Identification and Characterization of Three Distinct Families of Glycoprotein Complexes in the Envelopes of Human Cytomegalovirus

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Several disulfide-linked glycoprotein complexes were identified in the envelope of human cytomegalovirus (HCMV). These glycoprotein complexes were fractionated by rate-zonal centrifugation in sucrose density gradients in the presence of detergents. Fractionated glycoproteins and complexes were immunoprecipitated with three different monoclonal antibodies specific for HCMV glycoproteins and a rabbit polyclonal antiserum prepared against detergent-extracted virion and dense-body envelope glycoproteins. Three distinct families of disulfide-linked glycoprotein complexes were observed and designated glycoprotein complex gcI, gcII, and gcIII. The gcI family, recognized by monoclonal antibody 41C2 under nonreducing conditions, consisted of three complexes with approximate molecular masses of 250 to 300, 190, and 160 kilodaltons (kDa). These complexes consistently sediment more rapidly than other HCMV glycoproteins or complexes in sucrose density gradients. Upon reduction of the gcI family, two size classes of glycoproteins with average molecular masses of 93 to 130 and 55 kDa were observed. The gcII family was recognized by monoclonal antibody 9E10. Under nonreducing conditions, as many as six electrophoretic forms were observed for gcII. When reduced, the major component of the gcII family was a heterogeneous glycoprotein designated gp47-52. The gcIII family was recognized by monoclonal antibody 1G6. It consisted of a complex of approximately 240 kDa without reduction of disulfide bonds. When reduced, two glycoprotein size classes with average molecular masses of 145 and 86 kDa were observed. Polyclonal antiserum R-7 reacted strongly with the gcI and gcIII families, but weakly with the gcII family.

Human cytomegalovirus (HCMV) is a ubiquitous member of the herpesvirus group which infects a majority of the population by adulthood. Primary infection of an immunocompetent host by HCMV is usually mild or asymptomatic, and the virus establishes a latent state which may persist throughout life without sequelae. In immunocompromised hosts, such as organ transplant recipients or patients with acquired immunodeficiency syndrome, primary HCMV infection or reactivation of a latent infection can lead to severe disseminated disease which is frequently life threatening. In addition, congenital HCMV infection can cause abnormalities ranging from learning disorders to severe psychomotor retardation and death (for reviews, see references 9 and 18).

A more thorough understanding of the immune response to HCMV may allow for better control of infections in the susceptible host. In this regard, it is important to identify the viral antigenic determinants capable of inducing an effective immune response. Human convalescent-phase serum and murine monoclonal and rabbit polyclonal antibodies specific for HCMV glycoproteins are known to neutralize HCMV infectivity in vitro (1, 2, 6, 7, 10-12, 14, 16, 20, 21, 23). These antibodies recognize several HCMV-specified glycoproteins present in infected cells (3, 15-17, 19-22, 24, 26) and in purified virions and dense bodies (1, 2, 5-7, 10-12, 14, 23). Using monoclonal antibodies, several investigators have reported the presence of disulfide-linked glycoprotein complexes in the outer envelope of virions and dense bodies (1, 5, 7, 10, 12). One such complex consists of at least two glycoproteins of 50 to 58 and 93 to 130 kilodaltons (kDa) (1,

5, 7, 10, 12, 20). The 93- to 130-kDa glycoprotein is not the precursor to the 50- to 58-kDa glycoprotein (3). The proteins within this complex have epitopes which are targets for complement-dependent virus-neutralizing antibody (1, 20). The gene encoding the 50- to 58-kDa glycoprotein has been mapped and sequenced (4, 13). This gene has sequence homology with herpes simplex virus gB, Epstein-Barr virus gB, varicella-zoster virus gpII, and pseudorabies virus gpII.

Two different monoclonal antibodies have been described which can neutralize the infectivity of HCMV in the absence of complement: 9E10 (10) and 1G6 (21). Antibody 9E10 recognizes a family of glycoprotein complexes from Towne strain purified virions whose major component was a diffusely migrating glycoprotein designated gp50-52 (10). On the other hand, antibody 1G6 immunoprecipitates a glycoprotein of 86,000 Da from both AD169 and Towne strain HCMV-infected cells (19, 21).

In the work described in this report, the virion-associated glycoproteins and glycoprotein complexes of HCMV were radiolabeled with [³H]glucosamine and fractionated by rate-zonal centrifugation on sucrose density gradients in the presence of detergents. The fractionated glycoproteins and complexes were immunoprecipitated with monoclonal antibodies specific for gp55 (41C2), gp47-52 (9E10), and gp86 (1G6). The immunoprecipitates were analyzed by nonreducing and reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Three immunologically distinct families of glycoprotein complexes with different sedimentation properties in sucrose gradients were observed and designated gcI, gcII, and gcIII. The reduced components of gcI were gp55 and a diffuse species designated gp93-130.

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The major component of gcII was gp47–52. The components of gcIII were gp86 and a diffuse species designated gp145.

MATERIALS AND METHODS

Virus and tissue culture. Culture of human foreskin fibroblast cells and propagation of HCMV Towne strain have been described previously (25). Cells were infected at multiplicities of 5 to 10 PFU/ml. Virions and dense bodies were harvested from tissue culture fluid and purified as described previously (25); they were found to be free of cellular contaminants as judged by electron microscopy and SDS-PAGE analysis of [³⁵S]methionine-labeled preparations.

Radioisotope labeling. HCMV-infected cells were pulse-labeled for 12 to 24 h at 72 to 96 h postinfection with 50 μ Ci of [³H]glucosamine per ml (33 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) as described previously (25).

Rate-zonal centrifugation. Purified virions and dense bodies were solubilized for 5 min in radioimmunoprecipitation (RIPA) buffer (phosphate-buffered saline [pH 7.4] containing 1% Nonidet P-40 0.5% sodium deoxycholate, 0.1% SDS, 100 μ g of phenylmethylsulfonyl fluoride per ml, and 0.02% sodium azide) and then centrifuged at 12,000 $\times$ g for 15 min. Alternatively, virion and dense-body membranes were solubilized in phosphate-buffered saline containing 1% Nonidet P-40 and centrifuged as above. Supernatants were layered on 5 to 25% sucrose density gradients containing RIPA buffer or 1% Nonidet P-40. Centrifugation was carried out at 34,000 rpm in an SW-41 swinging-bucket rotor (Beckman Instruments, Inc., Fullerton, Calif.) for 18 h at 22°C. Fractions were collected, and portions from each fraction were either analyzed by SDS-PAGE or subjected to immunoprecipitation prior to SDS-PAGE.

Immunoprecipitation. Monoclonal antibody 41C2, which immunoprecipitates HCMV glycoproteins gp55 and gp93–130, and monoclonal antibody 9E10, which immunoprecipitates gp47–52, were described previously (10). Monoclonal antibody 1G6, which reacts with an infected-cell glycoprotein of 86 kDa, was also described previously (21). Rabbit polyclonal antiserum R-7 reactive with detergent-extracted virion and dense-body envelope glycoproteins was described previously (23). Human convalescent-phase serum 218 was a gift from L. Frenkel, University of Cincinnati.

The biotinylation of monoclonal antibody 41C2 and immunoprecipitation with streptavidin-agarose (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) were described previously (8). Immunoprecipitation with monoclonal antibodies 9E10 and 1G6, as well as polyclonal antiserum R-7 and human convalescent-phase sera, was done with protein A-Sepharose (Pharmacia, Uppsala, Sweden) as described previously (8).

SDS-PAGE. The electrophoresis of glycoproteins in SDS-polyacrylamide gels and the subsequent fluorography of the gels were described previously (23). Disulfide-linked glycoprotein complexes were analyzed in 7% gels in the absence of reducing agents, and reduced glycoproteins were analyzed in 8 or 9% gels in the presence of 5 to 10% 2-mercaptoethanol. ¹⁴C-labeled molecular weight standards were obtained from Bethesda Research Laboratories.

RESULTS

Immunoprecipitation of virion-associated glycoproteins. A series of immunoprecipitations were performed to demonstrate the reactivity of the immunological reagents used in this study. HCMV-infected cells were pulse-labeled with [³H]glucosamine, and virions and dense bodies were purified from tissue culture supernatants as described previously

(25). The envelope glycoproteins were solubilized and subjected to immunoprecipitation with three different monoclonal antibodies and two different polyclonal antisera. The immunoprecipitates were then analyzed by SDS-PAGE under reducing conditions.

Monoclonal antibody 41C2 immunoprecipitated two size classes of glycoproteins designated according to molecular weight as gp93–130 and gp55 (Fig. 1, lane 1). The majority of [³H]glucosamine label appeared in gp93–130. Monoclonal antibody 9E10 reacted with a heterogeneous species designated gp47–52 and with an unidentified species of greater than 200,000 Da (lane 2). Monoclonal antibody 1G6 immunoprecipitated two size classes of glycoproteins designated gp86 and gp145 (lane 3).

When the [³H]glucosamine-labeled glycoproteins were immunoprecipitated with a rabbit polyclonal antiserum (R-7) prepared against detergent-extracted virion envelope glycoproteins, a complicated profile was observed (lane 4). A similar profile was observed when the glycoproteins were immunoprecipitated with human convalescent-phase serum 218 (lane 5). Both antisera reacted strongly with gp55, gp93–130, gp86, and gp145 on the basis of electrophoretic mobility. The antisera reacted weakly with glycoproteins resembling gp47–52 when compared with monoclonal antibody 9E10. Preimmune rabbit sera failed to immunoprecipitate any detectable viral glycoproteins (lane 6). None of the antibodies tested reacted with [³H]glucosamine-labeled uninfected cell glycoproteins (data not shown).

Rate-zonal centrifugation of the HCMV glycoproteins and complexes. To further characterize the HCMV virion envelope complexes, we subjected them to rate-zonal centrifugation in sucrose density gradients. The viral glycoproteins were radiolabeled with [³H]glucosamine and detergent extracted from the envelopes of purified virions and dense

FIG. 1. Immunoprecipitation of the HCMV glycoproteins with monoclonal and polyclonal antibodies. Glycoproteins were radiolabeled with [³H]glucosamine, and the virions and dense bodies were purified. The glycoproteins were solubilized and immunoprecipitated with monoclonal antibodies to gp55 (41C2), gp47–52 (9E10), and gp86 (1G6) or with rabbit polyclonal antibodies (R-7) or human convalescent-phase serum (serum 218), as described in the text. The immunoprecipitated glycoproteins were subjected to electrophoresis in an SDS–8% polyacrylamide gel under reducing conditions. Lanes: 1, anti-gp55 (41C2); 2, anti-gp47–52 (9E10); 3, anti-gp86 (1G6); 4, rabbit polyclonal antiserum R-7; 5, human convalescent-phase antiserum 218; 6, preimmune rabbit serum. The molecular weights of the glycoproteins (gp), as well as the protein molecular weight standards (lanes St), are designated.

bodies. The solubilized glycoproteins and glycoprotein complexes were fractionated in sucrose density gradients in the presence of detergents, as described in Materials and Methods. Figure 2A demonstrates the sedimentation profile of the mixture of glycoproteins and complexes. Two peaks of radioactivity were observed. The shorter peak (Fig. 2A, fraction 5) represents a population of glycoproteins which migrated faster in sucrose gradients than those in the larger peak did (Fig. 2A, fractions 7 to 10).

Glycoproteins in the individual gradient fractions were analyzed by electrophoresis in an SDS-9% polyacrylamide gel under reducing conditions (Fig. 2B). At least five distinct glycoprotein size classes were detected. These glycoproteins were designated according to molecular weight as gp145 (Fig. 2B, lanes 7 to 9), gp93-130 (lanes 4 and 5), gp86 (lanes 7 and 8), gp55 (lanes 4 and 5), and gp47-52 (lanes 7 to 10). Little variation in the sedimentation profile was noted when the glycoproteins were solubilized with 1% Nonidet P-40 alone (data not shown).

Immunoprecipitation of the fractionated gcl family. The gradient-fractionated glycoproteins and complexes were immunoprecipitated with monoclonal antibody 41C2 and analyzed by nonreduced and reduced SDS-PAGE. Figure 3A demonstrates the profile of fractionated envelope glycoproteins and complexes after immunoprecipitation with monoclonal antibody 41C2. A sharp peak of radioactivity was observed (Fig. 3A, fraction 5) which corresponds to the faster-migrating peak observed in Fig. 2A. When the immunoprecipitates were analyzed by electrophoresis in an SDS-7% polyacrylamide gel under nonreducing conditions, three complexes of approximately 250 to 300, 190, and 160 kDa were observed (Fig. 3B, fraction 5). The 250- to 300-kDa species was the most abundant on the basis of the [³H]glucosamine label. The 190- and 160-kDa species were more apparent after prolonged exposure of the autoradiogram (not shown). We have designated these three complexes the gcl family. When the complexes were reduced

with 2-mercaptoethanol and analyzed in an SDS-9% polyacrylamide gel, two glycoprotein size classes designated gp93-130 and gp55 were observed (Fig. 3C, fractions 5 and 6). The results described above confirm the findings of Farrar and Greenaway, who used a single monoclonal antibody to an approximately 52-kDa glycoprotein to immunoprecipitate from virion envelopes three surface-iodinated complexes of sizes similar to those reported in our study (5). Our data suggest that the three complexes in the gcl family are physically associated, since they cosediment at a rapid rate in sucrose density gradients when both nonionic and ionic detergents are present.

Immunoprecipitation of the fractionated gcII family. Initial studies with monoclonal antibody 9E10 described its reaction with two related glycoprotein complexes isolated under nonreducing conditions from HCMV envelopes (10). To further study these complexes, [³H]glucosamine-labeled envelope glycoproteins were fractionated in sucrose density gradients and immunoprecipitated with 9E10. Figure 4A demonstrates the profile of these fractionated and immunoprecipitated complexes, which we have designated gcII. The gcII family migrated more diffusely and slowly than the gcl family did (Fig. 3A) in sucrose gradients. When the immunoprecipitates were analyzed by electrophoresis in an SDS-7% polyacrylamide gel under nonreducing conditions, as many as six different electrophoretic forms were observed at molecular mass intervals ranging from 93 to >300 kDa (Fig. 4B, fractions 7 to 9). In contrast to gcl, the gcII family did not cosediment in sucrose gradients, which suggests that they were not physically associated. When the complexes were reduced, the major species observed was gp47-52 (Fig. 4C, fractions 7 to 9). Additional species were sometimes observed under reducing conditions. These species may represent incompletely reduced complexes, since they were not always observed, as demonstrated above (Fig. 1, lane 2).

Immunoprecipitation of the fractionated gcIII family. Preliminary experiments with polyclonal antiserum R-7 (see

FIG. 2. Rate-zonal centrifugation of the HCMV glycoproteins and glycoprotein complexes. Glycoproteins were radiolabeled with [³H]glucosamine, and virions and dense bodies were purified. The glycoprotein complexes were solubilized and fractionated in 5 to 25% sucrose density gradients in the presence of detergents as described in the text. (A) Sedimentation profile of the [³H]glucosamine-labeled glycoproteins and complexes after rate-zonal centrifugation. Fraction 1 is at the bottom of the gradient. (B) Fluorogram of the glycoproteins after electrophoresis in an SDS-9% polyacrylamide gel under reducing conditions. Density gradient fraction numbers are indicated. The molecular weights of the glycoprotein size classes (gp), as well as the protein molecular weight standards (lane St), are designated.

FIG. 3. Fluorograms of fractionated and immunoprecipitated gC1. The viral glycoprotein complexes were prepared and fractionated by rate-zonal centrifugation as described for Fig. 2 and subjected to immunoprecipitation with monoclonal antibody 41C2. (A) Sedimentation profile of fractionated and immunoprecipitated gC1. (B) Fractions 4 through 6 were immunoprecipitated and subjected to electrophoresis in an SDS-7% polyacrylamide gel under nonreducing conditions. (C) Fractions 5 through 7 were immunoprecipitated and subjected to electrophoresis in an SDS-9% polyacrylamide gel under reducing conditions. The molecular weights of the complexes, the glycoproteins (gp), and the protein molecular weight standards (lane St) are designated.

below) suggested that a third glycoprotein complex exists in HCMV envelopes which was not recognized by monoclonal antibodies specific for the coimmunoprecipitating gp55 and gp93-130 or gp47-52. Therefore, the gradient-fractionated glycoproteins and complexes were immunoprecipitated with monoclonal antibody 1G6, which is specific for an 86-kDa glycoprotein detected in HCMV-infected cells (21). Figure 5A demonstrates the sedimentation profile of fractionated glycoproteins and complexes after immunoprecipitation with 1G6. A peak of radioactivity was observed in fraction 7. When the immunoprecipitates were analyzed by electrophoresis in a 7% gel under nonreducing conditions, a discrete species of 240 kDa was observed (Fig. 5B, fractions 7 and 8). In addition, small amounts of gp86 were present. Under reducing conditions, two glycoproteins designated gp145 and

gp86 were observed at approximately equal levels (Fig. 5C, fractions 7 and 8). When the 240-kDa species was excised from nonreducing SDS-polyacrylamide gels and then examined by reducing SDS-PAGE, both gp145 and gp86 were present (data not shown). Thus, the 240-kDa complex observed under nonreducing conditions appears to be a disulfide-linked heterodimer containing both gp145 and gp86. We have designated this HCMV glycoprotein complex as gCIII.

Immunoprecipitation of the fractionated glycoproteins and glycoprotein complexes with a polyclonal antiserum. The results presented above demonstrated the existence of three immunologically distinct families of glycoprotein complexes in the envelopes of HCMV. To test for additional complexes or uncomplexed glycoproteins, the gradient-fractionated glycoproteins and complexes were immunoprecipitated with a

FIG. 4. Fluorogram of fractionated and immunoprecipitated gCII. The viral glycoprotein complexes were prepared and fractionated as described for Fig. 2 and subjected to immunoprecipitation with anti-gp47-52 monoclonal antibody (9E10). (A) Sedimentation profile of fractionated and immunoprecipitated gCII. (B) Fractions 7 through 9 were immunoprecipitated and then subjected to electrophoresis in an SDS-7% polyacrylamide gel under nonreducing conditions. (C) Fractions 7 through 9 were immunoprecipitated and subjected to electrophoresis in an SDS-7% polyacrylamide gel under reducing conditions. The molecular weights of the complexes and glycoproteins (gp), as well as the protein molecular weight standards (lane St), are designated.

FIG. 5. Fluorogram of fractionated and immunoprecipitated gCIII. The glycoprotein complexes were prepared and fractionated as described for Fig. 2 and subjected to immunoprecipitation with anti-gp86 monoclonal antibody (1G6). (A) Sedimentation profile of fractionated and immunoprecipitated gCIII. (B) Fractions 6 through 9 were immunoprecipitated and subjected to electrophoresis in an SDS-7% polyacrylamide gel under nonreducing conditions. (C) Fractions 7 through 9 were immunoprecipitated and subjected to electrophoresis in an SDS-9% polyacrylamide gel under reducing conditions. The molecular weights of the complexes and glycoproteins (gp), as well as the protein molecular weight standards (lanes St), are designated.

polyclonal antiserum (R-7) prepared against detergent-extracted virion envelope glycoproteins. This polyclonal antiserum was previously shown to neutralize viral infectivity and to react with virus-specified antigens in the membranes of infected cells and virions and dense bodies (23, 26).

Figure 6A is a profile of the gradient-fractionated glycoproteins and glycoprotein complexes after immunoprecipitation with R-7. The immunoprecipitates were analyzed by SDS-PAGE under nonreducing conditions in a 7% gel (Fig. 6B) and under reducing conditions in an 8% gel (Fig. 6C). R-7 reacted strongly with the gCII family of 250 to 300, 190, and 160 kDa (Fig. 6B, fraction 5), as well as the gCIII 240-kDa species (Fig. 6B, fractions 7 and 8). In addition, uncomplexed glycoproteins of 145 and 86 kDa were observed under nonreducing conditions (Fig. 6B, fraction 8). As above, under reducing conditions the major components

of the gCI family were gp93-130 and gp55 (Fig. 6C, fraction 5), whereas the components of gCIII were gp145 and gp86 (Fig. 6C, fractions 7 and 8). Prolonged exposure of the autoradiograms in Fig. 6B and C indicated that polyclonal antiserum R-7 reacted weakly with the fractionated gCII family (data not shown).

DISCUSSION

Our results show that three immunologically distinct families of disulfide-linked glycoprotein complexes can be extracted from the envelopes of HCMV virions and dense bodies and fractionated in sucrose density gradients. We have designated these families gCI, gCII, and gCIII on the basis of their immunoprecipitations with monoclonal antibodies 41C2, 9E10, and 1G6, respectively. The gCI family,

FIG. 6. Fluorogram of fractionated glycoproteins and complexes after immunoprecipitation with a polyclonal antiserum prepared against virion and dense-body envelope glycoproteins. The glycoprotein complexes were prepared and fractionated in sucrose density gradients as described for Fig. 2 and immunoprecipitated with a rabbit polyclonal antiserum (R-7). (A) Sedimentation profile of the fractionated glycoproteins and complexes after immunoprecipitation with R-7. (B) Fractions 5 through 8 and 10 were immunoprecipitated with R-7 and subjected to electrophoresis in an SDS-7% polyacrylamide gel under nonreducing conditions. (C) Fractions 5 through 9 were immunoprecipitated with R-7 and subjected to electrophoresis in an SDS-9% polyacrylamide gel under reducing conditions. The molecular weights of glycoproteins (gp) and complexes, as well as the protein molecular weight standards (lanes St), are designated.

which appears to have at least one component (gp55) related to HSV gB (4, 13), has been observed by other investigators (1, 5, 7, 10, 12, 20). We observed three complexes with molecular mass estimates of 250 to 300, 190, and 160 kDa, similar to those described by Farrar and Greenaway (5). These three complexes comigrated at a relatively high rate in sucrose density gradients in the presence of detergents. Other virion complexes with similar or greater apparent molecular masses as estimated by SDS-PAGE (e.g., gcII) migrated at lower rates under identical centrifugation conditions. This suggests that the three complexes which make up the gcI family remain tightly associated after solubilization of virion envelopes.

The gcII family was initially described by Kari et al. (10), who used high-performance liquid chromatography along with immunoprecipitation to isolate complexes of 93 and 450 kDa from virion envelopes. In our present study, under nonreducing conditions as many as six different species were observed, ranging from 93 to >300 kDa. Under reducing conditions, the major species was gp47–52. Additional species of greater apparent molecular mass were occasionally observed under reducing conditions. Biochemical and immunological analysis of these higher-molecular-weight species has demonstrated that they are related to gp47–52 (data not shown). We propose that they represent incompletely reduced complexes, since these complexes isolated from the infected cell contain only gp47–52 (data not shown). We have recently cloned the viral gene encoding gp47–52. The gene product that was expressed *in vitro* was specifically immunoprecipitated by monoclonal antibody 9E10, as well as by human convalescent-phase serum (D. R. Gretch, B. Kari, R. Gehrz, and M. F. Stinski, submitted for publication). Thus, gp47–52 represents a bona fide viral gene product, and the gcII complexes contain various combinations of gp47–52.

The 240-kDa gcIII has not been previously described. Rasmussen et al. immunoprecipitated an 86-kDa glycoprotein from HCMV-infected cells by using monoclonal antibody 1G6 (19, 21), but the complex was not observed, since it is present in the infected cells in small amounts (D. R. Gretch and M. F. Stinski, unpublished data). In our current study, gcIII was immunoprecipitated with 1G6, polyclonal antiserum R-7, and human convalescent antiserum (not shown). This 240-kDa complex appeared to be a heterodimer of two glycoproteins designated gp145 and gp86. Alternatively, the gp145 and gp86 may exist within two separate 240-kDa complexes which were both immunoprecipitated by 1G6. Uncomplexed glycoproteins of 145 and 86 kDa were recognized by R-7, and small amounts of uncomplexed gp86 were recognized by 1G6. The relationships between the 145- and 86-kDa glycoproteins are not known at present.

We have recently examined the glycoprotein complexes as they exist within the HCMV-infected cell (Gretch et al., unpublished data). Precursor complexes of the gcI and gcII families were present at high levels. In contrast, gcIII was detected at very low levels in infected cells, although uncomplexed glycoproteins of 145 and 86 kDa were present (data not shown). Thus, formation of gcIII may occur late in virogenesis.

The three glycoprotein complexes described above are almost certainly important targets of the human immune response to HCMV infection. Monoclonal antibodies which recognize the gcII and gcIII families are capable of neutralizing the infectivity of HCMV in the absence of complement (10, 21). Antibodies specific for the gcI family require complement for neutralization (1, 4, 10, 20). The functional

roles of these glycoprotein complexes are uncertain at present. The gcI family contains the gp55 gene product, which has predicted amino acid sequence homology with herpes simplex virus gB, Epstein-Barr virus gB, varicella-zoster virus gpII, and pseudorabies virus gpII (4). This suggests that the gcI family may play a role in the fusion of viral and cellular membranes. Speculation of the roles of the gcII and gcIII is premature without further experimentation.

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