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Cytomegalovirus infection in infancy: virological and immunological studies

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SUMMARY

Immunological and virological studies on 18 infants with cytomegalovirus (CMV) infection were performed. Eleven of these infants were studied on multiple occasions over a period of 1 year. The patients were divided into three clinical groups based on the probable time of infection and the resulting variation in clinical presentation. General parameters of cell-mediated immunity as determined by E-rosette formation and lymphocyte proliferative responses to mitogens and antigens were found to be normal. Quantitation of CMV excretion in urine, CMV-specific immunofluorescent (IF) and complement-fixing (CF) antibody titres and CMV-specific cell-mediated immune responses were done on all patients at approximately monthly intervals. Throughout the study period all patients continued to excrete CMV despite the presence of high antibody titres to the virus. CMV-specific lymphocyte proliferative responses were absent or diminished in 15 of the 18 patients. The immunological and virological status of all patients was similar regardless of the clinical manifestation of infection.

INTRODUCTION

Cytomegalovirus (CMV) is known to cause congenital infection ranging from asymptomatic excretion to a systemic illness resulting in intrauterine growth retardation, microcephaly and psychomotor retardation. In addition, acquisition of the virus in the perinatal period or later in infancy may result in haematological, respiratory or hepatic disorders. Recently we reported a CMV-specific cellular immune defect in four infants with congenital infection (Gehrz *et al.*, 1977). In the present study we have extended our initial observations in a longitudinal prospective analysis of the virological and immunological status of 18 CMV-infected infants. These infants varied as to time of onset of infection and clinical presentation.

MATERIALS AND METHODS

Patients. Eighteen patients, aged 2 weeks to 46 months with documented clinical and/or virological evidence of CMV infection, were referred to the investigators. Approval for the study was obtained from the University of Minnesota Committee on the Use of Human Subjects in Research, and informed consent was obtained from the legal guardian(s) of each infant prior to participation in the study. Patients were seen in our clinic at approximately monthly intervals

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during the study period. CMV cultures of urine and throat, general parameters of cell-mediated immunity and CMV-specific cellular and humoral immunity were assayed each time.

Virology/serology. Urine samples and throat swabs were cultured for the presence of live CMV on a diploid human foreskin fibroblast cell line. CMV-specific serum antibody titres were determined by indirect immunofluorescent (IF) and complement-fixing (CF) techniques.

Cell-mediated immune studies. Lymphocyte proliferative responses to mitogens (phytohaemagglutinin, PHA; pokeweed mitogen, PWM; concanavalin A, Con A) and five common antigens (*Candida albicans*, *C. albicans*; streptokinase–streptodornase, SK/SD; mumps; tetanus toxoid, TET; purified protein derivative, PPD) as well as to purified CMV antigen were assayed as previously described (Gehrz *et al.*, 1977). Briefly, mononuclear cells were isolated by density-gradient centrifugation and 10^5 T cells, as determined by E-rosette formation, were placed in microtitre wells along with either control medium, mitogen or antigen. One microcurie of tritiated thymidine was added to each well 18–24 hr before harvesting onto glass-fibre filter paper and the filters were counted in a liquid scintillation spectrophotometer. Results are expressed as counts per minute (c.p.m.) $\pm$ 1 standard deviation.

RESULTS

In Table 1 our 18 patients are divided into three groups based on clinical presentation. Congenital infections could not always be documented by the strict criterion of a positive viral culture during the first week of life. Nevertheless, the presenting findings clearly indicated that the patients were

Table 1. Clinical presentation of 18 study patients with congenital or perinatal CMV infection

	Group 1: cytomegalic inclusion disease (CNS damage at birth)					Group 2: symptomatic CMV infection at birth (without CNS damage)								Group 3: late-onset CMV infection (after 28 days of life)				
	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Age at entrance to study (months)	23	10	1	3	1	46	5	1	12	3/4	2	3	1/2	18	2	5	5	4
Clinical findings																		
Intrauterine growth retardation	+		+	+	+													
Microcephaly	+	+	+	+	+													
Psychomotor retardation	+	+	+	+														
Sensorineural hearing loss	+	+	+	+		+												
Learning disabilities	+	+	+	+		+		+										
Chorioretinitis	+		+															
Hepatosplenomegaly		+	+	+	+		+	+	+	+	+	+	+	+	+		+	
Jaundice		+				+	+	+	+	+		+	+	+			+	
Hepatocellular dysfunction	+			+	+	+	+	+	+	+			+	+	+	+	+	
Thrombocytopenia			+		+		+	+		+		+	+					
Haemolytic anaemia																+	+	
Pneumonitis				+				+							+	+	+	+

* Patient No.

infected at some point during gestation. Patients 1–5, presumably chronically infected during pregnancy, presented with cytomegalic inclusion disease (CID), manifested by intrauterine growth retardation, microcephaly and psychomotor retardation. Patients 6–13 presented in the newborn period with systemic symptoms of acute CMV infection. They were symptomatic at the time of birth with hepatosplenomegaly (seven of eight), jaundice (seven of eight) and thrombocytopenia (five of eight). Patients 14–18 were asymptomatic during the first 28 days of life, presenting later in infancy with diffuse interstitial pneumonia (four of five), hepatitis (four of five) and haemolytic anaemia (two of five). These may represent either perinatal infections or infections acquired later in infancy.

In Table 2 both general parameters of cellular immunity, as measured by numbers of E-rosette-positive cells and mitogen-induced lymphocyte proliferation, and antigen-specific cellular immune responses are shown. These data are representative responses for each patient throughout the study period. All of the patients had normal numbers of E-rosette-positive lymphocytes. The lymphocyte proliferative responses to PHA, PWM and Con A fell within the normal range for each mitogen in most cases. Patients 9 and 13 showed elevated responses to PWM, patient 10 showed an elevated response to PHA and patients 17 and 18 both showed decreased PWM responses. However, the responses of these patients to the other two mitogens tested were within normal limits. Thirteen of the 18 patients developed positive lymphocyte proliferative responses to at least one antigen during the study period. The five who did not were studied only during the first 5 months of life and presumably had not yet been exposed to the antigens tested.

Table 3 shows the initial immunological and virological studies on these patients. Viral cultures of urine and/or throat, CMV-specific IF and CF antibody titres, and parameters of general and

Table 2. Lymphocyte proliferative responses of 18 infants with active CMV infection to mitogens and antigens

Patient	E rosettes	PHA	PWM	Con A	*C. albicans*	SK/SD	Mumps	TET	PPD
Group 1: cytomegalic inclusion disease									
1	63*	106,280†	33,955	104,651	3,544‡	−1,554	25,662	47,251	14,578
2	63	121,183	27,818	115,029	541	−1,129	18,452	32,765	−900
3	53	111,893	56,182	113,327	−3,792	1,419	−481	−1,714	−786
4	43	107,297	32,112	135,453	70,724	30	17,824	37,491	10,174
5	58	102,288	48,939	94,725	−1,016	−1,128	181	5,206	−396
Group 2: symptomatic newborns, no overt CNS damage									
6	58	125,996	51,556	130,889	161,737	74,625	101,406	111,120	35,120
7	62	121,040	37,850	137,862	25,047	619	237	97,914	16,566
8	49	127,940	28,327	100,728	64,237	27,228	3,361	34,132	12,865
9	48	119,291	65,519	102,802	43,759	−1,912	−2,508	17,496	−1,920
10	60	162,343	50,205	91,552	1,077	119	−301	527	617
11	54	115,640	30,651	136,200	38,900	15,602	23,493	24,204	30,387
12	53	121,556	41,552	116,637	542	−422	9,958	17,001	−353
13	47	96,258	66,347	114,093	−2,706	−224	−2,015	3,573	3,393
Group 3: late-onset CMV infection in infancy									
14	58	151,989	41,233	119,301	17,665	449	−257	32	385
15	54	94,544	44,951	119,456	7,301	556	−809	25,033	285
16	46	133,292	41,553	122,915	−5,760	3,645	1,691	75,931	−4,360
17	66	97,542	25,293	129,214	3,391	−2,352	−3,716	−1,406	1,466
18	58	123,996	17,959	121,996	3,336	261	6,262	14,019	9,992

* Normal ranges for E rosettes (Fleischer *et al.*, 1975): 1 week to 18 months = 50·2 ± 8·7%; 18 months to 10 years = 56·9 ± 5·9%

† Expressed as mean incorporation of tritiated thymidine in c.p.m. over background tissue culture control of triplicate results. Normal ranges for mitogens in our laboratory: PHA = 129,092 ± 23,010; PWM = 41,334 ± 11,656; Con A = 115,926 ± 25,138.

‡ A positive proliferative response to antigens is ⩾ 10,000 c.p.m. over background.

Table 3. Initial virological and immunological studies on 18 infants with active CMV infection

Patient	Age at initiation of study	Quantitation of CMV excretion/0·1 ml urine	CMV excretion/throat	CMV-specific antibody titre* IF	CMV-specific antibody titre* CF	CMV-specific lymphocyte proliferation c.p.m. ± 1 s.d.
Group 1: cytomegalic inclusion disease						
1	23 months	10^1	−	40	32	18,302 ± 5,690†
2	10 months	10^1	−	160	16	2,071 ± 1,606
3	1 month	10^1	+	640	256	3,894 ± 1,103
4	3 months	+	+	160	128	399 ± 587
5	1 month	10^4	+	80	16	−271 ± 776
Group 2: symptomatic newborns, no overt CNS damage						
6	46 months	+	n.d.	80	16	−313 ± 298
7	5 months	+	+	5,120	256	1,833 ± 494
8	1 month	10^3	n.d.	320	64	2,490 ± 675
9	12 months	+	−	640	16	2,104 ± 3,930
10	3 weeks	+	+	1,280	256	25,537 ± 3,454
11	2 months	10^3	+	80	16	20,998 ± 5,941
12	3 months	$10^{4·5}$	+	320	1,024	−197 ± 295
13	2 weeks	$10^{3·5}$	+	320	128	1,809 ± 2,050
Group 3: late-onset CMV infection in infancy						
14	18 months	+	n.d.	10	32	5,154 ± 3,291
15	2 months	+	+	80	4	3,783 ± 2,849
16	5 months	+	n.d.	40	16	5,154 ± 3,806
17	5 months	+	n.d.	160	64	−3,675 ± 607
18	4 months	10^5	+	40	32	2,823 ± 3,930

* IF and CF antibody titres are expressed as the reciprocal of the serum dilution.

† Expressed as mean incorporation of tritiated thymidine in c.p.m. over background tissue culture control ± 1 s.d. of triplicate results.

n.d. = Not done.

CMV-specific cellular immunity were evaluated on all patients. CMV was recovered from the urine of all infants and from the throat of 10 of the 13 who were cultured. All infants had positive IF and/or CF antibody titres. At the time of initial study, 15 of 18 infants had diminished or absent cell-mediated immune responses to CMV. There appeared to be no difference in CMV-specific immunity among the three clinical groups of patients.

Immunological and virological studies on 11 infants diagnosed early in life were performed on multiple occasions over the first year. CMV titres in the urine were determined on 25 samples from 11 infants during the first 12 months of life. Titres ranged from 10^2 to 10^7 $TCID_{50}$/0·1 ml and there was no significant change during this period. High CF and IF antibody titres to CMV persisted in these infants throughout the study period. The data in Fig. 1 show that throughout the 12-month study period, CMV-specific cellular immunity remained depressed in the population of infants compared with a control population of adults with serological evidence of prior CMV infection. In most cases, no significant proliferation over tissue culture control background was observed.

DISCUSSION

In our study, infants with congenital CMV, like those originally reported by Hanshaw (1971), excreted virus for months or years despite the presence of high antibody titres to the virus. We previously reported a CMV-specific cell-mediated immune defect in four CMV-infected infants

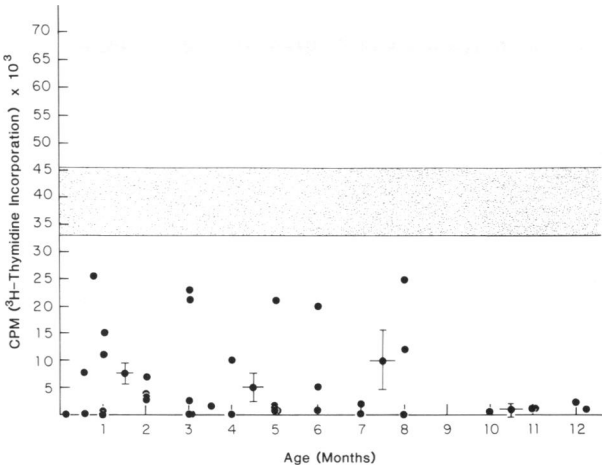

Fig. 1. Cumulative sequential studies of CMV-specific cell-mediated immunity of 11 infants during the first 12 months of life. Two or more determinations were performed on each infant. Mean c.p.m. ± standard error of the mean are shown for 3-month intervals. Shaded area depicts the 95% confidence interval of CMV-specific cell-mediated immunity for seropositive adult controls.

(Gehrz *et al.*, 1977), suggesting that cellular immunity plays a critical role in host defence against this virus. We have now extended our earlier observations to 18 CMV-infected infants, 11 of whom were followed on multiple occasions over the first year of life.

Our patients can be separated into three distinct clinical syndromes which may reflect both the mode of transmission and duration and extent of infection. Our first group of patients is typical of CID resulting from transplacental transmission of CMV following primary maternal infection. The clinical manifestations of CID include intrauterine growth retardation, microcephaly with or without intracranial calcifications, chorioretinitis, skeletal dysplasia and psychomotor retardation, which indicate long-standing infection. Our second group of infants presented with evidence of systemic viral infection at the time of delivery and therefore reflect intrauterine maternal–fetal transmission. While most of these patients showed gradual recovery with no detectable sequelae, one developed progressive neurological dysfunction over the first 4 years of life, a second presented at 7 weeks with extrahepatic biliary atresia, and a third developed severe hearing loss during the first year of life. Our third group developed acute illnesses later in infancy, including pneumonia, hepatitis, thrombocytopenia and haemolytic anaemia.

We observed that in 15 of 18 CMV-infected infants, there was a depression of CMV-specific cellular immunity, despite the presence of normal numbers of E-rosette-positive cells and the ability to respond to mitogens and at least one common antigen in a lymphocyte proliferation assay. We also have demonstrated that this CMV-specific defect is present not only in infants with CID, but also in those with late-gestation or perinatally acquired infection. Furthermore, longitudinal, prospective studies on 11 of these patients indicate that the defect persists for at least the first year of life, associated with continued viral excretion and high antibody production. This is in contrast to observations on CMV-infected adults who, after an initial phase of suppression of CMV-specific cell-mediated immunity, developed cellular immunity to the virus which persisted for more than a year along with significant titres of CMV-specific antibody (Levin *et al.*, 1979; Ten Napel & The, 1980).

Defective CMV-specific cellular immunity in congenital CMV infection has now been demonstrated by several other investigators. Starr *et al.* (1979) showed a decrease in both CMV-specific lymphocyte proliferation and interferon production in six viruric children. Reynolds *et al.* (1979) also demonstrated diminished or absent responses in 30 infants with either fetal or neonatal infection. Tamura *et al.* (1980) found age-related differences in the cell-mediated immune response to CMV, with infants under 1 year of age having responses significantly lower than those of older children and adults. The responses of infants excreting virus were lower than those of

seropositive infants without evidence of active infection, although the differences were not statistically significant. These results are compatible with our findings.

The inability of CMV-infected infants to mount a cell-mediated response to CMV is not due to a general depression of cell-mediated immunity such as that observed in acute CMV-mononucleosis patients (Rinaldo, Black & Hirsch, 1977; Levin *et al.*, 1979). Defective T-lymphocyte function is suggested by the inability of lymphocytes from these patients to respond selectively to CMV using assays measuring lymphocyte proliferation, interferon production and migration inhibitory factor production (Fiorilli *et al.*, 1978; Reynolds *et al.*, 1979; Starr *et al.*, 1979). However, Rola-Pleszczynski *et al.* (1977) have shown that some CMV-infected infants have normal CMV-specific cytotoxicity, suggesting that a subpopulation of effector T lymphocytes may be capable of responding to the virus. Since these patients are able to synthesize CMV-specific antibody, it is highly improbable that they lack a clone of T lymphocytes capable of recognizing the viral antigen.

The depressed proliferative responsiveness to CMV may represent an active infection of the lymphocyte population by replicating virus. The generalized immunosuppression observed in CMV-mononucleosis patients would support this hypothesis. However, we did not observe a generalized immunosuppression in our infants. An alternative explanation might be that the immune response is actively inhibited by either humoral or cellular factors, such as specific antibody, antigen–antibody complexes or a CMV-specific suppressor cell population. We have previously described an inverse correlation between high CMV-specific antibody titres and CMV-specific cell-mediated immunity in both mice and humans following infection with live, attenuated CMV (Howard *et al.*, 1978; Gehrz *et al.*, 1980). Since the defect in CMV-specific cellular immunity occurs in patients infected both early and late in gestation, as well as at the time of delivery or even later in infancy, it seems that the explanation of a state of immunological tolerance to the virus being established early in fetal life is inadequate to account for this phenomenon. The selective immune unresponsive state therefore may reflect an aberration in immunoregulation rather than a primary defect in cellular immunity. Further studies are needed to elucidate these possible mechanisms of immunoregulation and cellular interaction.

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