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Cytomegalovirus and child day care: risk factors for maternal infection

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To determine the rates and factors affecting cytomegalovirus transmission from children infected in day care to their seronegative mothers, we prospectively monitored 96 seronegative mothers. Of 46 seronegative mothers without infected children, 2 seroconverted. Among 50 mothers with infected children, 19 seroconverted and of these 19, 9 shed cytomegalovirus and all 9 shed the same isolate as their child. The annual seroconversion rate for these women was 30%, significantly higher than the 3% rate for mothers without infected children ($P < 0.001$; relative risk, 10.2; 95% confidence interval, 2.4, 43.8). Maternal infection was not

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associated with maternal age, race, duration of observation, duration of viral shedding by their children or the DNA pattern of each isolate but was associated with the age when a child's infection was identified. Only 3 of the 19 mothers who seroconverted had children older than 20 months of age (26, 28 and 28 months). Sixteen (57%) of 28 mothers with infected children 20 months of age or younger became infected compared with only 3 (13%) of 22 mothers with infected children more than 20 months ($P < 0.007$, Fisher's exact test, two tailed; relative risk, 3.9; 95% confidence interval, 1.3, 11.8). For mothers with infected children younger than 20 months of age the interval between identification of her child's infection and maternal infection ranged from 1 to 26 months (8 ± 6 (SD) months). Survival estimates revealed that mothers of infected children younger than 20 months of age acquired cytomegalovirus significantly more rapidly than mothers of older children (chi square, 9.34; $P < 0.0022$).

INTRODUCTION

Between 1 and 2% of newborns are infected with cytomegalovirus (CMV) *in utero*. The majority of these newborns are asymptomatic at birth and develop normally. Of the newborns infected *in utero,* however, 10% or less either have congenital CMV disease at birth and/or develop mental retardation or deafness. These infants with CMV disease are born of mothers who acquired a primary infection during pregnancy.[1-5] Thus preventing maternal infection during pregnancy is important.

Children who shed CMV are an important source of infection for seronegative mothers. Data linking CMV acquisition by children in day care and infection of their parents have been reported previously.[6-8]. To determine the important factors that contribute to CMV transmission from children to their parents we prospectively monitored parents of children who acquired CMV in day care.

METHODS

Subjects. From October, 1986, to October, 1989, 349 families with 397 children were monitored for CMV infection and 309 children provided 2 or more specimen sets for CMV culture. The children attended 1 of 3 urban nonprofit day-care centers in Richmond, VA, and the features of these centers and the families have been described.[9]

Protocol. Sequential urine and saliva specimens were obtained from each child and family member on an average of every 4 months (range, 3 to 5 months).

Sera were obtained from mothers at the beginning of the study or when their children were enrolled at the center. Sera were obtained from seronegative mothers whenever their children withdrew from day care, at least semiannually, whenever their child shed CMV and at the end of the study. Sera were assayed for immunoglobulin G to CMV by an enzyme immunoassay.[10]

This study was approved by the University committee for the conduct of human research and informed signed parental permission was obtained. Parents were informed of all culture results for their families.

Laboratory methods. Samples were cultured before and after concentration in duplicate on MRC-5 fibroblasts. Viral isolates from the first urine obtained were passed 2 to 4 times in MRC-5 fibroblasts and incubated for 48 hours with [^{32}P]orthophosphate. CMV DNAs were extracted by a modified Hirt procedure.[11] All isolates were endonuclease-digested with *Eco*RI and *Bam*III. Restriction fragments were separated by electrophoresis through 0.8% agarose as described previously.[11]

Statistical analysis. Comparisons were performed using the chi square test with Yates' correction, Fisher's exact or Student's *t* tests. The Spearman rank correlation was used. Survival analysis was performed using the SAS (SAS Institute, Inc., Cary, NC) Lifetest® and Lifereg® procedure and differences between survival estimates were tested using the log rank test for homogeneity.[12] Relative risk and 95% confidence intervals were calculated as described by Kleinbaum et al.[13]

RESULTS

At three day-care centers a total of 311 parents (seronegative and seropositive) provided 2 or more specimen sets for culture. Of the 311 parents 184 parents had children shedding CMV. Thirty-five (19%) of these 184 parents shed a CMV isolate with the same DNA pattern as the isolate acquired by their child in day care. None of 127 parents of uninfected children excreted a day-care center-transmitted isolate. One day-care center had a significantly lower rate (1%) of parents shedding CMV than did the other 2 centers (Table 1). Rates of CMV shedding by fathers and mothers were similar; however, for 7 families, parental infection could have occurred either from an infected spouse or a child.

Seronegative mothers. Mothers were monitored with viral isolation and serology. Of 46 mothers without infected children, 2 seroconverted during the study (annual rate, 3%) and none shed CMV. Among 50 mothers with infected children, 19 seroconverted and of these 19, 9 shed CMV. Each of the 9 mothers shed an isolate with the same DNA pattern as the isolate shed by her child. The annual seroconversion rate for the mothers with infected children was 30%, significantly higher than the 3% rate for mothers without infected children (chi square, 153, 1 d.f.; $P < 0.001$; relative risk, 10.2; 95% confidence interval, 2.4, 43.8).

The seroconversion rate for mothers of infected children was similar at each center (Table 1).

For seronegative mothers with infected children, CMV infection was significantly associated with the age when her child had become infected. Of the 9 seronegative mothers who shed CMV all had children who were identified as shedding CMV when younger than 20 months of age (range, 7 to 17 months). Only 3 of the 19 mothers who seroconverted had children over 20 months of age. If all 19 mothers who seroconverted acquired CMV from their child, then 16 (57%) of 28 mothers with infected children 20 months of age or younger became infected compared with only 3 (13%) of 22 mothers with infected children over 20 months ($P < 0.007$, Fisher's exact test, two tailed; relative risk, 3.9; 95% confidence intervals, 1.3, 11.8). Maternal infection was not associated with maternal age, race, duration of observation in the study, duration of viral shedding by their children or the DNA pattern of the isolate shed by the child (Table 2).

For 16 mothers who seroconverted and had infected children 20 months of age or younger, the mean age of the child when the child's infection was identified was 14 months (range, 7 to 20 months). The interval between identification of a child's infection and maternal infection ranged from 1 to 26 months (8 ± 6 (SD) months). Survival estimates shown in Figure 1 revealed that mothers of infected children identified under 21 months of age acquired CMV more often and rapidly than mothers of older children (chi square, 9.34; $P < 0.0022$).

For the 28 seronegative mothers with infected children younger than 21 months of age, maternal infection was not associated with either duration of viral shedding by their infants or the specific isolate shed. Although the interval between acquisition of CMV by a child and infection of the mother and the duration of viral shedding by her child were not correlated ($r = -0.2$; $P < 0.413$), all of the 19 children were shedding when their mothers seroconverted.

TABLE 1. Parental infection by day-care center

Parent Group*	No. of Parents Studied		
	Center 1	Center 2	Center 3
Fathers†			
Total	43	49	43
With infected children	30 (70)‡	34 (70)‡	15 (35)‡
Shedding CMV	5 (9)§	7 (14)§	1 (2)§‖
Seropositive mothers			
Total	20	37	23
With infected children	16 (80)‡	31 (84)‡	8 (35)‡
Shedding CMV	3 (15)§	10 (27)§	0 (0)§
Seronegative mothers			
Total	38	35	23
With infected children	19 (50)‡	21 (60)‡	10 (43)‡
Seroconverting	8 (21)§	8 (23)§	3 (13)§¶
Shedding CMV	5 (13)§	4 (11)§	0 (0)§

* Includes only parents submitting ≥2 specimen sets; average, 4 specimen sets per parent (range, 2 to 9).

† Two of the fathers at Day-Care Center 1 and five of the fathers at Day-Care Center 2 had wives who excreted the same isolates as their children.

‡ Numbers in parentheses, percent.

§ Numbers in parentheses, percent of total.

‖ Significantly fewer parents shed CMV at Center 3 compared to Center 1 or Center 2 (chi square, 13.9, 2 d.f.; $P < 0.001$).

¶ Not significantly lower than the maternal infection rates for seronegative mothers at Center 1 or Center 2; chi square, 0.8, 2 d.f.; $P > 0.1$).

TABLE 2. Cytomegalovirus infection among seronegative mothers

Maternal Characteristic	Children			
	Infected		Uninfected	
	<20* months	>20 months	<20 months	>20 months
No.	28	22	20	26
No. seroconverting	16 (57)†	3 (14)	1 (5)	1 (4)
No. shedding CMV	9 (32)	0 (0)	0 (0)	0 (0)
Age (years)	32 ± 3.8‡	32 ± 3.5	31 ± 3.7	32 ± 2.2
No.				
Caucasian	27	19	18	21
Black	1	2	1	2
Unknown	0	1	1	3
Months followed/mother	22 ± 10	17 ± 10	22 ± 10	16 ± 9
No. with spouse shedding	2	0	0	0
No. of children shedding the same isolate based on DNA pattern	18 (64)	13 (59)		
Months children shed virus	22 ± 10	16 ± 10		

* Child's age when first culture-positive.

† Numbers in parentheses, percent.

‡ Mean ± SD.

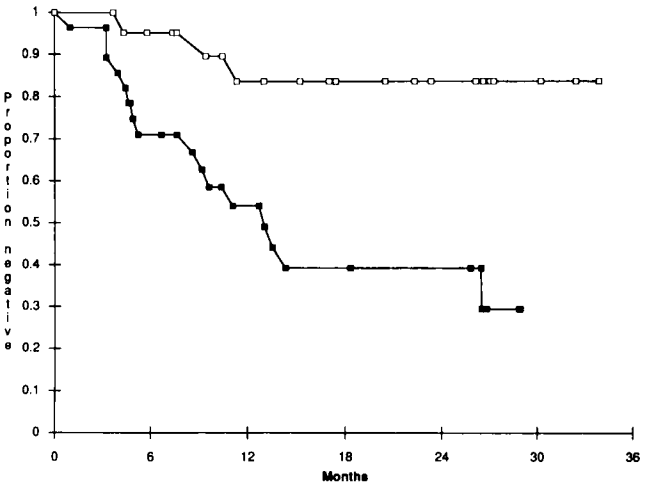

FIG. 1. Survival estimates for 50 seronegative mothers with infected children either ≤20 months of age (■) or >20 months of age (□). The two curves differed significantly using the log rank test for homogeneity (chi square, 9.34; $P < 0.0022$).

Seropositive mothers. Fifty-five mothers of 62 infected children were seropositive when their first serum sample was obtained. Of these 55 mothers 13 (24%) shed CMV. Each of these 13 mothers shed an isolate with the same DNA pattern as the isolate acquired by her child in day care. Maternal shedding was not associated with their child's age (Table 3). For the 13 mothers viral shedding either followed a primary infection acquired before the beginning of the study, with subsequent intermittent shedding, or followed reinfection with the isolate acquired from their children in day care. Of these 13 mothers 8 shed CMV in the first culture specimens obtained, 3 in their

TABLE 3. CMV shedding among seropositive mothers of infected children

Child's age* and first culture result	No. of Children†		
	Shedding CMV	With mothers shedding CMV‡	% with mothers shedding
Positive			
≤20 months	16	6	38
>20 months	23	7	30
Negative§			
≤20 months	11	0	0
>20 months	12	1	8

* Children's ages when first culture-positive.

† Includes 55 mothers of 62 children. Seven mothers (one of whom shed CMV) had 2 children excreting CMV. Mothers of children with initially positive cultures were observed for a mean of 22 ± 10 months and mothers of children with initially negative cultures for a mean of 21 ± 14 months. Both groups had an average of 4 cultures per mother.

‡ Infections were confirmed as day care-transmitted by restriction enzyme analysis.

§ Mothers of children with initially positive cultures shed CMV significantly more frequently than mothers of children with initially negative cultures; $P < 0.02$, Fisher's exact test, two tailed.

second and 2 in their third. Shedding of CMV by a seropositive mother was associated with whether her child shed before or after the study began. Children who shed CMV after the study began are more likely to have acquired CMV after the study began than to have shed intermittently.[9] Thirteen (33%) of 39 seropositive mothers with children infected before the beginning of the study shed CMV compared with only 1 (4.5%) of 22 seropositive mothers whose children shed CMV after the study began (Table 3). This suggests the majority of seropositive mothers shed CMV after a primary maternal infection.

DISCUSSION

The risk of maternal infection for seronegative mothers of infected children was increased 10-fold over that for mothers without infected children. Nearly half of the infected mothers shed CMV and each maternal isolate was the same as that shed by her child. Based on the seroconversion results, a seronegative mother's risk for infection was not related to the isolate shed by her child or the center attended. Therefore the risk for a seronegative mother is proportional to the risk of her child's acquiring CMV in day care. Mothers with children initially infected before 20 months of age had a 4-fold increased risk for CMV infection compared with mothers with children becoming infected after 20 months of age.

Annual seroconversion rates of greater than 10% have been observed previously for women in frequent contact with young children excreting CMV. Yeager[14] observed that 7 of 15 seronegative mothers of 15 newborns with transfusion-acquired CMV infections seroconverted in 1 year. Pass et al.[7] observed that 9 of 20 parents of shedding children younger than 18 months old seroconverted compared with 5 of 26 seroconversions for parents of older children ($P = 0.06$). Our results extend those of Pass et al. and demonstrate the significant risk associated with young children. Finally day-care workers caring for children younger than 2 years of age have the greatest risk for CMV acquisition.[15,16] The enhanced risk for mothers of younger children probably relates to the frequency and type of care these children require.

After a primary infection with CMV, reinfection with a second strain has been observed for transplant recipients, patients with acquired immunodeficiency syndome, those with multiple sex partners and most recently for children in day care.[9, 17-22] In this study there were 22 seropositive mothers who had children who acquired CMV in day care. The observation that only 1 of these mothers shed the same virus as her child suggests that previous infection may reduce the frequency of reinfection with a second strain.

Primary maternal CMV infection during the first 24 weeks of pregnancy places the fetus at greatest risk for permanent damage.[1-5] Primary maternal CMV infections during pregnancy are seldom recognized and many infants who develop neurologic sequelae are asymptomatic at birth. Thus the precise number of affected infants born annually is unknown. Assuming, however, that of the 4 million infants born in the United States annually 70% are born to upper or middle income mothers and, of these, 50% are seronegative and assuming, as observed by Stagno et al.,[1] that the seroconversion rate during pregnancy is 1.6% for seronegative mothers and the attack rate for significant handicaps is 20% for mothers infected in the first half of pregnancy, then approximately 2200 (range, 1500 to 3000) infants are born annually with significant neurologic handicaps caused by congenital CMV infection. In this study group 110 mothers had 2 children and of these 35 conceived when their older child was less than 24 months of age. Thirty-eight mothers were pregnant during the study, and of these 2 seroconverted during early pregnancy and 1 at or near conception. Given the low rate of symptomatic congenital disease after primary maternal infection during pregnancy, the broad spectrum and late onset of many manifestations, the low frequency of primary maternal infection, even for mothers of children in day care, and the wide range of infection rates among day-care centers, an increase in the symptomatic congenital infection rate nationally acquired in day care is likely to remain inapparent without active surveillance.

CMV infections are transmitted slowly even under conditions of prolonged and intimate contact. Nearly half of the mothers of infected children escaped infection. In addition pediatric health care workers do not acquire CMV from patients even if frequently exposed.[23-25] Prevention of CMV infection during pregnancy should be possible. Mothers with children younger than age 2 years in day care who are pregnant or anticipating pregnancy could be given the option for serologic testing of themselves and their child.

Serologic testing is simple, rapid, very accurate, and commercially available and can be performed on the serum obtained by a finger prick.[26] Seropositive children between 6 months and 2 years of age should be considered infectious. Seronegative mothers of seropositive children could be counselled to avoid "intimate contact" with their child during early pregnancy. Counseling would advise frequent hand-washing and if feasible, the use of gloves, especially when handling diapers or respiratory secretions, and avoidance of mouth to mouth contact. Whether such counselling and practices will reduce the risk of CMV acquisition is unknown. Such practices, however, if rigorously maintained should be successful.

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