Characterization of the Human Cytomegalovirus Envelope Glycoproteins

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SUMMARY
Virions of human cytomegalovirus were shown to contain two discrete membranes. An outer, loose fitting, membrane was sensitive to osmotic shock and could be partially removed by diluting buffered preparations of virions with water. Purified virions were shown, by SDS–polyacrylamide gel electrophoresis of virion membranes labelled by carbohydrate-specific procedures, to contain five glycoproteins with molecular weights of 52, 67, 95, 130 and 250, all \( \times 10^3 \). Digestion of virions with endoglycosidases revealed that there were structural differences between the carbohydrate portions of the glycoproteins. All five glycoproteins were recognized by antibodies present in pools of human convalescent sera.

INTRODUCTION
Human cytomegalovirus (HCMV) is a herpesvirus capable of producing a variety of clinical conditions in man which may arise either from a primary infection or from the re-activation of latent virus during periods of immunosuppression (Weller, 1971; Plummer, 1973). Although convalescent sera contain antibodies that recognize a large number of HCMV-specific polypeptides (Pereira et al., 1982), the antigens responsible for inducing the humoral or cell-mediated responses that are important for limiting or preventing HCMV disease have not yet been identified.

About 35 virus-specific polypeptides have been detected in virions (Kim et al., 1976; Gupta et al., 1977; Stinski, 1977, 1978; Gibson, 1983), but none of these has been fully characterized. However, some polypeptides have been shown to be glycosylated (Fiala et al., 1976; Kim et al., 1976; Stinski, 1976; Gibson, 1983). The glycoproteins, which are soluble in detergents, appear to be associated with the virion envelope (Stinski, 1976; Furukawa et al., 1984), which is probably derived from the nuclear and/or cytoplasmic membranes of infected cells (Smith & De Harven, 1973; Severi et al., 1979). The observation that antibodies directed against HCMV glycoproteins detect the corresponding antigens on internal and external membranes of infected cells supports this hypothesis (Stinski, 1976; Stinski et al., 1979).

This paper presents evidence for the presence of two separate layers within the envelope of the HCMV virion. It also describes the identification of glycoproteins from these membranes and the detection of differences in the carbohydrate moieties between some of the glycoprotein species.

METHODS
Antisera and enzymes. Anti-rabbit immunoglobulin prepared in goats and anti-human immunoglobulin prepared in rabbits were purchased from Miles Laboratories. Pooled HCMV-positive sera, obtained from convalescent adults, were a generous gift from Dr J. C. Booth, St Georges Hospital, London, U.K. Neuraminidase (acylneuraminyl hydrolase) from Vibrio cholerae was purchased from Behringwerke AG. Endoglycosidase D (endo-\( \beta \)-N-acetylglucosaminidase D) from Diplococcus pneumoniae and endoglycosidase H (endo-\( \beta \)-N-acetylglucosaminidase H) from Streptomyces plicatus were purchased from Miles Laboratories. Galactose oxidase (D-galactose:oxygen 6-oxidoreductase) was purchased from Sigma.

Virus production and purification. Human diploid embryo fibroblast cells (Flow 7000 and MRC5) were maintained at 37 °C in Dulbecco’s modified Eagle’s medium (DME) supplemented with 10% foetal calf serum

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(FCS). Confluent monolayers, at passage levels between 20 and 23, were infected with HCMV strain AD169, at a multiplicity of infection of 0:01. After virus adsorption for 1 h at 37 °C, the inoculum was replaced with DMEM containing 2% FCS and the infected cultures were incubated for 8 to 10 days when most of the cells showed an advanced cytopathic effect (c.p.e.). Culture supernatants were clarified by centrifugation (2000 r.p.m., 10 min) and used directly or stored at -80 °C. In some experiments, infected cell cultures, 5 to 6 days post-infection, were labelled for 24 to 72 h with L-[1-14C]fucose or D-[1-14C]glucosamine hydrochloride or D-[1-14C]galactosamine hydrochloride at 5 μCi/ml. Alternatively, infected cultures were labelled with 25 μCi/ml D-[6-3H]glucosamine hydrochloride or D-[2,6-3H]mannose.

Virions and dense bodies were collected from the clarified tissue culture supernatants by centrifugation in a Beckman SW28 rotor at 25000 r.p.m. for 30 min onto a cushion of 30% (w/v) potassium tartrate in Tris-buffered saline (TBS: 50 mM-Tris·HCl, 0·15 M-NaCl, pH 7·2). Material which banded at the cushion interface was diluted with TBS (2 vol.) and was then centrifuged once through a glycerol/tartrate gradient (Talbot & Almeida, 1977), in a Beckman SW41 rotor at 40000 r.p.m. for 20 min. Bands containing either virions or dense bodies were removed by aspiration, diluted with TBS (2 vol.) and used directly for electron microscopy (see below) or recovered by centrifugation in a Sorvall SS34 rotor at 14000 r.p.m. for 30 min. To isolate intracellular virus particles from infected cells, monolayers showing advanced c.p.e. were washed with phosphate-buffered saline (PBS), frozen and thawed three times and disrupted in a Dounce tissue homogenizer with 25 strokes of a tight fitting pestle. The homogenates were centrifuged in a Sorvall SS34 rotor at 10000 r.p.m. for 20 min and the intracellular virions purified from the supernatant fluids, as described above.

**Labeling in vitro.** Purified virions were resuspended in 500 μl PBS and labelled with 125I by the method of Hunter & Greenwood (1962) and unincorporated isotope was removed by centrifuging and washing the pellet with PBS. Immunoglobulin molecules were iodinated by a modification of the chloramine T method (Strange et al., 1971) and unincorporated isotope was removed by gel filtration through Sephadex G-25. Tissue culture cells were labelled with 131I methionine by incubation in DMEM containing 13 mg/l of methionine supplemented with radiolabeled methionine (20 μCi/ml) for 12 h. Unincorporated extracellular label was removed by washing the monolayer with PBS after which cells were lysed in detergent buffer (see below) and centrifuged in a Beckman SW50.1 rotor for 60 min at 50000 r.p.m. Virion glycoconjugates were labelled with tritiated borohydride after prior oxidation with sodium metaperiodate or galactose oxidase (Gahnberg & Hakomori, 1973). Virus suspended in 500 μl PBS (pH 7·4), was oxidized with either NaIO4 (2 mM at 0 °C for 10 min in the dark) or galactose oxidase (10 units for 30 min at 37 °C). Excess periodate was destroyed by adding 20 μl glycerol and enzymic activity was stopped by cooling to 0 °C. Tritiated sodium borohydride (10 mCi) was added to oxidized virus preparations and the solution incubated for 10 min at 0 °C. Excess tritiated borohydride was destroyed by the slow addition of 25% acetic acid (100 μl) at 0 °C and unincorporated isotope was removed by centrifuging and washing with PBS.

**Isolation and solubilization of virion envelope.** Fragments of virus envelope were obtained from osmotically shocked virions by diluting concentrated virus suspensions in PBS with 50 vol. water at 0 °C. After 30 min the disrupted virions and membrane fragments were labelled with 125I and the products analysed by centrifugation through glycerol/tartrate gradients, as described previously. Virions were extracted with detergent by dispersion in 200 μl 10 mM-Tris·HCl, 1 mM-CaCl2, 0·15 M-NaCl, 1% Triton X-100, 2 mM-phenylmethylsulphonyl fluoride, 1% ethanol, pH 7·3 (buffer A) and treatment with ultrasound (2 bursts of 30 s at 0 °C) in an ultrasonic water-bath. After 20 min at 0 °C the turbid suspension was centrifuged in a Sorvall SS34 rotor at 21000 r.p.m. for 40 min at 0 °C. Pelleted material was resuspended in PBS and used directly for electron microscopical examination; otherwise, both pellet and supernatant were stored at -80 °C prior to analysis.

**Preparation of antigen.** Antigens were prepared by dialysing freshly prepared Triton X-100 extracts of purified virus against 5000 vol. 0·1 M-acetic acid at 4 °C. Antigens were recovered by lyophilization and were then resuspended in PBS.

**Preparation and characterization of rabbit antiserum.** Two New Zealand white rabbits (15 to 20 weeks old) were injected intramuscularly with 100 μg resuspended antigen emulsified with an equal volume of adjuvant (total vol. 1 ml). The first injection was with complete adjuvant while the three successive injections, at 3-weekly intervals, were with incomplete adjuvant. Samples of blood were taken from the ear vein regularly and antibodies directed against HCMV membrane glycoproteins were assayed by immunoprecipitation (see below), using normal rabbit serum as a control. In virus neutralization assays (Reynolds et al., 1979) the hyperimmune rabbit serum gave a 60% plaque reduction at a 1:40 dilution, compared with 1:320 to 1:640 dilutions for convalescent human sera.

**Digestion with glycosidases.** Virions were resuspended in either 0·1 M-sodium acetate pH 5·5 containing endoglycosidase H (0·05 unit) or in 0·1 M-sodium phosphate pH 6·5 containing either neuraminidase (0·1 unit) or endoglycosidase D (0·05 unit). After incubation for 2 h (neuraminidase) or 6 h (endoglycosidase H or D) at 37 °C the treated virus was washed by centrifugation through cold PBS and extracted with buffer A before electrophoretic analysis of the detergent extracts.

**Immunoprecipitation and polyacrylamide gel electrophoresis.** Triton X-100-extracted material (40 μl) was pre-treated with normal rabbit serum (10 μl) for 1 h at 4 °C and non-specific immune complexes removed by
adsorption to Protein A-Sepharose beads [20 µl of a 50% (v/v) suspension in buffer A] for 1 h at 4 °C and centrifuged at 1500 r.p.m. for 2 min. The resulting supernatants were incubated with 20 µl of undiluted rabbit or human antisera for 12 h at 4 °C and the specific immune complexes adsorbed to Protein A-Sepharose, as described above. The beads were washed three times with buffer A and then resuspended in 200 µl 1% SDS, 1% 2-mercaptoethanol and heated for 5 min at 100 °C. The samples were electrophoresed in 8% linear or 3 to 12.5% gradient polyacrylamide gels in the presence of 0.1% SDS as described by Laemmli (1970). Fluorograms, prepared by the method of Bonner & Laskey (1974), and autoradiograms, consisting of dried gels, were exposed with Cronex L X-ray film (Dupont) at −80 °C.

**Electrophoretic transfers.** Polypeptides separated by SDS-PAGE were electrophoretically transferred to nitrocellulose membranes using Tris-glycine buffer (25 mM-Tris, 192 mM-glycine, pH 8.3) plus 20% methanol as originally described by Towbin et al. (1979). Non-specific protein binding sites were blocked by treating the nitrocellulose membranes with PBS containing 2% horse serum, 3% bovine serum albumin, 0.2% Tween 20, 0.02% sodium azide (buffer B) for 3 h at 4 °C. The membranes were incubated with 20 ml buffer B containing 0.2 ml rabbit anti-HCMV serum (see above) or 0.5 ml of pooled human HCMV-positive convalescent serum for 12 h at 4 °C. After three washes in buffer B (100 ml for 30 min at 4 °C with shaking) membranes were treated with 20 ml buffer B containing 10 µg/ml 125I-labelled anti-rabbit or anti-human immunoglobulin for 2 h at 37 °C. Unadsorbed antibody was removed by repeated washing with buffer B followed by PBS before drying and autoradiography.

**Electron microscopy.** Samples were adsorbed to Formvar-coated copper grids for 5 min with 1% sodium silicotungstate, pH 7.0 for 30 s. Grids were examined in a Philips EM400 T electron microscope at an accelerating voltage of 80 kV.

**RESULTS**

**Purification, morphology and stability of HCMV virions**

This study was facilitated by the use of cultures of human foreskin cells (Flow 7000) to produce the virus. These cells gave much higher yields of virions than MRC5 cells with a lower proportion of dense bodies. In most cases the glycerol/tartrate gradient system of Talbot & Almeida (1977) resolved the material obtained at the cushion interface into three bands and a pellet. The pellet contained dense bodies and some cellular material. A tight band, approximately half way down the gradient, contained virus particles (Fig. 1a) which were used in these studies. These preparations of virions were essentially free from cellular debris and dense bodies (Fig. 1b), which formed a diffuse band approximately two-thirds into the gradient. A band between the interface and the gradient contained membrane fragments plus a small number of virion-like particles (data not shown). Gradients obtained from culture supernatants harvested at very late times after infection, when many of the cells were becoming detached from the monolayer, contained an additional band, just above the virions. This comprised a mixture of unenveloped and partially degraded virions and probably corresponded to the non-infectious enveloped particles (NIEPs) of Irmiere & Gibson (1983). The purified virions used in this study were infectious for monolayers of human fibroblasts but their infectivity was not determined quantitatively.

Electron microscopy indicated that many virions were enclosed by an envelope (Fig. 1a) which in many cases could be seen alongside an uncoated virion (arrow 1). The envelope was fringed with spike-like structures, approximately 15 nm in length, which, by analogy with other enveloped viruses, probably contain glycoproteins. Some of the particles were impermeable to silicotungstate (arrow 2), whereas others were partially (arrow 3) or completely (arrows 4) penetrated by the stain with a corresponding highlighting of the nucleocapsid structure. In addition, two membranes were sometimes observed in the completely penetrated virions. Whereas one membrane was closely attached to the nucleocapsid (arrow 5), the other formed a loose-fitting outer envelope (arrow 6). Dense bodies did not contain this outer envelope and little or no surface structure was observed (Fig. 1b).

Treatment of virus preparations with water resulted in the removal of most of the outer envelope material. Centrifugation of the osmotically shocked virions through a glycerol/tartrate gradient allowed the separation of a membrane fraction, which contained fragments of various sizes (Fig. 1c), from partially degraded virions which possessed much less of the outer membrane structure (compare Fig. 1d with Fig. 1a). There was no significant increase in the number of virions penetrated by silicotungstate stain (Fig. 1d). This result indicates that the
Fig. 1. Electron micrographs of purified HCMV particles and envelopes. (a) Enveloped virion showing envelopes (arrow 1), particles not penetrated by silicotungstate (arrow 2), particles partially (arrow 3) or completely (arrow 4) penetrated by silicotungstate and inner (arrow 5) and outer (arrow 6) membrane of a virion. (b) Dense bodies. (c) Envelope fragments from (d) osmotically-shocked virions which were stripped of most of the outer envelope. (e) Intracellular virions. (f) Nucleocapsids obtained by extracting virions with Triton X-100. Bar markers represent 0.1 μm.
Fig. 2. Autoradiographs of $^{125}$I-labelled HCMV polypeptides separated by SDS-PAGE. (a) Virions; (b, d) Triton X-100-insoluble fraction; (c, e, f) Triton X-100-soluble fraction; (g) virion envelope fragments prepared by osmotic shock. Polypeptides were separated either in an 8\% (w/v) acrylamide gel (a to c) or in a gradient gel of 3 to 12.5\% (w/v) acrylamide (d to g). Numbers refer to mol. wt. ($\times 10^{-3}$).
membrane closely attached to the nucleocapsid (Fig. 1a, arrow 5) is osmotically stable. Some of the intracellular virus particles isolated from infected cells possessed a single osmotically stable membrane (Fig. 1e) suggesting that the virions acquire their outer envelope as they leave the infected cell.

**Extraction of the envelope components**

Extraction of virions with Triton X-100 removed the membrane structures to release intact nucleocapsids (Fig. 1f). The most abundant proteins in the detergent extract had mol. wt. of 23000, 30000, 52000, 67000, 95000, 130000 and about 25000 (Fig. 2). The largest protein species could be resolved into at least two components by electrophoresis in gradient polyacrylamide gels (Fig. 2). The Triton-insoluble fraction also contained small quantities of these proteins indicating that extraction was not completely effective. Three abundant proteins with mol. wt. of 35000, 64000 and 150000, plus several minor polypeptides, were not extracted from purified virions by Triton X-100 and these were assumed, therefore, to be associated with the nucleocapsid.

Fig. 3. Autoradiographs of 3H-labelled HCMV virion glycoproteins separated by SDS-PAGE. Virions were labelled with tritiated borohydride after prior oxidation with either sodium metaperiodate (a, c) or galactose oxidase (b, d). Polypeptides were separated either in an 8% (w/v) acrylamide gel (a, c) or in a gradient gel of 3 to 12.5% (w/v) acrylamide (b, d).
Fig. 4. Autoradiography of $^{125}$I-labelled HCMV virion proteins separated by SDS-PAGE showing the effect of glycosidase treatment on the mobility of Triton X-100-soluble components. (a, e) Triton X-100 extracts of virions; (b, c, d) Triton X-100 extracts from virions digested with neuraminidase, endoglycosidase D and endoglycosidase H, respectively. Samples were separated on an 8% (w/v) acrylamide gel.

The polypeptides of membrane fragments obtained from osmotically shocked virions were also analysed by SDS-PAGE (Fig. 2). Although membrane preparations produced in this way were variable with respect to yield and minor contaminants, they consistently contained polypeptides with mol. wt. 52000, 95000 and 130000 (Fig. 2). Therefore, these membrane fragments contained the same protein species as the detergent extract of the virions.

**Identification and characterization of virion glycoproteins**

The presence of glycoproteins in virions and purified envelopes was initially investigated by adding isotopically labelled sugars to infected cell cultures. Equivocal results were obtained with D-[3H]glucosamine, D-[3H]mannose, L-[14C]fucose and D-[14C]glucosamine due to low incorporation of the isotopes into virions. However, two polypeptides with mol. wt. of 67000 and 130000 were detected in virions labelled with D-[14C]galactosamine (data not shown).

Subsequent experiments involved the labelling of oxidized sugar residues within virion glycoproteins with sodium [3H]borohydride followed by electrophoretic analysis of the labelled glycoproteins on 8% linear or 3 to 12-5% gradient gels (Fig. 3). Oxidation of purified virus with sodium metaperiodate resulted in the subsequent labelling of five envelope proteins with mol. wt. of 52000, 67000, 95000, 130000 and 250000, and three nucleocapsid components, with mol. wt. of 35000, 64000 and 150000. Treatment of virus with galactose oxidase, which oxidizes unsubstituted hydroxyl groups on C6 of both galactose and N-acetylglactosamine moieties,
resulted in the subsequent labelling of only the five envelope components. We therefore conclude that these components are glycosylated and designate them gp52, gp67, gp95, gp130 and gp250. The metaperiodate-induced labelling of proteins with mol. wt. 35 000, 64 000 and 150 000 may be due to a non-specific oxidation of non-glycosylated components of the virion. The relatively high incorporation of label into gp67 and gp130 following galactose oxidase oxidation suggests that these glycoproteins are relatively rich in galactose and or N-acetylgalactosamine residues. The minor Triton-soluble proteins with mol. wt. of 23 000 and 30 000 were not labelled during these experiments and are therefore probably not glycosylated.

Envelope glycoproteins were further characterized by digesting 125I-labelled virions with glycosidases prior to Triton X-100 extraction and electrophoretic analysis (Fig. 4). Digestion with endoglycosidase D had no apparent effect on the mobilities of any of the glycosylated polypeptides. In contrast, digestion with endoglycosidase H reduced the mol. wt. of gp52 by about 2000 (Fig. 4), whereas digestion with neuraminidase slightly increased the molecular weights of gp95 and gp130. When detergent extracts of glycosidase-treated virions were electrophoresed through gradient gels no alteration in the molecular weight of gp250 components was observed (data not shown).

**Immunology of the glycoproteins**

Detergent-extracted components from purified virions were used to immunize rabbits and the resulting antiserum precipitated gp52, gp95, gp130 and gp250. The same glycoproteins were also immunoprecipitated by the convalescent human sera used in this study (Fig. 5). In control
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experiments no [35S]methionine-labelled proteins were detected when the rabbit hyperimmune serum was reacted with extracts of uninfected cells, indicating that the virus membrane preparations were essentially free from human antigens.

Western transfers of virion proteins separated by SDS–PAGE were also probed with these antisera (Fig. 5). In addition to the proteins present in the antigen (namely gp52, gp95, gp130 and gp250) the rabbit serum also recognized a polypeptide with a mol. wt. of 35000 which was not a major envelope component (see Fig. 3). In contrast, human antisera recognized most of the polypeptides present in the purified virus and although the envelope glycoproteins gave a significant reaction the major response was against the nucleocapsid components.

DISCUSSION

Examination of purified virus particles by electron microscopy showed that the virions are enclosed by a loose-fitting outer envelope which is osmotically fragile. Particles remaining after the physical removal of this envelope showed little surface detail and were not significantly penetrated by stain, indicating that an inner membrane was present and this could be seen in cross-section in some completely stained virions. The steps involved in the maturation of HCMV are poorly understood, although it is probable that the virus nucleocapsid first acquires a modified host cell nuclear membrane as it buds into the cytoplasm of the infected cell (Smith & De Harven, 1973). This membrane may then become further modified, for example, by the insertion of virus-specific glycoproteins to become the virus envelope. Alternatively, the virion may acquire a second membrane containing glycosylated proteins as it 'buds' through the cytoplasmic membrane systems (especially the Golgi apparatus) of the infected cell (Severi et al., 1979). We suggest that virus maturation involves firstly the envelopment of the nucleocapsid with a membrane derived from the cell nucleus and secondly the envelopment of this intracellular virus with an outer membrane either shortly before or as it leaves the infected cell.

Envelope components were purified from other virion components by extraction with non-ionic detergents (Stinski, 1976; Furukawa et al., 1984) or by osmotically shocking purified virus preparations. Five of the seven polypeptides extracted from purified envelopes were glycosylated and these were all recognized by human sera. Most of the previous studies on the glycoproteins of HCMV used virus preparations that were contaminated with dense bodies. A variable number of glycoproteins have been described, ranging from three (Fiala et al., 1976), four (Gibson, 1983), seven (Kim et al., 1976; Nowak et al., 1984) to eight (Stinski, 1976). By comparison with earlier reports we found no evidence for the glycosylated polypeptides with mol. wt. of about 22000 or 170000 (Kim et al., 1976), and our gp52 has a lower molecular weight than a glycoprotein with previously reported values of 57000 (Stinski, 1976; Gibson, 1983) or 58000 (Nowak et al., 1984). These differences may, to some extent, reflect variations between different strains of HCMV. We have also detected an additional high molecular weight glycoprotein, gp250, which represents a significant proportion of the 125I label in the detergent-solubilized fraction of purified HCMV virions. This protein together with gp95 and gp130 produces characteristic diffuse bands on polyacrylamide gels which may indicate microheterogeneity within the polysaccharide moieties. A similar high molecular weight glycoprotein, which contains a high proportion of carbohydrate and which plays a major role in the host response to virus infection, has been isolated and characterized from cells transformed with Epstein–Barr virus (Morgan et al., 1984). Our results regarding gp52, gp67, gp95 and gp130 agree well with the data of Nowak et al. (1984) who used radiolabelled concanavalin A to detect glycoproteins with mol. wt. of 58000, 68000, 94000 and 130000. The glycoproteins designated gp67 and gp130 were both labelled in vivo with [14C]galactosamine, the monosaccharide most commonly linked O-glycosidically to threonine or serine residues in glycoproteins. The efficient labelling in vitro of gp67 and gp130 after oxidation with galactose oxidase confirmed that these proteins contain a relatively high proportion of galactosamine or galactose. Recently, Clark et al. (1984) described a major virion component with a mol. wt. of 64000 from strain Towne which was rich in galactosamine. However, our results, obtained with virions labelled in vitro with 125I, indicated that gp67 was a minor membrane component of strain AD169 which migrated close to the major capsid protein of 64000 mol. wt.
Structural differences between some of the glycoproteins were observed following treatment of virions with glycosidases. Digestion with neuraminidase reduced the electrophoretic mobilities of gp55 and gp130, suggesting that these glycoproteins were extensively substituted with sialic acid residues which contribute to the electrophoretic mobility of sialo-glycoproteins. Digestion with endoglycosidase H affected only gp52, indicating that this glycoprotein contains N-glycosidically linked oligosaccharide. Endoglycosidase D, which also reacts with N-glycosidically linked oligosaccharides, had no apparent effect on any of the glycoproteins present in HCMV envelopes. This result may be due to the inaccessibility of carbohydrate substrates rather than their absence from these glycoproteins. Additional studies are required on the nature and the linkage of the carbohydrate moieties.

The glycoproteins of HCMV membranes were immunogenic in rabbits. The reaction of the immune rabbit sera with a protein having a mol. wt. of 35000, not observed in the Triton-extracted fraction, might be the result of a common epitope between this protein and one of the membrane glycoproteins. The immunogenic response in humans was directed primarily against the major capsid proteins with mol. wt. 64000 and 150000. This probably reflects the relative abundance of these proteins in the virion.

Glycoproteins from several herpesviruses are known to be immunologically important. For example, virus-neutralizing antibodies are induced by gp340 from Epstein-Barr virus (Thorley-Lawson & Geilinger, 1980; North et al., 1982) and glycoproteins, known to be structural components of the herpes simplex virion envelope, act as major antigenic stimuli for the host immune system (Cohen et al., 1978; Norrild, 1980; Spear, 1980). Herpesvirus glycoproteins therefore, have considerable potential as subunit vaccines. Further studies are needed to elucidate the importance of individual glycoproteins in inducing immunological protection against HCMV.

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