The Two Major Structural Phosphoproteins (pp65 and pp150) of Human Cytomegalovirus and Their Antigenic Properties

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SUMMARY

Human cytomegalovirus (HCMV) purified from cell culture contains two dominant structural phosphoproteins with apparent molecular weights of 65000 and 150000, designated as pp65 and pp150 respectively. The humoral immune response of infected individuals against pp65 is relatively weak and is not always detectable by Western blot analyses. This report shows that recent clinical isolates of HCMV do not necessarily have pp65 as a prominent constituent, suggesting that the low immune reaction is due to variable expression of the pp65 in natural infections. However, the HCMV strains tested in this study produced the large structural phosphoprotein (pp150) in about equal amounts. The pp150 is remarkably immunogenic, if compared with all other virion constituents; serum pools and individual sera from HCMV-infected patients recognized this particular protein intensively in immunoblot assays. Thus, phosphoprotein pp150 seems to be the primary polypeptide candidate for expression cloning in order to develop reagents for novel ways of HCMV diagnosis.

INTRODUCTION

Human cytomegalovirus (HCMV) is an important pathogenic agent in newborns after prenatal infection and in immunocompromised individuals. Serological diagnosis of HCMV needs improvement, since the commonly used ELISA detection systems for virus-specific IgG and IgM antibodies are based on poorly defined viral antigens. Purified HCMV particles have at least 25 structural proteins, of which several are modified by glycosylation or phosphorylation (Stinski, 1976; Kim et al., 1976; Pereira et al., 1982b, 1984; Gibson, 1983; Nowak et al., 1984a, b; Britt, 1984; Britt & Auger, 1985, 1986; Farrar & Oram, 1984). At least four phosphorylated structural proteins are found in HCMV (Gibson, 1983; Nowak et al., 1984a, b). One of these proteins has an apparent molecular weight of 65000 (65K) and is designated as pp65 (Nowak et al., 1984a) or lower matrix protein (Gibson, 1983). It is the predominant protein in virus particles purified from lytically infected human fibroblast cultures. A second major phosphorylated matrix protein is of 150K mol. wt. (pp150). Two minor phosphoproteins of HCMV have molecular weights of about 71K and 86K (Gibson, 1983; Nowak et al., 1984a). Non-infectious enveloped particles (NIEPs) have a similar pattern of structural phosphoproteins as infectious virions (Gibson & Irmieri, 1984). However, dense bodies, a second type of defective particles, largely consist of the major phosphoprotein pp65.

A number of studies have been done focusing on the antigenic properties of proteins from lytically infected cells using human sera and monoclonal mouse antibodies (Schmitz et al., 1980; Pereira et al., 1982a, 1983; Cremer et al., 1985; Zaia et al., 1986). Although initial studies have also been done to determine human antibody profiles against proteins of purified virions (Kim et al., 1983; Landini et al., 1985, 1986), far more work will be required using better defined virion proteins. This report demonstrates that the 150K structural phosphoprotein is outstanding among all virion constituents in eliciting a humoral immune response, whereas in contrast the antibody reaction against the phosphoprotein pp65 is highly variable.
METHODS

Viruses, cell culture and virion purification. The wild-type HCMV strains were isolated from urine of infected patients. Laboratory strain AD169 was provided by U. Krech, St. Gallen, Switzerland. For the propagation of viruses, human foreskin fibroblasts (HFF) were used following standard procedures. Purification of HCMV particles from the supernatant of infected cells was done by centrifugal separation in glycerol–tartrate gradients essentially as described by Talbot & Almeida (1977) and modified by Irmiere & Gibson (1983).

Human and rabbit antisera. Human hyperimmunesera, pooled and concentrated from about 1000 seropositive individuals, were provided by Biotest, Frankfurt, F.R.G. The individual HCMV antibody-positive sera were obtained from the diagnostic laboratories of this Institute. Antisera to HCMV proteins were raised in New Zealand white rabbits. The antigens for the injections were taken from gradient-purified virions, NIEPs, or dense bodies. The virion proteins were separated in preparative 8-5% polyacrylamide gels and stained with Coomassie Brilliant Blue. Appropriate bands were excised and pulverized, and the proteins were eluted with 0.1 M-NH4HCO3 pH 9.5 and 0.1% SDS. Each injection was estimated to contain approximately 500 μg of protein from NIEPs and dense body fractions, about 200 μg of 65K protein and approximately 20 μg of 150K protein. The initial doses were injected intracutaneously and subcutaneously at six to eight sites with an equal volume of complete Freund’s adjuvant (Sigma). The booster injections were given intramuscularly at monthly intervals using Freund’s incomplete adjuvant. The rabbit preimmune and immune sera were tested for recognition of HCMV proteins by ELISA and immunoblotting.

Radioactive labelling of viral proteins. Radiolabelling was started when viral cytopathic effect was about 60% (at 4 days post-infection) and continued for another 4 days until the virus was purified from culture supernatant. [35S]Methionine (50 μCi/ml) was added in methionine-free Eagle’s minimal essential medium, supplemented with 5% foetal calf serum; [32P], (50 μCi/ml) was added in complete medium. Radiochemicals were purchased from Amersham. The procedure used for the in vitro phosphorylation of HCMV proteins was essentially the same as described by Mar et al. (1981). Phosphorylation reactions were carried out at 24°C for 60 min with 10 μg of the specified protein using 7 μCi [32P]ATP in 200 μl of 50 mM-Tris–HCl buffer pH 8.0, containing 20 mM-MgCl2, 10 mM-DTT and 0.1% Nonidet P40.

Protein gel electrophoresis. Proteins of purified HCMV particles were denatured in SDS buffer (2% SDS, 10% 2-mercaptoethanol, 5% glycerol, 0.005% bromophenol blue, 50 mM-Tris–HCl pH 7.4; heated to 100°C for 3 min) and separated in denaturing 8-5% or 10% polyacrylamide gels essentially as described (Laemmli, 1970). In resolving gels, the ratio of methylene bisacrylamide to acrylamide was increased to 1:28 in order to separate the major capsid protein from the large phosphoprotein (Irmiere & Gibson, 1983). After electrophoresis, gels containing [35S]methionine were fluorographed by the method of Bonner & Laskey (1974). Gels containing unlabelled proteins were stained with Coomassie Brilliant Blue or with silver nitrate (Merril et al., 1981). Standard proteins of known molecular weight (Sigma) and Escherichia coli RNA polymerase (Boehringer) were run on the same gel.

Immunoblotting. For Western blot analysis, antigens from the acrylamide gels were electrophoretically transferred onto nitrocellulose (Towbin et al., 1979). Nitrocellulose sheets were cut into strips and blocked with NET buffer (150 mM-NaCl, 5 mM-EDTA, 50 mM-Tris–HCl pH 7.4, 0.25% gelatin, 0.05% Nonidet P40, 2% bovine serum albumin). After reaction with serum specimens (diluted from 1:30 to 1:200) the strips were incubated with horseradish peroxidase-coupled Protein A (Sigma) and staining was done with 40-chloro-l-naphthol and H2O2.

RESULTS

Expression of the major structural phosphoproteins

The proteins of purified HCMV particles from culture-adapted virus strains and recent clinical virus isolates have been found to be very similar by gel electrophoresis. Yet, a minimal difference in the migration velocity of the glycoprotein gp58 became apparent if envelope proteins from strains AD169 and Towne were compared by immunoprecipitation with monoclonal antibodies (Nowak et al., 1983). Fig. 1 shows the profiles of proteins from the prototype strain AD169 and a wild-type virus strain designated BR84. The viruses were purified from cell culture supernatants; structural proteins, about 0.6 to 1.5 μg per slot, were separated by 8-5% (w/v) polyacrylamide gel electrophoresis and stained with silver nitrate. Corresponding polypeptides appeared identical in electrophoretic mobility. However, a marked difference was recognized in the relative abundance of the major matrix phosphoprotein (pp65) if the clinical isolate BR84 and the laboratory strain were compared. Confirming numerous earlier studies with [35S]methionine labelling, the silver staining showed that pp65 is by far the most abundant structural protein in strain AD169. Even more of phosphoprotein pp65 was found in the dense body fraction (Fig. 1, lanes 3 and 4). However, the polypeptide of equivalent size appeared to be
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Fig. 1. Profiles of HCMV proteins from the prototype strain AD169 and a wild-type isolate BR84. Gradient-purified particles were loaded on an 8.5% running ('high-bis' gel), 5% stacking polyacrylamide gels, subjected to electrophoresis, and stained with silver nitrate. Lane 1, NIEP fraction of AD169 (0.6 µg); lane 2, virions of strain AD169 (0.6 µg); lanes 3 and 4, enriched dense bodies of strain AD169 (1.0 µg); lanes 5 and 6, isolate BR84 (1.5 µg and 0.6 µg) purified from the fifth passage; lane 7, wild-type isolate (1.0 µg), ninth passage. Left, mol. wt. markers (Sigma) of 205K, 116K, 97K, 66K, 45K, 29K and RNA polymerase subunits (Boehringer) of 160K, 150K and 38K.

a minor constituent of the wild-type strain BR84 (Fig. 1, lanes 5 and 6). Similarly, five other clinical isolates at low passage expressed the matrix protein pp65 to a low extent (data not shown). Low passage wild-type viruses did not have appreciable amounts of dense body particles. At higher passages, pp65 of the clinical isolates did increase in quantity (Fig. 1, lane 7). This indicates that the pp65 is not necessarily abundant in infectious HCMV particles, and the higher concentrations of this phosphoprotein correlate with the accumulation of dense body particles at higher cell culture passages.
Thus, it seems unlikely that the differences in pp65 concentration between fresh isolates and culture-adapted laboratory strains can be explained solely by variable incorporation of this protein into the matrix of complete virions.

Whereas the amounts of pp65 matrix protein appeared to be variable in HCMV particles (Fig. 1), the structural proteins of approximately 150K seemed to be rather constant (Roby & Gibson, 1986). Earlier studies had shown that one of these proteins, sometimes migrating as the upper band, is phosphorylated (pp150) (Nowak et al., 1984a, b). On the other hand, Gibson & Irmiere (1984) found the phosphorylated polypeptide (from the matrix) migrated faster than the non-phosphorylated protein (a major nucleocapsid protein) in polyacrylamide gels containing increased concentrations of methylene bisacrylamide ('high-bis'). To resolve the discrepancy, we analysed these labelled proteins in parallel on normal and 'high-bis' gels, combined with autoradiography and Western blotting. The phosphorylated protein sometimes migrated more slowly in the conventional gel system (Fig. 2, lanes 1 and 2). The two bands could not usually be discriminated by these gel conditions. However, the phosphoprotein was always identified as the lower band if 'high-bis' gels were used (Fig. 2, lanes 3 and 4).

Antibodies against the two major viral phosphoproteins in human sera

In order to identify immunoreactive virion polypeptides, total proteins from purified extracellular HCMV were electrophoresed and analysed by Western blot experiments with human sera. Fig. 3 (a) lane 1 shows the characteristic staining patterns with a pool of high titred anti-HCMV human sera. The most intense reaction was seen with the 150K phosphoprotein and within the area of glycoprotein gp58. On the other hand, the phosphoprotein pp65 was rather weakly recognized, in spite of large amounts of this matrix protein on the nitrocellulose strip. The low reactivity with pp65 was even more strikingly demonstrated with individual sera. Fig. 3 (a) lane 2 shows a Western blot performed under identical conditions as for in lane 1, using the serum (no. 40698) of a newborn with fatal cytomegalic inclusion disease. No antibody response was detectable against pp65. Similarly, some sera of children or adults hardly reacted with pp65 in Western blots, while others did under identical conditions (Fig. 4a, b). These patterns of reactivity did not correlate with particular clinical states (Table 1); however, there was a correlation with the titre in the complement fixation test.

All Western blots with human sera consistently showed stronger reactivity with 150K proteins than with any other structural protein of the virus (Fig. 3, 4 and Landini et al., 1985). To discriminate between immunoreactivities towards the 150K nucleocapsid protein and the matrix protein pp150, 'high-bis' gels were used in all subsequent experiments. As shown for instance in Fig. 3 (b) lanes 5 and 6, the strongest immune responses were always found with the faster migrating phosphorylated matrix protein pp150, irrespective of the stage of virus infection in serum donors (Table 1). In general, the pooled hyperimmune serum (Fig. 3a, lane 1) as well as individual sera consistently gave maximum reactions with pp150. These experiments showed that the phosphorylated matrix protein pp150, which is reproducibly found in the purified virus particles, is the virion protein that is always highly antigenic to the humoral immune system in natural human infection.

Antigenicity of the two major phosphoproteins in rabbits

Raising antibodies against structural viral proteins in rabbits not only yielded monospecific antisera for gene mapping, but also corroborated the relative immunogenic properties of the phosphoproteins. Sera from rabbits that had been immunized with the dense body fractions of HCMV-infected culture fluids reacted primarily with the matrix protein pp65 (Fig. 5a). However, a concentrated NIEP fraction induced a weak immune response against pp65, apparently due to the lower concentration of this particular protein in these defective particles. Again, the strongest reaction was seen against phosphoprotein pp150 (Fig. 5b). Immunization with gel-purified pp65 resulted in rapid seroconversion after two injections; the antibodies reacted with pp65 exclusively in Western blots (Fig. 5c). These experiments taken together show
that the matrix phosphoprotein pp65 is immunogenic. The weak antibody response in the course of natural infection is probably due to low amounts of pp65 in wild-type virions, and not the consequence of missing antigenic epitopes.

Further immunization experiments were performed with the two structural proteins of about 150K, the phosphorylated matrix protein and the major nucleocapsid protein (MCP). In the first series, the two proteins were injected simultaneously, and subsequently the two proteins were cut out separately and injected into two different rabbits. If both proteins were applied
Fig. 3. Immunoblot of HCMV virion proteins following electrotransfer to nitrocellulose (NC). HCMV virion proteins of strain AD169 were subjected to electrophoresis in a 'high-bis' polyacrylamide gel (in a 10% gel, in b 8.5%) electrotransferred to NC, and (a) assayed (lane 1) with a pool of high-titrated human antiserum (ELISA titre 1:50,000) and (lane 2) with a serum of a newborn with fatal cytomegalic inclusion disease (ELISA titre 1:2,560; serum 40698 in Table 1). The NC filters were incubated with Protein A coupled to horseradish peroxidase, followed by exposure to 4-chloro-l-naphthol. Abbreviations are as follows: 150K phosphoprotein (pp150), 65K matrix phosphoprotein (pp65), 58K glycoprotein (gp58, as described by Mach et al., 1986). (b) lane 3, [35S]methionine-labelled structural proteins of strain AD169, exposure after electrotransfer to nitrocellulose; lane 4, immune reaction of rabbit antiserum pp150 as shown in Fig. 5(d) (lane 3 represents the autoradiogram of this particular immunoblot); lane 5, immune reaction with an HCMV-positive serum (no. 40712); lane 6, immune reaction with a human serum negative by conventional serological procedures (no. 9/193); lane 7, immune reaction with serum 43136. M, mol. wt. markers (29K is not on the figure). Information on the human sera is given in Table 1.
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Fig. 4. Immune reaction of individual human sera with HCMV structural proteins separated by SDS–'high-bis' PAGE (8.5%ι) and electrotransferred to nitrocellulose. (a) High-titred sera were taken (complement fixation test, CFT ≥ 1:32) with the exception of the last lane, marked by a star (serum of a male patient with fatal acquired immune deficiency syndrome who shed the virus without titre in the CFT). (b) Low CFT-titred sera were taken from patients with different clinical symptoms. The immune reaction was performed as described for Fig. 3, and abbreviations are as in Fig. 3. More information about the human sera is given in Table 1.

Table 1. Listing of human sera from the patients examined with HCMV infections

<table>
<thead>
<tr>
<th>Fig.</th>
<th>Lane</th>
<th>Initials</th>
<th>Age</th>
<th>Sex</th>
<th>Lab. no.</th>
<th>Clinical status</th>
<th>Antibody titre in CFT</th>
<th>Reactivity in ELISAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>3(a)</td>
<td>2</td>
<td>S.R.</td>
<td>3 days</td>
<td>F</td>
<td>40698</td>
<td>Cytomegalic inclusion disease</td>
<td>1:8</td>
<td>+        +</td>
</tr>
<tr>
<td>3(b)</td>
<td>5</td>
<td>S.B.</td>
<td>34 years</td>
<td>F</td>
<td>40712</td>
<td>Healthy mother</td>
<td>1:16</td>
<td>+        -</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>J.G.</td>
<td>33 years</td>
<td>M</td>
<td>9/193</td>
<td>Healthy scientist</td>
<td>-</td>
<td>-        -</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>L.R.</td>
<td>17 years</td>
<td>M</td>
<td>43136</td>
<td>Kidney transplant recipient</td>
<td>1:32</td>
<td>+        +</td>
</tr>
<tr>
<td>4(a)</td>
<td>1</td>
<td>P.W.</td>
<td>32 years</td>
<td>M</td>
<td>50989</td>
<td>Kidney transplant recipient</td>
<td>1:16</td>
<td>+        +</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>L.G.</td>
<td>27 years</td>
<td>M</td>
<td>51710</td>
<td>Colitis</td>
<td>1:64</td>
<td>+        -</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>G.P.</td>
<td>46 years</td>
<td>M</td>
<td>53083</td>
<td>Reiter syndrome</td>
<td>1:64</td>
<td>+        -</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>S.H.</td>
<td>50 years</td>
<td>F</td>
<td>54206</td>
<td>Myocarditis</td>
<td>1:32</td>
<td>+        -</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>W.W.</td>
<td>25 years</td>
<td>M</td>
<td>45281</td>
<td>AIDS</td>
<td>-</td>
<td>+        -</td>
</tr>
<tr>
<td>4(b)</td>
<td>6</td>
<td>D.S.</td>
<td>10 years</td>
<td>F</td>
<td>45850</td>
<td>Retinitis</td>
<td>1:16</td>
<td>+        -</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>T.M.</td>
<td>30 years</td>
<td>F</td>
<td>45964</td>
<td>Arthritis</td>
<td>1:4</td>
<td>+        -</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>G.F.</td>
<td>42 years</td>
<td>M</td>
<td>48862</td>
<td>Fever</td>
<td>1:8</td>
<td>+        +</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>S.K.</td>
<td>17 years</td>
<td>M</td>
<td>46177</td>
<td>Acute lymphocytic leukaemia</td>
<td>-</td>
<td>+        -</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>N.K.</td>
<td>53 years</td>
<td>F</td>
<td>51332</td>
<td>Pericarditis</td>
<td>1:16</td>
<td>+        +</td>
</tr>
</tbody>
</table>
Fig. 5. Immune reaction of rabbit antisera with HCMV proteins separated by SDS-'high-bis' PAGE (8.5%) and electrotransferred to nitrocellulose. For the immune reaction sera were as follows: (a) rabbit antiserum raised against the dense body fraction of HCMV strain AD169; (b) rabbit antiserum obtained with the NIEP fraction; (c) serum raised against gel-purified pp65 (after second booster injection); (d) rabbit antiserum obtained with the structural proteins of about 150K (after four booster injections, the reaction is against pp150 exclusively); (e) rabbit antiserum raised against gel-purified protein p150 (after six booster injections, immune reaction shows pp150 and MCP150). The immune reaction was performed as described for Fig. 3.

simultaneously, antibodies against pp150 were raised significantly earlier (after four injections (Fig. 5d) instead of six injections). The antisera against purified pp150 remained monospecific and were raised after three booster injections, whereas immunization with gel-purified nucleocapsid protein p150 resulted in an immune serum after five booster injections, recognizing both pp150 and the nucleocapsid polypeptide (Fig. 5e); this was probably due to the difficulties in cutting out pp150 and MCP from preparative polyacrylamide gels separately without any cross-contamination prior to immunization. In summary, the matrix protein pp150 is particularly antigenic in rabbits. This is in line with the strong immunogenic properties of phosphoprotein pp150 in the natural infection of humans.

**DISCUSSION**

Human cytomegalovirus (HCMV) particles purified from supernatants of cell cultures contain two abundant structural phosphoproteins, designated pp65 and pp150 according to their apparent molecular weight in SDS–polyacrylamide gel electrophoresis. Both phosphoproteins have been described as constituents of the virion matrix (Gibson, 1983). The two proteins are specified by the HCMV genome, as the coding genes (in strain AD169) have been identified and their nucleotide sequence determined (Nowak et al., 1984a; Rüger et al., 1987; G. Jahn, T. Kouzarides, B. Fleckenstein & B. G. Barrell, unpublished results). The matrix phosphoprotein
pp65 is found in large amounts in cell culture-adapted laboratory HCMV strains. However, as shown in this study, pp65 is in low abundance in virions of recent clinical isolates gradient-purified from supernatants of infected cells. Continuous culture of clinical HCMV isolates resulted in the gradual accumulation of pp65-containing particles. The most plausible explanation seems to be that infectious virions have pp65 as a minor component, and the amount of this phosphoprotein correlates with the presence of dense bodies. Theoretically it is also possible that pp65 occurs exclusively in dense bodies, and whatever pp65 is found in the virion fraction is due to contamination by dense bodies. However, this seems not to be very likely, since we do not know of a proven precedent that a defined gene for a structural viral protein codes for components of defective particles only. The human immune response against pp65 is variable and is even undetectable in some individuals. Here we show that the phosphoprotein pp65 can be highly immunogenic upon inoculation of the isolated protein into rabbits. These results lead to the conclusion that the phosphoprotein pp65 is scarcely expressed in natural infection, and the extreme accumulation of this phosphoprotein in cytomegalovirus-infected cell cultures seems rather to be a cell culture artefact. Other known herpesviruses do not produce defective particles of the HCMV dense body type mostly consisting of a single defined matrix protein.

In the Towne strain of HCMV, the nucleotide sequence of the gene for a major 67K phosphorylated viral protein has been described (Davis & Huang, 1985). A different genome fragment coding for a weakly glycosylated abundant 64K protein was mapped by Pande et al. (1984), indicating that there are at least two different viral proteins with apparent molecular weights of about 65K. The genomic region of the Towne strain identified by Pande et al. (1984) is equivalent to the HCMV AD169 gene for pp65, which was described in detail in our laboratory (Nowak et al., 1984a; Rüger et al., 1987). Kim et al. (1983) have shown that clinical isolates of HCMV as well as laboratory strains could be captured by monoclonal antibodies against a structural protein of about 66K in a double antibody sandwich ELISA. These monoclonal antibodies have not been assigned to either of the two more recently defined proteins in this size range (Forman et al., 1985; Britt & Auger, 1985; Rüger et al., 1987). Thus, the experiments do not contradict the observed pronounced variability of pp65 in purified HCMV particles. Cremer et al. (1985) described the antibody response to cytomegalovirus polypeptides captured by different monoclonal antibodies in a solid-phase enzyme immunoassay. Other studies had shown that multiple bands of protein can be precipitated from HCMV-infected cell cultures using sera from patients with evidence of active virus infection (Pereira et al., 1982a, 1983; Zaia et al., 1986). Though it seemed to be in contrast to our observation that strong Western blot reactivity is limited to a small number of virion polypeptides, the discrepancy may most easily be explained by the fact that, in contrast to others, our study was done with gradient-purified virus particles. In a series of immunoblot experiments, Landini et al. (1985, 1986) showed recently that serum antibodies are directed against a limited number of individual cytomegalovirus structural polypeptides.

The high molecular weight phosphoprotein of HCMV (pp150) which is synthesized in equivalent amounts by wild-type and laboratory strains, is the most antigenic viral component in humans and rabbits. Although previous studies did not discriminate the antibody responses against pp150 and the nucleocapsid protein of similar size (Landini et al., 1985, 1986), we have shown here by reproducible separation of the two proteins in 'high-bis' gels that the stronger reactivity of all sera tested was directed against the phosphorylated matrix protein. The immunogenic nature of pp150 may be explained from the putative polypeptide secondary structure and the clustering of hydrophilic amino acids (Chou & Fasman, 1974; Hopp & Woods, 1981). Other herpesviruses that have been investigated do not possess such an antigenic phosphorylated structural protein in this size range. For instance, the strongest reactivities of herpes simplex virus-immune sera are directed against membrane glycoproteins such as gB (Bernstein et al., 1985; Kahlon et al., 1986) and against the major nucleocapsid protein VP5 of about 150K (Eberle & Mou, 1983). The matrix protein pp150 of HCMV or peptides thereof will be useful in the development of new diagnostic reagents. Thus, for instance, it was possible to use high-titred antisera against native and genetically engineered pp150 for in situ detection of
viral antigens in tissues (B. Borisch & G. Jahn, unpublished). It may also be possible to perform serodiagnosis with mixtures of selected viral proteins after expression in bacterial or eukaryotic cloning systems.

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REFERENCES


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