Morphology and Distribution of gp52 on Extracellular Human Cytomegalovirus (HCMV) Supports Biochemical Evidence that It Represents the HCMV Glycoprotein B

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SUMMARY

Glycoprotein gp52 exists within the mature human cytomegalovirus (HCMV) envelope in heterodimeric, disulphide-linked complexes with glycoproteins gp95 and gp130. Biochemical studies involving immunoprecipitations and Western blots have demonstrated that gp52 is the glycoprotein B (gB) homologue of HCMV but that gp95 and gp130 are probably separate gene products. The distribution of this putative gB on extracellular HCMV particles was revealed by high resolution electron microscopy of preparations labelled with a monoclonal antibody, F5, directly coupled to colloidal gold. F5-gold probes, specific for HCMV gp52, bind to the distal end of 12 nm long, slender spikes projecting from virion and dense body envelopes. Labelled spikes were most often present in closely packed, homogeneous clusters and were frequently present on envelope protrusions. The degree of labelling on individual HCMV particles was highly variable. Both the morphology and distribution of HCMV gp52 show strong similarity with that previously reported for the gB of herpes simplex virus. Other morphologically distinct spikes occur in the HCMV envelope but these were not recognized by F5-gold probes.
Fig. 1. Autoradiographs in (a) show that gp52, gp95 and gp130 of HCMV are immunoprecipitated by both F5 (lane 1) and anti-HCMV gB-VAC (lane 2). (b) A separate experiment shows reactivity of HCMV-positive human serum (lanes 1 and 2), anti-HCMV gB-VAC (lanes 3 and 4) and the MAb F5 (lanes 5 and 6) on Western blots of immunoaffinity-purified HCMV glycoproteins (lanes 1, 3 and 5) and semi-purified HCMV envelope preparations (lanes 2, 4 and 6). Glycoproteins are denoted only by their Mr $\times 10^{-3}$.

complexes which formed with either undiluted F5 hybridoma supernatant or anti-HCMV gB-VAC antiserum, were purified on Protein A-Sepharose and after reduction with 5% 2-mercaptoethanol, the individual glycoproteins were separated by PAGE and visualized by autoradiography. Both F5 and anti-HCMV gB-VAC were shown to immunoprecipitate gp52, gp95 and gp130 from HCMV envelope preparations (Fig. 1a, lanes 1 and 2). In addition, variable amounts of the protein of Mr 145K were occasionally precipitated by both antisera. This protein is thought to be the glycoprotein precursor of gp52 (described by Cranage et al., 1986) and is not thought to be incorporated into the immune complexes. Under the same conditions, no precipitation occurred using either normal mouse serum or normal rabbit serum.

Antibody reactivities were then compared by Western blotting of immunoaffinity-purified HCMV glycoproteins. Unlabelled, solubilized virion envelope components were adsorbed to immunoaffinity columns prepared with the purified F5 MAb coupled to Affi-Gel 10 (Bio-Rad). Glycine–NaCl–N-octyl glucoside, pH 2.5, allowed the elution of three disulphide-linked complexes. Individual glycoproteins were then separated by SDS–PAGE, electrophoretically transferred onto nitrocellulose sheets and allowed to react with anti-HCMV positive human sera, the F5 MAb or the anti-HCMV gB-VAC polyclonal antibody. Bands were visualized by incubation with the appropriate horseradish peroxidase-linked anti-IgG (Bio-Rad) and treated with 0.1% 4-chloro-l-naphthol until the colour developed.

The results are shown in Fig. 1 (b). HCMV-positive human serum (lanes 1 and 2) reacted with immunopurified glycoproteins gp52, gp95, gp130 and gp145 (lane 1) plus many other envelope components (lane 2). The anti-HCMV gB-VAC antiserum (lanes 3 and 4) was strongly reactive with immunopurified glycoproteins gp52 and gp145 but did not recognize gp95 and gp130 (lane 3). Similarly, both gp52 and its presumed precursor, gp145, were clearly recognized in the semi-purified HCMV envelope preparations treated in parallel (lane 4). The absence of cross-reactivity between gp52 and gps 95 and 130 using a polyclonal antiserum specific for gp52 and its precursor, indicates that gp95 and gp130 have no epitopes in common with gp52. There is therefore no direct product–precursor relationship between them and it is most likely that they are gene products completely distinct from gp52. The F5 MAb (lanes 5 and 6) reacts only weakly
Fig. 2 to 4. Fig. 2. An HCMV virion with a partially ruptured and collapsed envelope. F5-gold probes are easily identified at the distal ends of long (12 nm) slender spikes (indicated by arrows) projecting from the envelope. Fig. 3 and 4. F5-gold probes attached to unpenetrated HCMV virion (Fig. 3) and dense body (Fig. 4). Bar marker represents 100 nm, for Fig. 2, 3 and 4.

in Western blotting, but its specificity for gp52 could be seen in immunopurified preparations (lane 5). The absence of reactivity between F5 and gp145 may be due to incomplete processing of the epitope recognized, or there may simply be insufficient material present to be detected. Neither normal mouse serum nor normal rabbit serum reacted with any of the immunopurified HCMV glycoproteins nor with any of the components within HCMV envelope preparations.

Taken together, the data derived from immunoprecipitation and Western blot experiments demonstrate that F5 and anti-HCMV gB-VAC are specific for the same glycoprotein, and therefore it is evident that gp52 is synonymous with the glycoprotein shown by Cranage et al.
Short communication

Fig. 5. Virions are frequently asymmetric with an accumulation of dense, amorphous material (arrows) to one side of the nucleocapsid. F5-gold-labelled spikes seldom occur on the portion of envelope which overlies the dense material next to the nucleocapsid. There appears to be a genuine, reproducible difference in the number of labelled spikes present on individual HCMV particles. Bar marker represents 100 nm.

(1986) to be the gB homologue of HCMV. The HSV glycoprotein designated gB has been shown (Stannard et al., 1987) to have a distinctive morphology and to occur in virion envelopes in clusters of prominent 14 nm long spikes. A comparison of the nucleotide sequences and deduced amino acid sequences of the gB genes of HSV, HCMV and Epstein–Barr virus, showing all 10 cysteine residues present between the putative signal and anchor sequences to be perfectly aligned, led Cranage et al. (1986) to suggest that the extracellular portions of the proteins may possess a similar overall structure. In this study, immunogold labelling techniques were used to study the ultrastructure and distribution of gp52 on extracellular human cytomegalovirus particles.

HCMV strain AD169 was propagated in human embryonic fibroblast monolayers grown in MEM supplemented with 10% foetal bovine serum. Virus particles were concentrated from supernatant culture fluids by centrifugation at 48000 g for 90 min. The purified F5 MAb was freeze-dried and dissolved in distilled water at a concentration of 1-0 mg/ml and directly coupled to 4 to 8 nm colloidal gold particles. These electron-dense probes were allowed to react with the concentrated HCMV suspensions and samples were examined in a Hitachi 600 electron microscope after negative staining with 2% phosphotungstic acid. Details for the preparation of colloidal gold and gold-labelled antibodies, as well as the preparation of labelled or unlabelled
virus particles for electron microscopy, have been described previously (Stannard et al., 1987), and as stated then, care was taken at all times to ensure minimal damage to the virion preparations. F5-gold probes, specific for HCMV gp52, bound to the distal ends of 12 nm long slender spikes projecting from virion envelopes (Fig. 2 and 3) and dense body surfaces (Fig. 4). When the virion envelope was intact and closely held around the capsid (Fig. 3), resolution of the individual glycoprotein spikes was difficult. Because the morphological nature of the spikes was a prime consideration, attention was focused on the area of those particles where the envelope had partially collapsed thus allowing better visualization of the glycoprotein entities. For that reason most of the virions illustrated have also been penetrated by the negative stain to reveal, in addition, the distinctive nucleocapsid. Although F5-gold probes sometimes appeared to be randomly distributed, they were most often found in distinctive homogeneous clusters. It was noticed that both labelled and unlabelled virions were frequently asymmetric due to the accumulation of dense, amorphous material to one side of the nucleocapsid; F5-gold-labelled spikes were seldom found on that portion of the envelope which lay directly above the dense material next to the nucleocapsid (Fig. 5). The number of gp52 spikes present on individual HCMV particles was highly variable (compare Fig. 5a to Fig. 5c), which is thought to be a genuine difference and not simply due to incomplete labelling. There was often a coincidental envelope protrusion between 90° and 180° from the unilateral dense mass and clusters of gp52 spikes were frequently present on these envelope protrusions of various dimensions (Fig. 6a, b, c).

Protrusions on negatively stained enveloped viruses are often dismissed as artefacts, particularly when the virion in question occurs predominantly in a spherical form. This holds true for HCMV as well as other members of the herpesvirus group. However in our experience, envelope protrusions on HCMV (and other herpesviruses) occur with sufficient frequency to suggest that they should not be regarded as irrelevant. There are three main reasons for believing that the protrusions are genuine features of the HCMV envelope pleomorphism. Firstly, they were seen on freshly isolated virions from both cell cultures and in urine with equal frequency to that observed in specimens after prolonged storage at 4 °C, and the ratio of non-spherical to spherical virions could not be increased by adjustments of pH or severe centrifugal forces. Secondly, the diameters of the ‘heads’ of the intact virions with tail-like protrusions were in the same range as the diameters of the near-spherical virions without envelope protrusions. Distortion of the envelope to produce an artefactual tail or envelope extension would result in a considerable reduction in diameter which we did not observe. Thirdly, examination of ultrathin sections of infected human embryonic fibroblast monolayers, fixed in osmium and embedded in LR White resin, revealed intracellular HCMV virions with envelope extensions (Fig. 7) very similar to those seen on negatively stained particles. Although the majority of virions appeared spherical on thin sections the detection of a unilateral envelope protrusion would rely on a fortuitous angle of sectioning. It is also possible that changes in the envelope orientation might occur once the virions have been released from the cell. Hypothetically, envelope membrane extensions could, in an intracellular situation, be folded flat against the virion surface. It is of interest that on rare occasions inverted folds of the HCMV envelope membrane were seen, both in virions (Fig. 8) and dense bodies (Fig. 9). However any connection between these folds and the presence of envelope protrusions must at present remain a matter of speculation. Previous studies (Farrar & Oram, 1984) have reported the existence of apparent multiple layers of HCMV virion envelopes.

In addition to the slender gB spikes identified in this study, prominent spikes morphologically distinct from those labelled with F5-gold were often visible. The unlabelled spikes were much shorter and thicker than the gB spikes and were particularly noticeable on those virions where specific F5-gold labelling was sparse (Fig. 10).

The present study together with that already published on the morphology and distribution of various HSV-1 envelope glycoproteins (Stannard et al., 1987) illustrates that the gp52 spikes of HCMV are morphologically very similar, although not identical, to those of HSV-1 gB. Similarities include both their length (12 to 14 nm) and their clustered distribution on envelope
Fig. 6. Distinctive long spikes (labelled with F5-gold probes) are often found in homogeneous clusters (shown by arrows) which are frequently present on envelope protrusions of various dimensions (a, b and c). Protrusions occur on opposite sides of the virion to the area of accumulation of amorphous material next to the nucleocapsid. Bar marker represents 100 nm.

Fig. 7. Ultrathin sections of HCMV-infected fibroblasts show envelope protrusions (shown by arrows) to be present on intracellular HCMV virions seen in a cytoplasmic vesicle (a) and lying adjacent to the inner nuclear membrane after budding (b and c). The cytoplasm is denoted by Cy, nucleus by N and nuclear membrane by Nm. Bar marker represents 100 nm.
Fig. 8 to 10. Fig. 8 and 9. Inverted folds (indicated by arrows) are seen in the envelope membrane of an HCMV virion (Fig. 8) and dense body (Fig. 9). The dense body is also labelled with F5-gold probe. Fig. 10. Prominent spikes (shown by arrows), morphologically distinct from those labelled with F5-gold, are often visible. They are particularly noticeable on those virions with few gp52 spikes. Bar markers represent 100 nm.

protrusions. The cause, as well as the consequent effect, of this clustering is intriguing but remains to be elucidated. HCMV gp52 spikes, however, are more slender and lack the sturdy appearance of HSV-1 gB spikes. In addition, HSV-1 gB spikes have a characteristic T-shape in side view which is not a pronounced feature of the HCMV spikes. It should also be noted that despite extensive sequence homology between the gB genes of HCMV and HSV-1, we found no cross-reaction between the two viruses in immunogold electron microscopy. The HCMV MAb F5 did not react with HSV-1, nor did two MAbs specific for HSV-1 gB (I-59-2 and II-105) react with HCMV AD169 virions. Therefore the epitopes recognized by these MAbs are apparently not part of the conserved regions of the gB genes.

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