Structure and composition of a family of human cytomegalovirus glycoprotein complexes designated gC-I (gB)

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Mature monoclonal antibodies (MAbs) were made to the 52000 (gp52) and the 93000 to 130000 Mr (gp93-130) glycoproteins from a human cytomegalovirus (HCMV) glycoprotein complex designated gC-I or the gB homologue. MAbs recognizing either gp52 or gp93-130 could immunoprecipitate unreduced gC-I complexes from non-ionic detergent extracts of HCMV. Western blotting was performed with immunoaffinity-purified gC-I complexes which were reduced prior to analysis. MAbs made against gp52 recognized gp52 and a 158000 Mr glycoprotein (gp158). MAbs which recognized gp93-130 in a Western blot also reacted with gp158, which is a gC-I precursor glycoprotein. The origin of gp93-130 was demonstrated by the reactivity of our gp93-130 MAbs with a recombinant protein containing the N-terminal portion of the gB gene. These data are consistent with the hypothesis that gp52 and gp93-130 are generated from the same high Mr precursor by proteolysis. MAbs recognizing either gp52 or gp93-130 neutralized Towne strain HCMV, but MAbs recognizing gp52 required complement to neutralize whereas MAbs recognizing gp93-130 did not. It was also determined that gp93-130 and gp158 have detectable amounts of O-linked glycans but gp52 does not, showing a difference in the glycosylation of these glycoproteins. Analysis of gC-I disulphide bonds showed that two types were present, one which was very susceptible to reduction and a second which was less susceptible. These complexes could consist of very susceptible inter-complex disulphide bonds and less susceptible intra-complex disulphide bonds.

Introduction

Human cytomegalovirus (HCMV) represents a significant cause of morbidity and mortality in immunocompromised hosts (Ho, 1982). The envelope glycoproteins of viruses are often involved in the attachment to and penetration of the host cell and thus envelope glycoproteins of HCMV are likely to be important targets of the immune response to HCMV. It has been established that HCMV contains several families of disulphide-linked glycoprotein complexes as well as individual glycoproteins in its envelope (Gretch et al., 1988a; Kari et al., 1986). One of the families of complexes, which we designated gc-I (Gretch et al., 1988a), contains glycoproteins encoded by the gene designated as being the homologue of herpes simplex virus gB (Cranage et al., 1986). Thus, the gc-I designation identifies the family of complexes and gB the glycoproteins within the complexes. The gc-I family consists of three complexes which have Mr's of 150000, 180000 and 250000, respectively (Farrar & Greenaway, 1986). The 180000 Mr complex contained two glycoproteins with Mr's of 52000 (gp52) and 130000 (gp130), respectively, associated by disulphide bonds. Both the 150000 and the 250000 Mr complexes contained gp52 and a 93000 to 95000 Mr glycoprotein (gp93) associated by disulphide bonds. The 250000 Mr complex appears to be a dimer of the 150000 Mr complex (Farrar & Greenaway, 1986; Taylor et al., 1988). Pulse-chase studies using radioactive precursors and studies using inhibitors of glycosylation suggested that gp52, gp93 and gp130 arise from a single gene product (Britt & Auger, 1986; Britt & Vugler, 1989; Gretch et al., 1988b; Taylor et al., 1988). Analysis of these glycoproteins shows that gp52, which contains a transmembrane domain for anchoring it in the viral envelope (Spaete et al., 1988), is encoded by the carboxy-terminal region of the gB gene. Based on the studies described above, the N-terminal portion of the gene most likely codes for gp93-130, but this has not been established.

Since the exact relationships among the different glycoproteins of the gc-I complexes have not been fully defined we have further characterized them using murine monoclonal antibodies (MAbs). We were also
interested in investigating the potential importance of antibody responses to the various gC-I glycoproteins. The data presented in this report show that gp52 and gp93-130 arise from a single gene product. In addition, both gp52 and gp93-130 contain epitopes which are recognized by neutralizing MAbs.

The MAbs were also used to analyse the tertiary and quaternary structure of the gC-I complexes. It has been demonstrated that some viral envelope proteins do not exist in the membrane as disulphide-linked complexes but form these bridges after they are extracted from the viral envelope (Pinter et al., 1978). When extracted from the envelope of HCMV, gC-I consists of at least three complexes which differ in composition. As stated above, some complexes are heterodimers of gp52 plus gp93 and some appear to form multimers that are complexes containing two copies of gp52 and two copies of gp93. This suggested that two types of disulphide bridges were present, those between individual glycoproteins within complexes and those between complexes. We isolated the gC-I complexes under different conditions and determined that there are two types of disulphide bonds present in gC-I complexes. One type is very susceptible to reduction and appears to form inter-complex disulphide bonds. The second type is much more resistant to reduction and appears to form intra-complex disulphide bonds.

**Methods**

**Preparation of Towne strain HCMV.** Towne strain HCMV was grown on human skin fibroblasts as previously described (Kari et al., 1986) and virions were harvested from the culture medium. Cells were removed by low-speed centrifugation and the virions collected by ultracentrifugation. Virions were labelled by growth with [3H]GlCN (Amersham).

**Monoclonal antibodies.** MAbs to gp52 and gp93-130 were generated and characterized as described previously (Kari et al., 1986; Lussenhop et al., 1988). To make MAbs to gp93-130, gC-I was immunofluori purified as described below, purified gC-I complexes were reduced and individual proteins separated by SDS-PAGE. Proteins were detected by Coomassie blue staining. Glycoprotein gp93-130 was electroeluted from the gel and used as antigen to immunize mice and for screening of purified gC-I complexes. Proteins were eluted from the bound antibody by heating at 100 °C in sample solubilization buffer (0.1 M-Tris-HCl pH 6.8 containing 4% SDS) for 3 min to enable them to be electrophoresed. Proteins were electroeluted from the gel and used as antigen to immunize mice and for screening of hybridomas.

**Neutralization assays.** MAbs were purified by Protein G and tested for neutralizing activity against Towne strain HCMV in a plaque reduction assay as previously described (Lussenhop et al., 1988). Approximately 1000 to 2000 p.f.u. were used in a 36 mm dish. Viral neutralization was evaluated by using the formula, percent plaque reduction = [(average maximum p.f.u. − test p.f.u.)/average maximum p.f.u.] × 100, and is reported as the protein concentration required for a 50% reduction in plaque number.

**Generation of vaccinia virus gB recombinants and the C-terminal deletion mutant m165.** The construction of recombinant vaccinia virus was achieved by developed techniques (Mackett et al., 1987). Briefly, an XmaIII fragment (3125 bp) containing the gB gene obtained from Towne strain HCMV was cloned into the Smal site of the pSC11 vaccinia virus vector (from B. Moss, NIH, Bethesda, Md., U.S.A.). The procedure followed was originally described by Cranage et al. (1986) for HCMV strain AD169. The vector was then introduced into vaccinia virus through homologous recombination by transfection with the pSC11-gB recombinant and co-infection with wild-type vaccinia virus. The resulting thymidine kinase-negative recombinant virus was selected in HuTK-143 cells in the presence of 25 μg/ml of bromodeoxyuridine (BUDr). Viruses were stained with 320 μg/ml of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal). Blue plaques were selected and purified three times on HuTK-143 cells in the presence of BUDr. The C-terminal deletion mutant of gB, gBm165, was generated by infecting pSC11-gB recombinant-carrying *Escherichia coli* DH1 cells with 467·Tn5 phase. The plasmids carrying the Tn5 transposon were selected by kanamycin resistance and screened as described (de Brujin & Lupsik, 1984; Weber et al., 1987). The physical location of the Tn5 insertion was obtained by restriction enzyme analysis, which was shown to be able to map the insertion site to within 50 bp of the actual nucleotide. The precise site of insertion was determined by the dsDNA sequencing technique using a primer approximately 50 to 100 nucleotides from the insertion area. The gB mutant m165 used in this study had Tn5 inserted at nucleotide location 1539, which corresponded to amino acid 513.

**Preparation of cells infected with vaccinia virus recombinants for immunofluorescence studies.** Subconfluent HuTK-143 cells in chamber slides were infected with vaccinia virus recombinants including vaccinia-pSC11, vaccinia-gB and vaccinia-gBm165 at an m.o.i. of 0.1. At 2 days post-infection the cells were fixed with cold methanol-acetone (1:1), stained by incubation with either a gp52-specific MAb (41C2) or a gp93-130-specific MAb (3C2) and washed and incubated with fluorescein isothiocyanate-labelled goat anti-mouse IgG. Following a final wash, cells were examined with a Zeiss phase fluorescence microscope.

**Purification of gC-I from Towne strain HCMV.** The gC-I complexes were isolated by a modification of an immunoaffinity method using biotinylated MAbs and streptavidin-agarose (Gretch et al., 1987). Briefly, virions were extracted with a Tris–HCl buffer pH 7.5 containing 10 mM-NaCl and 10% NP40 and the extract was centrifuged to remove insoluble material. A biotinylated MAb was added to 1-0% NP40 extracts containing gC-I and incubated for 30 min before the addition of streptavidin-agarose. This mixture was allowed to react for an additional 45 min with constant mixing. The agarose beads were pelleted by centrifugation and then washed with phosphate-buffered saline (PBS) containing 0.1% NP40 and twice with PBS. Proteins were eluted from the bound antibody by heating at 100°C in sample solubilizer buffer (0.2 M-Tris–HCl pH 6.8 containing 4% SDS) for 3 min to enable them to be electrophoresed.

**SDS–PAGE and fluorography.** Radioactively labelled immunoprecipitated glycoproteins were separated by SDS–PAGE in 10% polyacrylamide gels using the method of Laemmli (1970). Radioactivity in gels was detected by fluorography using ENHANCE (New England Nuclear). Fluorographs were scanned with a Hoefer Scientific GS300 scanning densitometer.

**Western blot analysis.** A mini gel apparatus (Bio-Rad) was used for Western blotting. Glycoproteins were separated in 10% polyacrylamide gels. After electrophoretic transfer to nitrocellulose, the paper was blocked with 3% gelatin in Tris-buffered saline (TBS; 20 mM-Tris–HCl, 500 mM-NaCl pH 7.5). MAbs in ascitic fluid were diluted 1/500 in 1% gelatin in TBS. Diluted MAbs were incubated with the paper overnight, after which the paper was washed with TBS containing 0.05% Tween 20. Phosphatase-labelled goat anti-mouse IgG (Kirkegaard & Perry), diluted 1/1000 with 1% gelatin in TBS, was added and allowed to react for 1 h, the paper was washed and the substrate
5-bromo-4-chloro-3-indolyl phosphate/tetrazolium in 0.1 M-Tris buffer (Kirkegaard & Perry) was added. After visualization of bands, the reaction was stopped by immersion in water.

**Dithiothreitol (DTT) treatment of whole virus.** Aliquots of purified \(^{[3]H}\)GlcN-labelled virions were suspended in PBS (pH 7.5) containing 50 mM-DTT and allowed to react at room temperature for 30, 60, 90 or 120 min with constant mixing. Excess iodoacetamide was added, the reaction was left for 30 min and virions were collected by centrifugation. The pellet was washed to remove residual reagents. As a control, virions were also exposed to iodoacetamide without prior treatment with DTT. Virions were then extracted as described above and gC-I complexes immunoprecipitated. Immunoprecipitations were first performed with a gp52-specific MAb (41C2), followed by a gp93-specific MAb (3C2).

**Galactose oxidase labelling.** Purified extracellular virions were labelled with tritiated borohydride after treatment with galactose oxidase following the methods of Gahmberg & Hakomori (1973). Briefly, whole virions in PBS (pH 7.5) were treated with 10 units of galactose oxidase (Sigma) for 1 h at room temperature. Virions were then treated with 3-3 mCi of tritiated borohydride (600 mCi/mmol; New England Nuclear) for 30 min. Excess borohydride was destroyed with 25% acetic acid (100 µl) at 0 °C. Virions were then collected by centrifugation and washed with PBS.

**Results**

**Immunoprecipitation and Western blot analysis of gC-I complexes**

For immunoprecipitations, virions were grown with \(^{[3]H}\)GlcN, purified, extracted with 1-0% NP40 and insoluble material was then removed by centrifugation. These extracts were immunoprecipitated with MAbs recognizing gp52 (18F9) or gp93-130 (3C2 and 9F9) and an SP2 ascites was used as a negative control; the latter did not immunoprecipitate any glycoproteins (Fig. 1a, b, lanes 1). All gp52-specific or gp93-130-specific MAbs immunoprecipitated complexes with Mr's ranging from 150000 to 250000 as detected by SDS–PAGE under non-reducing conditions (Fig. 1a, lanes 2 to 4). These complexes tended to form broad bands when examined by SDS–PAGE. After reduction, glycoproteins with Mr's of 52000 (gp52), 93000 to 130000 (gp93-130) and 158000 (gp158) were detected (Fig. 1b, lanes 2 to 4). Serial immunoprecipitations of unreduced complexes were done with these three MAbs. Regardless of whether a gp52-specific or a gp93-130-specific MAb was used for the initial immunoprecipitation, little radioactivity was immunoprecipitated with the other glycoprotein, showing that these MAbs recognized the same gC-I complexes (Fig. 1c, lanes 1 to 3). These data also showed that these gC-I complexes all contained both gp52 and gp93-130.

To determine which individual gC-I glycoproteins were recognized by the MAbs, Western blot analysis was performed using immunoaffinity-purified gC-I as an antigen which had been reduced and its individual

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**Fig. 1.** Towne strain HCMV was labelled with \(^{[3]H}\)GlcN and extracted with 1-0% NP40. This extract was immunoprecipitated with the MAbs using Protein A-Sepharose which had been preincubated with goat anti-mouse IgG. After extensive washing with PBS containing 0-1% NP40, the proteins were eluted from the beads with SDS–PAGE sample solubilization buffer. Samples were subjected to electrophoresis under (a) non-reducing conditions using a 7% polyacrylamide gel or (b and c) reducing conditions using a 10% polyacrylamide gel. (a and b) Lanes 1, SP2; lanes 2, MAb 18F9; lanes 3, MAb 3C2; lanes 4, MAb 9F9. (c) Serial immunoprecipitations. An extract was first precipitated with MAb 3C2 and then with 18F9 (lane 1), with MAb 18F9 and then 3C2 (lane 2) or with MAb 9F9 followed by 3C2 (lane 3). SP2 was a negative ascites control. Numbers to the right indicate Mr's x 10^-3. MAb 18F9 is a gp52 MAb made using whole HCMV, whereas MAbs 3C2 and 9F9 are gp93 MAbs made using purified gp93.

**Fig. 2.** gC-I complexes were immunoaffinity-purified from a non-ionic detergent extract of Towne strain HCMV. Approximately 100 µg of gC-I was reduced and gC-I glycoproteins were separated by SDS–PAGE in a 9% polyacrylamide gel. Proteins were then electrophoosed onto nitrocellulose paper, the paper was cut into strips and the strips were incubated with the negative control SP2 or MAbs. Lane 1 shows a portion of the gel which was cut off and stained with Coomassie blue. Lane 2, SP2 negative ascites control; lanes 3 and 4, gp93-specific MAbs 3C2 and 9F9, respectively; lanes 5 to 7, gp52-specific MAbs 39E11, 9B7 and 18F9, respectively. Numbers to the right and left indicate Mr's x 10^-3.
Table 1. The amount of MAb needed to reduce the number of HCMV plaques by 50%.

<table>
<thead>
<tr>
<th>MAb</th>
<th>Subtype</th>
<th>MAb + C* (μg/ml)</th>
<th>MAb − C* (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3C2 (gp93)</td>
<td>IgG1</td>
<td>4-4</td>
<td>2-4</td>
</tr>
<tr>
<td>9F9 (gp93)</td>
<td>IgG1</td>
<td>4-2</td>
<td>2-4</td>
</tr>
<tr>
<td>18F9 (gp52)</td>
<td>IgG2b</td>
<td>3-6</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

* Amount of MAb in μg/ml needed to reduce the number of Towne HCMV plaques by 50% with complement (+ C*) or without complement (− C*). MAbs were purified with a Protein G column. Each MAb was tested in a range of 1 to 100 μg/ml of antibody with or without guinea-pig complement. Approximately 1000 p.f.u. of Towne HCMV were used in a 36 mm dish.

Reactivity of MAbs with gB recombinant proteins

Cells infected with vaccinia virus recombinants containing the entire gB open reading frame (ORF) (vaccinia-gB) and the C-terminal deletion of gB (vaccinia-gBm165), obtained by Tn5 transposition mutagenesis, was analysed by immunofluorescence using gp52 and gp93-130 MAbs. A C-terminal deletion was used to eliminate epitopes recognized by gp52 MAbs. The C-terminal portion of the gene encodes gp52, which starts at amino acid 461 and runs to the C terminus (Spaete et al., 1988). MAbs recognizing gp52 or gp93-130 did not detect viral antigens in cells infected with vaccinia-pSC11 which was used as a negative control (Fig. 3a, b). However, reactivity was detected with both gp52 and gp93-130 MAbs in cells infected with vaccinia-gB (Fig. 3c, d) and with gp93-130 MAbs in cells infected with vaccinia-gBm165 (Fig. 3e, f), which contained the first 513 amino acids of the N-terminal portion of gB according to restriction enzyme and sequence analysis. These data show that gp52 and gp93-130 are derived from the same gene and that the epitopes recognized by gp93-130 MAbs are located on the N-terminal portion of gB.

Neutralization analysis of murine MAbs

There have been several reports describing neutralizing MAbs which recognize gp52 (Banks et al., 1989; Britt, 1984; Britt et al., 1988; Lussenhop et al., 1988; Rassmussen et al., 1985). However, there have been no reports describing MAbs recognizing gp93-130 that neutralize HCMV. Therefore, we analysed our gp52 and gp93-130 MAbs for their ability to neutralize Towne strain HCMV, in the presence and absence of complement, in an in vitro plaque reduction assay. As we previously reported (Lussenhop et al., 1988), three MAbs recognizing continuous epitopes on gp52 (39E11, 9B7 and 18F9) neutralized Towne strain HCMV, but only in the presence of complement. Data for 18F9 are also presented in Table 1. Neutralization data for 39E11, 9B7
gp93-130 contains epitopes recognized by and 18F9 have been previously reported (Lussenhop et al., 1988). Therefore, both gp52 and gp93-130 neutralized Towne strain HCMV in the absence of complement (Table 1). Therefore, both gp52 and gp93-130 contain epitopes recognized by neutralizing MAbs but the mechanism of neutralization appears to be different.

gp93-130 is glycosylated differently from gp52

To determine whether gp52 and gp93-130 in the gC-I complexes were accessible on the surface of the virus and to characterize further the glycoproteins present in these complexes, extracellular virus was treated with galactose oxidase and oxidized residues were reduced with tritiated borohydride. This method was selected because it has been shown that gp93-130 contains O-linked oligosaccharides (Britt & Vugler, 1989; Kari & Gehrz, 1988). After labelling, virions were extracted with 1.0% NP40, insoluble material was removed by centrifugation and extracts were immunoprecipitated with a gp52 MAb (41C2), followed by a gp93-130 MAb (3C2).

Immunoprecipitates obtained from the NP40 extracts were examined by SDS-PAGE and fluorography under non-reducing and reducing conditions. Fluorograms were exposed for 5 days and 2 to 3 weeks in order to detect all changes in the relative amounts of the virions gC-I glycoproteins. This was particularly important with regard to gp52 which was not as heavily glycosylated as gp93-130. Examination of the control virus sample by SDS-PAGE under non-reducing conditions (Fig. 5a, lane 1), showed that it contained complexes with Mr's of 150000 to 250000. These data suggest that the disulphide bonds in the 250000 Mr complexes were not formed after extraction from the envelope of HCMV, but were originally present. However, when immunoprecipitates from DTT-treated virions were examined by SDS-PAGE, the 250000 Mr complexes were almost completely lost whereas the amount of the 150000 to 180000 Mr complexes were released from the virions by treatment with DTT so these initial supernatants were analysed for the presence of free gp93-130; none was detected (data not shown). The DTT-treated virions were then extracted with 1.0% NP40, insoluble material was removed by centrifugation and extracts were immunoprecipitated with a gp52 MAb (41C2), followed by a gp93-130 MAb (3C2).

Analysis of disulphide bonds in gC-I complexes

Previous analysis has suggested that the envelope of HCMV contains dimers of gC-I complexes (Farrar & Greenaway, 1986). These dimers were the species with the greatest Mr detected by SDS-PAGE, having an apparent Mr of 250000. We analysed the conditions under which these complexes could be obtained from virions to determine whether they cross-linked before or after isolation of the gC-I complexes. This was done by extracting gC-I complexes in the presence of iodoacetamide to prevent disulphide bond formation during isolation and by exposing virions to reducing agents prior to extraction of the gC-I complexes. To do this, virions were treated with DTT for 30, 60, 90 and 120 min, then by iodoacetamide, and collected by centrifugation. As a control, virions were treated with the same buffers and iodoacetamide but DTT was omitted. gp93-130 may have been released from the virions by treatment with DTT so these initial supernatants were analysed for the presence of free gp93-130; none was detected (data not shown). The DTT-treated virions were then extracted with 1.0% NP40, insoluble material was removed by centrifugation and extracts were immunoprecipitated with a gp52 MAb (41C2), followed by a gp93-130 MAb (3C2).

Fig. 4. Purified HCMV virions were labelled by treatment with galactose oxidase and reduction with tritiated borohydride. Tritium-labelled gC-I complexes were examined by SDS-PAGE under non-reducing or reducing conditions followed by fluorography. (a) Lane 1, control done by omitting galactose oxidase treatment; lane 2, gC-I complexes labelled and separated by SDS-PAGE under non-reducing conditions. (b) This figure is the same as (a) except that SDS-PAGE was done under reducing conditions. (c) gC-I complexes labelled by growing virus with [3H]GlcN were examined by SDS-PAGE for comparison under non-reducing conditions (lane 1) or reducing conditions (lane 2). Note that gp52 (lane 2) was labelled with [3H]GlcN, but was not labelled by galactose oxidase and tritiated borohydride treatment. Figures show Mr's × 10^2.
Fig. 5. HCMV virions labelled with [3H]GlcN were exposed to 50 mM-DTT for 0, 30, 60, 90 and 120 min (lanes 1 to 5). At the end of each time period, excess iodoacetamide was added. Virions were collected by centrifugation and extraction with 1% NP40. These extracts were immunoprecipitated with a gp52 MAb, 41C2. Immunoprecipitated gC-I complexes were examined by SDS-PAGE under non-reducing and reducing conditions followed by fluorography. (a) gC-I complexes examined under non-reducing conditions. This fluorogram was exposed for 3 weeks in order to detect the increase in free gp52 with increasing exposure of the virus to DTT. (b) A duplicate fluorogram which was only exposed for 5 days was scanned with a densitometer to detect changes in the relative amounts of the 250000 and the 150000 to 180000 M$_r$ complexes. The fluorogram lane scanned is indicated below each scan. The peak indicated by an arrow to the right in each scan indicates the point at which the scan was initiated on the fluorogram. Peak 1 is the 250000 M$_r$ complex, peak 2 the 150000 to 180000 M$_r$ complex, peak 3 gp93 and peak 4 gp52. (c) This figure is the same as (a) except reducing conditions were used and the fluorogram was exposed for only 2 weeks.

complexes increased in relative amount (Fig. 5a, b, lane 2). This occurred within 30 min of exposure to DTT. Some of the 150000 to 180000 M$_r$ complexes were still present after 120 min of exposure to DTT (Fig. 5a). This suggested that reduction of the disulfide bonds forming these complexes was much slower than reduction of disulfide bonds forming the 250000 M$_r$ complexes. However, a scan of a fluorogram exposed for 5 days showed that the amount of the 150000 to 180000 M$_r$ complexes decreased between 30 and 120 min of DTT exposure (Fig. 5b, lanes 2 to 5). As the relative amount of these complexes decreased, the relative amount of gp93-130 and gp52, which dissociated from the complexes, increased, as shown by SDS-PAGE performed under non-reducing conditions (Fig. 5a, b, lanes 2 to 5). The complete change in the relative amount of free gp52 was only clearly detected after a 3 week exposure of the fluorogram and thus some of the early increase in the amount of free gp52 was not detected by scanning a fluorogram exposed for 5 days (Fig. 5a, b). These data suggested that a portion of gp93-130 and gp52 remained associated with each other or with the gC-I complexes even though disulfide bonds between gp52 and gp93-130 were cleaved. Moreover, the non-covalent bonds between gp93-130 and gp52 were not disrupted by extraction from the viral envelope with a non-ionic detergent. When the gC-I complexes obtained from DTT-treated virions were reduced, the glycoproteins and their relative amounts were the same as that from the control virions which were not exposed to DTT prior to extraction (Fig. 5c). After immunoprecipitating extracts from the DTT-treated virions with a gp52 MAb, a gp93-130 MAb (3C2) was used. When this was done no additional radioactivity was immunoprecipitated (data not shown). These data showed that there was no free gp93-130 left in the extracts.

Discussion

The primary objective of this study was to characterize the relationships between the various glycoproteins and complexes in the family of HCMV glycoprotein complexes designated gC-I (Gretch et al., 1988a). Another objective was to determine the neutralizing activity of MAbs reactive with epitopes uniquely expressed on antigenically distinct glycoproteins in the gC-I complexes. Previous analysis has indicated that gC-I glycoproteins are derived from a single precursor glycoprotein reported to have an M$_r$ of 158000 to 170000 (Britt & Auger, 1986; Britt & Vugler, 1989; Gretch et al., 1988b; Taylor et al., 1988). After final processing events the M$_r$ of this glycoprotein is reduced to 138000 (Gretch et al., 1988b). This glycoprotein is a stable intermediate and is apparently cleaved to generate gp52 and gp93 (Gretch et al., 1988b). An additional gC-I glycoprotein has been described with an M$_r$ of 130000 (gp130) (Taylor et al., 1988). Glycoprotein gp130 may reflect alternative processing of gp138, which may involve differences in glycosylation or differences in proteolytic cleavage. The reactivity of our gp93-130 MAbs with vaccinia-gBm165 showed that gp93-130 was derived from the N-terminal portion of the gene encoding gp52. This result is consistent with previous work which suggested that gC-I glycoproteins are derived from a single gene product (Britt & Auger, 1986; Britt & Vugler, 1989; Gretch et al., 1988b; Taylor et al., 1988). In SDS–PAGE, gp93-130 tends to form a broad band, whereas gp52 tends to form a
The diffuse band formed by gp93-130 in SDS–PAGE may be due to the fact that gp93-130 is more heavily glycosylated than gp52 (Britt & Vugler, 1989). In addition, this diffuse band could contain gp93 and gp130, as described by others (Farrar & Greenaway, 1986). If this band contains both gp93 and gp130, then both glycoproteins lack the epitopes recognized by our gp52-specific MAbs. These epitopes were apparently removed by post-translational proteolytic cleavage of gp158 to produce the mature gp93-130. We were able to obtain neutralizing MAbs which recognized either gp52 or gp93-130 and, to our knowledge, there have been no previous reports of MAbs recognizing gp93-130 or of MAbs which recognize gp93-130 neutralizing HCMV. Furthermore, the mechanism of neutralization of our gp93-130 MAbs was different from that of our gp52 MAbs in that they did not require complement. We have also found that there are human antibody responses to both gp52 and gp93-130 (data not shown) which suggests that human immune responses to both gp52 and gp93-130 may be important in limiting infection with HCMV. The radioactive labelling experiments presented in this report showed that gp93-130 contained O-linked glycans, but that gp52 did not, which is consistent with previous observations (Britt & Vugler, 1989). It is interesting to note that both glycoproteins contain a significant amount of hydroxyamino acids, based on analysis of the gene encoding them (Spaete et al., 1988). Nonetheless, only gp93-130 contains significant amounts of O-linked glycans. Very little is known about the signals involved in O-glycosylation and thus, the glycoproteins of gC-I may provide a model for their study.

Our preparations of gC-I contained a glycoprotein of high $M_r$ designated gp158. After Coomassie blue staining, gp158 appeared to be a minor component relative to gp52 and gp93-130 and could therefore have been a precursor present due to contamination of the virus preparation with infected cell membranes. This appeared to be the case for two reasons. First, its $M_r$ was similar to that reported for gC-I precursor glycoproteins (Britt & Auger, 1986; Britt & Vugler, 1989; Gretch et al., 1988b) and, second, this glycoprotein was not detected when virions were purified by a combination of differential centrifugation and sucrose gradient purification (Gretch et al., 1988a, b; Kari et al., 1986). Glycoprotein gp158 did contain galactose and/or galactosamine residues, suggesting that it was at a late stage of processing. One explanation for these results is that gp158 was processed to a similar extent as gp52 and gp93-130 with the exception of the proteolytic step which would cleave it into the lower $M_r$ gC-I glycoproteins. These results also suggest that the proteolytic step which generates gp52 and gp93-130 occurs late in the processing of the gB gene product.

The glycoprotein complexes which compose the gC-I family found in the envelope of HCMV have $M_r$s which are heterogeneous. gC-I complexes with an $M_r$ of 250,000 appear to contain two copies of gp52 and two copies of gp93, which makes them a multimer of the 150,000 $M_r$ complexes which contain one copy of each of gp52 and gp93 (Farrar & Greenaway, 1986). These complexes do not appear to be artefacts of isolation. The inter-complex disulphide bonds forming the 250,000 $M_r$ complexes are much more susceptible to DTT than intra-complex disulphide bonds as almost complete reduction of inter-complex disulphide bonds was observed after 30 min, whereas intra-complex disulphide bonds were present after 120 min of exposure to DTT. The presence of disulphide bonds between viral glycoproteins which differ in their sensitivity to reducing agents has been reported for influenza virus (Selimova et al., 1988). In this case a gradual loss of infectivity was observed as the concentration of reducing agent increased, which suggested that maintenance of higher order structures was important to the functions of the surface glycoproteins. Because HCMV envelope glycoproteins other than gC-I were reduced at the same time as gC-I (data not shown), any loss of infectivity of HCMV could be attributed to disruption of more than one family of complexes. Thus, it would be difficult to determine the effect reduction of gC-I would have on infection. The reason for inter-complex disulphide bonds is not clear but they may be important in stabilizing these complexes and defining the surface architecture of HCMV, which is most likely to be important in maintaining the ability to infect a host cell. Although intra-complex disulphide bonds were more stable in the presence of DTT, there was still some reduction of these bonds. This was demonstrated by the fact that gp52 and gp93-130 could be dissociated by SDS–PAGE without further exposure to reducing agents. Thus, even though gp52 and gp93-130 were no longer disulphide-linked, they remained associated even after being extracted from the virion envelope with a non-ionic detergent. This implies that these glycoproteins have strong non-covalent bonds which were only disrupted by the harsh preparative conditions for SDS–PAGE. Analysis of the gene encoding gC-I glycoproteins has shown that gp52 contains a transmembrane domain which would anchor it in the envelope of HCMV (Spaete et al., 1988), whereas the portion of this gene encoding gp93-130 contains no transmembrane domain. It is interesting to speculate that gp93-130 may remain associated with the envelope of HCMV by its association with gp52. These associations apparently include both covalent and non-covalent bonds.

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References


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