PREVENTION OF CHILD-TO-MOTHER TRANSMISSION OF CYTOMEGALOVIRUS AMONG PREGNANT WOMEN

STUART P. ADLER, MD, JACK W. FINNEY, PHD, ANNE MARIE MANGANELLO, RN, AND AL M. BEST, PHD

Objective To determine if protective behavior prevents child-to-mother transmission of cytomegalovirus (CMV) during pregnancy.

Study design We studied 166 seronegative mothers (94% white women; mean age, 33 years) with a child <36 months of age attending a day care facility. Mothers, either pregnant or attempting pregnancy, were randomly assigned by day care center to either a control or intervention group. Mothers in the intervention group received instructions for hand washing, glove use, and for avoiding types of intimate contact with their child. The control group received no instructions or information about their serologic status or whether their child was shedding CMV.

Results In the intervention group, 7.8% of women (9 of 115) seroconverted, as did 7.8% of women (4 of 51) in the control group. Two independent predictors of maternal infection were (1) a child shedding and (2) a mother attempting pregnancy at enrollment. For 41 women attempting pregnancy at enrollment with a child shedding CMV, 10 of 24 became infected compared with only 1 of 17 women who were already pregnant at enrollment (P = .008).

Conclusions For seronegative women who already know they are pregnant, intervention may be highly effective for preventing CMV acquisition. *(J Pediatr 2004;145:485-91)*

Approximately 50% of infants born to mothers who acquire a primary cytomegalovirus (CMV) infection during pregnancy will be infected in utero and born with a congenital CMV infection.[1] Congenital CMV infection can result in mental retardation, deafness, and other neurologic handicaps.[2] Seronegative mothers with infected children acquire CMV infections at rates 20 to 25 times higher than other women, and at least half of seronegative mothers will become infected within 1 year after their child becomes infected.[3-6] Children younger than 3 years of age who acquire CMV after birth excrete CMV in urine and saliva for 6 to 42 months (mean, 18 months).[7] In the United States, approximately 60% of mothers of children in day care are seronegative and at least half of all young children are in group child care. Between 15% and 70% of all children in group day care acquire CMV infection.[8] Prevention of maternal infection during pregnancy is important for reducing the frequency of congenital infection.

See editorial, p 435.

Because vaccines against CMV are unavailable, it is important to determine whether modifying certain maternal behavior reduces the rate at which CMV-seronegative pregnant women acquire CMV from their children. We previously developed an intervention protocol that was feasible and acceptable to mothers and that could potentially change behavior for mothers of young children in day care.[9,10] In a trial with a small sample size, we found that the protocol was effective for pregnant women but ineffective for nonpregnant women using contraception. We sought to evaluate the effectiveness of the protocol in seronegative women who were either pregnant or not pregnant but attempting conception who had a child attending a child care center.

METHODS

Subjects

Subjects were mothers who were either pregnant or not pregnant but attempting conception (using no contraception) who had a child younger than 36 months of age

CMV Cytomegalovirus

From the Departments of Pediatrics and Biostatistics, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, and the Department of Psychology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

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Reprint requests: Stuart P. Adler, MD, Medical College of Virginia Campus, Virginia Commonwealth University, PO Box 980163, Richmond, VA 23298-0163. E-mail: sadler@hsc.vcu.edu.

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enrolled in 1 of 124 childcare centers in Central, Northern, and Eastern Virginia from 1999 through 2001. Informed consent was obtained from all subjects. The human experimentation guidelines of the US Department of Health and Human Services and those of Virginia Commonwealth University/Medical College of Virginia were followed in the conduct of the clinical research.

Protocol

Initially, child care centers were randomly assigned to one of three intervention groups. Subjects with a child enrolled at a specific center received an intervention based on the child care center assignment (to avoid the control group becoming informed of interventions). To achieve approximately equal sample sizes per group, the assignment of each center was randomized annually. The randomization was weighted on the basis of enrollment in the previous year. Families enrolled in one group did not cross over but remained in their group until termination of the study. Randomization was done with the use of SAS.[11] The three initial groups were

- Group 1. Control: Women in this group received basic information about CMV and the reasons for the study but no intervention. Women in this group were not told their serologic status or whether their child was shedding CMV.
- Group 2. Intervention: At an orientation session, mothers were given information about CMV infection and its potential complications. The women viewed an educational video demonstrating potential techniques for avoiding acquisition of CMV. Women were given detailed written and oral instructions for behavioral recommendations. Protective behavior included frequent hand washing (ie, after exposure to a child's bodily fluids and diaper changes, handling dirty laundry, touching the child's toys and other objects, and bathing the child) and wearing protective gloves (ie, during diaper changes and when handling the child's dirty laundry). Behavior to avoid included intimate contact with the child such as kissing on the mouth, sleeping together, sharing towels and washcloths, and sharing food or drink. Home visits every 3 months were conducted to assess adherence. Mothers estimated the percentage of times biweekly that they performed or avoided specific behavior on a 0% to 100% scale. After establishing a baseline, we discussed and answered questions about the procedures with each mother for approximately 15 minutes. Liquid soap and latex gloves were provided. Glove and soap use were monitored every 3 months for an indirect objective measure of adherence to the protective behavior. Mothers in group 2 knew their serologic status but were unaware if their child was shedding CMV.
- Group 3. Full intervention: Subjects in group 3 were treated in the same manner as subjects in group 2 except that they were told the results of the initial CMV culture for their child but were not told the results of subsequent cultures. Subjects in group 3 were told at enrollment that if their child was not shedding, there was a high probability that their child could begin to shed CMV at any time during the study or during pregnancy.

Measures

For women in the intervention groups, measures included the self-reported percentages of opportunities that they performed protective or avoidance specific behavior on a 0% to 100% scale. Every 3 months at a home visit, a research nurse provided new boxes of gloves and a new bottle of hand soap. For objective measures of adherence to protective behavior, the number of remaining gloves was counted, the amount of remaining soap was weighed, and the number of diapers remaining were counted.

Monitoring for CMV

For each woman, serum was collected every 3 months for 12 months or until delivery. All children in the home as well as participating fathers had urine and saliva obtained every 3 months for CMV culture. Serum was obtained from each participating father. All maternal sera were stored at −80° until a mother completed participation; seroconversion was then measured with the use of an enzyme immunoassay.[12] Neither the women nor the research nurses were aware of the results of virologic or serologic testing until the end of maternal participation.

Statistical Analysis

Logistic regression analysis was used to determine the factors associated with maternal seroconversion. Using the methods of Hosmer and Lemshow, unadjusted odds ratios were first inspected for potential inclusion in a model.[13] All those with an initial probability value of <.2 were included in the multivariate model. After removing nonsignificant factors and testing for 2-way interactions, a final model was identified.

The measures of adherence were compared by means of repeated-measures analysis of variance. Average daily adherence measures were also calculated for each subject to illustrate the magnitude of differences.

RESULTS

During the study, 234 eligible women were enrolled. Excluded from the data analysis were 42 women in the control group who at enrollment were CMV-seropositive and 26 initially seronegative women who failed to provide follow-up specimens. One hundred sixty-six women who were initially seronegative completed the study.

At the end of 1999, an interim data analysis showed that half of the children who shed CMV at any time during maternal participation were not shedding when their mothers were enrolled. Given this and the fact that the only difference between groups 2 and 3 was the mother's knowledge of her child's initial culture results, enrollment in group 2 was terminated and all mothers assigned to an intervention group were assigned to group 3.

Table I. Univariate analysis of variables associated with maternal seroconverison

Variable	Maternal seroconversion			Odds ratio		
	No	Yes	% Infected	*P* value	Estimate	95% CI
Maternal race (no. of subjects)						
Asian	1	0	0	1.0	ref*	
African American	4	0	0		N/A*	
Caucasian	143	13	8.3		N/A	
Other	2	0	0		N/A	
Unknown	3	0	0		N/A	
Maternal age at enrollment (mean years ±SD)	32.7 ± 3.9	32.6 ± 4.1		> .9	1/0	(0.9, 1.2)
No. of household children at enrollment (no. of subjects)						
1	150	12	7.4	.23	ref	
2	3	1	25		4.2	(0.2, 35.5)
3	0	0	0			
Pregnant at any time during enrollment (no. of subjects)						
Yes	129	11	7.8	> .9	ref	
No	24	2	7.7		1.0	(0.1, 3.9)
Conception prior to enrollment (no. of subjects)						
Yes	101	3	2.9	.0048	ref	
No	52	10	16.1		6.5	(1.9, 29.8)
Child's age at enrollment (mean months ±SD)	20.7 ± 6.1	16.8 ± 8.8		.0060	0.9	(0.8, 1.0)
Child shed CMV during maternal enrollment (no. of subjects)						
No	123	2	1.6	< .0001	ref	
Yes	30	11	26.8		22.6	(5.7, 151)
Child shed CMV at maternal enrollment (no. of subjects)						
No	133	8	5.7	.0246	ref	(1.2, 14.6)
Yes	19	5	20.8		4.4	
Intervention group (no. of subjects)						
Control (Group 1)	47	4	7.8	1.0	ref	
Intervention (Group 2)	22	1	4.3		0.5	(0.0, 3.9)
Full Intervention (Group 3)	84	8	8.7		1.1	(0.3, 4.4)
Location (no. of subjects)						
Eastern Virginia	40	2	4.7	.23	ref	
Central Virginia	67	5	6.9		0.7	(0.1, 3.3)
Northern Virginia	46	6	11.5		1.7	(0.5, 6.4)
Mother's knowledge of her child's initial culture (no. of subjects)						
Knew nothing	69	5	6.8	.0810	ref	
Knew negative	69	4	5.5		0.8	(0.2, 3.1)
Knew positive	15	4	21.1		3.7	(0.8, 15.6)
Father's serologic status (no. of subjects)						
Negative	38	3	7.3	.283	ref	
Positive	9	2	18.2		2.8	(0.3, 19.6)
Days to child's first positive CMV culture for 41 mothers with a child shedding CMV (mean ±SD)	54.7 ± 81	80.9 ± 142		.46		N/A
Duration of child's shedding for 41 mothers with a child shedding CMV (mean days ±SD)	169 ± 93	173 ± 130		.57	N/A	

*ref = reference cell for odds ratio. N/A = not appropriate

Table I lists the variables investigated by univariate analysis for association with maternal seroconversion. The following factors were significantly associated with maternal infection: a child shedding either at enrollment or at any time during maternal participation, a mother who was pregnant before enrollment, and child's younger age at the time of maternal enrollment.

All variables listed in Table I with a probability value of <.2 were used to develop a logistic regression model (Table II). Factors and 2-way interactions were removed until all were

Table II. Independent predictors of maternal infection

Child shed	Pregnant before enrollment	Mother's seroconversion		Adjusted odds-ratio exact*			
		No	Yes	%	estimate	95% CI	*P* value
No	Yes	85	2	2.3			
No	No	38	0	0.0	0.94	(—, 12.25)	.5734
Yes	Yes	16	1	5.9	2.62	(0.04, 53.25)	1
Yes	No	14	10	41.7	28.88	(5.39, 298.09)	< .0001

*Likelihood ratio χ^2 for the whole logistic regression model = 45.07, df = 3, $P < .0001$.

significant ($P < .05$). The model showed that two predictors interacted to produce a significant association with maternal infection. These were a child shedding CMV at any time and attempting pregnancy at enrollment. Not being pregnant at enrollment significantly increased risk of acquisition ($P < .0001$). Of 17 (5.8%) women with a child shedding CMV and who were pregnant before enrollment, only 1 became infected, compared with 10 of 24 (41.6%) women who were attempting conception at enrollment and whose a child shed CMV. As shown by survival analysis (Figure), the difference in infection rates between pregnant and nonpregnant women was not due to a shorter participation period for women pregnant at enrollment.

Regarding group assignment, intervention was unsuccessful for women not pregnant at enrollment, but only two pregnant women (with a child shedding CMV) were assigned to the control group to determine whether the intervention was directly responsible for the reduced infection rate for pregnant women.

Thirty-six (58%) of 62 women not pregnant at enrollment conceived. Of the 11 women who became infected and had a child shedding CMV, 10 were not pregnant at enrollment. Of these 10, 8 became pregnant after enrollment. Of these 8, 4 became infected within the 3 months before conception, one became infected just before conception or during the first trimester, and 3 became infected during the third trimester.

Fetal Infections

Of the 11 women with a child shedding CMV who became infected, 9 delivered a term newborn infant; of 7 infants tested, 5 were congenitally infected with CMV. Three infants were asymptomatic at birth and have remained asymptomatic through age 2 years. Of these three infants, one was born to a mother infected just before pregnancy, the second with a maternal infection during the second trimester, and the third with a maternal infection in the third trimester. Two infants had unilateral total neurosensory hearing deficit at birth; one was born to a mother who acquired a primary CMV infection within 3 months before pregnancy; and one was born of a mother who acquired a primary CMV infection either 2 months before pregnancy or 1 month after conception.

Measures of Adherence

Women who received an intervention were monitored for behavioral adherence. There were no significant differences between infected and uninfected women and between pregnant women and those attempting pregnancy (Table III). Although not statistically significant ($P = .14$), women pregnant at enrollment reporting kissing their child on the lips half as often as women who were not pregnant (Table III).

DISCUSSION

CMV-seronegative women with frequent contact with young children in group day care have between a 5- and 25-fold increased risk for acquiring CMV from these children compared with seronegative women not in contact with young children.[3-5,14-16] The consequences of CMV acquisition during pregnancy may be severe for the developing fetus.[2] Whether CMV transmission occurs through contact with contaminated environmental surfaces (fomites), saliva, urine, or respiratory droplets is unknown. Transmission through respiratory droplets is unlikely, but CMV survives on fomites, including diapers, toys, and the hands of day care personnel.[17-19] Improved hygiene, more frequent handwashing, or preventive measures should reduce the frequency of child-to-mother transmission.

In previous studies, only half of mothers who seroconvert shed CMV, but 95% of mothers who shed CMV shed an isolate with the same genome pattern as that excreted by their child, indicating that the young children are the source of maternal infection.[3,6,10] For these reasons, the end point for this study was maternal seroconversion.

Because random assignment was by day care center, proportional numbers of subjects were not necessarily enrolled in control and intervention groups. There were only two pregnant women assigned to the control group. It was, however, necessary to terminate the study because intervention before pregnancy was unethical if intervention during pregnancy was likely to be effective.

Our data suggest that intervention for pregnant women may have reduced the risk of acquiring CMV by 85%. In a previous study, 14 pregnant women were instructed on how to reduce their risks, but behavior was not reinforced nor success monitored; none of 14 pregnant women with a child

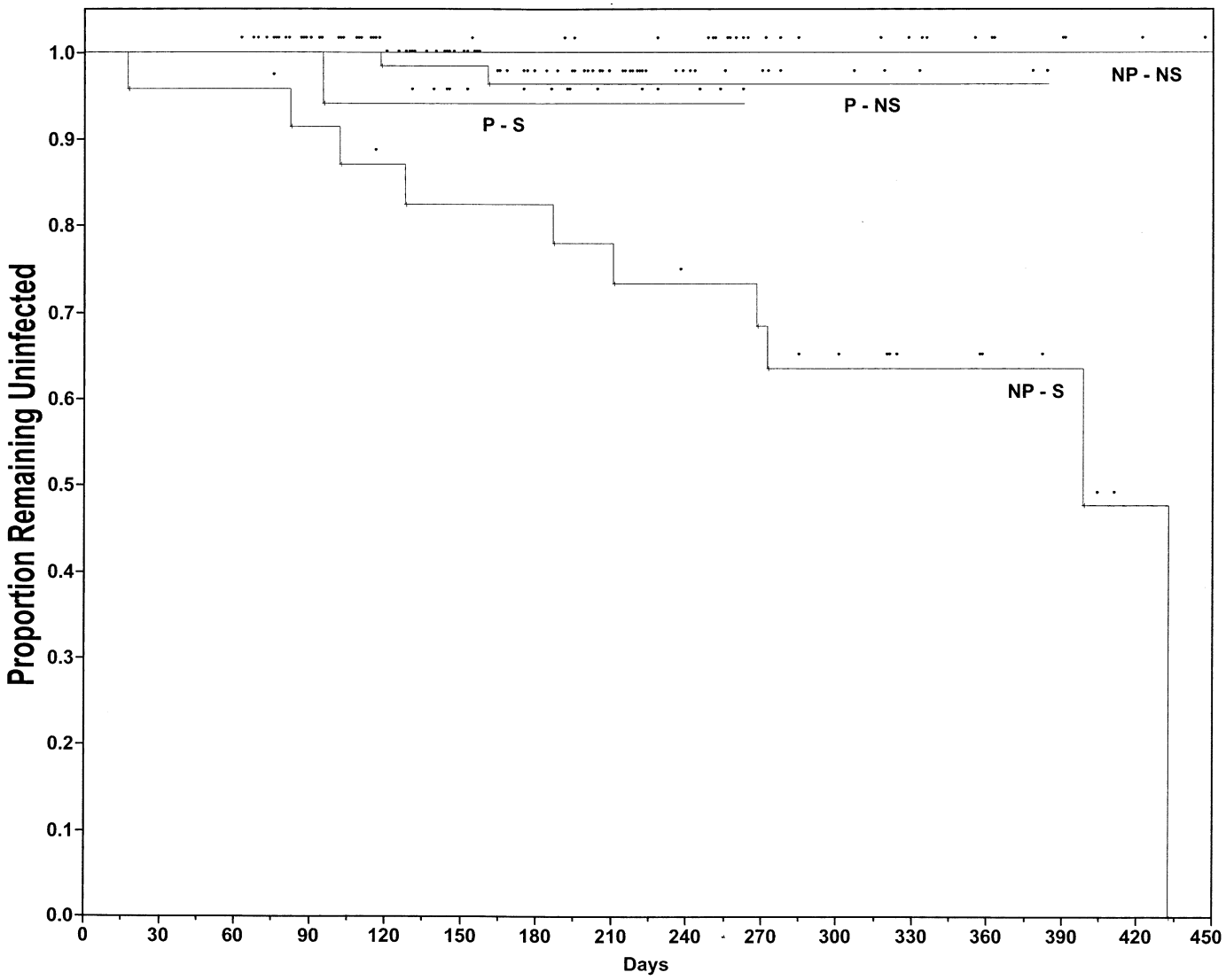

Figure. Kaplan-Meier survival analysis for 166 mothers remaining CMV-infected according to whether a mother was pregnant before enrollment or had a child shedding CMV: Mothers not pregnant at enrollment with a shedding child *(NP-S),* mothers not pregnant at enrollment without a shedding child *(NP-NS),* mothers pregnant at enrollment with a shedding child *(P-S),* and mothers pregnant at enrollment without a shedding child *(P-NS).* Mothers not pregnant at enrollment with a shedding child *(NP-S)* differed significantly from those of the other groups (log-rank $\chi^2 = 25.3$, $P < .0001$).

shedding CMV became infected, whereas 36% (14 of 39) of women who were neither pregnant nor attempting pregnancy became infected.[10]

It is unlikely that the low rate of infection for pregnant women was due to decreased sexual activity during pregnancy compared with women who were attempting pregnancy. The overall infection rate (42%) for the women with an infected child and attempting pregnancy was similar to the 36%, 45%, and 53% rates observed in previous studies for women with infected children but who were neither pregnant nor attempting conception.[3,6,10] For the women attempting pregnancy at enrollment, half of the maternal CMV infections occurred after the first trimester of pregnancy, a time when sexual activity should have diminished. There was no association between paternal seropositivity and maternal infection, and only 2 of 57 fathers who were tested shed CMV during the study. Of the two women with shedding husbands, one seroconverted and one did not.

We believe that intervention was successful in pregnant women because they were more motivated to alter behavior. Pregnant women were attentive, took notes, and asked questions, whereas the women who were not pregnant appeared much less engaged. This subjective observation was not reflected in an association between the subjective measures (self-reporting) or the objective measures (soap, glove, and diaper use) and infection rates. The self-reporting data were, however, only estimates obtained retroactively every 3 months and may not have reflected actual practice, and objective measures may not have targeted the behavior (hand washing) most associated with reduced transmission.

Table III. Monitoring of maternal child care activities according to maternal pregnancy status at enrollment

Variable	Mean ± SD/Subject		*P* value
	Not pregnant (N = 44)	Pregnant (N = 69)	
Objective measure			
Average no. of reports provided	5.0 ± 2.9	3.3 ± 1.9	.0002
Average no. of diapers/day	12.1 ± 6.8	14.1 ± 9.0	.22
Average g of soap/month	230 ± 150	232 ± 150	.93
Average no. of gloves/day	6.0 ± 6.3	8.1 ± 9.1	.21
Self reports			
Average no. of times biweekly			
Changed child's diaper	11.6 ± 6.8	13.6 ± 9.1	.23
Wore gloves while changing diapers	4.0 ± 7.8	4.2 ± 6.2	.89
Washed hands after helping child use potty chair or toilet	3.94 ± 7.7	4.3 ± 7.0	.80
Washed child's laundry	1.7 ± 1.4	2.2 ± 1.8	.10
Wore gloves while washing child's laundry	0.8 ± 0.9	0.9 ± 1.2	.48
Washed hands after washing child's laundry	1.4 ± 1.4	1.7 ± 1.7	.30
Bathed child	3.5 ± 2.1	3.4 ± 2.4	.96
Washed hands after bathing child	2.9 ± 2.1	2.9 ± 2.5	.93
Kissed child on lips	3.9 ± 8.5	2.1 ± 3.4	.14
Ate/drank after child	0.8 ± 2.1	0.5 ± 1.2	.41
Slept in the same bed with child	1.0 ± 1.4	0.9 ± 1.4	.72
Used towel or washcloth after child	0.4 ± 2.2	0.3 ± 1.2	.68
Percentage of time biweekly			
Washed hands after handling child's objects	64 ± 23	64 ± 27	.97
Washed hands after wiping child's nose	69 ± 23	62 ± 32	.22
Washed hands after spending a lot of time with child	63 ± 22	63 ± 27	.92

Reports from all mothers indicated that protective behavior and avoidance behavior were performed easily. None of the mothers were concerned about reducing the specific types of intimate contact with their children. Nearly all of the mothers said they were able to make and sustain changes for an average of 200 days per subject.

Results of this study suggest how to advise mothers with young children who are anticipating additional pregnancies. Mothers who are pregnant and have a child in day care could be given the option for serologic testing of themselves. Serologic testing is simple, rapid, accurate, available commercially, relatively inexpensive, and can be performed on the serum obtained by a finger prick. Seoronegative pregnant women could be counseled to use protective behavior (ie, frequent hand washing and the use of gloves) and to avoid intimate behavior (ie, mouth-to-mouth contact, sharing of utensils and food). The rate of CMV acquisition by children in child care is so high that testing the children is unlikely to be reassuring or helpful because the absence of CMV shedding by a child does not mean an absence of risk as long as the child remains in child care.

Our results suggest that counseling pregnant women will be effective. This could take place at the first obstetric visit, which frequently occurs within 6 weeks of conception. It is possible that more extensive maternal education, such as an intensive discussion of CMV neonatal morbidity and statistics of risk, could enhance motivation and reduce risk of infection for nonpregnant women. This approach, however, may not be highly effective or practical. A recent meta-analysis indicated strong fear appeals have only small influence on change of behavior.[20] Access to nonpregnant women would require a massive public education effort.

The use of large-group child care by mothers with children under age 5 years has been increasing over the last two decades and is anticipated to approach 75% by 2005, with an estimated one million child care centers in the United States. Our data provide a reasonable estimate on the risk for seronegative-pregnant white women with a child under age 3 years in large-group day care. We observed that without regard to intervention, 7.8% of the seronegative women seroconverted, and the overall congenital infection rate was 3%. These rates are nearly identical to those of a study in Birmingham, Alabama, of 604 initially seronegative women (7.8% annualized seroconversion rate) with at least one prior pregnancy who were predominately black, poor, and young (average age, 23 years).[21] In the Alabama study, the source of maternal infection was not determined; however, it is unlikely that many of the women had children enrolled in day care. The similarity of the rates of maternal seroconversion and congenital infection between the white, older, and more affluent population we studied compared with the Alabama population suggests that contact with young children in the

home may be the major source of CMV acquisition for seronegative women regardless of racial or socioeconomic factors.

Congenital CMV infection can result from primary maternal infection or from reinfection or reactivation of latent virus in women with antibody to CMV before pregnancy. Most cases of congenital CMV infection and disease result from primary maternal infection, and the disease caused by primary infection is more severe than that caused by reinfection or reactivation.[2,21] Some women with antibody to CMV before pregnancy, however, can deliver congenitally infected infants with central nervous system disease.[22-24] Whether this is associated with reactivation or reinfection is unknown, but there is indirect evidence for association of reinfection causing congenital disease in women with preconceptional antibody.[25] If reinfection of seropositive pregnant women is associated with congenital disease, immunization of seropositive women is unlikely to be effective. Behavioral intervention may be the only preventive measure available to seropositive pregnant women with young children in day care and thus may have broad public health impact.

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