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

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(Accepted 19 June 1985)

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.

Human cytomegalovirus (HCMV) is the largest of the human herpesviruses with a DNA genome of molecular weight $1.5 \times 10^8$, which codes for at least 30 structural proteins of the virion and a large number of infected cell-specific polypeptides (Kim et al., 1976; Stinski, 1978). In the infected cell, HCMV polypeptides are synthesized in a regulated cascade of immediate early (IEA), early (EA) and late (LA) antigens (Stinski, 1978), and at least four immediate early (IE) and 16 early polypeptides can be detected by immunoprecipitation with HCMV antibody-positive human sera (Blanton & Tevethia, 1981). The majority of HCMV polypeptides are currently poorly characterized, although an abundant 72K to 75K IE polypeptide and a 64K to 67K polypeptide constituting the major structural protein of the virus are clearly defined (Stinski, 1978; Goldstein et al., 1982). Recently, the use of monoclonal antibodies has facilitated the characterization of the glycoproteins of HCMV (Pereira et al., 1984) and neutralizing epitopes (Britt, 1984) have been defined. In this paper we report on a series of monoclonal antibodies directed against early and late HCMV polypeptides and their cross-reactivity between virus isolates.

Hybridoma lines secreting HCMV-specific antibody were produced using standard techniques to fuse NS1 myeloma cells with spleen cells from BALB/c mice primed with either virions and dense bodies purified by velocity centrifugation on sorbitol gradients (Stinski, 1976), or with nuclei from HCMV-infected cells. Hybrids were screened against both uninfected and HCMV-infected fibroblasts by ELISA or by indirect immunofluorescence (IIF) and cells producing HCMV-reactive antibody were cloned by limiting dilution. From the original fusions, 17 cloned hybridoma lines were established which produced antibodies recognizing either EA or LA. Many of these clones were similar in terms of their immunoglobulin isotype and behaviour in ELISA, IIF and Western blotting. Four representative antibodies are described below.

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...
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 diaminobenzidine. 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.

67K mol. wt. on Western blots of LA and purified virions. This polypeptide was only observed when viral proteins were separated under reducing conditions and is probably equivalent to the major 64K to 67K matrix protein (Kim et al., 1976). The major 67K late protein of HCMV was also inapparent when HCMV-infected cells or virions were studied by SDS–PAGE under non-reducing conditions and the gel stained with Coomassie Brilliant Blue (our unpublished observations). Monoclonal antibody H7/12 also recognized a single 67K polypeptide on Western blots of HCMV LA and virions, but it is unclear whether H7/4 and H7/12 recognize the same epitope, as direct labelling of the monoclonal antibodies and competition experiments have not yet been performed. The characteristics of the monoclonal antibodies described are summarized in Table 1.

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. 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 A280. 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


Fig. 2. Western blotting analysis of purified virions of different HCMV isolates. Lane 1, AD169; lane 2, Davis; lane 3, Kerr; lane 4, West; lane 5, Hallasay. Blots were probed with diluted ascites (1:50) of monoclonal antibodies H7/4 (a), H7/12 (b) and H9/2 (c) and the molecular weights of polypeptides were determined by comparison with molecular weight markers as described in Fig. 1. Monoclonal antibody H9/2 failed to detect a 43K polypeptide on blots of isolate West (c, lane 4). Identical blots were negative when probed with an irrelevant control monoclonal antibody.


*(Received 8 March 1985)*