preclude cell-specific differences in the carbohydrate moiety of gB. The 112K to 125K gB synthesized in the three cell lines could contain major differences in the carbohydrate moiety both at the compositional level and in size and yet not be detectable by this gel procedure. A 1% change in size would not be observed in a 10-5% acrylamide gel. We therefore, hydrolysed the MAb-immunoprecipitated 35S-labelled gB with increasing concentrations of V-8 protease to determine whether different glycopeptides were generated from gB derived from each cell line. Fig. 8 demonstrates several similarities and differences in the peptides generated by V-8 hydrolysis of gB synthesized in the three HSV-1-infected cell lines. The hydrolysis of pgB/gB derived from each cell line generated four major groups of protein fragments. These groups were similar in the three cell lines; however, the molecular weights of groups III and IV appeared to be slightly greater in the peptides derived from HEp-2 cells as compared to the Vero- and Meg-derived species. The peptide(s) generated in group II were distinctly different when derived from the Vero cells, compared to the Meg and HEp-2 cell lines. Some of the V-8 protease-generated bands are difficult to ascribe to pgB/gB since other labelled species co-precipitated with gB, particularly the doublet around 68K in the HSV-1-infected Meg cells (Fig. 8).

The proteolytic digestion of gD derived from HSV-1-infected Vero and Meg cells (Fig. 9) supported the initial differences described in Fig. 7. At all levels of V-8 protease employed the highest molecular weight species of glycosylated gD derived from Vero cells remained heterogeneous in appearance while the two high molecular weight forms of glycosylated gD made in the Meg cells remained as discrete bands. Also the 38K fragment generated from gD made in Vero appeared to be composed of a heterogeneous mixture of higher molecular weight species not detected in the corresponding fragment generated from gD made in Meg cells. Slight differences in the molecular weight of peptides generated in groups II and III were detected;
Fig. 7. Electrophoretic pattern of immunoprecipitated [\(^{35}\)S]methionine-labelled proteins from HEp-2 (lane 1), Vero (lane 2) (both labelled 24 to 48 h p.i.) and Meg (lane 3) (labelled 4 to 28 h p.i.) cells infected with HSV-1 at a m.o.i. of 5. The proteins from equal numbers of cells were immunoprecipitated with MAb to glycoprotein D. The immunoprecipitated proteins were electrophoresed on a 10.5% SDS-polyacrylamide gel and visualized by fluorography.

however, it is not clear at the present time how significant these differences are. A 23K species was generated from V-8 protease-treated gD made in Vero cells with no corresponding species generated from the Meg-derived gD.

DISCUSSION

The results of the studies in this report demonstrate that a human blood-derived cell line, Meg, with both megakaryocyte-like and B cell-like properties can readily support HSV-1 and -2 replication. Unlike other blood-derived cell lines, HSV infection of the Meg cell results in the complete replication \textit{de novo} of the virus leading to cell lysis as is common to other permissive cell lines. The HSV-1 and -2 proteins synthesized in this infected cell line appear to be very similar to those produced in HEp-2 and Vero cell lines. However, some quantitative and qualitative differences were observed in the electrophoretic protein patterns generated from the three infected cell lines labelled with [\(^{35}\)S]methionine or [\(^{35}\)H]mannose. These differences indicate that the regulation of viral protein synthesis and post-translational processing may be both species- and tissue-specific. The analysis and characterization of these differences in the infected cell and how they might relate to the mature virion are in progress.

The time course of maximal viral protein and glycoprotein synthesis observed in the three cell lines indicates that the Meg cell is more sensitive to, or more readily supports, a rapid replication of HSV-1 than HEp-2 or Vero cells. The total number of viable virions produced per cell is greater in the Vero and HEp-2 cells than the Meg cells (data not shown). It is unlikely that these
Fig. 8. Electrophoretic pattern of *S. aureus* V-8 protease-hydrolysed [35S]methionine-labelled proteins that were immunoprecipitated with MAb to gB. The gB was immunoprecipitated from equal numbers of HEp-2 (H), Vero (V) and Meg (M) cells and hydrolysed with increasing amounts of V-8 protease: lanes 1, 0 μg V-8; lanes 2, 100 μg V-8; lanes 3, 250 μg V-8; lanes 4, 500 μg V-8. The proteins were electrophoresed on a 10-5% SDS-polyacrylamide gel and visualized by fluorography.

The HSV gB and gD have been implicated in regulating viral adsorption, entry and replication (DeLuca et al., 1982; Little et al., 1981; Manservigi et al., 1977; Sarmiento et al., 1979; Spear et al., 1978). We therefore focused on these two species, as the regulatory function of these glycoproteins may be involved in the different time course of viral protein synthesis observed in the three infected cell lines. As would be expected the apparent differences between gB and gD synthesized in the two human cell lines, HEp-2 and Meg, are small. However, the differences between the non-human cell line, Vero, and the human cell lines are readily evident. The gB synthesized in all three cell lines infected with both HSV-1 and -2 migrated similarly in an acrylamide gel (data not shown). However, distinct differences were observed in the HSV-1 gD made in Vero cells as compared to the HSV-1 gD made in the two human cell lines (Fig. 7). The heterogeneously glycosylated species made in Vero cells spans the 59K to 62K range (Fig. 7 and 9) while in the two human cell lines two distinct high molecular weight species are immunoprecipitated with the anti gD MAbs. It is not clear, from the present studies, if the
apparent 62K species made in the HEp-2 and Meg cells is equivalent to the previously reported 59K gD molecule (due to differences in gel analysis) or if it is a contaminant. The detection of the heterogeneously migrating gD made in Vero cells spanning a 59K to 62K range would argue against the 62K species being a contaminant. It is interesting to note that glycosylated gD is synthesized at a relatively high level 24 to 48 h p.i., unlike the findings of Johnson & Spear (1984). Differences may be due to the HSV strain employed.

Differences in gB synthesized in the three infected cell lines were obvious only after hydrolysis and analysis of the peptides/glycopeptides generated. Four major groups of peptides were generated upon S. aureus V-8 protease hydrolysis of gB derived from HSV-1-infected cell lines. Several smaller species would also be generated but these were poorly resolved by these procedures. The HSV-1 gB fragments in group II and III (Fig. 8) appear to be more readily generated from gB made in the Meg and Vero cell than gB made in HEp-2 cells. The fragments in group IV are clearly most easily generated in the Vero cell. These selective differences on hydrolysis by V-8 protease may be due to protection by different carbohydrate complexes, different protein conformations dependent upon the carbohydrate moiety and/or different complex formation with the precipitating antibody complex.

It is possible that the gB protease-generated peptides of similar molecular weight in all three cell lines are poorly or non-glycosylated fragments and therefore represent polypeptides that are genetically coded for solely by the virus. The protease-generated gB fragments that are very different, derived from the Vero rather than the human cell lines, are probably heavily glycosylated. Since glycosylation is probably host cell-directed, differences observed in these glycopeptides would represent species-specific and possibly tissue-specific post-translational
modifications. This does not obviate the possibility that some viral glycosyltransferases are also involved in the process. Current studies are being directed at the more costly labelling of the glycoproteins in their carbohydrate moiety to evaluate more clearly which of the peptides generated upon hydrolysis are glycosylated.

The results obtained with gD made in all three HSV-1-infected cells (Fig. 7) and upon hydrolysis (Fig. 9) clearly demonstrate that the highest molecular weight species made in the Vero cell are glycosylated to varying extents resulting in a very heterogeneous band. The gD made in the two human cell lines, on the other hand, contain very distinct species in the same molecular weight range. These differences are maintained even after hydrolysis with the V-8 protease. The portion of the gD molecule made in Vero cells containing the apparently heterogeneously sized carbohydrate moiety is hydrolysed to a 38K fragment still retaining the variously sized carbohydrate groups. The corresponding fragment generated from gD made in the Meg cells appears as a discrete band with a slightly lower molecular weight. Work is in progress to determine whether the heterogeneously glycosylated gD species are associated with the mature virion or whether only selected species are ultimately associated with the virus membrane.

Further analysis of the carbohydrate composition, sequence and amino acid linkage is required to establish the specific nature of the observed differences in gB and gD and to determine whether more subtle differences exist in the gB and gD synthesized in the two human cell lines. Differences in the two human cell lines would help to establish the existence of tissue specificity in the glycosylation reactions as well as the apparent species specificity recognized in these studies. Tissue-specific regulation of the glycosylation reactions could play a subtle but significant role in determining function and antibody recognition of these glycoproteins made in vitro compared with tissue in vivo. The further definition of species- and tissue-specific post-translational modifications of HSV glycoproteins should expand our knowledge of the herpetic infectious process and could ultimately lead to a means to modulate it.

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