JOURNAL OF VIROLOGY, Aug. 1985, p. 274–280
0022-538X/85/080274-07$02.00/0

Vol. 55, No. 2

Viral Polypeptides Detected by a Complement-Dependent Neutralizing Murine Monoclonal Antibody to Human Cytomegalovirus

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Received 4 February 1985/Accepted 16 April 1985

Murine monoclonal antibodies were produced which coimmunoprecipitated, under reducing conditions, 130,000- and 55,000-dalton (Da) polypeptides from cells infected with human cytomegalovirus (CMV) strain AD169. A 92,000-Da species, possibly a biosynthetic intermediate, was also detectable. One of the monoclonal antibodies, 15D8, neutralized CMV AD169 only in the presence of guinea pig complement. A second monoclonal antibody, 14E10, coimmunoprecipitated the 130,000- and 55,000-Da polypeptides but did not neutralize viral infectivity. By sequential immunoprecipitation, both monoclonal antibodies have been shown to recognize the same polypeptides. Monoclonal antibody 15D8 detected the 130,000- and 55,000-Da polypeptides in five of six clinical strains and three laboratory strains tested. The 14E10 monoclonal antibody detected the 130,000-Da protein in four of six CMV clinical isolates and in strain AD169 but did not immunoprecipitate any polypeptides from extracts of cells infected with either Towne or Davis laboratory strains. In kinetic studies, the synthesis of the 130,000-Da polypeptide preceded the appearance of the 55,000-Da polypeptide. In infected cells radiolabeled with a pulse of L-[^{35}S]methionine, the isotope was initially detected in the 130,000-Da polypeptide but could be chased into the 55,000-Da polypeptide. These polypeptides exist in the intracellular and extracellular virus as disulfide-linked multimers. Extracellular virus contained a high-molecular-weight (greater than 200,000 Da) multimer composed entirely of 55,000-Da polypeptides. In extracts from infected cells an additional high-molecular-weight multimer was detected consisting of disulfide-linked 130,000-Da polypeptides.

The immune mechanisms which restrict human cytomegalovirus (CMV) infections are thought to involve both humoral and cellular reactions. Data from several laboratories have pointed to the importance of antibodies in protection from generalized CMV disease, particularly in the immunocompromised host (3, 9, 20). The possibility of vaccination against CMV is currently being studied. At present, only live, attenuated virus strains are available as immunogens. The efficacy of these vaccines is controversial (14) and of questionable use in certain patient populations, for example, young women of childbearing age.

The development of an effective subunit vaccine for CMV is dependent upon information regarding the immunogenic polypeptides which play a role in disease resistance. Envelope glycoproteins, thought to be major targets for virus-neutralizing antibody and potentially important in host defense, are now being identified. We have previously reported the characteristics of an 86,000-molecular-weight protein which is detected by virus-neutralizing monoclonal antibody (16). The identification of major glycoproteins which are the targets for viral neutralization is also being pursued by several other groups. A 64,000-dalton (Da) polypeptide is thought to be the abundant tegument protein interfacing the virion and envelope (5). This polypeptide, representing about 70% of the total viral protein, has been purified by high-pressure liquid chromatography and shown to stimulate low titers of neutralizing antibody after hyperimmunization of rabbits (21). However, these investigators state that the 64,000-Da polypeptide is probably not a major surface antigen since whole virions stimulate neutralizing antibody titers considerably higher than the isolated 64,000-Da polypeptide. Others have used neutralizing antibodies produced by hybridomas to detect multiple polypeptides within the infected cell (12, 13) or on extracellular virions (1). The biosynthetic relationships among the several polypeptides detected by a single monoclonal antibody are unclear, especially because each antibody reacts with a single epitope. In this study we report the absolute complement dependence of virus neutralization by murine monoclonal antibodies which detect two major viral polypeptides of 130,000 Da (p130) and 55,000 Da (p55) synthesized by both laboratory and clinical strains of CMV. The p130 appears to be a precursor to p55, which is a constituent of extracellular virus. A 92,000-Da species (p92) which could represent an intermediate form of the two polypeptides is detected both on intracellular and extracellular viruses. The monomer polypeptides p130 and p55 can be detected as self-linked, disulfide-bonded multimers.

MATERIALS AND METHODS

Viruses and cells. The laboratory strains of CMV used were AD169, Towne, and Davis. The clinical strains of CMV were isolated from the urine of congenitally infected newborns or that of patients undergoing cardiac transplantation. CMV was propagated with human embryonic lung (HEL) cells (15). Simian CMV (strain CSG) was purchased from the American Type Culture Collection (Rockville, Md.) and

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TABLE 1. Plaque-reducing concentrations of 15D8 hybridoma IgG and effect of 2% guinea pig complement

Antibody	Concn (μg/ml) for 50% plaque reduction of strain[a]			
	AD169		CSG	
	+ (76)	− (69)	+ (110)	− (99)
Ascites fluid IgG	80	>280	140	ND[b]
Cell culture IgG	20	>280	>280	>280

[a] +, Presence of complement; −, absence of complement. Numbers in parentheses are the average plaque numbers from two replicates inoculated with virus and complement but without antibody.
[b] ND, Not done.

propagated in HEL cells. PFU titers of strain CSG were 10^6 PFU/ml.

Monoclonal antibodies. The procedures for the fusion and selection of CMV-neutralizing monoclonal antibodies, as well as for the purification of gamma globulin (IgG), have been described (16). The monoclonal antibody 15D8 was selected on the basis of its ability to neutralize CMV infectivity. Seven other monoclonal antibodies, selected on the basis of binding in a radioimmunoassay, immunoprecipitated both p130 and p55 from strain AD169 but had no neutralizing activity. One of these antibodies, 14E10, was used for additional experiments. 15D8 IgG was prepared from both ascites fluid and serum-free tissue culture of hybridoma cells. Hybridoma cells in tissue culture were propagated in HB 102 medium (Hana Biologicals, Berkeley, Calif.) to a saturation density of 4×10^5 cells per ml in 100 ml of medium per 175-cm^2 tissue culture flask. IgG was isolated from both the tissue culture supernatants and ascites fluid by ammonium sulfate precipitation. Both 15D8 and 14E10 belong to the IgG2a subclass of immunoglobulins as determined by radial immunodiffusion with specific antibody to murine immunoglobulin (Tago, Inc., Burlingame, Calif.).

Virus neutralization. The plaque reduction assay for neutralizing antibody to CMV has been described (16). Sonicates of infected cells were clarified and used as cell-free virus in neutralization assays.

Radiolabeling of CMV-infected cells. Unless otherwise indicated, cells grown in minimal essential medium were radiolabeled with L-[^{35}S]methionine and extracted under conditions previously described (16). For some experiments, intracellular and extracellular labeled viruses were harvested separately. Intracellular extracts were made from 10^7 cells in 1 ml of lysis buffer (140 mM NaCl, 20 mM Tris-Tris hydrochloride [pH 8.3], 1% Nonidet P-40, 0.5% deoxycholate, 1 mg of chicken ovalbumin per ml, 0.2 mM phenylmethylsulfonyl fluoride, 100 U of aprotinin per ml). Extracellular virus was labeled for 3 days in minimal essential medium with 1% of the normal methionine concentration and 1 mCi of L-[^{35}S]methionine per 2×10^7 infected cells. The supernatant from virus-infected cells was centrifuged for 1 h at 200,000 × *g*. The virus from 100 ml of supernatant was resuspended in 0.5 ml of lysis buffer.

Immunoprecipitation and SDS-PAGE of radiolabeled CMV-infected cell extracts. Methods for immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) have been described (16). For both 15D8 and 14E10, approximately 4.2 μg of IgG was used for immunoprecipitation. Immunoprecipitates were analyzed under both reducing and nonreducing conditions for some experiments. For reduction, immunoprecipitates were eluted from staphylococcus A cells into sample buffer (62.5 mM Tris base [pH 6.8], 3% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.02% [wt/vol] bromphenol blue). Nonreduced immunoprecipitates were eluted into sample buffer without 2-mercaptoethanol.

Immunofluorescence. Methods for immunofluorescent staining have been reported (16).

RESULTS

Virus neutralization by 15D8 IgG. The neutralizing activity of IgG isolated from both mouse ascites fluid and tissue cultures of 15D8 hybridoma cells is shown in Table 1. The neutralization was totally dependent upon the addition of guinea pig complement (GIBCO Laboratories, Grand Island, N.Y.). The IgG derived from cultures of hybridoma cells grown in serum-free medium was slightly more potent in neutralizing CMV than was that derived from ascites fluid. The concentration of antibody from tissue cultures required to diminish simian CMV infectivity was 10-fold higher than that required for AD169. The difference in IgG concentrations from ascites fluid, which neutralized simian and human CMVs, was less than twofold.

Even with concentrations of tissue culture-derived IgG of 100 μg/ml and higher, a 70% reduction of the virus challenge was maximum (data not shown). Higher concentrations of IgG gave questionable specific effects since strain CSG was also inactivated (Table 1). The neutralization of laboratory and clinical strains of CMV by tissue culture IgG is shown in Table 2. Laboratory strain Davis was resistant to neutralization by 15D8, as was clinical strain D. However, the susceptibility of strain Towne and clinical strains A, E, and F was similar to that of strain AD169. Clinical strains B and C did not produce plaques under agar overlay; therefore, the neutralization suceptibility could not be determined.

Viral polypeptides detected by 15D8 and 14E10 IgGs in both laboratory and clinical strains of human CMV. 15D8 and 14E10 IgGs were used to immunoprecipitate L-[^{35}S]methionine-labeled extracts of six clinical CMV strains (less than 10 passages) and three laboratory strains (Davis, Towne, and AD169). 15D8 strongly immunoprecipitated two proteins, p130 and p55, as well as a smeared band (p92) from all laboratory strains and five of six clinical strains (Fig. 1). The intensity of the immunoprecipitated p92 showed some variability among different antigen preparations. The two coimmunoprecipitated polypeptides are designated hereafter as p130/p55, and p92 will be referred to separately. It is noteworthy that clinical strain D was neither neutralized nor immunoprecipitated by 15D8. The p130/p55 proteins were immunoprecipitated from AD169 by 14E10. However, 14E10 immunoprecipitated only p130 from four of six clinical

TABLE 2. Neutralization of CMV clinical and laboratory strains by 15D8 IgG

Strain	PFU[a] in control	μg/ml of IgG for 50% PFU reduction
AD169	76	30
Towne	92	28
Davis	127	>280
Isolate[b]		
A	24	8
D	23	>280
E	14	2.8
F	65	7

[a] Average plaque number from two replicates inoculated with virus and 2% guinea pig complement and incubated without antibody.
[b] Letters refer to isolates shown in Fig. 1.

strains and did not immunoprecipitate any proteins from Towne or Davis laboratory strains (Fig. 1). In immunoprecipitates with 14E10 as antibody, p92 was observed faintly with AD169 and not with other strains. No immunoprecipitates were detectable with normal mouse serum and intracellular extracts of AD169 (data not shown).

To determine whether both 15D8 and 14E10 were reacting with the same polypeptides, L-[^{35}S]methionine extracts of AD169-infected cells were sequentially immunoprecipitated with 15D8 followed by 14E10 (Fig. 2). Antigen extracts were immunoprecipitated initially with 100 times more antibody than was normally used. Antigen-antibody complexes were removed, and the supernatant was reimmunoprecipitated with 30 times more antibody than was normally used. After initial immunoprecipitation with 15D8 (lane 1), no polypeptides could be detected in the supernatant after a second immunoprecipitation with either 15D8 (lane 2) or 14E10 (lane 3). In contrast, initial immunoprecipitation with 14E10 (lane 4) almost completely removed the polypeptides capable of reacting in a second immunoprecipitation with 14E10 (lane 5) but not with 15D8 (lane 6).

No specific bands of immunoprecipitation were observed when L-[^{35}S]methionine-labeled intracellular extracts of strain CSG were immunoprecipitated with either 15D8 or 14E10 under the same conditions as those described for CMV (data not shown).

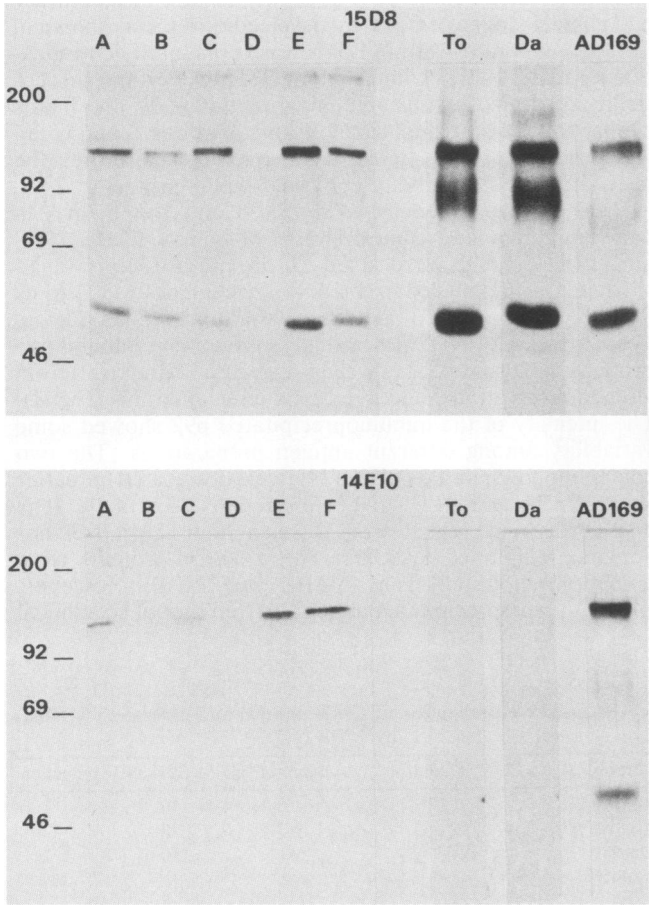

FIG. 1. Immunoprecipitation of clinical isolates (A to F) and laboratory strains Towne (To), Davis (Da), and AD169 by IgG (5 μg) from hybridomas 15D8 and 14E10. CMV-infected cells were radiolabeled with L-[^{35}S]methionine before immunoprecipitation and SDS-PAGE. Positions of molecular weight markers (10^3) are indicated on the left.

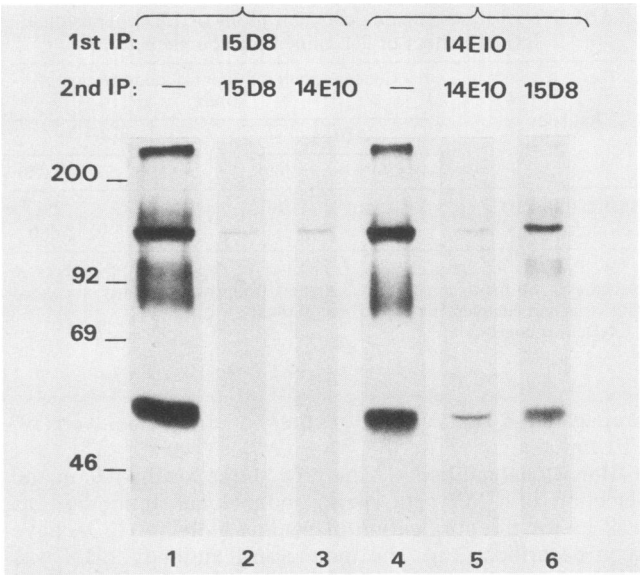

FIG. 2. Sequential immunoprecipitation with hybridomas 15D8 and 14E10 of L-[^{35}S]methionine-labeled extracts of CMV-infected cells. Radiolabeled infected-cell extracts were initially immunoprecipitated with approximately 100 μg of IgG. Immune complexes were then removed, and supernatants were immunoprecipitated a second time with 15D8 and 14E10 for SDS-PAGE.

Analysis of biosynthesis of p130/p55 by immunofluorescence and immunoprecipitation. For immunofluorescence, cover slips of HEL cells were infected at a multiplicity of infection

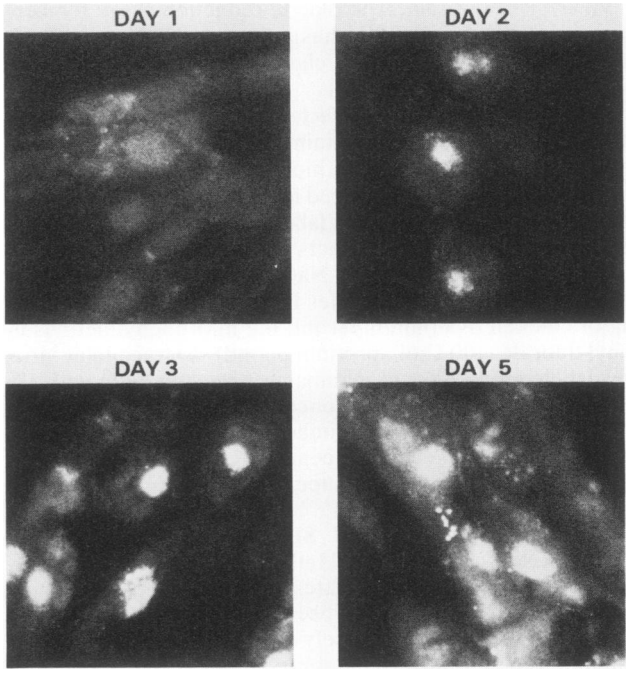

FIG. 3. Biosynthesis of p130/p55 in CMV-infected cells studied by indirect immunofluorescence with 0.16 μg of 15D8 IgG per ml. Cultures were harvested on days 1, 2, 3, and 5 after infection.

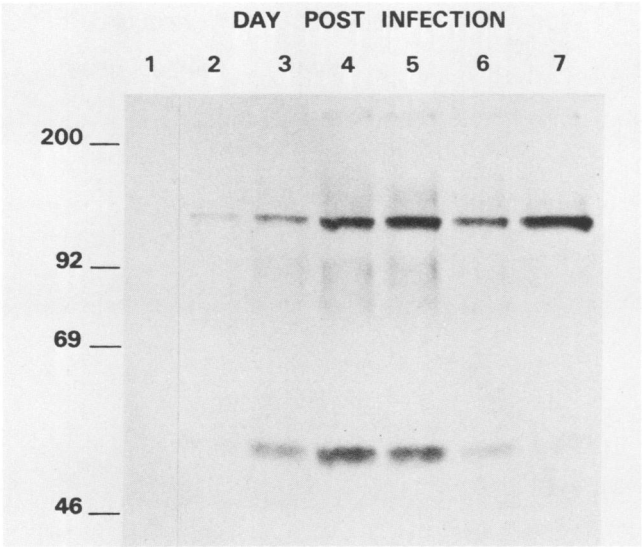

FIG. 4. Biosynthesis of p130/p55 in CMV-infected cells studied by immunoprecipitation with 15D8 IgG of L-[^{35}S]methionine-labeled infected cells. Infected cultures were labeled for 4 h with 500 μCi of L-[^{35}S]methionine on days 1 through 7 after infection. Viral polypeptides were immunoprecipitated with approximately 10 μg of 15D8 IgG for SDS-PAGE.

of 1 and harvested daily for 5 days for staining with approximately 0.16 μg of 15D8 IgG per ml (Fig. 3). Immunofluorescence was first detected in the cell cytoplasm on day 2 after infection. The perinuclear area of fluorescence became brighter during the replication cycle (day 3) but even after 5 days was still local rather than diffuse.

The time of p130/p55 synthesis was determined by immunoprecipitation. Cultures were infected at a multiplicity of infection of 0.1 and harvested after radiolabeling daily for 7 days. Both polypeptides were detectable at 2 days after infection (Fig. 4). The incorporation of label was most intense on days 4 and 5. After day 5 the amount of p55 decreased. The intensity of label in the p130 species remained relatively constant between days 4 and 7.

To determine whether the decrease in intracellular p55 and p92 reflected the release of virus from infected cells, extracellular virus was labeled with L-[^{35}S]methionine and immunoprecipitated with 15D8. Both the p55 and p92 were detected on extracellular virus (Fig. 5, lane 4). The thin bands observed at approximately 135,000 and 66,000 Da were not virus specific since they were also observed after immunoprecipitation of extracellular virus with normal mouse serum (Fig. 5, lane 7).

The biosynthetic relationship between the coimmunoprecipitating polypeptides was investigated by pulse-chase analysis. On day 4 after CMV infection, cultures were pulsed with L-[^{35}S]methionine for 15 min, washed copiously with Hanks balanced salt solution, and replenished with minimal essential medium without methionine and containing 1% dialyzed fetal calf serum. Cultures were harvested and immunoprecipitated with 15D8. Polypeptides were then reduced for SDS-PAGE (Fig. 6B). Immediately after pulse, the isotope was detected predominantly in p130. The p55 species was faintly visible by 1 h and was detected maximally between 2 and 4 h. As the label increased in p55, it decreased in p130. The p92 species appeared coincidentally with p55.

SDS-PAGE of p130 and p55 with and without reduction. L-[^{35}S]methionine-labeled extracts of CMV, either as intracellular or extracellular virus, were immunoprecipitated with 15D8 IgG and electrophoresed, both with and without reduction of disulfide bonds (Fig. 5). Lanes 1 and 4 show the typical pattern of immunoprecipitation of reduced intracellular (p130/p55) and extracellular (p55) viral polypeptides. Without reduction, intracellular viral proteins appeared as a broad, high-molecular-weight band of greater than 200,000 Da (data not shown). When gels were run for an additional 1 h after the bromphenol dye front had reached the bottom, two closely migrating bands could be detected (lane 2). It should be noted that the reduced-molecular-weight markers do not accurately reflect the true size of nonreduced samples because of different rates of migration. Hence, when gels were overrun to resolve the nonreduced sample, the markers migrated to a position well below the separated bands. Nonreduced extracellular viral proteins gave only a single high-molecular-weight band even when the gels were overrun (lane 5). This band migrated to the same position as the upper band of the doublet from the intracellular virus.

The contribution of p130 and p55 to the nonreduced high-molecular-weight proteins was investigated in three different experiments. First, the nonreduced high-molecular-weight proteins from both infected cells and extracellular virus shown in Fig. 5 were extracted from the gel in sample buffer containing 2-mercaptoethanol and reanalyzed by SDS-PAGE. Both the gel slice and the sample buffer were loaded for SDS-PAGE under reducing conditions. The doublet from the intracellular extract (Fig. 5, lane 2) yielded both p130 and p55 upon reduction (lane 3). The single band from the extracellular virus, corresponding to the upper band of the doublet (lane 5), yielded only the p55 upon reduction (lane 6). Second, immunoprecipitates of the pulse-labeled infected cultures previously described were electrophoresed under nonreducing conditions (Fig. 6A) but without overrunning. Immediately after the 15-min pulse the lower band of the high-molecular-weight doublet was visible. At 2 h after the pulse, and coincidentally with the detection of p55 under reducing conditions, the upper band of the high-molecular-

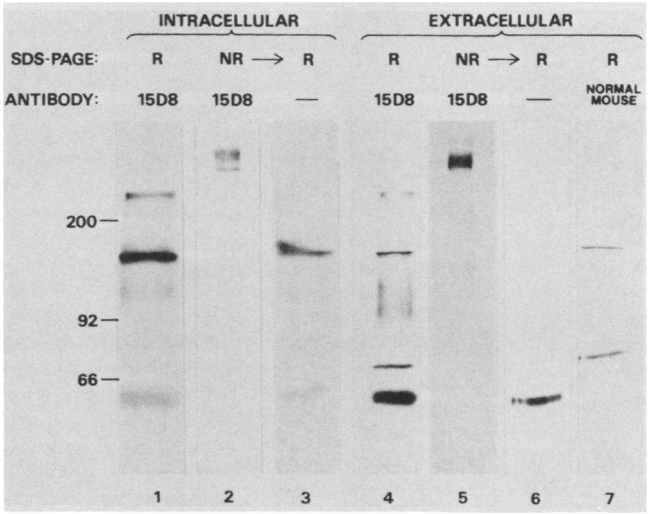

FIG. 5. Immunoprecipitation and SDS-PAGE of intracellular and extracellular CMV polypeptides. L-[^{35}S]methionine extracts were immunoprecipitated with either 15D8 IgG or undiluted normal mouse serum. SDS-PAGE was done under both reducing (R) and nonreducing (NR) conditions. The nonreduced samples were rechromatographed as shown in lanes 3 and 6, respectively, under reducing conditions (NR→R).

weight doublet was detectable. Third, 14E10 IgG, which detects only p130 on reduced clinical strains, was used to immunoprecipitate clinical isolate E (Fig. 7) for SDS-PAGE. Only the lower band of the doublet was detected under nonreducing conditions. The sharply defined upper band represents the gel origin. In contrast, electrophoresis of the immunoprecipitate of 15D8 with clinical isolate E showed both p130 and p55 under reducing conditions, and both bands of the high-molecular-weight doublet were seen in nonreduced samples.

DISCUSSION

Our data show that a murine monoclonal antibody, reactive with two coimmunoprecipitating CMV polypeptides, will neutralize CMV infectivity only in the presence of exogenous guinea pig complement. These findings are consistent with the well-described complement requirement for the neutralization of CMV by some sera (6, 19) but not by others (10). The reason for differences in complement requirement for the neutralization by monoclonal antibodies to different polypeptides is not clear. The complement dependence of 15D8 is in marked contrast to our experience with monoclonal antibody to the 86,000-Da polypeptide, which neutralizes completely and in low (1 μg/ml or lower) concentrations without added complement. Both of these monoclonal antibodies are IgG2a isotypes. It is of interest that the need for complement is also evident in the polyvalent sera produced after immunization of guinea pigs with p130/p55 which had been purified by monoclonal antibody affinity columns (L. Rasmussen, J. Mullenax, R. Nelson, and T. C. Merigan, Virology, in press). Whether the human antibody repertoire for recognition of viral antigenic determinants is similar to that of the mouse is not known. Although the mouse system is useful for initial characterization of potentially important antigenic determinants, the in vivo relevance of antibodies to p86 and p130/p55 in humans must be determined.

The antigenic sites on p130/p55 which are targets for neutralizing antibody could be either sparse or functional only in combination with antibody of sufficiently high affinity. Which of these two factors is most important for neutralization is not clear from our data. There is evidence supporting both possibilities. Our finding that only one of our eight hybridoma clones to the p130/p55 polypeptides secreted neutralizing antibody suggests that there are unique antigenic determinants, perhaps variable among CMV strains, which are involved in neutralization. Specifically, the neutralizing 15D8 reacts by immunoprecipitation with p130/p55 on most clinical and laboratory strains used in this study. Hybridoma 14E10 has no neutralizing activity and probably recognizes epitopes different from those recognized by 15D8, since it failed to detect p55 on clinical strains used in the study and was nonreactive with p130/p55 on the Towne and Davis laboratory strains. Our data also suggest that the nonneutralizing 14E10 has lower affinity for p130/p55 than the neutralizing 15D8 does. The IgG from 15D8 was capable of immunoprecipitating p130/p55 from lysates reacted originally with excess 14E10 antibody, but the IgG from 14E10 was not. Whether high-affinity antibod-

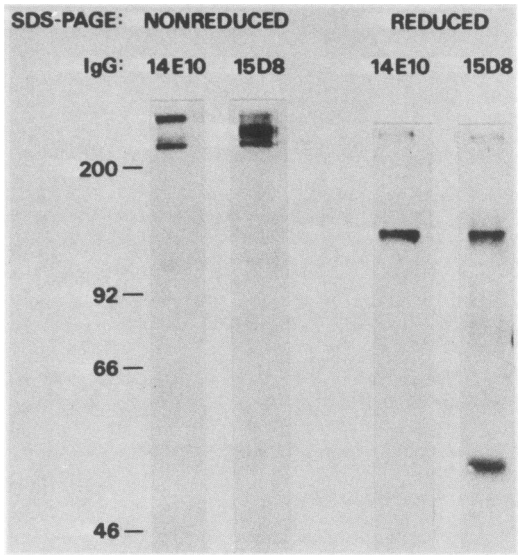

FIG. 7. Intracellular polypeptides of clincal isolate E immunoprecipitated by 5 μg each of 14E10 and 15D8 IgGs and analyzed by SDS-PAGE under nonreducing and reducing conditions.

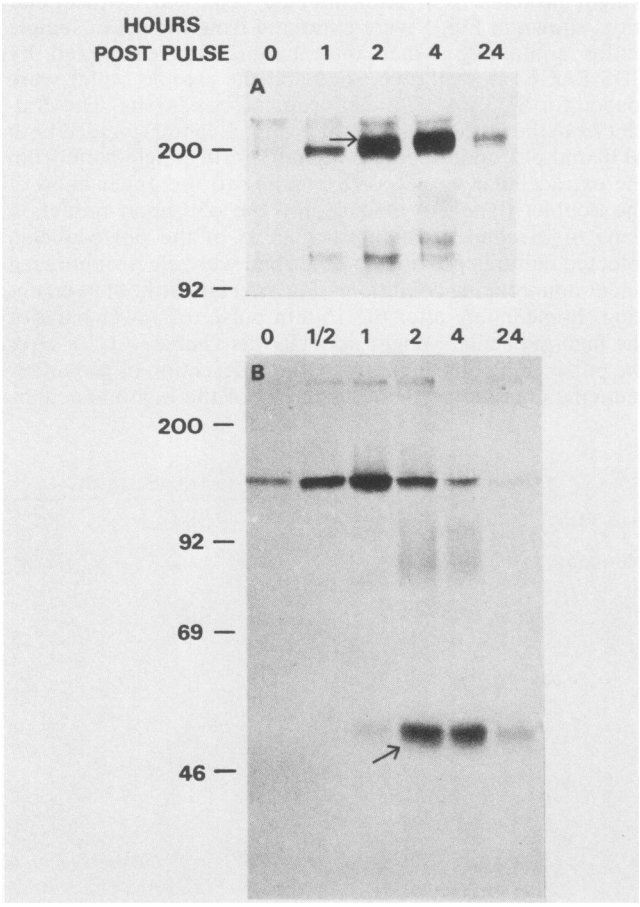

FIG. 6. Pulse-chase labeling of CMV-infected cells. Multiple 4-day-old CMV-infected cultures (approximately 2×10^6 cells) were pulsed for 15 min with 1 mCi of L-[^{35}S]methionine. After copious washing with Hanks balanced salt solution, cultures were harvested at the times shown postpulse, immunoprecipitated with 10 μg of 15D8 IgG, and analyzed by SDS-PAGE without (A) and with (B) reduction of polypeptides. The coincidental appearance of the upper band of the high-molecular-weight doublet detected under nonreducing conditions and p55, detected under reducing conditions, is indicated by the arrows. Molecular weights are 10^3.

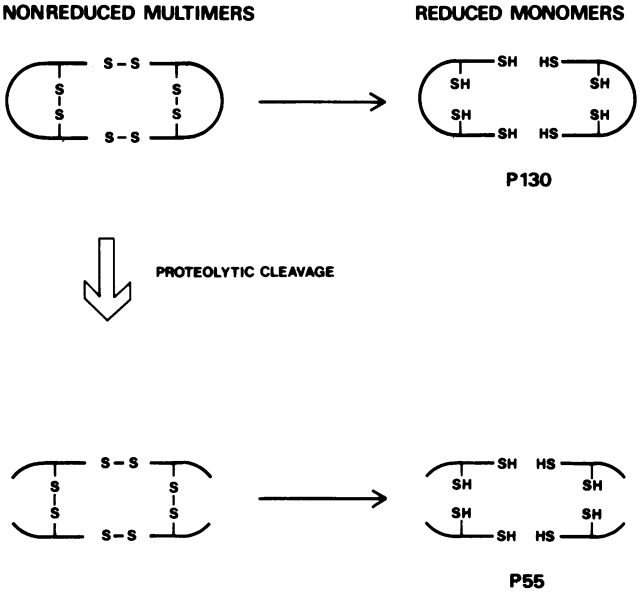

FIG. 8. Proposed structures and cleavage pattern of p130 and p55 in reduced and nonreduced form.

ies to epitopes on the p130/p55 other than those detected by 15D8 would be effective in neutralizing virus is not known.

The presence of nonspecific murine IgG in antibody from ascites fluid could account for its lower neutralizing specificity when compared to tissue culture-derived antibody. The specific activity of IgG from hybridomas to reovirus proteins has also been shown to be greater from tissue cultures than from ascites fluid (8).

It is likely that p130 is the precursor to p55, which is then assembled into mature, enveloped virions. Daily harvests of radiolabeled infected cells show that the synthesis of p130 precedes that of p55. The decrease in label in intracellular p55 in these kinetic studies is accompanied by the detection of p55 in extracellular fluids. By pulse-chase analysis of CMV-infected cells, radiolabel is incorporated first into p130 and can be chased into p92 and p55 over a 4-h period. The relationship of p92 to p130 and p55 is unclear. The p92 appears to be a form of p55 for the following reasons. First, in kinetic studies it was detected when the p55 was most abundant (Fig. 4 and 6). Second, it was not immunoprecipitated by 14E10, which detects only the p130 on clinical isolates, but it was visible in immunoprecipitates with 15D8 which reacts with both p130 and p55 (Fig. 1). Third, the p92 was detectable in preparations of extracellular virus which contained no detectable radiolabeled p130 (Fig. 5). Definitive data on relationships between these polypeptides are currently being sought in our laboratory by peptide mapping. Product-precursor relationships among herpesviruses have been investigated most thoroughly with herpes simplex virus (17) where nonglycosylated precursor polypeptides give rise to fully glycosylated envelope glycoproteins. Recently a similar scheme has been proposed for CMV glycosylated polypeptides (13). Whether the p92 is a glycosylated intermediate in the biosynthesis of p130/p55 or an aggregate, perhaps heavily glycosylated, of p55 remains to be elucidated.

The high-molecular-weight forms of the CMV polypeptides detected by 15D8 under nonreducing conditions are two closely migrating bands of greater than 200,000 Da. The upper band of the high-molecular-weight doublet consists of multimers of p55 self-linked by disulfide bonds, while multimers of p130, similarly linked, are confined to the lower band. The evidence for this is as follows. First, in pulse-chase experiments the diffuse upper band of the high-molecular-weight doublet appeared simultaneously with p55, detectable when polypeptides were reduced. Second, the high-molecular-weight doublet from intracellular extracts could be extracted from the gel and subsequently reduced to yield both p130 and p55. When the extracellular nonreduced polypeptide (the upper band of the doublet) was extracted and reduced, only p55 was seen. Third, 14E10 detected p130 in clinical strains under reducing conditions and only detected the lower band of the doublet in nonreduced samples. It is unlikely that the high-molecular-weight polypeptides seen without sample reduction represent artifacts since they were still observed when 1 mM dithiothreitol was included in cell extracts to prevent the formation of nonspecific aggregates (data not shown). Disulfide-bond-linked glycoproteins on the virion envelope have been reported in other herpesviruses, e.g., herpes simplex virus (4, 22), varicella-zoster virus (7), pseudorabies virus (H. Hampl and T. Ben Porat, Abstr. 6th Cold Spring Harbor Meet. Herpesviruses, p. 67, 1982), and CMV (1). Whether this type of disulfide bond linkage is relevant to the biological activity of the polypeptide, as is true for some viruses, is not known. With enveloped RNA viruses, disulfide bonds have been shown to be important for maintaining conformational determinants necessary for immunogenicity (2). Also, cleavage of the disulfide bonds of the F protein on the virion envelope of paramyxoviruses is necessary for infectivity (18).

A model of processing of p130 and p55 which accounts for all major species seen with both reducing and nonreducing SDS-PAGE is presented in Fig. 8. The precursor p130 multimer is linked by both interchain and intrachain disulfide bonds. Proteolytic cleavage of p130 at a site not involving the intrachain bonds would yield four disulfide-bond-linked p55 monomers. Reduction of the uncleaved precursor would produce monomers of p130. Monomers of p55 would be formed when the disulfide bonds of the cleaved product were reduced. The slightly higher molecular weight of the p55 nonreduced multimer as compared with the p130 nonreduced multimer could be the result of glycosylation on the p55 multimer.

The coimmunoprecipitating polypeptides described in our study are similar in size to those reported by other groups on both intracellular (9) and extracellular (1) viruses. However, neither we nor Nowak et al. (11) were able to demonstrate the presence of the precursor p130 on extracellular virions. The basis of these differences is not clear but will probably be resolved by further analysis of the epitope spectra of these related CMV polypeptides.

ACKNOWLEDGMENTS

We gratefully acknowledge the help of Pat Chen in performing the virus neutralization assays, the continued assistance of Margaret Nelson, and the expert secretarial assistance of Lucile Lopez.

This study was supported by Public Health Service grant AI-05629 from the National Institutes of Health.

LITERATURE CITED

1. **Britt, W.** 1984. Neutralizing antibodies detect a disulfide-linked glycoprotein complex within the envelope of human cytomegalovirus. Virology **135**:369–378.
2. **Cloyd, M. W., B. Chesebro, J. Portis, and M. Weir.** 1982. MCF-specific murine monoclonal antibodies made against

AKR-247 MCF virus recognize a unique determinant associated with gp70-p15(E) complex. J. Virol. **41**:1112–1117.
3. **Condie, R., and R. O'Reilly.** 1984. Prevention of cytomegalovirus infection by prophylaxis with an intravenous, hyperimmune, native, unmodified cytomegalovirus globulin: randomized trial in bone marrow transplant recipients. Am. J. Med. **30**:134–141.
4. **Eberle, R., and R. Courtney.** 1982. Multimeric forms of herpes simplex virus type 2 glycoproteins. J. Virol. **41**:348–351.
5. **Gibson, W.** 1981. Structural and non-structural proteins of strain Colburn cytomegalovirus. Virology **111**:515–537.
6. **Graham, B., Y. Minamishima, G. Dreesman, H. Haines, and M. Benyesh-Melnick.** 1971. Complement-requiring neutralizing antibodies in hyperimmune sera to human cytomegalovirus. J. Immunol. **107**:1618–1630.
7. **Grose, C., D. Edwards, K. Weigle, W. Friedrichs, and W. McGuire.** 1984. Varicella-zoster virus-specific gp140: a highly immunogenic and disulfide-linked structural glycoprotein. Virology **132**:138–146.
8. **Lee, P., R. Hayes, and W. Joklik.** 1981. Characterization of antireovirus immunoglobulins secreted by cloned hybridoma cell lines. Virology **108**:134–146.
9. **Meyers, M., J. Leszczynski, J. Zaia, M. Flournoy, B. Newton, D. Snydman, G. Wright, M. Levin, and E. Thomas.** 1983. Successful prevention of cytomegalovirus infection after marrow transplantation with cytomegalovirus immune globulin. Ann. Intern. Med. **98**:442–446.
10. **Mocarski, E., and M. Stinski.** 1979. Persistence of the cytomegalovirus genome in human cells. J. Virol. **31**:761–775.
11. **Nowak, B., C. Sullivan, P. Sarnow, R. Thomas, F. Bricout, J. Nicolas, B. Fleckenstein, and A. Levine.** 1984. Characterization of monoclonal antibodies and polyclonal immune sera directed against human cytomegalovirus virion proteins. Virology **132**:325–338.
12. **Pereira, L., M. Hoffman, D. Gallo, and N. Cremer.** 1982. Monoclonal antibodies to human cytomegalovirus: three surface membrane proteins with unique immunological and electrophoretic properties specify cross-reactive determinants. Infect. Immun. **6**:924–932.
13. **Pereira, L., M. Hoffman, M. Tatsuno, and D. Dondero.** 1984. Polymorphism of human cytomegalovirus glycoproteins characterized by monoclonal antibodies. Virology **139**:73–86.
14. **Quinnan, G., M. Delery, A. Rook, W. Frederick, J. Epstein, J. Manischewitz, L. Jackson, K. Ramsey, K. Mittal, S. Plotkin, and M. Hilleman.** 1984. Comparative virulence and immunogenicity of the Towne strain and a nonattenuated strain of cytomegalovirus. Ann. Intern. Med. **101**:478–483.
15. **Rasmussen, L., D. Kelsall, R. Nelson, W. Carney, M. Hirsch, D. Winston, J. Preiksaitis, and T. Merigan.** 1982. Virus-specific IgG and IgM antibodies in normal and immunocompromised subjects infected with cytomegalovirus. J. Infect. Dis. **145**:191–199.
16. **Rasmussen, L., R. Nelson, D. Kelsall, and T. Merigan.** 1984. Murine monoclonal antibody to a single protein neutralizes the infectivity of human cytomegalovirus. Proc. Natl. Acad. Sci. U.S.A. **81**:876–880.
17. **Sarmiento, M., and P. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. IV. Conformation of the virion glycoprotein designated VP7(B_2). J. Virol. **29**:1159–1167.
18. **Scheid, A., and P. Choppin.** 1977. Two disulfide-linked polypeptide chains constitute the active F protein of paramyxoviruses. Virology **80**:54–66.
19. **Waner, J., and T. Weller.** 1978. Analysis of antigenic diversity among human cytomegaloviruses by kinetic neutralization tests with high-titered rabbit antisera. Infect. Immun. **21**:151–157.
20. **Winston, D. J., R. B. Pollard, W. G. Ho, J. G. Gallagher, L. E. Rasmussen, S. N. Huang, C. H. Lin, T. G. Gossett, T. C. Merigan, and R. P. Gale.** 1982. Cytomegalovirus immune plasma in bone marrow transplant recipients. Ann. Intern. Med. **97**:11–17.
21. **Zaia, J., B. Clark, Y. Ting, R. Vanderwal-Urbina, L. Troianello, L. Balce-Directo, and R. Eberle.** 1984. Production, detection and characterization of antibody to 64-66,000 dalton glycoprotein of HCMV. Birth Defects Orig. Artic. Ser. **20**:498–499.
22. **Zweig, M., C. Heilman, Jr., H. Rabin, and B. Hampar.** 1980. Shared antigenic determinants between two distinct classes of proteins in cells infected with herpes simplex virus. J. Virol. **35**:644–652.