Monoclonal Antibodies Recognizing Early and Late Antigens of Human Cytomegalovirus: Heterogeneity of Polypeptides Recognized between Virus Isolates

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SUMMARY

The characteristics of four human cytomegalovirus (HCMV)-specific monoclonal antibodies as assessed by ELISA, immunofluorescence, immunoprecipitation and Western blotting are described. Two antibodies recognized a 67K late polypeptide of HCMV, one recognized 43K and 79K polypeptides present early and late in HCMV-infected cells, and the fourth identified a 72K early nuclear protein of HCMV. The antibodies recognized these antigens in all HCMV isolates tested by immunofluorescence and ELISA, but demonstrated inter-isolate variations in polypeptides recognized by Western blotting.

Monoclonal antibodies were initially characterized by their reactivity with HCMV IEA, EA and LA by ELISA and IIF. Cells expressing HCMV IEA were produced by infecting human embryonic fibroblasts at high m.o.i. and culturing for 3 h in the presence of cyclohexamide (50 μg/ml) and then for a further 3 h with actinomycin D (10 μg/ml), but without cyclohexamide. Cells expressing HCMV EA were produced by culturing infected cells for 4 days in the presence of phosphonoformate (200 μg/ml), and LA by culture of infected cells for 4 days in the absence of...
Fig. 1. Analysis of the specificity of anti-HCMV monoclonal antibodies by immunoprecipitation and Western blotting. Western blots of uninfected (U), early (E) and late (L) HCMV-infected cell antigens and gradient-purified virions (V) were probed with monoclonal antibodies H7/4 (a) and H9/2 (b). (c) Monoclonal antibody H11 was used in the immunoprecipitation of 3S-labelled uninfected, immediate early, early and late cell antigens. Molecular weights were determined by comparison with Coomassie Brilliant Blue staining of unlabelled molecular weight markers (Pharmacia). These were phosphorylase B (94000), bovine serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), soybean trypsin inhibitor (20100) and α-lactalbumin (14400).

inhibitors. Monoclonal antibodies H7/4 (IgG3) and H7/12 (IgM) were specific for HCMV LA by both ELISA and IIF, and gave predominantly cytoplasmic fluorescence by IIF. Antibody H9/2 (IgG1) reacted strongly with both EA and LA by ELISA, and weakly with uninfected cell antigen. This antibody produced only poor cytoplasmic staining by IIF and also showed weak diffuse staining of uninfected fibroblasts, reflecting the ELISA observations. Monoclonal H11 (IgG1) recognized both EA and LA by ELISA, but not IEA or uninfected cells. IIF demonstrated that the antigen recognized by H11 was located in the nucleus of phosphonoformate-blocked cells, and could be detected as early as 6 h post-infection. At later times (>48 h) in permissively infected cells this antigen was also detected in the cytoplasm. None of the antibodies described cross-reacted with herpes simplex type 1 or varicella-zoster virus-infected cells by IIF.

The antigens recognized by these monoclonal antibodies were further characterized by Western blotting, and immunoprecipitation, essentially as described by Goldstein et al. (1982) except that Western blots were blocked with 3% bovine haemoglobin, and antigens visualized with peroxidase-coupled anti-mouse immunoglobulin (Dako, Copenhagen, Denmark) and dianinobenzidine. Monoclonal antibody H11 specifically immunoprecipitated a single polypeptide of approximately 72K mol. wt. from both early and late HCMV-infected cell extracts (Fig. 1), and identified a polypeptide of the same mol. wt. on Western blots (data not shown). By Western blotting antibody H9/2 identified polypeptides of 43K and 79K mol. wt. in both early and late infected cells, and 43K and 67K mol. wt. polypeptides in purified virions (Fig. 1). It is possible that the 79K polypeptide seen in the infected cell may be processed to a 67K form during maturation of the virion, although this and the question of the inter-relationship of the 79K and 43K polypeptides would require peptide mapping studies and pulse-chase experiments to resolve the issue. In contrast to ELISA and IIF results, no binding of H9/2 to uninfected cells was observed on Western blots, suggesting that either the ELISA and IIF data reflect non-specific binding to uninfected cells or that the relevant epitope on uninfected cells may be denatured during SDS-PAGE. Antibody H7/4 identified a single polypeptide of
Table 1. Summary of characteristics of anti-HCMV monoclonal antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Isotype*</th>
<th>Neutralization†</th>
<th>Antigen (mol. wt.)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>H7/4</td>
<td>IgG3</td>
<td>-</td>
<td>Late (67000)</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>H7/12</td>
<td>IgM</td>
<td>-</td>
<td>Late (67000)</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>H9/2</td>
<td>IgG1</td>
<td>-</td>
<td>Early (43000, 79000)</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>H11</td>
<td>IgG1</td>
<td>-</td>
<td>Early (72000)</td>
<td>Nuclear</td>
</tr>
</tbody>
</table>

* The immunoglobulin isotype of monoclonal antibodies was determined by double radial immunodiffusion against anti-mouse immunoglobulin subclass sera (Miles Laboratories).
† Neutralization was assessed by plaque reduction after a 1 h incubation of HCMV AD169 with monoclonal antibody supernatant or ascites either alone or in the presence of rabbit complement.

Although a study of the kinetics of neutralization of different HCMV isolates using polyclonal antisera suggested that there might be antigenic variation between isolates (Waner & Weller, 1978), recent studies using IIF and immunoprecipitation with monoclonal antibodies have failed to detect antigenic variation between HCMV isolates (Pereira et al., 1982; Amadei et al., 1983). In this study we examined the reactivity of the monoclonal antibodies described above with three laboratory isolates of HCMV (AD169, Davis and Kerr) and three clinical isolates (West, Hallasay and Blakie) using the techniques of IIF, ELISA and Western blotting.

Monolayers of Flow 5000 human embryonic fibroblasts were infected with the six HCMV isolates, and fixed in acetone when c.p.e. was advanced. By IIF, all four monoclonal antibodies recognized all six virus isolates, although the intensity of staining varied. This probably reflects different rates of virus replication among the HCMV isolates. To avoid possible difficulties arising from varied rates of virus replication, ELISA assays were performed using sorbitol gradient-purified virion and dense body preparations of each isolate. Protein concentrations were estimated by measuring the A_{280}. ELISA plates were coated with 100 ng/well of each isolate by overnight incubation at 4 °C in 0.1 M-bicarbonate buffer pH 9.5. Titration by ELISA of ascitic fluids of monoclonal antibodies H7/4, H7/12 and H9/2 at dilutions of 1/400 to 1/3200 indicated that all three monoclonal antibodies showed equal reactivity with the five HCMV isolates tested (isolate Blakie was not used as insufficient virus was obtained for velocity gradient purification).

Reactivity of these three antibodies with the five virus isolates was further assessed by Western blotting. Polypeptides of purified virions/dense bodies were separated by SDS–PAGE on linear 10% polyacrylamide gels (approx. 20 µg protein/lane), transferred to nitrocellulose by Western blotting, and probed with diluted ascites of the three monoclonal antibodies. As can be seen in Fig. 2, H7/4 (a) and H7/12 (b) identified a 67K molecular weight polypeptide with all five virus isolates, but additional polypeptides of 68K and 55K were observed with the Davis isolate (lanes 2), and a 71K polypeptide was observed with the Kerr isolate (lanes 3). These additional polypeptides presumably reflect precursor or processed forms of the 67K protein. With four of the five isolates tested, antibody H9/2 (c) detected polypeptides of 67K and 43K molecular weight, as described for Fig. 1. However, the 43K polypeptide was not detected on blots of isolate West (lane 4), reflecting either the absence of the 43K polypeptide from this isolate, or antigenic variation of the relevant epitope.

Therefore, although the monoclonal antibodies described in this paper were cross-reactive between HCMV isolates by IIF and ELISA, variation of individual polypeptides between
isolates was detected by Western blotting. The question of whether antigenic variation exists between HCMV isolates is important in the context of both vaccine production and diagnosis. The data presented in this and other publications (Pereira et al., 1982; Amadei et al., 1983) suggest that there is unlikely to be major antigenic variation between HCMV isolates, but the results presented in Fig. 2 indicate that lesser variations between isolates may be detected by Western blotting.

The use of monoclonal antibodies as an aid to diagnosis of HCMV by early confirmation of tissue culture isolation has recently been described (Griffiths et al., 1984). Preliminary studies indicate that monoclonal antibody H11 which gives distinct nuclear immunofluorescence at an early stage of infection before the onset of cytopathic effect and cross-reacts with 12 HCMV isolates so far tested, may be of use in such a diagnostic assay (P. D. Griffiths, personal communication).

REFERENCES


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