Biochemical and immunological analysis of discontinuous epitopes in the family of human cytomegalovirus glycoprotein complexes designated gC-I

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The envelope of human cytomegalovirus contains a family of disulphide-linked glycoprotein complexes designated gC-I which contain two glycoproteins of 52000 \( M_r \) (gp52) and 93000 to 130000 \( M_r \) (gp93-130). Epitopes recognized by several of our gC-I gp52-specific monoclonal antibodies (MAbs) were previously assigned to three domains based on reactivity with gC-I in a competitive binding assay. In this report, we have used additional gC-I MAbs to characterize three distinct discontinuous epitopes in the gC-I complexes. Two of these epitopes were in Domain I and one in Domain III. These epitopes were resistant to proteolysis, heat denaturation and SDS treatment. However, the discontinuous epitopes were lost after reduction of disulphide bonds. After digestion of gC-I complexes with chymotrypsin, two fragments of 43000 (43K) \( M_r \) and 34000 (34K) \( M_r \) were obtained which contained all discontinuous and continuous epitopes recognized by our gp52 MAbs. The \( M_r \) of these fragments could not be reduced further by longer digestion or by use of other proteases such as trypsin or pronase. The 43K fragment contained N-linked oligosaccharides not detected in the 34K fragment. These oligosaccharides may have prevented a complete proteolytic digestion so that the 34K fragment was not always obtained. It was established that 80 to 90% of the mass of these fragments was contributed by gp52. Thus the discontinuous epitopes were composed primarily of gp52 and not gp93-130.

Introduction

Human cytomegalovirus (HCMV) is a member of the herpes family of viruses and is an important pathogen of man. Infection with HCMV is usually asymptomatic and self-limiting in normal individuals. However it can cause severe birth defects in the developing foetus if contracted in utero and can be devastating in immunosuppressed patients (Ho, 1982). The envelope of HCMV contains at least three families of disulphide-linked glycoprotein complexes (Gretch et al., 1988a; Karl et al., 1986). Many of the glycoproteins in these complexes are recognized by human anti-HCMV sera (Kari & Gehrz, 1990). Thus, immune responses to these glycoproteins are likely to be important in preventing or limiting an HCMV infection. One of these families of complexes, which we designated gC-I (Gretch et al., 1988a), contains glycoprotein complexes which range in \( M_r \) from 150000 to 250000 (Kari et al., 1990; Farrar & Greenaway, 1986). The gC-I complexes contain glycoproteins encoded by the gB gene of HCMV (Cranage et al., 1986). In Towne strain HCMV, the gB gene product is synthesized as a large precursor glycoprotein that is cleaved to generate two glycoproteins of 52000 (gp52) and 93000 to 130000 (gp93-130) \( M_r \) (Kari et al., 1990; Britt & Vugler, 1989; Gretch et al., 1988b; Britt & Auger, 1986). Because gC-I complexes are large disulphide-linked structures they have the potential to contain many continuous and discontinuous epitopes. Both gp52 (Lussenhop et al., 1988) and gp93-130 (Kari et al., 1990) contain epitopes recognized by neutralizing murine monoclonal antibodies (MAbs). However, most MAbs made using whole virus as antigen have recognized gp52 (Banks et al., 1989; Karl et al., 1986; Britt, 1984; Rasmussen et al., 1984). Many of these MAbs recognize epitopes that map between amino acids 400 and 700 on gp52 (Banks et al., 1989; Utz et al., 1989).

Previously we mapped the epitopes on gp52 recognized by several of our MAbs (Lussenhop et al., 1988). Using a competitive binding ELISA, we were able to assign the epitopes recognized by the MAbs into three domains based on the ability of the MAbs to inhibit the binding of other MAbs. A model is shown in Fig. 1 which illustrates how the three domains and their epitopes could be arranged based on the competitive binding ELISAs. Domain I contained epitopes recognized by MAbs 41C2, 26B11 and 39E11. These epitopes were both continuous (41C2 and 39E11) and discontinuous (26B11). Domain II contained only continuous epitopes recognized by MAbs 9B7, 18F9 and 34G7. Both
Domains I and II contained epitopes recognized by neutralizing MAbs. In addition we observed that MAbs recognizing epitopes in Domain II augmented the binding of MAbs recognizing epitopes in Domain I. Domain I MAb 39E11 inhibited the binding of Domain II MAbs 9B7 and 18F9 suggesting that the epitopes recognized by these MAbs were where the two domains overlapped. MAbs 39E11, 9B7 and 18F9 were the only MAbs to neutralize HCMV. Neutralizing MAbs are indicated with an N in the figure. Discontinuous and continuous epitopes are also indicated.

**Methods**

**Preparation of HCMV and MAbs.** HCMV was grown on human skin fibroblasts, harvested and purified as described (Kari et al., 1986). Towne, Toledo and AD169 strains of HCMV were used. Virus was labelled with either [3H]arginine (Arg) or [3H]glucosamine (GlcN) (Amersham). MAbs to gp52 (Lussenhop et al., 1988) and gp93-130 (Kari et al., 1990) were generated and characterized as described.

**Simultaneous two-antibody-binding assay.** This assay was done as previously described (Lussenhop et al., 1988). Briefly, purified Towne strain HCMV or whole gC-I were coated onto Immunolon 96-well microtitre plates. Some of the MAbs were biotinylated as described (Gretch et al., 1987). A fixed amount of biotinylated MAb and varying amounts of a second unlabelled MAb were added simultaneously to each well and incubated for 90 min at room temperature. Wells were washed, and peroxidase-labelled streptavidin was added and incubated for 90 min. After washing, the substrate o-phenylenediamine was added and the AC40 was read.

**Proteolysis.** Extracellular virus was solubilized with 10% NP40 in 50mM-Tris–HCl buffer pH 7.4 containing 150 mM-NaCl. Insoluble material was removed by centrifugation at 16000 g for 30 min. The extract was collected and protein content determined with the BCA protein assay (Pierce). The extract was digested with chymotrypsin (Sigma) at an enzyme:protein ratio of 1:50 for 24 h at room temperature. Proteolysis was terminated by addition of PMSF.

**Purification of whole gC-I and gC-I chymotrypsin fragments.** Whole gC-I complexes or gC-I proteolytic fragments were isolated by an immunoaffinity method using biotinylated MAbs and streptavidin–agarose as previously described (Gretch et al., 1987). Briefly, a biotinylated gp52-specific MAb (41C2) was added to 10% NP40 extracts containing gC-I complexes or gC-I chymotrypsin fragments. The MAb was incubated with the extracts for 30 min before adding 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 PBS containing 0.1% NP40 and twice with PBS. Whole gC-I or gC-I chymotrypsin fragments were eluted from the bead complex by heating at 100 °C in sample solubilization buffer for 3 min (0.2 M-Tris–HCl pH 6.8, containing 4% SDS).

**CNBr cleavage of gC-I chymotrypsin fragments.** Fragments which had been immunoprecipitated were eluted from the streptavidin–agarose beads with 70% formic acid. The beads were pelleted by centrifugation and the supernatant was collected. Excess CNBr was added to the supernatant and the reaction mixture was placed under an atmosphere of nitrogen. The reaction was allowed to proceed at room temperature for 24 h in the dark. The reaction was stopped by the addition of water. Samples were dried under vacuum twice before solubilization in SDS-PAGE sample solubilization buffer.
Table 1. Simultaneous two-antibody-binding assay

<table>
<thead>
<tr>
<th>Competing unlabelled MAb/</th>
<th>Inhibition/augmentation (%)*</th>
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<tr>
<td>Domain I epitope type</td>
<td>Domain II</td>
</tr>
<tr>
<td>41C2† Continuous</td>
<td>9B7†</td>
</tr>
<tr>
<td>30C7/Discontinuous</td>
<td>18F9†</td>
</tr>
<tr>
<td>38B11/Discontinuous</td>
<td>34G7†</td>
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| 41C2† Continuous          | +99 ± 2                     |
| 30C7/Discontinuous        | +91 ± 7                     |
| 38B11/Discontinuous       | +97 ± 4                     |
| 26B11/Discontinuous       | +96 ± 2                     |
| 39E11/Continuous          | -90 ± 0                     |
| 41C2† Continuous          | +306 ± 46                   |
| 30C7/Discontinuous        | +209 ± 80                   |
| 38B11/Discontinuous       | +208 ± 52                   |
| 26B11/Discontinuous       | -76 ± 1                     |
| 39E11/Continuous          | -91 ± 3                     |
| 41C2† Continuous          | +153 ± 13                   |
| 30C7/Discontinuous        | +105 ± 3                    |
| 38B11/Discontinuous       | +119 ± 0                    |
| 26B11/Discontinuous       | -68 ± 11                    |
| 39E11/Continuous          | -94 ± 3                     |
| 41C2† Continuous          | +39 ± 8                     |
| 30C7/Discontinuous        | +17 ± 5                     |
| 38B11/Discontinuous       | +30 ± 4                     |
| 26B11/Discontinuous       | +38 ± 7                     |
| 39E11/Continuous          | +47 ± 2                     |

* Inhibition is indicated by a minus sign and augmentation by a plus sign. Values are the percentage increase or decrease in A490 of two separate experiments plus and minus the mean.
† Biotinylated MAbs, 41C2, 9B7, 18F9 and 34G7, were incubated with HCMV in the presence of another unlabelled MAb. Binding of the biotinylated MAb was determined in an ELISA.

SDS-PAGE and fluorography. Radioactively labelled glycoproteins were separated by SDS-PAGE in straight 10% polyacrylamide gels or in 5 to 15% polyacrylamide gradient gels using the method of Laemmli (1970). Radioactivity in gels was detected by fluorography using Enhance (New England Nuclear).

Western blotting. Chymotrypsin gC-I fragments were separated in 10% polyacrylamide gels and electroblotted onto nitrocellulose membranes with a 0.2 μm pore size. After electroblotting, the paper was blocked with 3% gelatin in Tris-buffered saline (TBS) (20 mM-Tris-HCl, 500 mM-NaCl, pH 7.5; MAb in ascites fluid were diluted 500-fold and human sera were diluted 30-fold in 1% gelatin in TBS. Human sera were obtained from congenitally infected infants and from convalescent adults. MAb and human sera were incubated with the paper overnight. The paper was washed with TBS containing 0.05% Tween 20. Phosphatase-labelled goat anti-mouse IgG or goat antihuman IgG (Kirkegaard & Perry), diluted 1000-fold 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-HCl buffer was added. When the bands became visible, the reaction was stopped by immersing in water.

Deglycosylation. N-glycanase (Genzyme) was used according to the manufacturer's directions to remove N-linked oligosaccharides from unreduced gC-I chymotrypsin fragments.

Results

Simultaneous two-antibody-binding assay

We have previously established that Domains I and III each contained at least one discontinuous epitope based on loss of reactivity after reduction of disulphide bonds. We had also established that three other gC-I-specific MAb (30C7, 43C8 and 38B11) recognized discontinuous epitopes (Lussenhop et al., 1988). Therefore, a simultaneous two-antibody-binding assay was used to determine whether the epitopes recognized by these MAb were contained in the domains that we previously described. All three MAb augmented the binding of Domain II MAb 9B7, 18F9 and 34G7 and inhibited the binding of Domain I MAb 41C2 (Table 1). These data placed the epitopes recognized by these MAb in Domain I. The ability of MAb 30C7, 18F9 and 34G7, which recognized discontinuous epitopes in Domain I, to augment the binding of Domain II MAb was the same as that of Domain I MAb 41C2 which recognized a continuous epitope. A fourth MAb (26B11) which recognized a discontinuous epitope in Domain I inhibited the binding of Domain II MAb 9B7 and 18F9, but augmented the binding of Domain II MAb 34G7 (Table 1). Thus, Domain I contained at least two discontinuous epitopes, one recognized by MAb 30C7, 43C8 and 38B11, and the other by MAb 26B11.
isolated from the Toledo and Towne strains of HCMV (Fig. 2b, lanes 1 and 2) and an $M_r$ value of 80000 to 116000 when isolated from the AD169 strain of HCMV (Fig. 2b, lane 3). gC-I isolated from the Toledo and Towne strains also contained a less abundant glycoprotein with an $M_r$ of 158000 (Fig. 2b, lanes 1 and 2). This glycoprotein is a precursor to gp52 and gp93-130 and was most likely to be present in the virus preparation due to contamination with cellular membranes (Kari et al., 1990). With strain AD169, this glycoprotein had a lower $M_r$ (Fig. 1b, lane 3).

The NP40 extracts containing gC-I complexes were digested with chymotrypsin for 24 h. Kinetic studies showed that the digestion was complete in 24 h (data not shown). After digestion with chymotrypsin, gC-I fragments were immunoprecipitated with a gC-I gp52-specific MAb 41C2. Two fragments were immunoprecipitated when all three strains were used. Under non-reducing conditions, these fragments were 43000 (43K) and 34000 (34K); representative data for AD169 and Towne are shown in Fig. 3(a), lanes 1 and 2. Thus, although the $M_r$ values of the gC-I glycoproteins varied between strains, those of the chymotrypsin fragments did not. When gC-I was labelled with $^{[3]H}$GlcN, the 43K and 34K fragments were equally labelled (Fig. 3a, lanes 1 and 2). However, when gC-I was labelled with $^{[3]H}$Arg or when the fragments were stained with Coomassie blue, the 43K fragment was more abundant and the 34K fragment was a minor component (Fig. 3a, lanes 3 and 4). Longer digestion with chymotrypsin, or use of trypsin or pronase instead of chymotrypsin did not further reduce the $M_r$ values of these fragments or produce a single fragment (data not shown).

After reduction of disulphide bonds in the 43K and 34K fragments, one abundant glycoprotein was detected with an $M_r$ of 34000 (representative data for Towne strain HCMV is shown in Fig. 3b, lanes 1 and 2). This glycoprotein labelled efficiently with either $^{[3]H}$Arg or $^{[3]H}$GlcN. The next most abundant glycoprotein had an $M_r$ of 9000 (Fig. 3b). At least two minor polypeptides were detected which had $M_r$ values between 28000 and 34000 (Fig. 3b).

**Biochemical characterization of the 34K and 43K fragments**

The 34K and 43K fragments were analysed to determine their differences. The number of peptides in each unreduced fragment was determined by a two-dimensional system. The 34K and 43K fragments labelled with $^{[3]H}$Arg were obtained from Towne strain HCMV and first separated by SDS-PAGE under non-reducing conditions. The unreduced fragments were cut from the gel, then each fragment was reduced with 2-mercaptoethanol (2-ME) and subjected to SDS-PAGE a second time. When this was done, the 43K fragment was found to contain polypeptides of $M_r$ 30000 to 34000 and 9000 (Fig.
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Fig. 4. (a) gC-I chymotrypsin fragments were labelled with [3H]Arg and isolated from Towne strain HCMV. The 43K and 34K fragments were first separated by SDS-PAGE under non-reducing conditions (lane 2). The individual fragments were cut from the gel and reduced with 2-ME prior to SDS-PAGE a second time. Lane 1, peptides obtained after reduction of the 43K fragment. Lane 3, peptides obtained after reduction of the 34K fragment. (b) Unreduced gC-I fragments were digested with N-glycanase to determine whether there were differences in the accessibility of N-linked oligosaccharides in the 43K and 34K fragments. Lane 1, undigested [3H]Arg-labelled 43K and 34K fragments. Lane 2, [3H]Arg-labelled 43K and 34K fragments digested with N-glycanase. Lane 3, undigested [3H]GlcN-labelled 43K and 34K fragments. Lane 4, [3H]GlcN-labelled 43K and 34K fragments digested with N-glycanase. (c) Whole virions, labelled with [3H]GlcN, were reduced with DDT and alkylated to eliminate inter-complex disulphide bonds. These virions were extracted with NP40, then the extract was digested with chymotrypsin and immunoprecipitated with gp52-specific MAb 41C2.

4a, lanes 1 and 2). The 34K fragment contained a 28000 to 30000 M₉ polypeptide (Fig. 4a, lanes 2 and 3). The 34K fragment probably contained other small peptides which either did not contain Arg residues or were too small to be retained in the gel. Thus, a major difference between the 43K and 34K fragments was the presence in the 43K fragment of a polypeptide of 9000 M₉.

Another possible difference in the 34K and 43K fragments was in their polysaccharide content. For instance, Savvidou et al. (1981) described a human IgG1 κ MAb protein in which some L chains were glycosylated at asparagine 107 and some were not. Thus, it was possible that not all gC-I glycoprotein molecules contained the same number of N-linked oligosaccharides. The presence of additional polysaccharides could prevent complete proteolysis. Therefore, we treated the 34K and 43K fragments with N-glycanase. This glycosidase is capable of removing a variety of N-linked oligosaccharides from proteins. Both [3H]Arg- and [3H]GlcN-labelled fragments were used in these experiments. When this was done, the M₉ of the 43K fragment was reduced by about 4000, whereas the 34K fragment did not show a significant reduction in M₉ (Fig. 4b, lanes 1 and 2). Furthermore, the GlcN label was lost from the 43K fragment as a band at 39K appeared. This occurred with little or no loss of GlcN label from the 34K fragment (Fig. 4b, lanes 3 and 4). Therefore, the unreduced 43K fragment contained N-linked oligosaccharides which were susceptible to N-glycanase and the 34K fragment did not. These data showed a difference in the N-glycosylation of these two fragments which could have affected their susceptibility to proteolysis.

We have also shown that gC-I complexes contain both intra-complex disulphide bonds and inter-complex disulphide bonds (Kari et al., 1990). However, only the gC-I complexes of M₉ 250000 contained the inter-complex disulphide bonds. This difference in higher order structure could also account for differences in the extent of proteolysis which occurred among the complexes. Therefore, extracellular virus was exposed to reducing agents to eliminate inter-complex disulphide bonds (Kari et al., 1990). The virions were then extracted with NP40 to solubilize gC-I complexes, which were digested with chymotrypsin. The fragments were immunoprecipitated with MAb 41C2. When this was done, the 43K and the 34K fragments were still obtained (Fig. 4c). Thus, the presence of gC-I complexes with inter-complex disulphide bonds did not influence the extent of proteolysis.

Immunological characterization of the 34K and 43K fragments

The fragments generated by chymotrypsin digestion were analysed to determine whether they contained the continuous and discontinuous epitopes recognized by our gC-I gp52-specific MABs and were reactive with human anti-HCMV sera. After digestion with chymotrypsin the 34K and 43K fragments could be immunoprecipitated by MAbs which recognized epitopes in all three domains, including those which recognized discontin-
uous epitopes (MAb 11B4, Domain III) (Fig. 5a, lanes 1 to 3). In addition the 34K and 43K fragments were immunoprecipitated by human anti-HCMV sera (Fig. 4b, lanes 1 to 3). Interestingly, the polyvalent human sera did not immunoprecipitate any other fragments. Thus, the only [3H]GlcN-labelled epitopes left intact and capable of eliciting a human antibody response were those contained in the 34K and 43K fragments.

**SDS treatment and Western blot analysis of the 34K and 43K fragments**

The 34K and 43K fragments were treated with SDS to determine whether SDS treatment would alter the epitopes recognized by our gp52-specific MAbs and prevent their binding. The 34K and 43K fragments were isolated by immunoaffinity chromatography as described in Methods. The fragments were boiled in SDS and separated by SDS-PAGE prior to electrobobting onto nitrocellulose membranes. These membranes were probed with MAbs and with human anti-HCMV sera. All gp52-specific MAbs recognizing epitopes in all three domains were reactive with the 34K and 43K fragments (representative data for several MAbs are shown in Fig. 6a). These included Domain I and Domain III MAbs which recognized discontinuous epitopes. In addition, all human anti-HCMV sera tested reacted with these fragments (Fig. 6a). The nitrocellulose membranes were also probed with MAbs which recognized gC-1 gp93-130, and these MAbs did not react (data not shown). Thus, the epitopes recognized by these MAbs were removed by proteolysis. The 34K and 43K fragments were also reduced prior to Western blotting to determine the importance of disulphide bonds in the binding of our MAbs and human anti-HCMV sera. After reduction of disulphide bonds, three polypeptides were detected by Coomassie blue staining which had M\_ values of 34000, 30000 and 28000 (Fig. 6b, lane 1). The 34000 M\_ polypeptide was most abundant. After reduction of disulphide bonds, MAbs recognizing discontinuous epitopes failed to react, indicating the importance of the
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data showed that the intra-complex disulphide bonds are important in maintaining resistance to proteolysis with chymotrypsin.

**CNBr treatment of the 34K and 43K fragments**

The 34K and 43K fragments were also treated with CNBr to determine whether methionine residues were important for the binding of our MAbs which recognized continuous epitopes in the 43K and 43K fragments. Immunoaffinity-purified fragments were treated with CNBr and then prepared for Western blotting under non-reducing conditions. MAbs 41C2 and 39E11 which recognized continuous epitopes in Domain I still reacted strongly with two fragments with M_r of 38000 and 30000 (Fig. 7, lanes 2 and 3). However, MAbs which recognized continuous epitopes in Domain II showed weak (18F9 and 34G7) to negative (9B7) reactivity with these fragments (Fig. 7, lanes 4 to 6). These data showed that a methionine residue was an important part of the epitope recognized by Domain II MAb 9B7, but less important to the epitope recognized by Domain II MAb 34G7. Moreover, although Domain II was cleaved by CNBr treatment, it remained associated with Domain I when the fragments were examined under non-reducing conditions. These data suggested that there is a disulphide bridge between Domains I and II.

**Discussion**

The gC-I family of complexes contains protease-resistant fragments which contain all the continuous and discontinuous epitopes recognized by our gC-I gp52-specific MAbs. The 34K and 43K fragments contained these epitopes although they represented only 20 to 30% of the total mass of the gC-I complexes. On the other hand, 80 to 90% of the mass of the 34K and 43K fragments was contributed by gp52. This conclusion was made based on the fact that the 28000 to 34000 M_r polypeptides obtained after reduction of the 34K and 43K fragments all reacted with gp52-specific MAbs that recognized continuous epitopes (Fig. 6b, 41C2, 39E11, 9B7, 18F9 and 34G7). This showed that most of the mass of the 34K and 43K fragments came from gp52 and not from gp93-130.

Western blot analysis was also done with gC-I that had been subjected to complete reduction prior to proteolysis with chymotrypsin. This was done to determine whether intra-complex disulphide bonds played a role in protecting epitopes recognized by our gp52 MAbs from proteolysis. To do this, gC-I was immunoaffinity-purified, reduced with DTT and alkylated with iodoacetamide. The reduced gC-I glycoproteins were subjected to proteolysis with chymotrypsin and the entire digest was prepared for Western blotting. When this was done, all gp52-specific MAbs recognizing continuous or discontinuous epitopes lost reactivity (data not shown). These disulphide bonds in maintaining these epitopes (Fig. 6b, 11B4 and 26B11). Furthermore, one of the human sera tested also lost reactivity after reduction of disulphide bonds (Fig. 6b, A4), suggesting that this individual tended to make antibodies which recognized the discontinuous epitopes in the 34K and 43K fragments. It is interesting to note that the 28000 to 34000 M_r polypeptides obtained after reduction of the 34K and 43K fragments all reacted with gp52-specific MAbs that recognized continuous epitopes (Fig. 6b, 41C2, 39E11, 9B7, 18F9 and 34G7). This showed that most of the mass of the 34K and 43K fragments came from gp52 and not from gp93-130.
glycopeptides contained in these fragments, did not vary among the strains of HCMV tested. This was the case because the majority of the mass of these fragments was obtained from gp52 and not gp93-100.

At least two factors appear to play a role in the protease resistance of the chymotrypsin fragments. To some extent, the polysaccharide content of the 43K fragment may prevent it from being reduced to 34K by proteolysis. Inter-complex disulphide bonds in the 250 000 M_r gC-I complexes did not influence the extent of proteolysis with chymotrypsin. However, the conformation established by intra-complex disulphide bonds probably plays a major role in limiting proteolysis since reduction of all disulphides prior to proteolysis eliminates the protease resistance of the epitopes recognized by our gp52 MAbs.

The 34K and 43K fragments contained at least three discontinuous epitopes. One discontinuous epitope was in Domain III and was recognized by MAb 11B4. The other two discontinuous epitopes were in Domain I. One was recognized by MAb 26B11 and the other one by MAbs 30C7, 43C8 and 38B11. The discontinuous epitopes in Domains I and III were resistant to proteolysis, heat denaturation and treatment with SDS. However, reduction of disulphide bonds eliminated these epitopes, showing their dependence on cysteine residues and disulphide bonds. One adult human serum sample also lost reactivity to the 43K and 34K fragments after reduction of disulphide bonds, suggesting that most of the antibodies made by this individual recognized discontinuous epitopes in the gC-I fragments. However, this individual did make antibodies which recognized continuous epitopes on undigested gp93-130 and other continuous epitopes on undigested gp52 (data not shown). This is an important observation because the Domain III epitope is a discontinuous epitope. This epitope is recognized by a non-neutralizing MAb (11B4) which blocked the binding and antiviral activity of all other neutralizing MAbs recognizing Domains I and II (Lussenhop et al., 1988). Thus, antibodies made to this epitope could benefit the virus and not the host. This has implications for the construction of a subunit vaccine involving gC-I glycoproteins, in that certain antibody responses to discontinuous epitopes on gp52 might actually make some individuals more susceptible to HCMV infection. Thus, it might be preferable to use the entire gB glycoprotein or limit the vaccine to the N-terminal region of gB encoding gp93-130, which contains epitopes recognized by neutralizing murine MAbs (Kari et al., 1990) and is recognized by human antibodies (Kari & Gehrz, 1990).

Another distinction was made between epitopes in Domains I and II. Continuous epitopes in Domain II were affected by CNBr treatment, whereas continuous epitopes in Domain I were not. Thus, a methionine residue was important in maintaining the continuous epitopes in Domain II, particularly that recognized by Domain II MAb 9B7. The external portion of gp52 extends from amino acids 461 to 714 (Spaete et al., 1988). We have established that our gp52 MAbs do not react with an N-terminal recombinant gB glycoprotein which included amino acids from the N terminus to amino acid 513 (Kari et al., 1990). Thus, the continuous epitopes recognized by our MAbs are between amino acids 513 and 714 on gp52. Two major neutralizing domains have been mapped to this portion of gp52 (Banks et al., 1989) and another neutralizing epitope has been more precisely mapped between amino acids 608 and 625 on gp52 (Utz et al., 1989). It is clear from the results in this study that both methionine and cysteine residues are important to the epitopes recognized by our MAbs. Between amino acids 514 and 611 in gp52, amino acid residues 551, 574 and 611 are cysteines, and amino acid residues 542 and 565 are methionines (Spaete et al., 1988). Domains I and II remained associated after CNBr cleavage of methionine residues. One way for this to occur would be through cross-linking of domains with cysteine residues. In this regard, methionine residue 565 is bracketed by cysteine residues 551, 574 and 611. Thus, if gp52 were cleaved at methionine 565 much of the molecule could still be held together if intra-chain disulphide bridges were formed between these cysteine residues. Based on this information, it seems likely that the epitopes recognized by our MAbs would at least be contained in a region of gp52 from amino acids 514 to 635. The gp52 peptides obtained by proteolysis were also glycosylated, and this portion of gp52 contains three N-glycosylation sites.

The 43K fragment contained a 9K M_r glycoprotein not detected in the 34K fragment. This glycoprotein may play a role in limiting the proteolysis of the 43K fragment so that the 34K fragment was not obtained. The origin of the 9K glycoprotein is uncertain, but it may be derived from gp93-130. We are currently attempting to make MAbs which recognize the 9K glycoprotein to identify its origin in the gC-I complexes.

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