Review article

Human cytomegalovirus structural proteins

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Introduction

Members of the Herpesviridae family are viruses with a core containing the viral DNA in the form of a torus, a capsid (100 nm in diameter and composed of 162 capsomers), a tegument, which is a structure of variable thickness between the capsid and the envelope, and an envelope. The envelope is the trilaminar outer covering which contains numerous protrusions of spikes consisting of virally encoded glycoproteins. The resulting diameter of the virion varies from 120 to 300 nm.

Herpesviruses have been divided into three subfamilies on the basis of biological properties. The Alphaherpesvirinae are herpesviruses with a variable host range, short replication cycle, rapid spread in cultured cells, a lytic effect on infected cells and a tropism for establishing a latent infection in sensory ganglia. The Betaherpesvirinae have a restricted host range, a longer replication cycle, an infection which progresses more slowly in cultured cells and a latent state that can be established in numerous cells and tissues. The Gammaherpesvirinae have a restricted host range, an in vitro replication confined mainly to lymphoblastoid cells (members of this subfamily can infect either B or T cells) where infection does not reach the final stage of viral progeny production. Latency can be established in lymphoid tissues.

Herpesviruses are widely distributed in nature and among nearly 100 herpesviruses already discovered, seven have been isolated from humans. Herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2, varicella-zoster virus (VZV) (or human herpesvirus 3) are members of the Alphaherpesvirinae subfamily. Human cytomegalovirus (HCMV) (or human herpesvirus 5), human herpesvirus 6 and 7 are members of the Betaherpesvirinae subfamily. Epstein–Barr virus (EBV) (or human herpesvirus 4) belongs to the Gammaherpesvirinae subfamily (for review see Roizman, 1993).

HCMV is a ubiquitous virus that can cause severe morbidity in immunocompromised patients and congenitally infected newborns (for review see Alford & Britt, 1993). HCMV has the largest genome among the herpesviruses (240 kbp). One genome has been completely sequenced (Chee et al., 1990; EMBL accession number X17403) and contains 208 predicted open reading frames (ORFs) encoding more than 100 polypeptides, most of which are not well-characterized (Chee et al., 1990; Mocarski, 1993; Stinski, 1990). During the past decade, a large number of HCMV envelope glycoproteins and internal structural proteins have been identified by laboratories throughout the world using a variety of immunological, biochemical and molecular techniques. In many instances, the proteins isolated by biochemical techniques have altered properties with respect to the characteristics predicted from the primary translation products. For example, alternative transcriptional or translational events often result in multiple protein products and many of the proteins undergo extensive post-translational modifications including glycosylation, phosphorylation, myristylation or sulphation. In addition, proteolytic processing has been shown to alter some structural proteins. The currently known glycoproteins are recognized as disulphide-linked complexes in the virion envelope and some of these complexes require stable or transient association with other viral and/or cellular proteins for their maturation. Analysis of the HCMV DNA sequence from the AD169 strain by Chee et al. has lead to a widely accepted alphanumeric nomenclature for the predicted ORFs based on their location in the unique and repeat regions of the genome. Various nomenclatures for HCMV polypeptides have evolved based on physicochemical characteristics (i.e., Mr, association in disulphide-linked complexes, glycosylation and phosphorylation); structural and functional properties (i.e., location in the virion, temporal expression at various times of infection, role in virus infectivity, replication and virion assembly); or homology with proteins in other human and animal herpesviruses (i.e., alphabetical designations based on homology with HSV glycoproteins). However, these nomenclatures have been confusing because of protein heterogeneity among
HCMV strains, technical differences in methods used in various laboratories, lack of homologue proteins in many cases and limited knowledge of protein structure and function.

In response to the need to adopt a uniform nomenclature of HCMV proteins, two nomenclature workshops have been held in the past year. An attempt was made to reach a consensus opinion on the nomenclature of those structural proteins whose ORF has been identified. The First Human CMV Nomenclature Workshop for structural proteins took place on April 19th, 1993 at the Fourth International Cytomegalovirus Conference held at the Institut Pasteur, Paris, and was chaired by Drs M. P. Landini and R. R. Spaete. The deliberations of this workshop have been reported in the Proceedings of the IV International Cytomegalovirus Workshop, Excerpta Medica International Congress Series (Landini & Spaete, 1993). A second workshop extended the discussion to non-structural proteins and was chaired by Dr Edward S. Mocarski at the 18th International Herpesvirus Workshop held in Pittsburgh, Pennsylvania, U.S.A., July 25 to 30, 1993. A consensus nomenclature generally acceptable to CMV investigators has emerged from these meetings. In brief, the groups (more than 100 individuals from all over the world) recognized that the alphanumeric ORF designation developed by the MRC (Cambridge) sequencing group (Chee et al., 1990), offered the most useful starting point. Particularly so when relational databases are developed to access the CMV literature. The information contained in the ORF designation was expanded in the following manner: (i) The prefix ‘p’ would be used to designate proteins not known to be phosphorylated or glycosylated, followed by the ORF designation. (ii) The prefixes ‘pp’ and ‘gp’ are used for indicating a phosphoprotein or glycoprotein, respectively. (iii) A descriptor added in parenthesis may be used to complete the designation of the protein. For example, the major capsid protein would be designated ‘pUL86 (MCP)’ and this designation would be used at least once in the title, abstract and key words. ‘MCP’, ‘pUL86’, ‘ICP4’, ‘p150’ or any other name could then be used in the body of the text at the investigator’s discretion. The descriptor may change as the function of the respective protein becomes better understood. (iv) Small letter designations are used as suffixes to name additional ORFs that are found to encode proteins. For example, the protein expressed in the region of UL98 and UL99, but which is composed of parts of each, is designated ‘pUL98a’. This suffix should also provide for the case where insertion of DNA in another strain reveals the existence of other ORFs. The purposes of this review are: (i) to provide a comprehensive survey of the current knowledge of the gene products; protein structure, synthesis and pro-
cessing; functions; and monoclonal antibody (MAb) reagents for the HCMV envelope glycoproteins, viral capsid proteins and tegument proteins; and (ii) to review designations for these proteins in light of the consensus reached on nomenclature in order to facilitate communication among investigators working in the field and the scientific community as a whole. Prototype MAbs, along with their reference laboratories, have been identified as reagents that may be useful in standardizing future studies in other laboratories. A schematic illustration of the relative positions of the HCMV ORFs encoding the structural proteins discussed in this review is shown in Fig. 1.

**HCMV structural proteins**

**Capsid proteins**

Although HCMV virions contain many proteins (Edwards, 1976; Kim et al., 1976; Stinski, 1977, 1978; Gupta et al., 1977; Fiala et al., 1976; Landini & Michelson, 1988), the capsids of HCMV, like those of other herpesviruses, are composed of a relatively simple set of proteins (Schmitz et al., 1975; Gibson, 1983; Landini & Michelson, 1988; Sedarati & Rosenthal, 1988; Britt, 1991).

Nucleocapsid assembly has been studied by Gibson and colleagues using the simian CMV strain Colburn (for review see Gibson, 1991). PreB capsids have the simplest structure, are present exclusively in the nucleus of infected cells, and contain at least three major protein species [the major capsid protein pUL86, the minor capsid protein (pUL46) and the smallest capsid protein (M, 12K)]. Pre-B capsids undergo processing to become stable B capsids. These particles have, in addition to the proteins of preB capsids, a 36K to 38K protein (pUL80) which is probably involved in DNA packaging and/or nucleocapsid envelopment. B capsids are the most immature particles recovered from the cytoplasm of CMV-infected cells. Once formed, B capsids are thought to become filled with DNA and mature into C capsids (Lee et al., 1988). This transition involves elimination of the assembly protein from the nucleocapsid and acquisition of the tegument proteins. These particles, after envelopment by the Golgi vacuoles and lamellae (Severi et al., 1988; Smith, 1980; Stackpole, 1969), and egress from the cell, become the mature, infectious virions. However non-infectious enveloped particles (NIEP) are often observed in extracellular virus preparations and they appear to be enveloped B capsids.

For the purposes of this review, only the three best characterized capsid proteins (i.e. pUL86, pUL80 and pUL46), whose coding regions have been identified (UL86, UL80 and UL46, respectively) will be taken into consideration. Antibodies to these proteins do not exhibit virus-neutralizing activity and are unable to bind to either extracellular virus or the surface of virus-infected cells (Rasmussen, 1990; Britt, 1991).

(i) **pUL86**

A protein of $M_r$ 150K to 155K, also called ICP4 (Infected Cell Protein 4) and MCP (Major Capsid Protein) is the main structural element of the viral capsid and constitutes approximately 90% of its total protein content (Gibson, 1983). In the extracellular viral particle there are two proteins of apparent $M_r$ 150K (one is the phosphorylated structural component of the viral tegument, and the other is the most abundant capsid protein). Differential migration in gels with high bis to acrylamide ratios can separate the two proteins (Irmiere & Gibson, 1983; Jahn et al., 1987b). The coding region of the capsid protein, pUL86 (MCP), was identified by comparing the protein sequence with the respective sequences of HSV, EBV and VZV (Chee et al., 1989). Monospecific antisera and a MAb directed against pUL86 cross-reacted with VP5 of HSV-1 as shown by immunoblotting and immunofluorescence (Rudolph et al., 1990a). The predicted length of pUL86 is 1370 amino acids. Northern blot analysis with subclones of its coding region detected a large mRNA of about 8 kb (Rudolph et al., 1990b).

HCMV pUL86 has been prokaryotically expressed in three portions as β-galactosidase fusion proteins, covering about 75% of the ORF (Rudolph et al., 1990a). One fusion protein encoded by nucleotides 101 to 1243, a second encoded by nucleotides 144 to 3089 and a third encoded by nucleotides 2624 to 3793 were tested with human sera and the second protein was found to be immunodominant. Furthermore a rabbit antiserum to the second fusion protein cross-reacted with VP5 of HSV. It has been determined that the degrees of nucleotide sequence identity between HCMV UL86 and the ORFs coding for the major capsid proteins of the other members of the *Herpesviridae* family are the following: EBV, 29%; VZV, 23%; HSV, 25% and HHV-6, 43% (Lawrence et al., 1990).

HCMV pUL86 can also be considered the CMV-group common antigen. In fact, using a monospecific rabbit antiserum to pUL86, Weiner & Gibson (1981) found that all primate CMVs examined contain a single cross-reactive protein band that ranged in $M_r$ from 144K to 153K.

In vivo immune response. Specific antibodies are produced during natural infection. However, this protein is not a strong immunogen.

Specific immunological reagents. Rudolph et al. (1990a) described rabbit antisera to three recombinant
proteins containing portions of pUL86 (amino acids 33 to 414, 648 to 1029 and 874 to 1264). At least two murine MAbs to pUL86 have been obtained (Pereira et al., 1982a; Rudolph et al., 1990a) and one (28-4 produced and characterized by Britt) was shown to react with an epitope localized between amino acids 33 and 414 (Rudolph et al., 1990a). See Table 1.

(ii) pUL80a

This protein is abundant in the immature viral capsid but absent from the mature virion. However it can be detected easily in extracellular virion preparations as it is present in B capsids, which can undergo envelopment and egress from the infected cell (Irmiere & Gibson, 1983, 1985). Its coding region is located at the 3' end of a larger ORF that contains a set of four nested, in-frame, 3'-coterminial genes, referred to as UL80 (Robson & Gibson, 1989; Shenk et al., 1991; Welch et al., 1991a; EMBL accession no. X17403). Each of these nested genes give rise to a separate mRNA and corresponding protein (Welch et al., 1991a). HCMV pUL80a is phosphorylated and its 40K to 45K precursor is synthesized at late times after infection, moves slowly into the nuclear fraction and undergoes maturational cleavage which eliminates its carboxy-terminal portion.

Table 1. Summary of HCMV structural protein-specific monoclonal antibodies

<table>
<thead>
<tr>
<th>Protein</th>
<th>Antisera (source)*</th>
<th>Monoclonal antibodies (source)*</th>
<th>Epitope (amino acids)</th>
<th>References†</th>
</tr>
</thead>
<tbody>
<tr>
<td>pUL86</td>
<td>Anti-SH1 (ra)</td>
<td></td>
<td>33-414</td>
<td>Rudolph et al. (1990a)</td>
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<tr>
<td></td>
<td>Anti-FS1 (ra)</td>
<td></td>
<td>648-1029</td>
<td>Rudolph et al. (1990a)</td>
</tr>
<tr>
<td></td>
<td>Anti-SS1 (ra)</td>
<td></td>
<td>874-1264</td>
<td>Rudolph et al. (1990a)</td>
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<td></td>
<td></td>
<td></td>
<td>28-4 (mu)</td>
<td>Britt (p.c.); Rudolph et al. (1990a)</td>
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<td></td>
<td></td>
<td></td>
<td>CH14, CH23 (mu)</td>
<td>Pereira et al. (1982a)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>10.8.20 (mu)</td>
<td>Michelson (p.c.)</td>
</tr>
<tr>
<td>pUL80a</td>
<td>962 (ra)</td>
<td></td>
<td>103-373</td>
<td>Lindenmaier et al. (1990); Landini et al. (1991a)</td>
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<tr>
<td></td>
<td>888 (ra)</td>
<td></td>
<td>53-226</td>
<td>Lindenmaier et al. (1990)</td>
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<td></td>
<td>Anti-C1 (ra)</td>
<td></td>
<td>C' terminal</td>
<td>Welch et al. (1991a)</td>
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<tr>
<td></td>
<td>Anti-N1 (ra)</td>
<td></td>
<td>N' terminal</td>
<td>Welch et al. (1991a)</td>
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<td></td>
<td></td>
<td></td>
<td>30K + 16K</td>
<td>Baum et al. (1993)</td>
</tr>
<tr>
<td>pUL46</td>
<td>None described</td>
<td></td>
<td></td>
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<tr>
<td>pUL48</td>
<td>Anti-XP1 (ra)</td>
<td></td>
<td>X2-16 (hu)</td>
<td>Bradshaw et al. (1991)</td>
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<tr>
<td>pUL32</td>
<td>None available</td>
<td></td>
<td></td>
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<tr>
<td>pUL82</td>
<td>Anti-p65 (ra)</td>
<td></td>
<td>583-646</td>
<td>Jahn et al. (1987a); Jahn &amp; Mach (1990)</td>
</tr>
<tr>
<td></td>
<td>Anti-p65 (ra)</td>
<td></td>
<td>555-705</td>
<td>Jahn et al. (1987a); Jahn &amp; Mach (1990)</td>
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<tr>
<td></td>
<td>Anti-p68 (mo)</td>
<td></td>
<td>555-705</td>
<td>Yamanaka et al. (1992)</td>
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<tr>
<td>ppUL83</td>
<td>Anti-p65 (ra)</td>
<td></td>
<td>9220 (mu)</td>
<td>Shimokawa et al. (1988)</td>
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<td></td>
<td>Anti-p68 (mo)</td>
<td></td>
<td>4D1, 7B4, 7D2, 8E3, 8E10 (mu)</td>
<td>Britt &amp; Vigler (1987)</td>
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<td></td>
<td>2A6G8, 1C3G1, 4C1F7, 4D5G8 (mu)</td>
<td>Shuster et al. (1985)</td>
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<td>28-19, 28-103, 28-77 (mu)</td>
<td>van der Bij et al. (1988b); Greife et al. (1992)</td>
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<td></td>
<td>95-28, 95-12 (mu)</td>
<td>Kim et al. (1983)</td>
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<td>H74, H7-12 (mu)</td>
<td>Gerna et al. (1992); Revello et al. (1992)</td>
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<tr>
<td>ppUL99</td>
<td>Anti-C3 (ra)</td>
<td></td>
<td>108-253</td>
<td>Britt &amp; Auger (1985); Plachter et al. (1990)</td>
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<td></td>
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<td></td>
<td>Z01 (hu)</td>
<td>Lucas et al. (1988); Amadei et al. (1983)</td>
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<tr>
<td>ppUL65</td>
<td>Anti-P2-1 (ra)</td>
<td></td>
<td>283-288</td>
<td>Rodgers et al. (1985)</td>
</tr>
<tr>
<td>ppUL98a</td>
<td>Anti-P2-1 (ra)</td>
<td></td>
<td>208-216/280-285</td>
<td>Chardonne et al. (1983)</td>
</tr>
<tr>
<td>pUL56</td>
<td>Anti-p130 (hu)</td>
<td></td>
<td>12-26</td>
<td>Pereira et al. (1982a)</td>
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<td></td>
<td></td>
<td>381-425</td>
<td>Hoshi et al. (1987); Shimokawa et al. (1988)</td>
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<td>Sutherland et al. (1987)</td>
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<td>Young et al. (1989)</td>
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<td>Redmond et al. (1986)</td>
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<td>Kitamura et al. (1990)</td>
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<td>Ohlin et al. (1991)</td>
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<td>Martiinez &amp; St Jeor (1986)</td>
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<td>Re et al. (1985); Meyer et al. (1988)</td>
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<td>Pereira et al. (1982a)</td>
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<td>Davis et al. (1984)</td>
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<td>Lahijani et al. (1991); Lahijani &amp; Otteson (1992)</td>
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<td>Bradshaw et al. (1989)</td>
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<td>Bogner et al. (1993)</td>
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* Abbreviations: ra = rabbit; mo = mouse; hu = human (affinity purified antibodies).
† Abbreviation: p.c., personal communication.
(Gibson et al., 1990; Shenk et al., 1991). It has been determined that the proteinase responsible for matura-
tional cleavage of pUL80a precursors is encoded by the
longest gene in UL80 (Welch et al., 1991b). Baum et al.
(1993) have recently reported that the UL80-encoded
85K polyprotein is cleaved at three proteolytic sites to
yield proteins of M1, 50K, 30K, 16K and 13K. The UL80
polyprotein is cleaved at the following three sites: (i) at
the N-terminus of the assembly protein domain; (ii)
between the 30K and 50K proteins and (iii) within the
30K protease itself, which yields the 16K and 13K
proteins. The protease activity resides in the 30K protein
derived from the N-terminal domain of the precursor
polyprotein and the autocleavage may be a mechanism
to inactivate the protease.

Using two rabbit hyperimmune sera (962 and 888),
pUL80a was localized by immunoelectron microscopy
inside the viral capsids both in the nucleus and cytoplasm
(Landini et al., 1991b).

In vivo immune response. The in vivo immune reactivity
to pUL80a is mainly due to IgM (Landini et al., 1985,
1986) and in several cases of secondary acute infection
(endogenous reactivations or exogenous reinfections),
IgM antibodies reacting exclusively with tegument p150
and capsid pUL80a are detectable (Landini et al., 1986).
Furthermore it was found that there is a common
antigenic epitope shared by a linear determinant at the
carboxy-terminal half of pUL80a and a cell membrane
component of M', 60K (mp60) which is recognized by
IgM in sera from patients with primary HCMV infection
(Landini et al., 1991c).

Specific immunological reagents. Rabbit antisera to
pUL80a (N and C termini) has been reported by Welch
et al. (1991b), and rabbit antisera to fusion proteins of
pUL80a containing amino acids 103 to 373 and 57 to 226
have been generated by Lindenmaier et al. (1990). Rabbit
antisera to the 30K plus the 16K proteins were obtained
by Baum et al. (1993). No MAbs have been described
that are directed against this protein. See Table 1.

(iii) pUL46
A lower M1 protein, which is less abundant than p155, is
also present in complete capsids and has been called the
minor Capsid Protein (mCP). By analogy with HSV-1,
this protein might be involved in anchoring viral DNA
into capsids (Braun et al., 1984). In HCMV, this protein
has been predicted to be encoded by UL46, whose 290
amino acid product (Chee et al., 1990) is probably
related to a 34K to 37K structural protein characterized
in both human and simian CMV (Gibson, 1983; Irmiere
& Gibson, 1985).

No information is available on its in vivo immuno-
genicity and no specific antibodies have been described.

**Tegument proteins**

The viral tegument is the region located between the
capsid and the envelope. The details of virion maturation
and acquisition of the tegument in HCMV-infected cells
are not clearly established. HCMV tegument is formed
by at least seven proteins of Mrs 212K, 150K, [71K and
65K], [28K and 58K], and 67K. Two less abundant
proteins of 80K and 24K have also been tentatively
designated as tegument constituents. With the exception
of p212, each tegument protein is phosphorylated (for
reviews, see Gibson, 1983; Landini & Michelson, 1988;
Jahn & Mach, 1990). The function of the tegument
proteins remains unclear, although comparisons with
other herpesviruses predict that they may play important
roles in viral gene regulation, in modification of host cell
metabolism and in the envelopment process. HCMV
tegument proteins do not seem to induce the production
of monoclonal or polyclonal antibodies with either
neutralizing activity or the capacity to bind to virus or
virus-infected cells (Britt, 1991).

For the purpose of this review, only the six best
categorized tegument proteins (i.e., p212, p150, p71
and p65, p28 and p67) whose coding regions have been
identified (UL48, UL32, UL82 and UL83, UL99 and
UL65, respectively) will be taken into consideration.

(i) pUL48
Recently, Bradshaw et al. (1991) studied the epitope
specificities of human-derived monoclonal antibodies.
The epitopes were characterized by immune screening of
an HCMV genome library constructed in λgt11. The
selected clones were sequenced and sequences were
matched with ORFs from the complete genome sequence
in GenBank. These authors found an antibody reacting
with a previously unrecognized viral protein. In fact,
MAb X2-16 identified an antigen of M1, 216K in infected
cell lysates both by immunoprecipitation and immuno-
blotting. On immunoblots, a closely spaced doublet was
often observed, but it was not apparent by immuno-
precipitation. Immunoblot analysis of purified virions
and dense bodies showed that the UL48 gene product,
which is recognized by MAb X2-16, is a structural
component of the viral particle. Carbohydrate labelling
experiments and the use of deglycosylating enzymes did
not indicate a glycosylated molecule. Two sequenced
clones showed > 97.9% nucleotide sequence identity
with the ORF UL48, which has counterparts in other
human herpesviruses [i.e., HSV (UL36) and EBV
(BPLF1)]. The smaller clone encodes amino acid residues
583 to 646 within the 2241 amino acid ORF from nucleo-
tide 62921 through 70057 coding for a predicted protein of \( M_r \) 253K. The apparent \( M_r \) of the UL48 gene product was consistently about 36K lower than predicted. Whether this reflects an actual initiation site different from the predicted site, cleavage during processing, or aberrant migratory pattern on acrylamide gels is unclear.

It is interesting that in the HSV system, a temperature sensitive mutation in the gene coding for the homologous protein affects the release of viral DNA from capsids and the onset of viral DNA synthesis (Batterson et al., 1983; McGeoch et al., 1988).

In vivo immune response. During natural infection, antibody reactivity to proteins with \( M_r \)'s higher than 150K is frequently observed. However this reactivity has not been determined to be directed to pUL48.

Specific immunological reagents. MAbs to pUL48 (Bradshaw et al., 1991) have been described. See Table 1.

(ii) pUL32

As previously mentioned, there are two proteins with apparent \( M_r \)'s of 150K (the phosphorylated structural component of the viral tegument and the most abundant capsid protein) which can be distinguished by differential migration in gels with a high bis to acrylamide ratio (Irmiere & Gibson, 1983; Jahn et al., 1987b). The tegument phosphoprotein p150 constitutes approximately 20% of the virion protein content (Irmiere & Gibson, 1983). This phosphoprotein also contains O-linked carbohydrates (Benko et al., 1988). The gene encoding p150, UL32, was mapped by screening a bacteriophage \( \lambda \)gt11 cDNA expression library with monospecific rabbit antiserum and was mapped to HindIII fragments J and N of HCMV strain AD169 (Jahn et al., 1987a). The gene is transcribed into a late 6.2 kb RNA.

The reading frame for p150, deduced from computer analysis, gives rise to a polypeptide of 1048 amino acids. Protein secondary structure analysis revealed multiple \( \beta \)-pleated sheets in hydrophilic clusters, providing a possible explanation for the immunogenic properties of the polypeptide (Jahn et al., 1987a). Recently, Zipeto et al. (1993) have identified a variant HCMV strain containing an in-frame three nucleotide deletion in UL32 that caused the absence of an aspartic acid residue in the corresponding protein. Attempts to plaque-purify the deletion mutant were unsuccessful, suggesting that the variant was growth defective. HCMV pUL32 could therefore be essential for viral replication.

In vivo immune response. Tegument pUL32 is the strongest immunogen present in the viral particle (Gibson & Irmiere, 1984; Landini et al., 1985, 1986; Jahn et al., 1987b). High antibody titres to this protein have been found repeatedly irrespective of the stage of infection in nearly 100% of HCMV-seropositive subjects. Furthermore, all four subclasses of IgG are induced by this polypeptide (Landini et al., 1988a) and the overall immune response persists for years after convalescence when antibody to the other HCMV polypeptides has disappeared (Landini et al., 1985, 1986). However, early during the course of naturally occurring primary CMV infection (Landini et al., 1988b) as well as after infection of seronegative individuals with the Toledo strain vaccine (Gönczöl & Plotkin, 1990), the antibody response to pUL32 was either very low or absent. The entire amino acid sequence of HCMV tegument pUL32 consisting of 1048 amino acid residues was divided into 95 overlapping 20 amino acid peptides that were synthesized on polyethylene rods. The rods were subjected to enzyme immunoassay with either pooled HCMV-positive or -negative human sera. When conjugated with BSA, two peptides (those between amino acids 617 and 636 and 595 and 614) gave a positive reaction. IgG was present in 90 and 100% of HCMV-positive human sera, respectively, in a good correlation with the results obtained by complement fixation (Novák et al., 1991). In another study, several overlapping peptides were synthesized at the carboxy-terminus of pUL32 and tested with different human sera. An important IgM-binding region (able to react with IgM present in 83% of IgM-positive sera) was localized in thelast 38 amino acids of the molecule (Landini et al., 1991a). Several recombinant proteins have been obtained that express portions of pUL32 and most of them were shown to react strongly with HCMV-seropositive human sera. (Scholl et al., 1988; Ripalti et al., 1989; Landini et al., 1989, 1990; Plachter et al., 1990, 1992).

Specific immunological reagents. A monospecific antiserum has been produced against the native pUL32 (Weiner & Gibson, 1981). Another monospecific antiserum has been produced against fusion proteins containing amino acids 555 to 705 of pUL32 (Jahn et al., 1987a; Jahn & Mach, 1990). MAbs to pUL32 have been described (Jahn & Mach, 1990; Yamanaka et al., 1992). See Table 1.

(iii) ppUL82 and (iv) ppUL83

A phosphorylated protein of \( M_r \) 65K and another phosphorylated protein of 71K, which migrates more slowly, are present in the tegument of HCMV and were named lower and upper matrix protein because of their apparent mobility in PAGE (Gibson, 1983). There is no immunological cross-reactivity between the upper and the lower matrix protein (pp71 and pp65, respectively).
The lower matrix protein (p65) has also been referred to as 69K matrix-like protein (Irmiere & Gibson, 1983), pp65 (Nowak et al., 1984a), pp64 (Clark et al., 1984; Pande et al., 1984), p66 (Landini et al., 1985, 1986), and ICP27 (Geballe et al., 1986). The genes coding for p65 and p71 are located in tandem between approximately 0.51 and 0.53 map units (UL82 and UL83). HCMV p65 or ppUL83 is encoded by the 5' terminal part of an abundant bicistronic, unspliced 4 kb mRNA (Somogyi et al., 1990), whereas ppUL82 (p71 or the upper matrix protein in the phosphorylation studies of Roby & Gibson, 1986), corresponds to the single translational reading frame from a rare unspliced 1.9 kb mRNA that is coterminal with the 4 kb transcript (Ruger et al., 1987). The mRNA encoding ppUL83 in HCMV Towne appears to be produced efficiently both early and late in infection, but it is not translated at high levels until the late phase (Geballe et al., 1986; Depto & Stenberg, 1989). Preliminary experiments have suggested that ppUL83 is a protein kinase (PK) but this has not been proven directly (Somogyi et al., 1990). Pande et al. (1990) isolated and purified ppUL83 from virions and dense bodies by FPLC. Analysis showed very few differences between the proteins from strains AD169 and Towne and suggested that ppUL83 is encoded by an unspliced message. A MAb (9220) and immunoelectron microscopy were used to localize ppUL83 to the matrix of the dense bodies (Landini et al., 1987). Recent clinical isolates of HCMV display considerable quantitative variations in the expression of ppUL83 (Siqueira-Linhares et al., 1981; Klages et al., 1989). Klages et al. (1989) found that a reduced production of ppUL83 correlates with reduced amounts of its transcript in wild-type strains compared to the high level of expression found in the laboratory strain AD169. Other studies do not seem to support this finding completely (Faucon-Biguet et al., 1986; Grefte et al., 1993). Britt & Vugler (1987), used ppUL83-specific murine MAbs and polyclonal monospecific antisera to show that ppUL83 is degraded into three proteins with Mr's of 52K, 51K and 50K that are found only in the infected cell and not in purified virions. HCMV ppUL83 was shown to be one of the viral antigens in great abundance in complement-fixing antigen (Kim et al., 1977; Fiala et al., 1978). It is transported to the nucleus of HCMV-infected cells immediately after infection (Yamauchi et al., 1985), and can be detected in significant amounts before immediate early (IE) gene expression (Geballe et al., 1986; Grefte et al., 1992; Revello et al., 1992). Schmolke et al. (1993) have shown that cells can acquire ppUL83 from non-infectious dense bodies as well as infectious virions, and that ppUL83 is efficiently transported to the nucleus in the absence of viral gene expression. In contrast, translocation of endogenously synthesized ppUL83 from the nucleus into the cytoplasm occurs late in infection and appears to require the presence of other viral and or virus-induced proteins. This antigen is present in polymorphonuclear leukocytes of patients with HCMV viraemia and is the antigen on which the antigenaemia test is based (van der Bij et al., 1988a; Grefte et al., 1992).

HCMV ppUL82 is not as abundant in infected cells as ppUL83, but its importance was recently established. In fact, it was shown to be able to trans-activate promoters containing upstream ATF- or AP1-binding sites (Liu & Stinski, 1992). Mutations in the carboxy-terminal region of UL82 eliminated trans-activation. The IE promoter of HCMV was also enhanced 20-fold by the UL82 product. Therefore this protein might represent the structural protein (or one of the structural proteins) necessary to trans-activate IE gene expression.

In vivo immune response. Although little is known of the in vivo immune response to ppUL82, reactivity to ppUL83 is one of the most widely studied so far. HCMV ppUL83 appears to be one of the most immunogenic structural antigens of HCMV (Schmidt et al., 1980; Pereira et al., 1982a, 1983; Lery et al., 1982; Cremer et al., 1985; Landini et al., 1985, 1986; Jahn et al., 1987b). The ppUL83-specific antibody response is very high during the acute phase of infection, as well as during early convalescence, but decreases thereafter (Pereira et al., 1982b, 1983; Forman et al., 1985; Landini et al., 1985, 1986; Jahn et al., 1987b; Porath et al., 1987; Gold et al., 1988; Landini et al., 1988b). A study aimed at analysing the appearance of serum IgG and IgM against HCMV structural proteins in sequential sera from renal transplant recipients during the first 1 to 3 months of primary infection has shown that very often the first antibodies to appear are specific for ppUL83 (Landini et al., 1988b). Peripheral blood mononuclear cells (PBMC) from HCMV-immune donors also proliferate and produce interleukin-2 (IL-2) when stimulated with purified ppUL83 (Forman et al., 1985), and helper T cell clones have been obtained that respond to this protein (Liu et al., 1988a, b). This protein is also one of the viral antigens recognized as target antigens for cytotoxic T lymphocytes (CTL) as shown by blocking cytotoxicity with specific MAbs (Charpentier et al., 1986). Recently, Riddell and coworkers (unpublished results), have shown that the majority of CD8+ human leukocyte antigen (HLA) class I-restricted cytotoxic T cell clones generated by repeated stimulation of PBMC from immune donors with autologous HCMV-infected fibroblasts, reacted with ppUL83.

Specific immunological reagents. Two rabbit (Shimokawa et al., 1988; Pande et al., 1991) and one mouse
(Britt & Vugler 1987), anti-ppUL83 antisera have been described. Furthermore, a large number of murine MAbs (Amadei et al., 1983; Britt & Auger, 1985; Chardonnet et al., 1983; Gerna et al., 1992; Greffie et al., 1992; Kim et al., 1983; Lucas et al., 1988; Michelson et al., 1984, 1985; Pereira et al., 1982a; Plachter et al., 1990; Redmond et al., 1986; Revello et al., 1992; Rodgers et al., 1985; Shuster et al., 1985; van der Bij et al., 1988b) as well as human MAbs (Hoshi et al., 1987; Foung et al., 1989; Kitamura et al., 1990; Ohlin et al., 1991; Sutherland et al., 1987; Shimokawa et al., 1988) to this protein have been described (see Table 1). A murine MAb to ppUL82 was described by Nowak (1984b), but the hybridoma is no longer available. No other immunological reagents specific for this protein have been described.

(v) ppUL99

The gene (UL99) coding for this extremely hydrophilic and highly immunogenic structural protein was mapped on the genome of AD169 (Meyer et al., 1988). A MAb (P2G11) specific for the UL99, 28K polypeptide (Re et al., 1985) was used to screen a cDNA library constructed from poly(A)+ RNA of HCMV-infected cells in the prokaryotic expression vector Agtl1. Hybridization of cDNA with cosmid and plasmid clones mapped the gene to the HindIII R fragment (map unit 0-63 to 0-65) of the AD169 strain. The gene is transcribed into two late 1-3 to 1-6 kb RNAs (Meyer et al., 1988; Martinez et al., 1989; Depto & Stenberg, 1992). The nucleotide sequence of the coding region was determined. Parts of the ppUL99 polypeptide were expressed in Escherichia coli as hybrid proteins fused to β-galactosidase, these were readily and specifically recognized by HCMV-seropositive human sera. Antibodies raised against fusion proteins specifically immunoprecipitated ppUL99 from infected cells. In vitro phosphorylation experiments showed that p28 is phosphorylated. The gene coding for ppUL99 has also been expressed in insect cells utilizing a recombinant baculovirus (Giugni et al., 1992). The recombinant ppUL99 was specifically recognized by antibodies to native ppUL99, including HCMV-seropositive human sera. Using MAb P2G11, the antigen was localized by immunoelectron microscopy at the external surface of viral capsids, both in the cytoplasm and in the extracellular space (Landini et al., 1987). Intranuclear capsids were always negative.

In vivo immune response. The in vivo antibody response against ppUL99 is almost solely IgG, and proceeds in a similar fashion to the response to the tegument p150 (high IgG titres lasting for many years after the convalescence), although the antibody titres detected in patients’ sera are generally lower (Landini et al., 1985, 1986; Zaia et al., 1986; Hay et al., 1987; Shimokawa et al., 1987; Meyer et al., 1988). ppUL99 has also been suggested as a target antigen for CTL as shown by blocking cytotoxicity with specific MAbs (Charpentier et al., 1986).

Specific immunological reagents. A rabbit monospecific antiserum that recognizes the product of the HindIII R fragment containing UL99 has been described (Martínez & St Jeor, 1986), as well as murine MAbs (Re et al., 1985; Meyer et al., 1988; Pereira et al., 1982a; Pande et al., 1988) (see Table 1).

(vi) pUL65

Another internal protein has an approximate Mr of 67K, is phosphorylated and is expressed from a spliced transcript (1-9 kb) that includes a portion of UL65. Its expression may be differentially regulated by post-transcriptional processes (Davis et al., 1984; Davis & Huang, 1985; Goin & Stinski, 1986). The possibility that this protein has a PK activity has been suggested.

No information is available on its in vivo immunogenicity and a specific MAb has been described (Davis et al., 1984).

Internal proteins with a still undefined location

(i) ppUL98a

Analysis of the HindIII R fragment identified two short ORFs (ORF2-1, ORF2-2) in addition to the ORF coding for ppUL99 (Lahijani et al., 1991, 1992). Their initiation codon does not follow Kozak’s rules which have been used to predict the potential number of ORFs in the HCMV genome (Chee et al., 1990). The majority of this region is located upstream from the transcription initiation site of the 1-3 kb mRNA specifying ppUL99. A synthetic peptide representing a hydrophilic region in the amino terminus of ORF 2-1 was chemically synthesized and antibody was raised against the peptide. This antibody reacts with a protein of Mr 58K in HCMV-infected cells starting from 62 to 96 h post-infection (p.i.). However ORF 2-1 is capable of coding for a protein of Mr 14K. The discrepancy between the sequence-predicted size and the observed protein cannot be explained by post-transcriptional modification alone. Recent data support the hypothesis that the stop codon separating the two ORFs is missed, thereby generating an extended ORF capable of coding for a 32K protein. Subsequent post-translational modification could then yield a protein of Mr 58K.

No information is available on its in vivo immunogenicity.
Specific immunological reagents. A rabbit monospecific antiserum has been obtained against amino acids 12 to 26 of ppUL98a (Lahijani et al., 1991, 1992) (see Table 1).

(ii) pUL56

Studying the reactivity of a human MAb (X-16), Foung et al. (1989) found a protein of Mr approximately 96K in the viral particles. Inserts from recombinant Agt11 clones identified with X-16 have been sequenced (Bradshaw et al., 1991) and shown to match the CMV genome library in the UL56 ORF. This ORF is read in the reverse direction from nu 86019 to 83458 on the AD169 genome and encodes a protein of 850 amino acids with a predicted Mr of 95 870. This predicted Mr corresponds to the apparent Mr of the protein reacting with X-16. A transcript of 8.5 kb was previously identified in this region and hypothesized to encode the homologue of HSV ICP18.5 (Spaete et al., 1988). The Towne sequence has been deduced and shown to possess a 29.7% overall amino acid sequence identity with HSV ICP18.5. The sequence has been published electronically and the GenBank/EMBL accession number is M74112. The UL56 gene is one of the eight genes located near the DNA polymerase within a block conserved throughout the herpesviruses. Earlier studies with HSV had suggested that the product of UL56 is required for maturation, transport or membrane insertion of glycoproteins. In this regard it is interesting to point out that human MAb X-16 has neutralizing activity (Foung et al., 1989). However, this hypothesis has not been supported by results obtained later with the pseudorabies virus protein homologue of HSV ICP18.5 (Mettenleiter et al., 1993) and with HSV ICP18.5 (Tengelsen et al., 1993). Both groups conclude that this protein is necessary for viral replication and plays an essential role in the process of mature capsid formation (most likely in the cleavage of replicative concatameric viral DNA and its encapsidation).

A protein of Mr 130K recently described as the UL56 product (Bogner et al., 1993) is likely to represent the same protein. Analysis under non-reducing conditions suggested that p130 forms high Mr disulphide-linked complexes of about 300K.

In vivo immune response. This protein seems to be immunogenic in vivo as specific antibodies are found in human sera (Bogner et al., 1993). However the development of the specific immune response has not been studied.

Specific immunological reagents. A MAb (X-16) specific for this protein has been described (Foung et al., 1989) and affinity-purified human antibodies have been obtained (Bogner et al., 1993) (see Table 1).

Other internal proteins

In addition to the proteins described above, a number of proteins predicted from the nucleotide sequence of the HCMV genome are similar to known or suspected herpesvirus structural proteins. The predicted HCMV UL48, UL77 and UL104 products are similar to the products of HSV UL36, a protein involved in DNA release from capsids, UL25 involved in capsid assembly, and UL6 a structural component of the capsid, respectively (for review see Roizman & Sears, 1990). Since the HCMV proteins have not yet been characterized, they are not taken into consideration in this review.

Envelope glycoproteins

Analysis of the sequence of the AD169 strain of HCMV has identified approximately 55 ORFs (ORFs) encoding potential glycoproteins, although the extent of transcription and translation of most of these ORFs is unknown (Chee et al., 1990; for a recent review see Mocarski, 1993). Immunoprecipitation of HCMV or detergent extracts of virion envelopes with human convalescent sera and HCMV-specific polyclonal antibodies has identified a large number of structural polypeptides (Kim et al., 1976; Pereira et al., 1982b, 1983; Stinski, 1976, 1978). Additional glycoproteins have been identified in lysates of HCMV-infected cells at early and late times after infection, which include precursor and mature structural glycoproteins as well as non-structural viral glycoproteins that may be involved in regulation of viral or host cell events. Thus far, only two glycoproteins have been moderately well characterized in the envelopes of virions, gpUL55 (gB) and gpUL75 (gH), the homologues of HSV gB and gH, respectively (also reviewed by Mocarski, 1993). Several additional glycoproteins have been mapped to viral ORFs (see Table 3). These include gpUL4 (gp48), gpUL16, gpUL18 (MHC), gpUL115 (gL), gpUS10 and gpUS11. Several individual glycoproteins or putative glycoproteins have been reported but relatively little is known regarding their structure and function (Britt & Auger, 1985; Michelson et al., 1989). Since their coding ORFs have not been assigned, these proteins will not be discussed further in this review.

The characterization and classification of HCMV glycoproteins has been complicated by a number of factors. Several glycoprotein complexes have been identified in the virion envelope, each containing two or more constituent glycoproteins associated by disulphide bonds (Benko & Gibson, 1986; Gretch et al., 1988a; Pereira et al., 1984; Stinski, 1976, 1977). Obviously, the make-up of these complexes is a reflection of the experimental conditions used to identify them. Some complexes are composed of dimers or multimers of glycoproteins derived by endoproteolytic cleavage of a
Table 2. Summary of HCMV glycoprotein-specific monoclonal antibodies

<table>
<thead>
<tr>
<th>Glycoprotein</th>
<th>MAb</th>
<th>Epitope</th>
<th>Neutralizing activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>gpUL55</td>
<td>C41, C176, C197</td>
<td></td>
<td>(+ C')*</td>
<td>Tomiyama et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>C202, C242, C279 (hu)</td>
<td></td>
<td>(-C')</td>
<td>Tomiyama et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>C141, C208 (hu)</td>
<td></td>
<td></td>
<td>Compton (1993)</td>
</tr>
<tr>
<td></td>
<td>27-28</td>
<td></td>
<td></td>
<td>Britt &amp; Vugler (1992); Kniess et al. (1991)</td>
</tr>
<tr>
<td></td>
<td>27-39</td>
<td>Oligomer</td>
<td></td>
<td>Rasmussen et al. (1985)</td>
</tr>
<tr>
<td></td>
<td>14E10</td>
<td></td>
<td></td>
<td>Meyer et al. (1990, 1992)</td>
</tr>
<tr>
<td>gB-N-terminal</td>
<td>C23 (hu)</td>
<td>69-77/78</td>
<td>(-C')</td>
<td>Meyer et al. (1990, 1992)</td>
</tr>
<tr>
<td></td>
<td>CH86</td>
<td>(DC1)</td>
<td>(+C')</td>
<td>Basgoz et al. (1992); Meyer et al. (1992)</td>
</tr>
<tr>
<td></td>
<td>CH408</td>
<td>27-84 (DC1)</td>
<td>(+C')</td>
<td>Basgoz et al. (1992)</td>
</tr>
<tr>
<td></td>
<td>CH45-1, CH386-3, CH404-4, CH412-2</td>
<td></td>
<td></td>
<td>Ohlin et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>ITC88</td>
<td>71-78</td>
<td>(+/-C')</td>
<td>Qadri et al. (1992)</td>
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<td></td>
<td>CH177-3 (253-1, 358-5, 382-2, 388-4, 395-1, 92-1, 105-7, 112-1)</td>
<td>411-447 (D1)</td>
<td>(-C')</td>
<td>Banks et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>3C2 (9F9)</td>
<td>34,37,39</td>
<td>2%78</td>
<td></td>
</tr>
<tr>
<td>gB-C-terminal</td>
<td>15-D8</td>
<td>101-163</td>
<td>(-C')</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7-17</td>
<td>556-615 (AD-1)</td>
<td>(-C')</td>
<td></td>
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<tr>
<td></td>
<td>C1/F5</td>
<td>27-27, 27-156</td>
<td>(-C')</td>
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<td></td>
<td>27-180, 27-160</td>
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<td></td>
<td>SDZ 89-104 (hu) [EV1-15]</td>
<td>549-645</td>
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<tr>
<td></td>
<td>CH244-4 (130-9, 143-13)</td>
<td>447-476 (D2a)</td>
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<td>CH424-1 (432-1, 434-1, 436-1, 442-1, 446-2)</td>
<td>476-618 (D2b)</td>
<td>(-C') except 424-1 and 434-1 nt (+C')</td>
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<td>CH51-4, CH114-5, CH409-2</td>
<td>618-645 (D3)</td>
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<td>CH58-2 (216-2, 340-4, 381-1, 385-3, 402-5, 410-3)</td>
<td>716-906 (DC2)</td>
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<td>CH28-2 (405-1, 421-5) [aa833-852] (DC3)</td>
<td>878-898; 405-1 and 421-5</td>
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<td></td>
<td>41C2, (2B11)</td>
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<td></td>
</tr>
<tr>
<td>gB-C-terminal (continued)</td>
<td>39E11, (9B7, 18F9)</td>
<td>(+C')</td>
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<tr>
<td></td>
<td>34G7, 11B4</td>
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<tr>
<td></td>
<td>ITC33, ITC34, ITC39, ITC48, ITC52, ITC63B, ITC63C (hu)</td>
<td>484-650</td>
<td>(+/-C') except ITC33, ITC34 and ITC39</td>
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<tr>
<td>gpUL75 (gH)</td>
<td>1G6</td>
<td>Site B</td>
<td>(-C')</td>
<td>Cranage et al. (1988); Rasmussen et al. (1984, 1988)</td>
</tr>
<tr>
<td></td>
<td>14-4b</td>
<td>Site B</td>
<td>(-C')</td>
<td>Simpson et al. (1993); Urban et al. (1992)</td>
</tr>
<tr>
<td></td>
<td>SDZ 89-109 (hu) [EV2-7]</td>
<td></td>
<td></td>
<td>Ehrlich et al. (1987, 1988)</td>
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<tr>
<td></td>
<td>HCMV-16</td>
<td>c2</td>
<td>(-C')</td>
<td>Cranage et al. (1988)</td>
</tr>
<tr>
<td></td>
<td>5, 33, 341, 442</td>
<td>Site A</td>
<td>(-C')</td>
<td>Baboonian et al. (1989)</td>
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<td></td>
<td>115</td>
<td>Site AB</td>
<td>(-C')</td>
<td>Simpson et al. (1993)</td>
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<tr>
<td></td>
<td>109</td>
<td>Site B</td>
<td>(-C')</td>
<td>Simpson et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>AP86-SA4</td>
<td></td>
<td>(-C')</td>
<td>Urban et al. (1992)</td>
</tr>
<tr>
<td>gcII</td>
<td>9E10</td>
<td></td>
<td>(-C')</td>
<td>Kari et al. (1986, 1990b)</td>
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<td></td>
<td>8B4, 26E2</td>
<td></td>
<td></td>
<td>Kari et al. (1986, 1990b)</td>
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<td></td>
<td>15G5 (23B10, 25C8, 27B4, 40B7)</td>
<td></td>
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<td>gp93</td>
<td>N2</td>
<td></td>
<td>(-C')</td>
<td>Michelson et al. (1989)</td>
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<td>gp65</td>
<td>14-9</td>
<td></td>
<td>(+C')</td>
<td>Britt &amp; Auger (1985)</td>
</tr>
</tbody>
</table>

* +/- C' denotes whether the antibody required complement for neutralizing activity.
single precursor glycoprotein (Britt & Vugler, 1989; Kari et al., 1990a; Spaete et al., 1988, 1990), whereas others appear to contain multiple glycoproteins (encoded by separate viral genes) which associate late in the replicative cycle to form complexes in the viral envelope (Kari et al., 1990b). Still other HCMV glycoproteins [i.e., gpUL75 (gH)] may associate with virus-encoded [i.e., gpUL115 (gL); (Kaye et al., 1992b; Spaete et al., 1993)] or transiently with cellular proteins [i.e., glucose-regulated proteins (GRP) and fibroblast growth factor receptor (FGFr) in the endoplasmic reticulum (ER) (Britt & Vugler, 1992; Spaete et al., 1991, 1993)] to facilitate their transport through the ER and Golgi. Thus, the nomenclature system, which identifies individual glycoproteins with a unique polypeptide sequence encoded by a specific ORF, responds to the need to identify the constituents of glycoprotein complexes which contain multiple, sometimes unrelated glycoproteins. Characterization of the complexes is particularly important inasmuch as they constitute the major targets for host immune responses.

Classification of the HCMV glycoproteins and complexes has been further complicated by the heterogeneity of individual glycoproteins (as judged by mobility differences in gel systems) within and between HCMV strains expressed in various cell lines; similarity in Mr's between unrelated glycoproteins and complexes; and technical differences in methods employed to determine Mr's. For example, the HCMV virion envelope contains a complex of Mr 93000 Da [gcII(93)]; disulphide-linked glycoproteins of 93K to 116K in gpUL55 (gB) complexes; and a non-complexed glycoprotein of 93K, all of which co-precipitate from unreduced detergent extracts of purified HCMV virions as a single broad band when human sera or polyclonal antisera are used. Similarly, several glycoproteins have been identified with Mr's in the range of 47K to 63K, including the C-terminal cleavage product of gpUL55 (gB); glycoprotein complex II (gcII); and gpUL4 (gp48). To further complicate matters, many of these glycoproteins and complexes migrate as broad bands. Differences in the extent of glycosylation of potential glycosylation sites and differences in late post-translational processing of the oligosaccharide moieties result in a heterogeneous family of glycoprotein species in SDS–PAGE gels. Moreover, sequence polymorphism among strains and cell-specified differences in post-translational processing (including cells of similar and diverse lineage) contribute to differences in the Mr's of mature glycoproteins encoded by the same gene. Additional confusion has resulted from differences in experimental conditions used to purify and separate the HCMV glycoproteins and complexes. Important considerations include the HCMV strain, cell line used to propagate the virus, purification method (i.e., biochemical, immunoaffinity, molecular expression), and electrophoretic conditions (i.e., use of reducing agents; gel concentration and use of linear or gradient gels in SDS–PAGE). The availability of HCMV-specific MAbs and expression of individual gene products in recombinant systems has helped to clarify many of these issues. Nevertheless, only a small number of the total predicted HCMV glycoproteins have been identified.

Similar to other human and animal herpesviruses, the HCMV envelope glycoproteins are presumed to be involved in virus adsorption to cellular receptors (i.e., attachment); fusion with the plasma membrane and penetration into the cytoplasm; virion assembly; and egress of progeny virus from the infected cell. Although selective deletion of genes encoding certain herpesviral glycoproteins impairs viral replication in vitro, it is presumed that their gene products (i.e., glycoproteins and complexes) are essential to the biology of the virus in vivo. It appears that more than one glycoprotein may serve the critical functions of adsorption, fusion/penetration, assembly and egress. Until recently, functional analysis of HCMV glycoproteins has been complicated by the difficulty in establishing transfected cell lines that contain individual essential glycoprotein genes, and which are permissive for HCMV deletion mutants (Compton, 1993). HCMV glycoproteins exhibiting structural homology with glycoproteins of other herpesviruses (i.e., gB and gH) have been presumed to serve similar functions, although this has not been proven directly. Putative cellular receptors for HCMV are only beginning to be characterized (Adlish et al., 1990; Beersma et al., 1991; Kari & Gehrz, 1993; Keay & Baldwin, 1991; Keay et al., 1989; Neyts et al., 1992; Nowlin et al., 1991; Söderberg et al., 1993a, b; Taylor & Cooper, 1989, 1990), and the role of individual HCMV glycoproteins as ligands for virus attachment and penetration is not known.

(i) gpUL55 (gB)

The gpUL55 (gB) glycoprotein complex is a major target for virus-neutralizing antibody in the virus as well as in complexes isolated from viral membrane preparations (Britt et al., 1990; Gönczöl et al., 1990, 1991; Marshall et al., 1992; Rasmussen et al., 1985). There is general agreement that the polypeptides identified as gp58, gB, gp55-116, and gcI (Britt, 1984; Britt & Auger, 1986; Britt & Vugler, 1989; Brücher et al., 1990; Cranage et al., 1986; Farrar & Greenaway, 1986; Farrar & Oram, 1984; Gretch et al., 1988a; Kari et al., 1986, 1990a; Law et al., 1985; Lehner et al., 1991; Mach et al., 1986; Nowak et al., 1984b; Radsak et al., 1990; Rasmussen et al., 1985, 1988; Spaete et al., 1988; Stannard et al., 1989; Taylor et al., 1988), and described in these reports are from the family of glycoproteins originally designated gA by Pereira et al. (1984).
The gpUL55 (gB) ORF encodes a 906 or 907 amino acid primary translation product depending on the virus strain (Cranage et al., 1986; Spaete et al., 1988). The gpUL55 (gB) envelope glycoprotein is a disulphide-linked glycoprotein complex resulting from endoproteolytic cleavage of a fully glycosylated 150K to 158K precursor (Britt & Auger, 1986; Gretch et al., 1988a; Mach et al., 1986; Spaete et al., 1988). Cleavage occurs at R<sub>460</sub> of gB near a motif of basic residues commonly found at processing sites, R/R−X−K/R−K/R (Barr, 1991; Spaete et al., 1988, 1990). The 93K to 116K N-terminal cleavage product represents the ectodomain of the type I glycoprotein and the 55K C-terminal cleavage contributes to the transmembrane portion of the complex (Basgöz et al., 1992; Meyer et al., 1990, 1992). Maturation of gB is conventional for many viral envelope glycoproteins in that the nascent peptide is cotranslationally modified in the ER by the addition of high mannose sugars, the molecule oligomerizes and is transported to the Golgi complex where the sugars are modified to the complex type, and where endoproteolytic cleavage, mediated by a calcium-dependent cellular protease, occurs (Britt & Auger, 1986; Britt & Vugler, 1989; Gretch et al., 1988b; Spaete et al., 1990). Finally, the oligomeric forms are transported to the surface of infected cells and virions (Britt & Vugler, 1992; Kari et al., 1990a). High mannose forms of a gB precursor can also be detected on the nuclear membranes of HCMV-infected fibroblasts (Radsak et al., 1990). The N-terminal cleavage product contains both N- and O-linked sugars, whereas the C-terminal cleavage product contains only N-linked sugars (Britt & Vugler, 1989; Gretch et al., 1988b; Kari et al., 1990a). Oligomerization appears to take place in the ER shortly after synthesis, and proceeds through a series of folding intermediates that are influenced by resident ER proteins (Britt & Vugler, 1992).

The gB ORF derives from a single coding region (UL55) in the unique long (U<sub>5</sub>) region of the HCMV genome that exhibits a low level of nucleotide identity with the genes encoding the gB glycoproteins of HSV, EBV and gpII of VZV (Cranage et al., 1986; Mach et al., 1986). These genes presumably represent a primordial gene family conserved among herpesviruses which involves biological functions critical to the evolution and survival of these viruses. The gB gene of HSV has been shown to be essential for viral replication in vitro, and HSV gB is involved in fusion of the virus with the cell membrane (Roizman & Sears, 1990). The EBV homologue encoded by the BALF4 ORF provides an interesting contrast to the gBs of both HCMV and HSV in that this 110K glycoprotein is not processed in the Golgi and is not detected on the plasma membrane of EBV-infected cells or on the virion envelope. EBV gB appears to be localized to the inner and outer nuclear membrane and to the ER (Gong et al., 1987). Although it has not been formally demonstrated, it is assumed that gpUL55 (gB) will also prove to have an essential function. Recent studies with HCMV gB (and gH) have provided the first evidence of function by demonstrating a role for gB in cell penetration, transmission of infection from cell-to-cell, and in the fusion of cells (Navarro et al., 1993; Rasmussen et al., 1991b; Simpson et al., 1993).

Because gB is the major target for virus-neutralizing antibodies, it has become the leading subunit vaccine candidate (Britt et al., 1988; Gönczöl et al., 1986; Marshall et al., 1990; Spaete et al., 1988, 1990; Wells et al., 1990). Much work has been directed at defining immunologically relevant epitopes on the protein (for example see Silvestri et al., 1991). These reports have been abstracted as MAb reactivities in Table 2. In addition, an ELISA using purified gB epitopes (amino acids 484 to 650: AD1; and amino acids 100 to 207: AD2), has been successfully set up for the detection of neutralizing antibodies (Kropff et al., 1993). Expression of gB in various recombinant expression systems has been directed at evaluating gB as an antigen (Britt et al., 1988; Spaete, 1991). In addition to E. coli and yeast expression systems, gB has been expressed in baculovirus vector systems (Spaete, 1991; Wells et al., 1990). Mammalian cells infected with adenovirus vectors and vaccinia–UL55 recombinant viruses (Berenci et al., 1993; Britt et al., 1988; Cranage et al., 1986; Liu et al., 1991; Marshall et al., 1990), or transfected with UL55 under the control of heterologous eukaryotic promoters (Spaete et al., 1988, 1990) express glycoproteins and complexes of similar or identical M<sub>s</sub>s to those found in HCMV-infected fibroblasts. Studies of sequence variation in gB among HCMV clinical isolates have their genesis in the desire to estimate the difficulties that will be encountered in using a single strain of gB as an antigen in an outbred population (Chou & Dennison, 1991; Chou, 1992a; Lehner et al., 1991; Roy et al., 1993).

Much recent effort has shown that HCMV reacts with a putative cellular receptor protein of M<sub>s</sub> approximately 30K to 34K (Adlish et al., 1990; Nowlin et al., 1991; Rasmussen et al., 1991b; Taylor & Cooper, 1989, 1990). More recently, evidence has been presented that the CD13 cell surface marker plays a role in cellular attachment and penetration of HCMV (Söderberg et al., 1993a, b). It is not currently known which of the envelope glycoproteins is responsible for this interaction.

(ii) gpUL75 (gH)

A disulphide-linked complex (gcIII) with an M<sub>s</sub> of 245K has been described by Gretch et al. (1988a), which contains the 86K glycoprotein originally reported by...
Rasmussen et al. (1984) as p86, and an antigenically unrelated glycoprotein of M, 145K (gp145). The 86K glycoprotein was subsequently shown to be the product of the UL75 ORF (Cranage et al., 1988; Pachl et al., 1989). The gene encoding gp145 and the properties of this glycoprotein are unknown.

The HCMV gene encoding gpUL75 (gH) exhibits homology with the gH gene of HSV, BXLF2 of EBV and gpIII of VZV (Cranage et al., 1988). Although gpUL75 (gH) can be detected readily in lysates of mammalian cells transfected with UL75, the glycoprotein is retained in the ER (Kaye et al., 1992b; Spaete et al., 1991, 1993). Surface expression appears to require association of gH with an ‘escort’ protein of viral or cellular origin in the ER. The gene product encoded by the UL115 ORF has recently been shown to facilitate surface expression of gpUL75 (gH), and thus appears to be the functional homolog of gL in HSV (Hutchinson et al., 1992; Kaye et al., 1992b; Spaete et al., 1993).

The UL75 ORF encodes a gene product of 742 or 743 amino acids depending on the strain of HCMV (Cranage et al., 1988; Pachl et al., 1989). The gene product is modified by the addition of complex N-linked carbohydrates (Kaye et al., 1992b; Rasmussen et al., 1988; Spaete et al., 1993). Observed differences in the sensitivity of gpUL75 (gH) to treatment with endoglycosidase H (endo H) are probably due to the use of lysates as compared with supernatants in the recombinant systems used to express gpUL75 (gH). However, in virus-infected cells the endo H-sensitive forms are confined to the nuclear compartment (Bogner et al., 1992). These workers provide evidence that four of the six potential N-linked glycosylation sites are used and that virion gpUL75 (gH) is endo H resistant.

Sequence comparisons of gpUL75 (gH) from clinical isolates reveals a greater degree of sequence conservation than has been observed for gB among these same strains (Chou, 1992b). The close association with gpUL115 (gL) or gp145 may be a factor in this apparent constraint on gpUL75 (gH) sequence divergence.

The HSV gH gene is essential for replication, and HSV gH appears to mediate fusion of the virion envelope with the cell plasma membrane in the steps of fusion/penetration and egress (Roizman & Sears, 1990). It is assumed that HCMV gpUL75 (gH) will also prove to be essential for replication. Preliminary work has shown that as for other herpesvirus gH homologues, antibodies to gpUL75 (gH) can prevent cell-to-cell spread of the virus in vitro (Rasmussen et al., 1991b; Simpson et al., 1993).

HCMV gpUL75 (gH) has been shown to be a target for the human immune response as well as being capable of inducing complement-independent neutralizing antibodies in mice and guinea pigs (Rasmussen et al., 1984, 1988, 1991a). When the IgG subclass distribution against gpUL75 (gH) and gpUL55 (gB) epitopes was studied, a high prevalence of IgG3 was found directed to epitopes encoded by amino acids 15 to 142 of gpUL75 (gH) (Urban et al., 1994). A catalogue of the MAbs with specificity for gpUL75 (gH) is listed in Table 2.

A cellular receptor protein of M, 92.5K has been identified (on human and non-human cells of diverse origin) which can be co-precipitated with purified gpUL75 (gH) and is detected by an anti-idiotypic MAb2 that mimics gpUL75 (gH) (Keay et al., 1989; Keay & Baldwin, 1991; Rasmussen et al., 1991b). Furthermore, pre-incubation of fibroblasts with the gH MAb2 prevents virus fusion but not attachment (Keay & Baldwin, 1991).

(iii) gpUL4 (gp48)

A viral glycoprotein of M, 48K (gp48) has been detected in detergent extracts of purified virions by Western blot using a monospecific antibody prepared against synthetic peptides predicted to be B cell epitopes in the amino acid sequence from UL4, an HCMV early gene (Chang et al., 1989a). This region is controlled by three temporally regulated upstream promoters (Chang et al., 1989b) as well as coding information contained in the second of three short upstream ORFs found in the transcript (Degnin et al., 1993; Schleiss et al., 1991). The highest levels of the glycoprotein accumulate at late times. The primary translation product is predicted to be 152 amino acids and could potentially encode a protein of 17K. The observed M, of 48K is accounted for by N-glycan modification and predicted O-glycan modification (Chang et al., 1989a). Deletion mutants lacking UL4 have demonstrated the non-essential nature of this gene in cell culture (Ripalti & Mocarski, 1991; Takekoshi et al., 1987, 1991). The function of this structural glycoprotein is currently unknown.

(iv) gpUL110 (gM)

An M, 45K membrane protein was identified in HCMV envelope preparations using anti-envelope serum (Lehner et al., 1989). The anti-envelope serum was used to screen a ght1 library and a positive clone mapped to the UL100 ORF. Hydrophatic analysis has revealed a protein with multiple membrane-spanning domains having features consistent with an integral membrane protein, hence the protein was initially termed IMP.

The polypeptide encoded by UL100 has homology with the BBRF3 gene of EBV, UL10 of HSV and ORF50 of VZV (Lehner et al., 1989). Recently, the UL10 gene product of HSV has been characterized as the viral glycoprotein, gM (Baines & Roizman, 1993). The HSV UL10 ORF has been shown to be non-essential for replication in tissue culture (Baines & Roizman, 1991),

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Table 3. Consensus report from the nomenclature workshop for HCMV structural proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Literature designation</th>
<th>Location</th>
<th>ORF</th>
<th>HSV homologue (gene)</th>
<th>Name</th>
<th>Descriptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>p155</td>
<td>ICP4, MCP, pp150, 153K</td>
<td>Capsid</td>
<td>UL86</td>
<td>VPS (UL19)</td>
<td>pUL86</td>
<td>MCP (major capsid protein)</td>
</tr>
<tr>
<td>p37</td>
<td>AP, p36, p38, p35</td>
<td>Capsid</td>
<td>UL80a</td>
<td>VP22a (UL26)</td>
<td>pUL80a</td>
<td>AP (assembly protein)</td>
</tr>
<tr>
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<td>mCP</td>
<td>Capsid</td>
<td>UL46</td>
<td>VP19c (UL38)</td>
<td>pUL46</td>
<td>mCP (minor capsid protein)</td>
</tr>
<tr>
<td>p212</td>
<td>HMWP, pp200, p216</td>
<td>Tegument</td>
<td>UL48</td>
<td>VP1 (UL36)</td>
<td>pUL48</td>
<td>HMWP (high molecular weight protein)</td>
</tr>
<tr>
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<td>BPP, p149</td>
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<td></td>
<td>ppUL32</td>
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<td>Tegument</td>
<td>UL82</td>
<td></td>
<td>ppUL82</td>
<td>UMP, IE-TAP (upper matrix protein, immediate early transactivating protein)</td>
</tr>
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<td>ICP27, LMP, pp65, pp66, gp64, p69, p68, PK68</td>
<td>Tegument</td>
<td>UL83</td>
<td>p (UL11)</td>
<td>ppUL83</td>
<td>LMP (lower matrix protein), PK (protein kinase)?</td>
</tr>
<tr>
<td>p67</td>
<td>p25, p32</td>
<td>Tegument</td>
<td>UL65</td>
<td></td>
<td>ppUL65</td>
<td>PK (protein kinase)</td>
</tr>
<tr>
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<td></td>
<td>Tegument</td>
<td>UL99</td>
<td></td>
<td>ppUL99</td>
<td>PK (protein kinase)</td>
</tr>
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<td>UL56</td>
<td>ICP18.5 (UL28)</td>
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<td>gB</td>
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<td>UL100</td>
<td>gM</td>
<td>gpUL100</td>
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<td></td>
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</tr>
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<td>UL16</td>
<td></td>
<td>gpUL16</td>
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</tbody>
</table>

and on this basis it is assumed that the HCMV gene product will also prove to be dispensable for growth in cell culture.

Glycoproteins with a still undefined location

(i) gpUL115 (gL)

The demonstration that gL forms a complex with gH in HSV-infected cells (Hutchinson et al., 1992), prompted the search for a CMV homologue in the published sequence (Chee et al., 1990). The UL115 ORF was identified as a putative homologue on the basis of both limited sequence identity and conserved position in the genome (Kaye et al., 1992b; Spaete et al., 1993). The gene product of the UL115 ORF is predicted to encode a late viral gene product of 278 amino acids with one N-linked glycosylation site. In immunoprecipitation experiments in the infected cell using an anti-gH MAb, a coprecipitated molecule is seen as a doublet of approximately 32K. The N-linked site is utilized, but following deglycosylation by N-glycanase, gpUL115 (gL) was not reduced to a single species indicating possible O-linked glycosyl additions.

Co-expression of gH and gL in a variety of recombinant systems has revealed that these proteins form a disulphide-linked complex, although the stoichiometry of the association in the virus has not been established. The expected presence of gL in the virion envelope is also yet to be demonstrated. As discussed above, formation of the complex results in cell surface expression of gpUL75 (gH). The fact that the UL115 ORF from the HCMV Towne strain is 99% identical to the AD169 strain at both the nucleotide and amino acid level (GenBank accession no. L01422 and EMBL accession no. X17403, respectively), also supports the notion discussed above that evolutionary constraints could be operating to preserve conformation of both gH and gL.

(ii) gpUL18 (MHC)

The ORF designated UL18 exhibits significant nucleotide sequence identity with the class I α-chain genes of both HLA in man and H-2 in mouse (Beck & Barrell, 1988). The predicted glycoprotein is of particular interest because of previous studies suggesting that HCMV binds to β2-microglobulin (Grundy et al., 1987). Eukaryotic expression of UL18 indicates that a viral glycoprotein of Mr 67K can be obtained which co-expresses with β2-microglobulin (Browne et al., 1990). However, there is no direct evidence that this glycoprotein exists in the virion envelope to act as a ligand for β2-microglobulin on the cell surface to serve as a potential receptor mechanism for HCMV (Beersma et al., 1991). There is also no evidence that binding of the UL18 product in the HCMV-infected cell to β2-microglobulin disrupts assembly and expression of HLA class I molecules (Browne et al., 1992). Recently, a UL18 deletion mutant strain of HCMV has been shown to infect and replicate in cell
culture at levels similar to those of wild-type HCMV, suggesting that the gene product is non-essential for growth in vitro (Browne et al., 1992).

(iii) gpUS10

The US6 family (Chee et al., 1990), corresponds to family 2 described by Weston & Barrell (1986), and contains six ORFs predicted to be membrane glycoproteins, US6 through US11. It has been proposed that certain members of this family contribute to the virion glycoprotein complex II (gcII). Three glycoprotein complexes designated gcII, have been isolated from the envelope of HCMV by Kari and coworkers and have Mr's of 93K, 130K and > 250K (Gretch et al., 1988, 1989; Kari & Gehrz, 1988, 1992; Kari et al., 1986, 1990b). These complexes are related since they are all recognized by the same MAbs. They contain two groups of glycoproteins that are biochemically and antigenically distinct. In SDS-PAGE group 1 glycoproteins form a broad band with Mr's of 47K to 63K (grp1(47-63)) and are recognized by group 1 MAb (Kari et al., 1989, 1990b). The most abundant group 2 glycoproteins have Mr's of 39K to 48K (grp2(39-48)), 90K (grp2(90)), 130K (grp2(130)) and 200K to 250K (grp2(200)) and are recognized by group 2 MAb (Kari et al., 1990b). How this diverse family of glycoproteins and complexes is generated has not been determined.

HCMV mutants have been isolated that show the dispensable nature of the US6 gene cluster for growth in cell culture (Jones et al., 1991; Jones & Muzithras, 1992; Kollett-Jons et al., 1991). Some of these deletion mutants have been used to show that US6 family members do not contribute to the composition of gcII (T. Jones, personal communication).

In particular, the US10 ORF is predicted to encode a primary translation product of 185 amino acids with characteristics of a glycoprotein. The US10 ORF has been expressed and shown to encode a glycosylated gene product of Mr 22K (T. Jones, personal communication). It is not clear at this time whether this glycoprotein is a virion component.

(iv) gpUS11

As discussed above, the US11 ORF is a member of the US6 family. The US11 ORF is predicted to encode a primary translation product of 215 amino acids with the characteristics of a glycoprotein. The US11 ORF has been expressed and shown to encode a glycosylated gene product of Mr 32K (T. Jones, personal communication). The Mr of the US11 gene product was reduced to 25K in the presence of tunicamycin, suggesting that the mature glycoprotein contains N-linked carbohydrate (T. Jones, personal communication). As is the case for gpUS10, it is not clear at this time whether the gpUS11 glycoprotein is a virion component.

(v) gpUL16

Kaye et al. (1992a), have characterized the gene product of the UL16 ORF as an Mr 50K type I membrane glycoprotein. The UL16 ORF is predicted to encode a primary translation product of 230 amino acids with eight potential N-linked glycosylation sites and having no homologues in current data bases. These workers have shown that gpUL16 is synthesized with early kinetics but accumulates to maximum levels at late times after infection. HCMV gpUL16 is not detectable in purified enveloped virions. Finally, they created a lacZ insertion mutant that disrupted expression of gpUL16 and thus demonstrated that the gene was not essential for growth in human fibroblasts in culture.

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