INFECTION AND IMMUNITY, June 1982, p. 924-932
0019-9567/82/060924-09$02.00/0
Vol. 36, No. 3

Monoclonal Antibodies to Human Cytomegalovirus: Three Surface Membrane Proteins with Unique Immunological and Electrophoretic Properties Specify Cross-Reactive Determinants

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Received 23 December 1981/Accepted 5 February 1982

Seventy-seven clones of hybridomas selected for reactivity by immunofluorescence with human cytomegalovirus (CMV)-infected cells were produced by fusing mouse myeloma cells with the spleen cells of mice immunized with CMV strain AD169. The clones were classified into seven groups on the basis of the electrophoretic properties of the polypeptides immune precipitated from extracts of CMV-infected cells. Studies on the three groups of monoclonal antibodies directed against CMV surface membrane antigens showed the following. Clones in each group were differentiated by immunoglobulin subclass, neutralizing activity, and reactivity with the antigenic domains of proteins exposed on the surface membranes of intact CMV-infected cells. Monoclonal antibodies in each group precipitated one slowly migrating protein and multiple faster migrating forms which shared antigenic determinants. The first group of monoclonal antibodies precipitated four glycosylated polypeptides with apparent molecular weights of 130,000, 110,000, 100,000, and 60,000. Monoclonal antibody CH51 of this group neutralized infectious virus but failed to react with antigenic domains on the surfaces of infected cells. The second group of monoclonal antibodies precipitated four polypeptides with apparent molecular weights of approximately 66,000, 55,000, 50,000, and 46,000. Monoclonal antibodies CH65 and CH134 in this group had neutralizing activity and reacted with antigenic domains of proteins exposed on the surface of CMV-infected cells. The third group of monoclonal antibodies precipitated four polypeptides with apparent molecular weights of 49,000, 48,000, 34,000, and 25,000. Serological analysis of 15 naturally occurring CMV strains with a panel of monoclonal antibodies to surface membrane proteins showed that the antigenic determinants reactive with the antibodies tested were conserved in all of the strains. Monoclonal antibodies to surface membrane proteins on CMV-infected cells may prove to be valuable reagents for identification of virus isolates.

Human cytomegalovirus (CMV), a member of the herpesvirus family, specifies more than 50 infected-cell polypeptides (6, 27). According to the time at which their peak rates of synthesis occur, the viral polypeptides may be classified as early, intermediate, and late. The structural proteins of CMV are produced in abundance late in infection during assembly of progeny virions. As many as 30 polypeptides have been associated with CMV virions; a number of these are glycosylated and comprise the virion envelope (4, 6, 13, 22, 25). Like herpes simplex virus (HSV) glycoproteins, the glycoproteins of CMV are inserted into membranes of infected cells and share antigenic determinants with those in the virion envelope (2, 11, 24, 28). CMV glycoproteins contain the major immunological determinants of the virus and elicit the production of neutralizing antibody (5, 18a, 25, 26). Viral glycoproteins have been shown to be the major antigens immune precipitated from extracts of CMV-infected cells by human convalescent sera (18a).

A number of studies focused on the biochemical and immunological properties of CMV to determine the extent of variation among strains. Epidemiologically unrelated strains of CMV have been shown to differ in restriction enzyme cleavage sites of their DNAs (8, 9, 10, 12). Comparison of the electrophoretic properties of the structural polypeptides of different strains, however, failed to show extensive variability, although minor differences were found (7, 23). Complement fixation tests and neutralization

tests have suggested that CMV strains may differ in immunological determinant sites (10, 29). In a recent study, we showed that antibodies which precipitate viral glycoproteins predominate in human sera and that the viral glycoproteins contain antigenic determinants that are shared among different strains of CMV (Pereira et al., submitted for publication).

In this study, we produced monoclonal antibodies to CMV to characterize the antigenic determinant sites of the viral proteins. Seven groups of monoclonal antibodies identified polypeptides with different immunological and electrophoretic properties. A panel of monoclonal antibodies to surface membrane proteins on CMV-infected cells reacted with different strains. The data indicated that monoclonal antibodies to shared antigenic determinants may be useful reagents for identification of CMV isolates.

MATERIALS AND METHODS

Cell culture and media. Human fetal diploid lung (HFDL) cells were grown in fortified Eagle minimal essential medium (MEM) containing twice the standard concentration of vitamins and amino acids, supplemented with 10% fetal bovine serum. The NS-1 cell line was derived from MOPC-21, a BALB/c myeloma cell line (14). NS-1 cells and the hybrids derived by fusing them with mouse spleen cells were grown in RPMI 1640 supplemented with 20% fetal bovine serum.

Virus strains. Isolation and properties of CMV strain AD169 (CMV AD169) were described elsewhere (21). CMV strains MA, TD, WD, MC, and other isolates used in this study were obtained from specimens submitted to the Viral and Rickettsial Disease Laboratory at the California Department of Health.

Generation and selection of hybridomas producing antibody to CMV. Procedures for producing immunoglobulin-secreting hybrid cell lines were described by Oi and Herzenberg (16). Briefly, BALB/c mice were immunized with CMV-infected cells which were sonicated and emulsified in complete Freund adjuvant. After 4 weeks, the mice were injected intraperitoneally with CMV-infected cells disrupted by sonication in phosphate-buffered saline (PBS). After 3 days, the spleens were removed, and the cells were fused with an equal number of NS-1 cells by using polyethylene glycol. Hybrids were selected in culture media containing hypoxanthine, aminopterin, and thymidine (15). After 2 weeks, the culture fluids of viable hybrids were screened by immunofluorescence on cells infected with CMV strain AD169 (20). Hybridomas producing antibody to CMV were cloned by limiting dilution and cocultured with BALB/c thymocytes. Clones which continued to produce antibody were expanded and injected into mice for propagation as ascites tumors.

Characterization of monoclonal antibodies. Immunoglobulins of the monoclonal antibodies were characterized by Ouchterlony double diffusion tests with rabbit anti-mouse immunoglobulin (Miles Laboratories). Surface membrane immunofluorescence tests were done on CMV AD169-infected HFDL cell monolayers grown on cover slips at 96 h postinfection. Unfixed, infected-cell monolayers were incubated with culture fluids harvested from viable hybridomas. After 30 min, the cells were washed with PBS and stained with fluorescene-conjugated goat anti-mouse immunoglobulin (Antibodies, Inc.). Controls were uninfected HFDL cells. Plaque-reduction neutralization tests were done as previously described (20).

Radiolabeling infected-cell proteins. Confluent monolayers of HFDL cells were infected with 5 to 10 PFU per cell with CMV AD169 and other strains before radiolabeling at 4 days postinfection. To radiolabel the infected-cell polypeptides, cultures were replenished with MEM containing 1/10 the normal amount of methionine, but supplemented with 50 μC of [^{35}S]methionine per ml (specific activity, 1,200 mCi/mmol). Radiochemicals were purchased from New England Nuclear Corp.

Preparation of radiolabeled antigen. To prepare large amounts of antigen for immune precipitation reactions with monoclonal antibodies, confluent monolayers of HFDL cells in 32-oz (ca. 960 ml) bottles were infected with 10 PFU of CMV AD169 per cell. To prepare radiolabeled antigens from different strains, the cells were grown in 25-cm^2 bottles and infected with 10 PFU per cell. Infected cells were radiolabeled with [^{35}S]methionine (50 μC/ml) in media with 1/10 the normal amount of methionine at 96 to 120 h postinfection. At the end of the radiolabeling period, the cells were washed with cold PBS, dislodged from the glass, and centrifuged. Infected cells were extracted with 1% Nonidet P40 and 1% sodium deoxycholate (Sigma Chemical Co.) and sonicated. Infected-cell extracts were centrifuged at 24,000 rpm in an SW27.1 rotor for 1 h at 4°C to remove insoluble proteins.

Immune precipitation tests. Radiolabeled CMV AD169 antigen (0.01 to 0.05 ml) was mixed with 0.5 ml of culture fluid from hybridomas positive by immunofluorescence tests. Mixtures were incubated for 1 h at 37°C, followed by 1 h of incubation with rabbit anti-mouse immunoglobulin G (IgG) (Miles Laboratories). Protein A-Sepharose (Sigma Chemical Co.) was added to collect the immune precipitates. To remove unreacted antigen, the immune complexes that adsorbed to Sepharose beads were washed repeatedly with PBS containing 0.1% Nonidet P40 and 0.1% sodium deoxycholate.

Preparation of samples for electrophoresis. Immune precipitates and radiolabeled infected cells were prepared for electrophoresis in polyacrylamide gels by using the same methods. Samples were denatured and solubilized by heating at 80°C in the presence of sodium dodecyl sulfate (SDS) and β-mercaptoethanol. SDS-polyacrylamide gel electrophresis was done in a discontinuous buffer system containing 0.1% SDS. The separation gel contained 9% acrylamide cross-linked with *N,N*-diallytartardiamide. Reagents for polyacrylamide gel electrophoresis were purchased from Bio-Rad, Inc.

RESULTS

Selection and characterization of monoclonal antibodies to CMV. Seventy-seven hybridomas

producing monoclonal antibodies to CMV were selected on the basis of reactivity in indirect immunofluorescence tests with cells infected with CMV AD169. Immunofluorescence patterns obtained with monoclonal antibodies of different specificities are illustrated in Fig. 1. Figure 1A shows the reaction of monoclonal antibody CH23, which immunofluoresced with an antigen accumulating in the nucleus of CMV-infected cells. Monoclonal antibodies CH51 (Fig. 1B) and CH19 (Fig. 1C) reacted with antigens throughout the cytoplasm of infected cells.

Monoclonal antibodies positive in immunofluorescence tests were reacted in immune precipitation tests with [^{35}S]methionine-labeled extracts of CMV-infected cells. The electrophoretic mobility of CMV-infected cell polypeptides that immune precipitated with selected monoclonal antibodies are shown in Fig. 2. Based on electrophoretic mobilities of immune-precipitated polypeptides, the hybridomas were divided into seven different groups (Table 1). Group A, exemplified by monoclonal antibodies CH14 and CH23, precipitated a polypeptide of 150,000 molecular weight. Group B, exemplified by monoclonal antibody CH86, precipitated two polypeptides with apparent molecular weights of 130,000 and 60,000. Trace amounts of two additional polypeptides with molecular weights of 110,000 and 100,000 were also precipitated by all of the monoclonal antibodies in this group. Monoclonal antibody CH75 represented group C; it precipitated a polypeptide of 96,000 molecular weight. Group D, represented by antibodies CH41 and CH64, precipitated a polypeptide of 74,000 molecular weight. Monoclonal antibodies CH40, CH134, and CH65, representative of group E, precipitated a polypeptide with a molecular weight of 66,000. Two additional faster migrating polypeptides of 50,000 and 46,000 molecular weight and trace amounts of a fourth polypeptide of 55,000 molecular weight were also detected in the precipitates. Group F, containing monoclonal antibodies CH16, CH13, and CH20, precipitated three bands with apparent molecular weights of 49,000, 48,000, and 25,000. A fourth polypeptide of 34,000 molecular weight was precipitated in trace amounts by all monoclonal antibodies in this group. Group G, containing monoclonal antibody CH19, precipitated a polypeptide of approximately 20,000 molecular weight.

Reactivity of monoclonal antibodies directed against cell surface antigens specified by CMV. The following experiments were designed to characterize monoclonal antibodies reactive with surface membrane proteins specified by CMV. Monoclonal antibodies were differentiated by reactivity with glycosylated polypeptides,

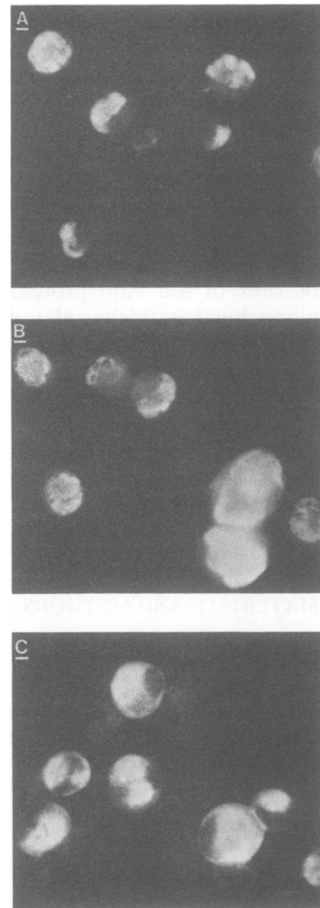

FIG. 1. Photomicrographs of immunofluorescence reactions of monoclonal antibodies with CMV-infected cells. (A) CH23; (B) CH51; (C) CH19. ×470.

immunofluorescence with surface membranes of CMV-infected cells, subclass of immunoglobulin produced, and neutralizing activity.

In a previous study, we identified the major glycoproteins produced in CMV-infected cells by immune precipitation tests with convalescent human sera (18a). Monoclonal antibodies in groups B, E, and F precipitated polypeptides comigrating with glycoprotein bands in profiles of electrophoretically separated [^{14}C]glucosamine-labeled CMV-infected cells. Monoclonal antibodies in each of the groups immune precipitated several glycosylated polypeptides with different electrophoretic mobilities. The 10 clones comprising group B precipitated four polypeptides which comigrated with glycoprotein bands with apparent molecular weights of 130,000, 110,000, 100,000, and 60,000. Group E contained 29 clones which precipitated four polypeptides comigrating with glycoproteins

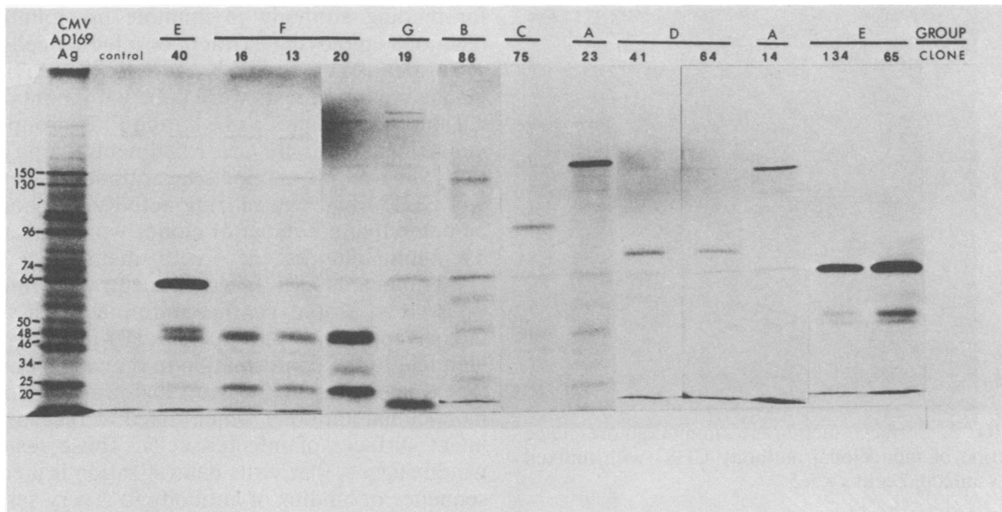

FIG. 2. Autoradiogram of electrophoretically separated [^{35}S]methionine-labeled polypeptides (molecular weight × 10^3) immune precipitated from CMV-infected cell extracts by monoclonal antibodies.

with apparent molecular weights of 66,000, 55,000, 50,000, and 46,000. The five clones in group F precipitated four polypeptides comigrating with glycoproteins of 49,000, 48,000, 34,000, and 25,000 molecular weight.

To differentiate the monoclonal antibodies in groups B, E, and F, we tested for immunofluorescence reactions with surface membranes of CMV-infected cells. The staining pattern obtained with monoclonal antibody CH83 from

TABLE 1. Apparent molecular weights of polypeptides immune precipitated from CMV-infected cells by monoclonal antibodies

Group	Monoclonal antibodies (clone no., CH)	Polypeptide apparent molecular wt	Comments
A	14, 23, 31, 33, 34, 39, 42, 43, 46, 53, 58, 61, 63, 70, 73, 74, 78, 80, 87, 117, 121, 124	150,000	Nucleocapsid protein
B	28, 44, 45, 51, 86, 99, 105, 112, 114, 143	130,000, 110,000,[a] 100,000,[a] 60,000	Four bands (glycosylated)
C	25, 26, 49, 75, 98, 103	96,000	
D	41, 64, 107	74,000	
E	12, 27, 29, 30, 35, 38, 40, 55, 65, 67, 69, 77, 83, 84, 85, 88, 89, 90, 91, 92, 94, 95, 96, 100, 101, 106, 122, 134, 142	66,000, 55,000, 50,000,[a] 46,000	Four bands (glycosylated)
F	13, 16, 20, 102, 104	49,000, 48,000 34,000,[a] 25,000	Four bands (glycosylated)
G	19	20,000	

[a] Immune precipitated in trace amounts.

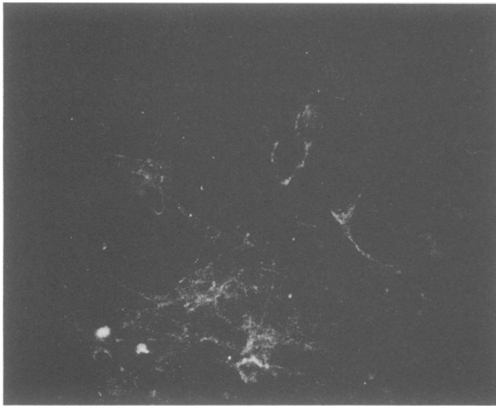

FIG. 3. Surface membrane immunofluorescence reaction of monoclonal antibody CH83 with unfixed CMV-infected cells. ×565.

group E is shown in Fig. 3. The antigen appeared as discrete foci of immunofluorescence scattered over the surface membranes of infected cells. Comparative tests done with selected clones showed that some monoclonal antibodies in each group reacted by immunofluorescence with intact infected-cell membranes, whereas other antibodies did not react with antigenic determinants on the cell surface (Table 2). The results indicated that some monoclonal antibodies were directed to antigenic domains exposed on the cell surface and some were not. Antibodies which failed to react by immunofluorescence with intact infected-cell membranes were probably directed against hydrophobic antigenic domains embedded in the cell membrane. These domains would be inaccessible to the corresponding antibody in intact cells but available for binding antibody in immune precipitation reactions employing extracts of infected cells.

TABLE 2. Reactivity of monoclonal antibodies to viral glycoproteins in immunofluorescence tests with intact surface membranes of CMV-infected cells

Group	Class	Monoclonal antibodies (clone no., CH)	Immunofluorescence reaction
B	a	28, 44, 143	+
	b	45, 51, 112, 114	−
E	a	12, 30, 65, 77, 83, 89, 90, 92, 134	+
	b	27, 35, 38, 67, 69, 85, 88, 91, 94, 95, 96, 102, 122, 142	−
F	a	13, 16	+
	b	20, 104	−

To identify clones with neutralizing activity, plaque reduction tests were done with monoclonal antibodies in groups B, E, and F. In group B, monoclonal antibody CH51 had neutralizing activity; in group E, monoclonal antibodies CH65, and CH134 had neutralizing activity. It should be noted that a number of clones which reacted by immunofluorescence with membranes of CMV-infected cells failed to neutralize infectious virus. Moreover, the conformation of surface glycoproteins on infected cells may not be identical to the conformation in the virion envelope as indicated by the neutralizing activity of a monoclonal antibody which failed to react with intact surfaces of infected cells. These results would suggest that virus neutralization is a consequence of binding of antibody to a very selective subset of antigenic determinant groups exposed on the surface of the virion.

Monoclonal antibodies in groups B, E, and F were differentiated by immunoglobulin class (Table 3). Most of the clones produced IgG1 and IgG2a. One clone in each group produced IgG2b, and one clone in group E produced IgM. All of the clones in group F produced IgG1.

Immune precipitation of viral polypeptides from cell extracts infected with different CMV strains. The purpose of these experiments was to study the electrophoretic and immunological properties of glycoproteins specified by different strains of CMV. Two series of experiments were done. In the first series of experiments, the electrophoretic properties of polypeptides synthesized by different strains were studied. Cells infected with four different strains of CMV were radiolabeled with [^{35}S]methionine at 4 to 5 days postinfection. Radiolabeled infected-cell extracts of the strains were disrupted in SDS and β-mercaptoethanol and electrophoresed in SDS-

TABLE 3. Immunoglobulins produced by cloned hybridomas reactive with CMV-infected cell surface proteins

Group	Monoclonal antibodies	Class
B	44, 45, 86, 99, 143	IgG1
	28, 114	IgG2a
	51	IgG2b
E	12, 27, 29, 55, 65, 67, 83	IgG1
	85, 89, 90, 94, 106, 134	IgG1
	35, 101, 122	IgG2a
	30	IgG2b
	91	IgM
F	13, 16, 20	IgG1

polyacrylamide gels. Profiles of electrophoretically separated polypeptides produced by different strains were compared with CMV AD169 as shown in Fig. 4. Except for differences in the amount of some polypeptides synthesized, e.g., the glycoprotein of 66,000 molecular weight that was synthesized by strain MC, the polypeptide patterns showed little variation and were similar to that of AD169.

In the second series of experiments, the antigenic properties of surface proteins synthesized by different strains were compared with those of CMV AD169 by immune precipitation tests with monoclonal antibodies. In these experiments, [^{35}S]methionine-labeled antigens prepared from each of the strains described above were immune precipitated with monoclonal antibodies in groups E and F. Electrophoretic profiles of polypeptides in immune precipitates obtained with each strain were identical to AD169 and are illustrated by strain WD shown in Fig. 5. The experiments showed that the monoclonal antibodies reacted with different strains which specified surface proteins with cross-reacting antigenic determinants.

Serological analysis of CMV strains with a panel of monoclonal antibodies to the viral glycoproteins. In a series of experiments, we analyzed 15 CMV isolates with a panel of monoclonal antibodies from groups B, E, and F. To diversify the panel, antibodies were chosen on the basis of immunoglobulin class, neutralizing activity, and reactivity in surface membrane immunofluorescence tests. In this way, we selected a variety of antibodies potentially reactive with different antigenic domains on each glycoprotein. The panel of antibodies was used to test 15 CMV isolates by immunofluorescence. Results of these tests showed that each monoclonal antibody in the panel reacted with all the CMV isolates. Additional tests with selected monoclonal antibodies done with CMV strain Towne showed that it also specified cross-reactive determinants. Properties of the monoclonal antibodies in the panel used for serological analysis of the strains are summarized in Table 4.

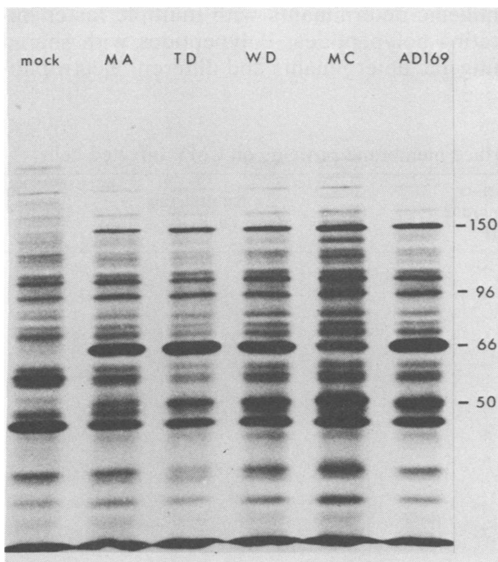

FIG. 4. Autoradiogram of electrophoretically separated [^{35}S]methionine labeled infected cell polypeptides (molecular weight × 10^3) specified by CMV isolates and strain AD169.

DISCUSSION

Monoclonal antibodies to CMV polypeptides. In this study, we generated hybridomas producing monoclonal antibodies to CMV to identify the major viral antigens and, in particular, the cell surface antigens specified by CMV in infected cells. We isolated 77 clones producing monoclonal antibodies to CMV AD169. The clones were divided into seven groups based on the electrophoretic mobility of polypeptides immune precipitated from CMV-infected cells. Four groups of clones precipitated viral polypeptides with apparent molecular weights of 150,000, 96,000, 74,000, and 20,000. The three remaining groups of clones, B, E, and F, reacted with surface antigens on CMV-infected cells, and each group reacted with a distinct class of polypeptides. The polypeptides precipitated by these groups of monoclonal antibodies comigrated with viral glycoproteins. All of the antibodies in group B precipitated a class of four polypeptides with apparent molecular weights of 130,000, 110,000, 100,000, and 60,000. Antibodies in group E precipitated a class of four polypeptides with apparent molecular weights of 66,000, 55,000, 50,000, and 46,000. The bulk of the monoclonal antibodies identified thus far fell into group E. Group F monoclonal antibodies precipitated a class of four polypeptides of 49,000, 48,000, 34,000, and 25,000 apparent molecular weights.

A number of monoclonal antibodies that were reactive with membrane proteins on CMV-infected cells were directed to antigenic determinants exposed on the cell surface. CH65 and CH134, monoclonal antibodies in group E, reacted with cell surface antigenic domains and had neutralizing activity. Although many of the antibodies reacted with antigenic domains protruding out of the plasma membrane, most of these failed to neutralize infectious virus. It is possible that the sites are cleaved off during the process of envelopement or that the antibodies are directed to sites not involved in virus neutralization. The data strongly suggest that neu-

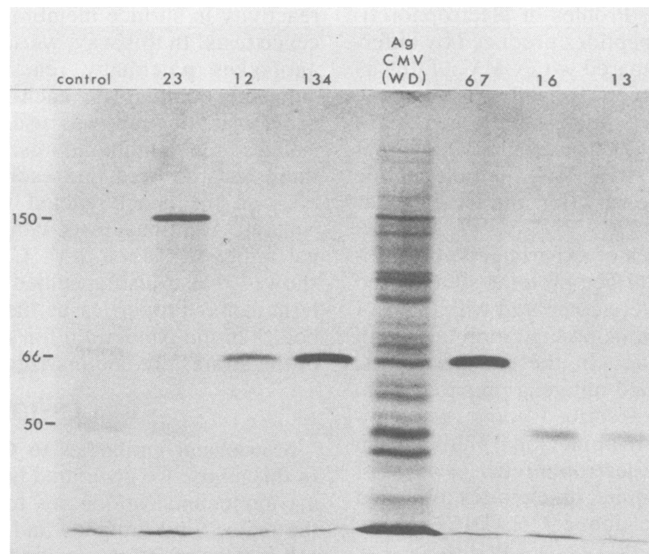

FIG. 5. Autoradiogram of [^{35}S]methionine-labeled polypeptides (molecular weight $\times 10^3$) immune precipitated from CMV strain WD-infected cell extracts by monoclonal antibodies.

tralization requires binding of antibody to particular antigenic sites on polypeptides of the virion envelope. It is noteworthy that monoclonal antibody CH51 of group B neutralized infectious virus but failed to react by immunofluorescence with proteins in intact infected-cell membranes. This finding indicated that the conformation of viral glycoproteins in the surface membrane of infected cells is different from the conformation in the virion envelope. It appears that the structure of surface membrane glycoproteins may be modified during envelopment, resulting in a change in the topological orientation of antigenic domains as evidenced by the presence of antigenic sites that are on the virion surface envelope but not detected on surface membranes of infected cells.

Antigenically related glycoproteins. Immune precipitation experiments with monoclonal antibodies showed that immunologically distinct surface proteins on CMV-infected cells shared antigenic determinants with multiple faster migrating polypeptides. Polypeptides with shared antigenic determinants and different electropho-

TABLE 4. Reactivity of monoclonal antibodies to surface membrane proteins on CMV infected cells

Group	Immunoprecipitated polypeptide (apparent molecular wt)	Clone (CH no.)	Surface membrane IF[a]	Class	Neutralizing activity	IF[a] with 15 CMV strains
B	130,000, 110,000, 100,000, 60,000	28	+	IgG2a	−	+
		44	+	IgG1	−	+
		51	−	IgG2b	+	+
		112	−	IgG1	−	+
		114	−	IgG2a	−	+
E	66,000, 55,000, 50,000, 46,000	12	+	IgG1	−	+
		65	+	IgG1	+	+
		90	+	IgG1	−	+
		91	−	IgM	−	+
		92	+	IgG1	−	+
		134	+	IgG1	+	+
F	49,000, 48,000, 34,000, 25,000	13	+	IgG1	−	+
		16	+	IgG1	−	+
		20	−	IgG1	−	+

[a] IF, immunofluorescence.

retic properties have also been precipitated by monoclonal antibodies to HSV types 1 and 2 (1, 3, 18, 19, 30; Pereira, *in* J. Hurrell, ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, in press). In most instances, faster migrating forms are partially glycosylated precursors to fully glycosylated products. Exceptions are those polypeptides which differ greatly in molecular weight where the smaller proteins may represent cleavage products of the more slowly migrating forms. Glycosylated polypeptides of intermediate electrophoretic mobilities were detected in the immune precipitates from CMV-infected cell extracts. Polypeptides with closely related electrophoretic mobilities are probably antigenically related precursors to more slowly migrating mature forms of the glycoproteins. However, with reference to the class of polypeptides precipitated by group B monoclonal antibodies, the 60,000-molecular-weight polypeptide is far too small to be a partially glycosylated precursor of polypeptides with molecular weights of 130,000 to 100,000. As indicated by studies with HSV proteins (18), the smaller polypeptide may be the cleavage product of a larger protein.

At the present time, it is not clear whether all of the antigenically distinct glycosylated polypeptides specified by CMV have been identified by the monoclonal antibodies thus far characterized. Stinski showed that CMV virions and dense bodies were composed of eight glycoproteins with different electrophoretic properties (26). In studies with convalescent sera, we found that eight electrophoretically distinct glycoproteins were precipitated from glucosamine-labeled CMV-infected cell extracts (18a). In the present study, we identified 11 polypeptide bands which comigrated with glycoproteins in CMV-infected cells. It is noteworthy that polypeptides with apparent molecular weights of 50,000 and 46,000 that were precipitated by monoclonal antibodies in group E had electrophoretic mobilities comparable to polypeptides with molecular weights of 49,000 and 48,000 that were precipitated by group F antibodies. The monoclonal antibodies distinguished four polypeptides which may have appeared as one broad band in immune precipitates obtained in the study with human convalescent sera (18a). Comigration of the bands would account for the different number of glycoproteins precipitated from CMV-infected cell extracts. We are currently studying antigenically related polypeptides with different electrophoretic properties to determine the nature of the relationship between faster migrating forms and more slowly migrating forms.

Serological analysis of CMV strains with a panel of monoclonal antibodies to surface membrane proteins. Immune precipitation tests with selected monoclonal antibodies showed that surface membrane proteins with immunological and electrophoretic properties similar to those of CMV AD169 were precipitated from radiolabeled extracts of cells infected with three different strains of CMV. More extensive analysis of 15 strains of CMV, including the Towne strain, in immunofluorescence tests with a panel of monoclonal antibodies to the surface proteins of CMV-infected cells showed that all of the strains specified cross-reactive antigenic determinants. The panel was comprised of monoclonal antibodies to different classes of cell surface proteins. Antibodies reactive with the same class of proteins were derived from differed clones and reacted with a variety of antigenic domains on the polypeptides. So far, we have failed to detect antigenic differences among strains which can be propagated in cell culture. In contrast, serological analysis of HSV types 1 and 2 showed that they differed in the expression of particular antigenic domains on the surface membrane glycoproteins (17).

Serological studies with human sera suggest that naturally occurring strains of CMV specify infected-cell glycoproteins with cross-reactive immunological determinants. Immune precipitation tests showed that human sera precipitated polypeptides specified by CMV AD169 (18a). Similarly, in a study comparing the antibody titers of human sera in immunofluorescence reactions with homologous and heterologous virus, no difference in endpoint titer was found (S. Stagno, unpublished results). In contrast, tests with polyvalent rabbit antisera produced against different strains of CMV showed differences in the kinetics of neutralization with homologous and heterologous virus (29). Additional studies with guinea pig antisera have shown that complement-fixing antibody titers in tests with different strains of CMV were significantly different (10). The differences may reflect cumulative reactions of mixtures of cross-reacting and virus strain-specific antibodies directed against a number of antigenic determinants on the viral polypeptides. The monoclonal antibodies used in this study identify strong antigenic determinants conserved among different strains in nature. Studies with a panel of antibodies to the antigenic determinants on three infected-cell surface proteins with different electrophoretic and immunological properties indicated that monoclonal antibodies may be useful reagents for serological identification of CMV isolates.

ACKNOWLEDGMENTS

These studies were supported by Public Health Service grant AI-15972 from the National Institute of Allergy and Infectious Diseases.

We thank Dale Dondero and Lyndon Oshiro for assistance with the photography.

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