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Cytomegalovirus-Infected Cell Polypeptides Immune-Precipitated by Sera from Children with Congenital and Perinatal Infections

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Congenital or perinatally acquired human cytomegalovirus (CMV) infections in children may be symptomatic or asymptomatic. In this study, we characterized the electrophoretic properties of CMV-infected cell polypeptides immune-precipitated by sera from children with different types of CMV infections from birth to 4 years of age. Sodium dodecyl sulfate-polyacrylamide gel analysis of immune precipitates formed with radiolabeled extracts of cells infected with CMV strain AD169 showed the following. (i) Electrophoretic profiles of CMV polypeptides immune-precipitated by sera from children with perinatal and congenital infections were similar. At least 11 polypeptides with apparent molecular weights of 150,000, 140,000, 110,000, 100,000, 74,000, 66,000, 50,000, 49,000, 34,000, 25,000, and 20,000 were precipitated. Antibody titer in anticomplement immunofluorescence tests and virus titer in urine correlated with the intensity of polypeptide profiles in autoradiograms. (ii) The initial immune response of children with symptomatic congenital infections was delayed as compared to that of children with asymptomatic congenital and perinatal CMV infections. Sera obtained serially from symptomatic children for years after birth continued to precipitate CMV polypeptides, whereas sera from children with subclinical congenital infections precipitated lesser amounts over time. (iii) Immune precipitates obtained with sera from CMV-infected patients and with monoclonal antibodies to CMV contained polypeptides with comparable electrophoretic and immunological properties.

Cytomegalovirus (CMV) infection is the leading cause of congenital viral infections, with an incidence averaging 1% of all live births (5, 13). An additional 5 to 10% of infants acquire CMV in the perinatal period as a result of mother-to-infant transmission. The majority of infants with congenital and perinatal CMV infections are asymptomatic, but some may display signs of either overt or mild disease at the outset. Late-appearing sequelae may occur in as many as 10 to 15% of infants with congenital CMV (5, 10, 12, 13). Studies have shown that transmission in utero can occur in women with high titers of neutralizing antibody to CMV and that strains with homologous antigenic and genetic properties can be transmitted to the fetus (13). Congenital CMV infections may be associated with both primary or recurrent maternal infection, but symptomatic cases are more likely to be the result of primary infection (11). Persistent virus replication occurs in children with CMV infections acquired early in life, as evidenced by excretion of virus in urine and saliva (15). Congenital CMV infection is also characterized by accelerated postnatal development of serum immunoglobulin G and M and circulating immune complexes (10, 13). Congenitally infected children have diminished or absent cell-mediated immunity to CMV antigens (6).

CMV glycoproteins, which contain the major antigenic determinants of the virus, are inserted into the membranes of infected cells and the virion envelope (18, 19). Immune-precipitation studies on sera with complement-fixing activity against CMV antigens have shown that late CMV-infected cell polypeptides, in particular the viral glycoproteins, are highly immunogenic in the human host (7). Studies with monoclonal antibodies to CMV identified three groups of glycosylated polypeptides with different antigenic and electrophoretic properties (8). The glycoproteins were located on the surface membranes of CMV-infected cells and contained neutralizing sites.

In the present study, we characterized the electrophoretic and immunological properties of

polypeptides precipitated from extracts of CMV-infected cells by sera from children with CMV infections. Analysis of sera obtained serially from children with symptomatic congenital CMV infections showed that they precipitated viral polypeptides for more prolonged periods of time than sera from children with asymptomatic congenital or perinatally acquired CMV infections. Groups of antigenically related polypeptides in immune precipitates formed by patient sera were identified by comparison with immune precipitates formed by monoclonal antibodies to CMV.

MATERIALS AND METHODS

Cells and virus. Human fetal diploid lung cells were grown in fortified Eagle minimal essential medium containing twice the standard concentration of vitamins and amino acids, supplemented with 10% fetal bovine serum. The isolation and properties of CMV strain AD169 have been published (1).

Monoclonal antibodies to CMV. Monoclonal antibodies CH13, CH19, CH23, CH41, CH51, and CH65 were produced against CMV strain AD169. Selection and properties of monoclonal antibodies to CMV were as described previously (8). Hybridomas were propagated in mice to produce ascites fluids of high antibody titer.

Patient population. The subjects studied included 12 children with congenital CMV infection, 6 with perinatal CMV infection, and 6 uninfected controls. Six of the congenitally infected infants were symptomatic at birth, their main clinical manifestations being hepatosplenomegaly, jaundice, and thrombocytopenia. Four subjects have developed major sequelae characterized by severe psychomotor retardation and sensorineural hearing loss. All patients have been prospectively studied at the Perinatal Infections Clinic, University of Alabama in Birmingham.

Sera. At least five serum specimens were collected from each subject between 2 months and 4 years of age. All sera tested had been stored at −20°C for varying periods.

Virology. For isolation of CMV, urine specimens were processed and examined as previously described (9). Specimens were obtained from all subjects within 1 week of birth and at preselected intervals afterwards. Titration of CMV in urine was performed with serial 10-fold dilutions, and endpoints were calculated as the 50% tissue culture infective dose by the Reed-Muench formula (9).

Serology. CMV strain AD169 was used as the source of antigen for all serological assays. Serum antibody titers to CMV were determined by using anticomplement immunofluorescence (ACIF) tests (4).

Preparation of radiolabeled antigens. Human fetal diploid lung cells were infected with 5 PFU of CMV strain AD169 per cell. Infected cells were radiolabeled from 72 to 96 h postinfection. Cultures were replenished with medium containing 1/10 the normal amount of methionine, but supplemented with 50 μCi of [^{35}S]methionine per ml (specific activity, 1,200 mCi/mmol; purchased from New England Nuclear Corp., Boston, Mass.). Infected cells were harvested, extracted with 1% Nonidet P-40 and 1% sodium deoxycholate (Sigma Chemical Co., St. Louis, Mo.), and centrifuged at 24,000 rpm in an SW27.1 rotor for 1 h at 4°C to remove insoluble proteins. Uninfected human fetal diploid lung cells were radiolabeled, and extracts were prepared as control samples.

Immune-precipitation tests. Radiolabeled extracts of CMV-infected cells (10 to 20 μl) were mixed with 100 μl of serum and incubated for 1 h at 37°C. Protein A-Sepharose (Sigma) was added to precipitate the immune complexes, followed by repeated washing with phosphate-buffered saline containing 0.1% Nonidet P-40 and 0.1% sodium deoxycholate. Immune-precipitation reactions with monoclonal antibodies were modified as follows. Mouse ascites fluids (1 to 50 μl) were mixed with radiolabeled extracts of CMV-infected cells and incubated for 1 h with rabbit anti-mouse immunoglobulin G (Miles Laboratories, Elkhart, Ind.).

Preparation of samples for polyacrylamide gel electrophoresis. Immune precipitates were denatured and solubilized by heating at 80°C in the presence of sodium dodecyl sulfate and β-mercaptoethanol. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was done in a discontinuous buffer system containing 0.1% sodium dodecyl sulfate. Separation and stacking gels contained 9.25% and 3% acrylamide, respectively, cross-linked with *N,N'*-diallyltartardiamide.

RESULTS

Polypeptides immune-precipitated by sera from CMV-infected children. The study population comprised 12 children with congenital CMV infections (6 symptomatic and 6 asymptomatic), 6 children with perinatally acquired CMV infections, and 6 uninfected controls. Clinical diagnosis, virus isolation from urine, and results of ACIF tests of selected patients are summarized in Table 1. All CMV-infected children had high antibody titers in ACIF tests and shed CMV in urine, whereas controls were negative by ACIF tests and virus isolation.

Two series of experiments were done to characterize the electrophoretic mobility of CMV-infected cell polypeptides immune-precipitated by sera from children with congenital and perinatally acquired CMV infections. In the first series of experiments, selected sera were reacted with radiolabeled extracts of uninfected cells and CMV-infected cells to identify the virus-specific polypeptides immune-precipitated. Sera 5 and 13, obtained from patients with congenital and perinatal CMV infections, respectively, precipitated at least 10 polypeptides from extracts of cells that had been infected with CMV strain AD169 and radiolabeled from 4 to 5 days postinfection (Fig. 1). Polypeptides with apparent molecular weights of 150,000, 140,000, 110,000, 100,000, 66,000, 50,000, 49,000, 25,000, and 20,000 were precipitated. The sera failed to react with polypeptides in radiolabeled extracts of uninfected human fetal diploid lung cells.

In the second series of immune-precipitation

tests, we characterized the polypeptides immune-precipitated by sera obtained between 12 and 15 months of age from four children with congenital symptomatic CMV infection, three children with congenital asymptomatic infection, and three children with perinatal CMV infection (Fig. 2). Sera from patients with congenital symptomatic infections (no. 1, 2, 3, and 4) immune-precipitated large quantities of polypeptides with apparent molecular weights of 150,000, 140,000, 110,000, 100,000, 74,000, 66,000, 50,000, 49,000, 34,000, 25,000, and 20,000. These polypeptides were also identified as the major antigens precipitated by convalescent-phase sera of adult patients from extracts of CMV-infected cells at 96 h postinfection (7). As shown previously, three polypeptides with apparent molecular weights of 140,000, 66,000, and 49,000, and faster-migrating forms antigenically related to them, comigrated with glycoprotein

TABLE 1. Children studied

Subject no.	Diagnosis	Age (mo)	ACIF titer	Virus titer (log)	Subject no.	Diagnosis	Age (mo)	ACIF titer	Virus titer (log)
1	Symptomatic, congenital	4	256	5.5	9	Perinatal	5	64	+
		7	256	4.7			10	16	4.3
		18	ND[a]	2.5			12	64	2.5
		27	64	0			16	16	0.5
		33	64	+			33	8	−
2	Symptomatic, congenital	2	256	4.5	10	Perinatal	3	64	4.8
		4	256	+			5	256	4.3
		8	ND	4.5			9	16	2.7
		20	ND	1.5			15	16	3.3
		47	16	ND			24	16	2.3
							48	8	−
3	Symptomatic, congenital	2	256	3.3					
		4	256	4.5	11	Perinatal	3	16	5.3
		12	ND	5.5			7	64	3.5
		23	64	+			13	64	+
							24	16	2.5
4	Symptomatic, congenital	2	256	+			49	64	+
		6	64	+					
		9	64	+	12	Perinatal	3	16	4.5
		13	256	ND			6	16	2.8
							9	64	3.5
5	Asymptomatic, congenital	2	16	5.3			12	16	3.7
		5	256	4.5			16	ND	3.5
		14	64	2.3			24	ND	−
		24	ND	0.3					
		48	64	−	13	Perinatal	1	64	+
							9	16	+
6	Asymptomatic, congenital	1	256	>5.5			12	ND	+
		4	ND	4.7			30	8	2.0
		6	256	ND			49	ND	1.5
		18	16	ND					
		30	64	−	14	Uninfected control	1	16	−
		42	16	−			6	−	−
							12	−	−
7	Asymptomatic, congenital	6	16	4.3			27	−	−
		12	8	+					
		18	16	ND	15	Uninfected control	2	−	−
		26	16	+			6	−	−
		30	−	ND			34	−	−
8	Asymptomatic, congenital	1	64	+					
		6	16	+					
		9	16	3.5					
		15	−	3.8					
		20	−	3.8					
		39	−	2.7					

[a] ND, Not determined.

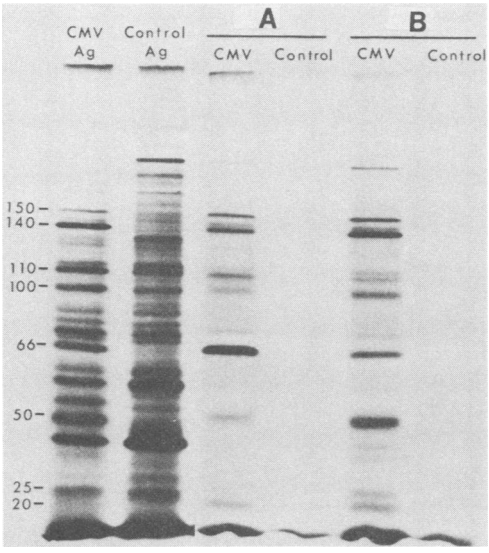

FIG. 1. Electrophoretic profiles of [^{35}S]methionine-labeled polypeptides in extracts of CMV-infected and uninfected cells (control) and immune precipitates. Electrophoretically separated polypeptides in immune precipitates formed by sera from (A) patient no. 5 (asymptomatic congenital) and (B) patient no. 13 (perinatal) with radiolabeled extracts of infected and control cells. Numbers at left give apparent molecular weight (10^3).

bands in CMV-infected cells (7, 8). Sera from asymptomatic, congenitally infected children (no. 6, 7, and 8) and from perinatally infected children (no. 9, 10, and 12) precipitated smaller amounts of polypeptides than did sera of symptomatic children. Polypeptides with apparent molecular weights of 74,000, 49,000, 34,000, and 25,000 were not detected or were present in trace amounts in immune precipitates formed by sera from children with asymptomatic congenital and perinatal CMV infections. Sera from perinatally infected children also failed to precipitate an infected-cell polypeptide with apparent molecular weight of 110,000. Results of ACIF tests showed that the sera contained antibody reactive with CMV-infected cells and were consistent with the results of immune-precipitation tests (Table 1).

Electrophoretic patterns of polypeptides immune-precipitated by sera obtained serially from children with congenital and perinatal CMV infection. Qualitative and quantitative differences in polypeptide profiles obtained with single sera became more pronounced when sequential specimens were studied. In this series of experiments, we characterized the polypeptides immune-precipitated from extracts of [^{35}S]methionine-labeled, CMV-infected cells by sera from children with congenital and perinatal CMV infections over time (Fig. 3 through 6). Immune precipitates obtained with sequential sera from congenital, asymptomatic patient no. 5, sera from patient no. 13 (perinatal CMV infection), and sera from uninfected control subjects no. 14 and 15 are shown in Fig. 3. Differences in the infected-cell polypeptides immune-precipitated by sequential serum specimens were noted. For sera from patient no. 5, the profile of CMV polypeptides precipitated at 2 months, most

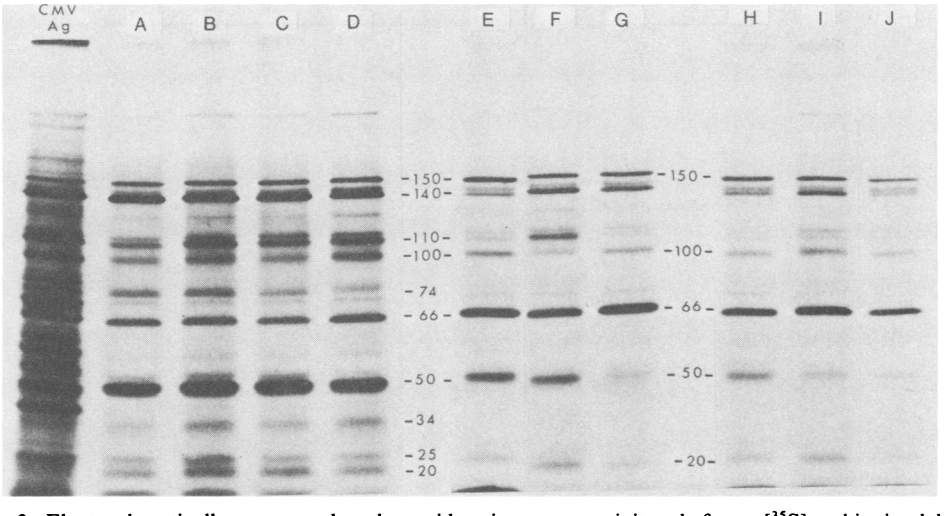

FIG. 2. Electrophoretically separated polypeptides immune-precipitated from [^{35}S]methionine-labeled, CMV-infected cell extracts. Sera are from children with congenital symptomatic (A through D), congenital asymptomatic (E, F, and G), and perinatal (H, I, and J) CMV infections. Numbers give apparent molecular weight (10^3). Patients: (A) 1, (B) 2, (C) 3, (D) 4, (E) 6, (F) 7, (G) 8, (H) 10, (I) 9, (J) 12.

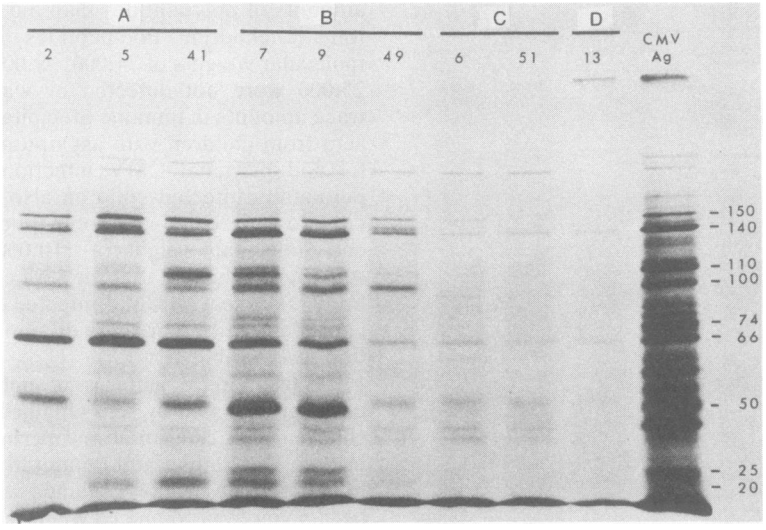

FIG. 3. Electrophoretically separated polypeptides immune-precipitated from [^{35}S]methionine-labeled extracts of CMV-infected cells by sera from (A) patient no. 5 (asymptomatic congenital), (B) patient no. 13 (perinatal), and (C and D) uninfected controls (no. 14 and 15, respectively). Numbers above profiles designate age in months when sera were obtained. Numbers at right give apparent molecular weight (10^3).

likely formed by maternal antibody transmitted before birth, contained a polypeptide with apparent molecular weight of 66,000 and trace amounts of others. Sera taken at 5 months showed an immune response to CMV infection and precipitated polypeptides with apparent molecular weights of 150,000, 140,000, and 66,000; however, polypeptide bands of 110,000, 74,000, 49,000, and 25,000 apparent molecular weight were not precipitated. At 41 months, the 110,000-apparent-molecular-weight polypeptide was detected. ACIF antibody titer correlated with results of immune-precipitation tests for this subject. Virus titer in urine declined during this period (Table 1). At 7 and 9 months, sera from patient no. 13 immune-precipitated a spectrum of CMV late polypeptides. Polypeptide bands precipitated in large quantities had appar-

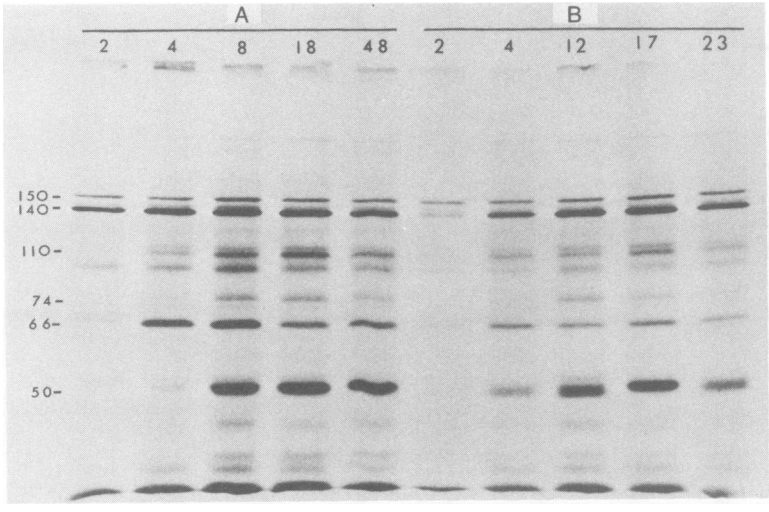

FIG. 4. Electrophoretically separated polypeptides immune-precipitated from [^{35}S]methionine-labeled extracts of CMV-infected cells by sera from patients (A) no. 2 and (B) no. 3 with symptomatic congenital CMV infections. Numbers above profiles designate age in months when sera were obtained. Numbers at left give apparent molecular weight (10^3).

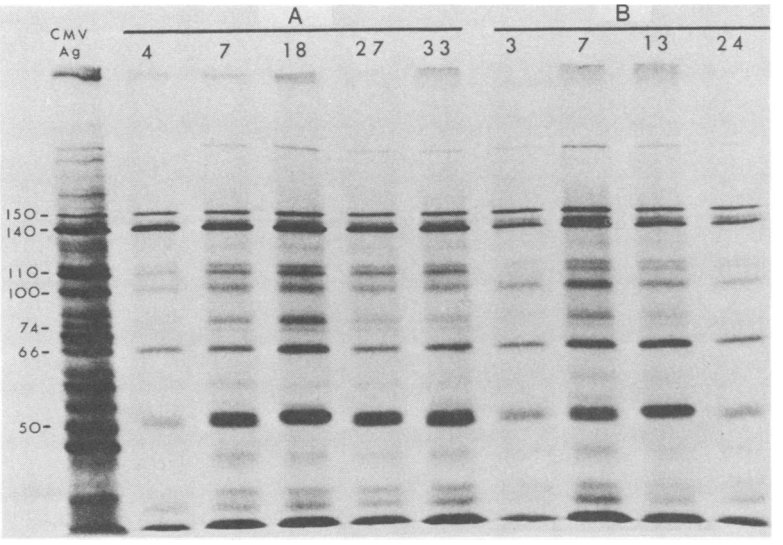

FIG. 5. Electrophoretically separated polypeptides immune-precipitated from [^{35}S]methionine-labeled extracts of CMV-infected cells by sera from patients (A) 1 and (B) 11 with congenital symptomatic and perinatal CMV infections, respectively. Numbers above profiles designate age in months when sera were obtained. Numbers at left give apparent molecular weight (10^3).

ent molecular weights of 140,000, 66,000, and 49,000. Serum obtained at 49 months precipitated only traces of CMV polypeptides with apparent molecular weights of 140,000 and 100,000. These results corresponded with ACIF tests and low virus titer in urine (Table 1). Sera from two uninfected controls (no. 14 and 15) failed to immune-precipitate CMV polypeptides, although the background varied in some experiments. All control sera gave similar results and were negative for antibody to CMV by ACIF tests (Table 1). No infectious virus was isolated from urine of control subjects.

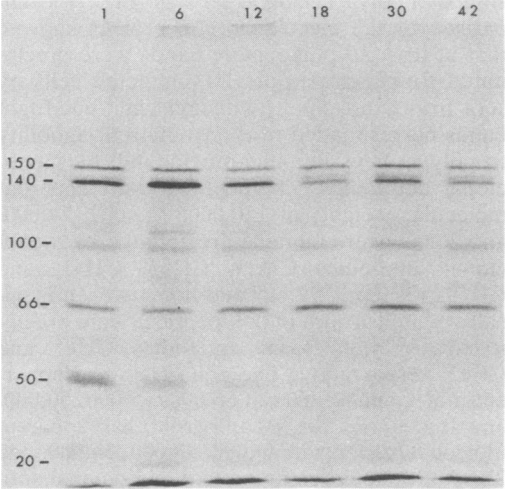

FIG. 6. Electrophoretically separated polypeptides immune-precipitated from [^{35}S]methionine-labeled extracts of CMV-infected cells by sera from patient no. 6 with asymptomatic congenital CMV infection. Numbers above profiles designate age in months when sera were obtained. Numbers at left give apparent molecular weight (10^3).

To further characterize the temporal appearance of antibody to CMV-infected cell polypeptides, we tested sera obtained serially from patients no. 1, 2, and 3, with symptomatic, congenital CMV infections, and sera from patient no. 11, who had perinatal CMV infection (Fig. 4 and 5). Electrophoretic analysis of precipitates with serially obtained sera from patient no. 2 revealed a strong antibody response beginning at 8 months and continuing to 48 months after birth (Fig. 4). Immune precipitates obtained with sera from two other symptomatic patients showed that precipitating antibody to CMV was present in high titers from 12 to 23 months and 7 to 33 months after birth (Fig. 4 and 5). Infected-cell polypeptides with apparent molecular weights of 150,000, 140,000, 110,000, 100,000, 74,000, 66,000, 50,000, 49,000, 34,000, 25,000, and 20,000 were contained in immune precipitates. Sera from two of the other three symptomatic, congenitally infected patients had similar polypeptide profiles (data not shown). Polypeptide profiles obtained with sera from perinatally infected subject no. 11 were similar to those obtained from congenitally infected children (Fig. 5). A strong precipitating antibody response to CMV polypeptides was found at 7

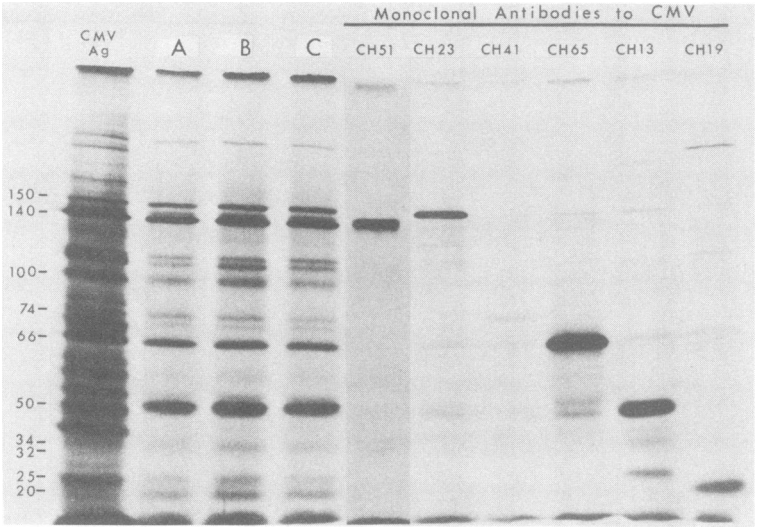

FIG. 7. Electrophoretically separated polypeptides immune-precipitated from [^{35}S]methionine-labeled extracts of CMV-infected cells by sera from congenital symptomatic CMV-infected children (A) no. 1, (B) no. 2, and (C) no. 3 and by monoclonal antibodies to CMV. Numbers at left give apparent molecular weight (10^3).

and 13 months, but it had declined appreciably at 24 months. Antibody was also detected by ACIF tests, and virus was isolated from urine (Table 1). This pattern was characteristic of five of six patients with perinatal CMV infection.

Figure 6 illustrates immune-precipitation tests with sera obtained serially from a patient (no. 6) with asymptomatic congenital CMV infection. Analysis of immune precipitates showed that CMV polypeptides were present in moderate amounts in tests with sera taken at 1 and 6 months after birth. Virus was isolated from urine at these times (Table 1). Sera taken at 12, 18, 30, and 42 months precipitated only trace amounts of CMV polypeptides. Antibody was detected by ACIF tests; however, no virus was isolated from urine (Table 1). Similar polypeptide profiles were obtained with sera from three of five patients with congenital, asymptomatic infections. Sera from another patient (no. 8) at 6 and 9 months gave similar polypeptide profiles, but sera taken at 30 and 42 months did not precipitate any detectable CMV polypeptides (data not shown). Antibody titer was negative by ACIF; however, virus was isolated from urine at a relatively high titer (Table 1).

Comparison of polypeptides immune-precipitated by sera from CMV-infected children and monoclonal antibodies to CMV. In this series of experiments, we analyzed the polypeptides in immune precipitates formed by monoclonal antibodies to CMV and sera from children (no. 1, 2, and 3) with symptomatic, congenital CMV infections. Monoclonal antibodies CH51, CH23, CH41, CH65, CH13, and CH19 were chosen as representative clones which react with six antigenically and electrophoretically distinct groups of CMV polypeptides (8). Antibodies CH51, CH65, and CH13 reacted with polypeptides which comigrated with viral glycoproteins. Immune precipitates obtained by reacting [^{35}S]methionine-labeled extracts of CMV-infected cells with patient sera and with monoclonal antibodies were electrophoresed in adjacent slots of a polyacrylamide gel slab (Fig. 7). Comparison of the electrophoretic profiles showed that at least 10 polypeptide bands were precipitated from extracts of CMV-infected cells by sera from congenitally infected children. Eight bands corresponded in electrophoretic mobility to polypeptides immune-precipitated by monoclonal antibodies. Polypeptides with apparent molecular weights of 150,000, 140,000, 74,000, and 20,000 were immune-precipitated by monoclonal antibodies CH23, CH51, CH41, and CH19, respectively. In addition, two antigenically related groups of polypeptides were precipitated by monoclonal antibodies CH65 and CH13, respectively. The first group had apparent molecular weights of 66,000, 55,000, 50,000, and 46,000, and the second group had apparent molecular weights of 49,000, 48,000, 34,000, and 25,000. It should be noted that monoclonal antibody CH51 precipitated four antigenically related glycoproteins with apparent molecular weights of 140,000, 110,000, 100,000, and 60,000. The faster-migrating bands do not incorporate large amounts of [^{35}S]methionine, and only the major band of 140,000 apparent molecular weight was detected in this autoradiogram.

DISCUSSION

CMV-infected cell polypeptides immune-precipitated by sera from children with CMV infections. Polyacrylamide gel analysis of immune precipitates formed by sera from children with CMV infections showed that at least 10 electrophoretically distinct polypeptides were precipitated specifically from extracts of CMV-infected cells but not from uninfected-cell extracts. Polypeptide profiles were comparable to those reported in an earlier study in which we characterized CMV polypeptides immune-precipitated by convalescent sera (7). The bulk of the immunogenic polypeptides in extracts of cells prepared with glycine buffer at late times after infection were structural proteins and glycoproteins. In the present study, CMV-infected cells were extracted with nonionic detergents. Under these conditions the extracts were enriched for soluble proteins and glycoproteins; however, many insoluble proteins may have been removed from the antigen preparations. Data obtained in both of these studies probably represent a minimum estimate for the number of CMV polypeptides that elicit an antibody response in the host.

Sera from infected children immune-precipitated the viral glycoproteins from CMV-infected cell extracts. As indicated by high titers of infectious virus recovered from urine, CMV was actively replicating at the time sera were obtained. The structural proteins, in particular glycoproteins in the membranes of CMV-infected cells, induced a strong antibody response during infection. In a previous study on monoclonal antibodies to CMV, we showed that three groups of glycoproteins with different antigenic and electrophoretic properties were immune-precipitated by monoclonal antibodies CH51, CH65, and CH13 (8). Glycoproteins with apparent molecular weights of 140,000 and 66,000 contained neutralizing sites. In the present study, we found that three major glycoprotein bands with apparent molecular weights of 140,000, 66,000, and 49,000 were contained in immune precipitates formed by sera from CMV-infected children. It is likely, therefore, that a large proportion of antibodies detected by ACIF, complement fixation, and neutralization tests reacted with the viral glycoproteins.

Immune reactivity of serial serum samples from children with symptomatic, congenital and asymptomatic CMV infections. Immune precipitates formed by sera taken at approximately the same age from patients with different types of CMV infections showed quantitative and qualitative differences in the polypeptide profiles (Fig. 2). Sera from symptomatic, infected children precipitated a greater number of polypeptides, and in larger quantities, than asymptomatic, congenital and perinatally infected patients. Differences observed between the groups were more pronounced upon analysis of serial serum samples. Two differences were noted. First, the appearance of CMV polypeptides in immune precipitates obtained with sera from symptomatic children with congenital CMV infections was delayed, in some cases for as long as 12 months after birth. As compared to profiles with sera from asymptomatic, congenital or perinatally acquired CMV infections, viral polypeptides were immune-precipitated over longer periods of time and in larger amounts by sera from children with symptomatic, congenital CMV infections. Second, immune precipitates obtained with sera from symptomatic, congenitally infected children contained large amounts of the 49,000-apparent-molecular-weight polypeptide and three faster-migrating polypeptide bands with apparent molecular weights of 48,000, 34,000, and 25,000 which are antigenically related to it. These polypeptides continued to be precipitated in large amounts by later serum specimens from most of the children with congenital, symptomatic CMV infections. Sera taken at comparable ages from most of the asymptomatic children failed to react with this group of polypeptides.

The severity of clinical symptoms in CMV-infected children may result from both virus replication and the host immune response. It was reported earlier that circulating immune complexes in children with symptomatic, congenital CMV infections were heavier, and deposits of immune complexes were found along the basal membrane of glomeruli (16). Our results showed that children with severe congenital infections continue to produce antibody reactive with CMV polypeptides synthesized during virus replication. Deposition of these immune complexes may cause tissue injury. Resolution of CMV infection and disappearance of virus from urine may occur more readily in children with an early immune response to CMV proteins, as indicated by analysis of polypeptides immune-precipitated by sera from children with congenital, asymptomatic infections. Cell-mediated immunity to CMV may be depressed in CMV-infected children and may be another factor influencing recovery (2, 9, 17). Furthermore, the lymphocyte transformation response of CMV-infected children, diminished during the first year of life, has been reported to improve with increasing age (6).

Immunoserological tests currently available for screening children with suspected CMV infections are limited. Testing for immunoglobulin M in cord blood by radioimmunoassay is accurate in only 85% of congenital CMV infections,

and the rate of false-positive reactions may be high depending on the test used (3, 14). Isolation of CMV from urine may not be possible in all cases, and the slow growth of virus may preclude rapid diagnosis of infection. In this study and previous reports, we have identified highly immunogenic CMV-infected cell polypeptides and glycoproteins. The data indicate that large amounts of antibody to CMV present in patient sera may be detected by using purified viral polypeptides as antigens in serological assays. Children who have suffered the most severe form of congenital CMV infections and no longer displayed manifest signs of infection still maintained antibody to CMV. Those at greatest risk may be identified by reactivity of antibody to CMV antigens, in particular to the 49,000-apparent-molecular-weight polypeptide. Development of more rapid and sensitive tests for diagnosis of CMV infections is especially important for identifying the large number of asymptomatic children who may develop sequelae later in life as a result of subclinical CMV infections.

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LITERATURE CITED

1. **Becker, P., J. L. Melnick, and H. D. Mayor.** 1965. A morphological comparison between developmental stages of herpes zoster and human cytomegalovirus. Exp. Mol. Pathol. **4**:11–23.
2. **Gehrz, R. C., S. C. Marker, S. O. Knorr, J. M. Kalis, and H. H. Balfour, Jr.** 1977. Specific cell-mediated immune defect in active cytomegalovirus infection of young children and their mothers. Lancet **i**:844–847.
3. **Griffiths, P. D., S. Stagno, R. F. Pass, R. J. Smith, and C. A. Alford.** 1982. Congenital cytomegalovirus infection: diagnostic and prognostic significance of the detection of specific immunoglobulin M antibody in cord serum. Pediatrics **69**:54–549.
4. **Griffiths, P. D., S. Stagno, D. W. Reynolds, and C. A. Alford.** 1978. A longitudinal study of the serological and virological status of 18 women infected with cytomegalovirus. Arch. Virol. **58**:111–118.
5. **Hanshaw, J. B.** 1971. Congenital cytomegalovirus infection: a fifteen year perspective. J. Infect. Dis. **123**:555–561.
6. **Pass, B. F., M. E. Dworsky, R. J. Whitley, A. M. August, S. Stagno, and C. A. Alford.** 1981. Specific lymphocyte blastogenic responses in children with cytomegalovirus and herpes simplex virus infections acquired early in infancy. Infect. Immun. **34**:166–170.
7. **Pereira, L., M. Hoffman, and N. Cremer.** 1982. Electrophoretic analysis of polypeptides immune precipitated from extracts of cytomegalovirus-infected cells by human sera. Infect. Immun. **36**:933–942.
8. **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. **36**:924–932.
9. **Reynolds, D. W., S. Stagno, and C. A. Alford.** 1979. Laboratory diagnosis of cytomegalovirus infections, p. 399–439. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections. American Public Health Association, Washington, D.C.
10. **Reynolds, D. W., S. Stagno, K. G. Stubbs, A. J. Dahle, M. M. Livingston, S. S. Saxon, and C. A. Alford.** 1974. Inapparent congenital cytomegalovirus infection with elevated cord IgM levels. N. Engl. J. Med. **290**:291–296.
11. **Stagno, S., R. F. Pass, M. E. Dworsky, R. E. Henderson, E. G. Moore, P. D. Walton, and C. A. Alford.** 1982. Congenital cytomegalovirus infection. The relative importance of primary and recurrent maternal infection. N. Engl. J. Med. **306**:945–949.
12. **Stagno, S., D. W. Reynolds, C. S. Amos, A. J. Dahle, F. P. McCollister, I. Mohindra, R. Ermocillo, and C. A. Alford.** 1977. Auditory and visual defects resulting from symptomatic and subclinical congenital cytomegaloviral and toxoplasma infections. Pediatrics **59**:669–678.
13. **Stagno, S., D. W. Reynolds, E. S. Huang, S. D. Thames, R. J. Smith, and C. A. Alford.** 1977. Congenital cytomegalovirus infection. Occurrence in an immune population. N. Engl. J. Med. **296**:1254–1258.
14. **Stagno, S., D. W. Reynolds, R. F. Pass, and C. A. Alford.** 1980. Breast milk and the risk of cytomegalovirus infection. N. Engl. J. Med. **302**:1073–1076.
15. **Stagno, S., D. W. Reynolds, A. Tsiantos, D. A. Fucillo, W. Long, and C. A. Alford.** 1975. Comparative serial virologic and serologic studies of symptomatic and subclinical congenitally and natally acquired cytomegalovirus infections. J. Infect. Dis. **132**:568–577.
16. **Stagno, S., J. E. Volanakis, D. W. Reynolds, R. Stroud, and C. A. Alford.** 1977. Immune complexes in congenital and natal cytomegalovirus infections of man. J. Clin. Invest. **60**:838–845.
17. **Starr, S. E., M. D. Tolpin, J. M. Friedman, K. Paucker, and S. A. Plotkin.** 1979. Impaired cellular immunity to cytomegalovirus in congenitally infected children and their mothers. J. Infect. Dis. **140**:500–505.
18. **Stinski, M. F.** 1976. Human cytomegalovirus: glycoproteins associated with virions and dense bodies. J. Virol. **19**:594–609.
19. **Stinski, M. F., E. S. Mocarski, D. R. Thomsen, and M. L. Urbanowski.** 1979. Membrane glycoproteins and antigens induced by human cytomegalovirus. J. Gen. Virol. **43**:119–129.