Epidemiology of Congenital Cytomegalovirus Infection: Maternal Risk Factors and Molecular Analysis of Cytomegalovirus Strains


To determine factors that influence the occurrence of congenital cytomegalovirus (CMV) infection, the authors surveyed prospectively 8,254 infants born in eastern Iowa between October 1989 and June 1994. The authors conducted a case-control study to identify maternal risk factors, matching each CMV-infected infant with three uninfected infants according to hospital and date of birth. CMV strains were compared by using the polymerase chain reaction (PCR) to identify common sources of infection. Of the 7,229 infants cultured successfully for CMV, 35 (0.48%) were congenitally infected. Mothers of CMV-infected infants were more likely to be single (odds ratio (OR) = 3.05, p = 0.016), to work in sales (OR = 4.93, p = 0.008), or to be students (OR = 5.01, p = 0.017). Conversely, women who worked in health-care professions were less likely to have a congenitally infected infant (OR = 0.14, p = 0.049). PCR analysis indicated 27 distinct strains of CMV, but two groups of infants (two infants per group) excreted strains with indistinguishable molecular patterns. One of these pairs of infants had older siblings who attended the same child-care center during their mothers’ pregnancies. The authors concluded that demographic and occupational factors influenced the risk of giving birth to an infant with congenital CMV infection. Many distinct CMV strains were identified, suggesting that major point source outbreaks had not occurred. Nonetheless, point source acquisition of CMV from child-care environments did account for some cases of congenital CMV infection in eastern Iowa. Am J Epidemiol 1998; 147:940-7.

child care; cytomegalovirus; infection, congenital; polymerase chain reaction

Approximately 30,000–40,000 infants are congenitally infected with cytomegalovirus (CMV) annually in the United States, and as many as 9,000 of these infants have long-term sequelae (1, 2). Consequently, CMV remains a major cause of mental retardation, learning disability, sensorineural hearing loss, and visual loss among children born in the United States. No vaccine is presently available to prevent the public health consequences of this infection.

Prior studies have provided important data regarding the incidence and long-term sequelae of congenital CMV infection (3), the risk of fetal transmission, the role of maternal immunity (4), and the epidemiology of CMV infection among young children (5–7). Children in group child care acquire CMV at high rates—approximately 10–20 percent annually versus 2–5 percent for children who do not attend group care (5–7). CMV-infected children excrete CMV for extended periods and therefore constitute a major reservoir of CMV (5–7). Consequently, the annual rates of CMV acquisition by women who work in child-care centers range from 8 percent to 20 percent as compared with 3–5 percent in the general population (8–10). The parents of young children in child care are also at increased risk of CMV infection (11).

In addition to becoming infected through contact with young children, women of reproductive age can acquire CMV from their sexual partners. Chandler et al. (12) studied the epidemiology of CMV infection among women attending a sexually transmitted disease clinic and observed that serologic evidence of prior CMV infection was closely associated with higher numbers of sexual partners and younger age at first sexual intercourse. Sociodemographic factors, such as urban residence or race, also affect the rate of
CMV acquisition (1). Other factors that influence the maternal risk of delivering a congenitally infected infant have not been determined precisely.

We conducted a population-based case-control study in eastern Iowa to identify potential risk factors among women who delivered infants infected congenitally with CMV. CMV strains isolated from congenitally infected infants were characterized by using polymerase chain reaction (PCR)-based methods to determine strain similarities and to identify point source acquisition of CMV infection.

MATERIALS AND METHODS
Surveillance for congenital CMV infection

The population consisted of infants and their families recruited from October 1989 through June 1994 from the four hospitals located in Cedar Rapids and Iowa City, Iowa. Congenital infection with CMV was determined by culturing a urine sample within the first 2 weeks of life. Urine was obtained from disposable diapers, sterile bags, or a urine collection system (Bard® SureCatch®, Bard Urological Division, Covington, Georgia). The majority of the urine samples were acquired from disposable diapers. After informed consent was obtained from a parent, the urine sample was cultured for CMV using modifications of methods described previously (10). The study was approved by the University of Iowa Human Subjects Committee.

Design of the case-control study and collection of epidemiologic data

Cases were defined as infants who excreted CMV during the first 2 weeks of life, whereas controls were defined as infants who had negative urine cultures for CMV. Infants were matched by hospital and date of birth. Sequential hospital numbers of study infants with negative cultures were used to identify potential controls. Study personnel contacted the parents of uninfected infants born on the same day as the cases and continued contacting parents of infants born on successive days until three controls were identified for each case.

Identical questionnaires were administered to the parents of case and control infants during a telephone or in-home interview. Parental information was gathered regarding age, education, marital status, occupation, sexual history, and drug or alcohol use. Family information was collected on the presence of siblings or other children in the home and on the use of out-of-home child care.

Detection of CMV

Urine samples were cultured in duplicate for CMV on monolayers of human foreskin fibroblast cells, as described previously (10). Cultures were observed for 4 weeks. CMV infection was established by detecting a CMV-specific cytopathic effect and by staining cell monolayers using indirect immunofluorescence and a CMV-specific monoclonal antibody (Microtrak; Syva Company, Palo Alto, California).

Analysis of CMV strains by PCR

Using modifications of methods described previously (13, 14), we extracted and amplified viral genomic and cellular DNAs by PCR, using primers for the phosphoprotein 65, a-sequence, glycoprotein B, and major immediate early (MIE) genes of human CMV (13-17). PCR products were characterized in agarose or polyacrylamide gels. DNA from the Towne strain of human CMV (courtesy of Dr. Mark Sutski, The University of Iowa, Iowa City, Iowa) was used as a positive control for PCR analysis, and DNA extracted from uninfected human foreskin fibroblast cells (Advanced Biotechnologies, Columbia, Maryland) was used as the negative control.

CMV strains were assigned a three-part descriptor derived from the a-sequence product size (designated A-H), glycoprotein B genotype (based on the schema of Chou and Dennison (17) and designated I-V), and MIE pattern (based on the restriction fragment length polymorphisms (RFLPs) and designated A-P). Strains with indistinguishable genotypes for all three gene regions were analyzed further by determining the RFLPs of the a-sequence product. RFLPs of the Hinfl and HaeIII digests of the MIE gene products were compared by computer using the Bio Image Whole Band Analyzer (Millipore, Ann Arbor, Michigan).

Strains were considered distinct if they exhibited differences in the size of the a-sequence PCR product, the glycoprotein B pattern, or the RFLPs for MIE or a-sequence gene regions. Conversely, CMV strains were considered indistinguishable if the a-sequence product size, glycoprotein B pattern, and RFLPs were identical.

Statistical analysis

Odds ratio estimates and 95 percent confidence intervals relating risk factors of interest to CMV case-control status were calculated on the basis of conditional logistic regression models. Because of the relatively small sample size in this study, all estimates and p values were obtained by using the exact methods available in LogXact (Cytel Software Corporation, Cambridge, Massachusetts, 1993). The methods included a conditional logistic regression analysis that accounted for matching conditions.
RESULTS

Incidence of congenital CMV Infection

During the study interval, 7,229 infants were cultured successfully for CMV, and 35 of those were positive, corresponding to a congenital infection rate of 0.48 percent. The hospitals at which these infants were born served a predominately white (94 percent), middle-class, semiurban population of approximately 250,000. The study successfully cultured 24.81 percent of the 29,134 liveborn infants delivered at the four hospitals from October 1989 through June 1994. All available infant-mother pairs were invited to participate, and fewer than 10 mothers declined. Because of shortened hospital stays, infants and mothers were frequently not available when the study team was at each site, or no urine was available for culture before infants were discharged from the hospital. Information on the baseline CMV serostatus of mothers prior to pregnancy was not available.

CMV-infected infants were detected at each study site (15 at site 1, four at site 2, eight at site 3, and eight at site 4). The parents of these infants resided in 17 eastern Iowa communities that ranged in population from approximately 500 to 150,000 (figure 1). The parents of 18 infants (51 percent) lived in Cedar Rapids, Iowa City, or the contiguous cities of Marion and Coralville, an urban area with a combined population of approximately 200,000. The annual incidence of congenital infection per site is summarized in table 1. The greatest number of CMV-infected infants was detected in March and December (six each), corresponding to incidence rates of 0.73 and 1.4 percent, respectively.

Identification of maternal risk factors for infection

To identify risk factors for delivering a congenitally infected infant, we compared detailed demographic and occupational data regarding the mothers of the 35 cases with the data for the mothers of the 105 controls. All control infants were born within 3 days of their respective cases. Univariate analysis (table 2) showed that women who delivered congenitally infected infants were more likely to be students (odds ratio (OR) = 5.01, p = 0.017), employed in sales occupations (OR = 4.93, p = 0.008), single (OR = 3.05, p = 0.016), or younger (OR for 5-year decrease = 1.57, p = 0.017).

By contrast, women who worked in health-care professions (as a physician, nurse, nurse’s aide, or laboratory technician) collectively had a reduced risk of delivering a congenitally infected infant (OR = 0.14,
However, analysis of certain occupations involving potential child contact suggested a possible effect on risk. The odds ratios were increased for women who worked as child-care providers (OR = 2.12, p = 0.223) or teachers (OR = 7.24, p = 0.172), although neither of these exposures achieved significance for the study population. Combining child-care providers and teachers into one risk factor was slightly more suggestive of a risk (OR = 2.36, p = 0.110).

Multivariate regression analysis was performed to determine whether the effect of working in sales occupations was confounding or being confounded by other predictor variables. When adjustments were made for sales in two-factor models, being a student retained its significance (adjusted OR = 4.78, 95 percent CI 1.15–23.48, p = 0.029) as did a lower age (adjusted OR = 1.51, 95 percent CI 1.02–2.32, p = 0.039), whereas the factors of being single and working in the health-care professions were no longer predictive (adjusted OR = 2.38, 95 percent CI 0.91–6.49, p = 0.080; and OR = 0.18, 95 percent CI 0.00–1.2, p = 0.115, respectively). However, when adjustments were made for working in sales, the combined risk factor of being either a teacher or a child-care provider became significant (adjusted OR = 3.12, 95 percent CI 1.07–9.12, p = 0.036).

In all analyses performed with sales work and one other risk factor, the adjusted p value for sales remained significant, with an adjusted odds ratio of at least 3.86. When the occupations of sales worker, student, and teacher/child-care provider were included in a three-factor model predicting CMV status, sales work and student status remained significant (p = 0.004 and p = 0.040); teacher/child-care provider was nearly significant (p = 0.051). Adding age to this model resulted in sales remaining significant (p = 0.008) and teacher/child-care provider remaining nearly significant (p = 0.059). However, the factors student and age did not retain significance (p = 0.123 and p = 0.208, respectively). The reported number of sexual partners was not predictive of CMV status (p = 0.95, p = 1.0, p = 1.0, and p = 0.80, respectively, for the numbers of sexual partners in the preceding 6, 12,

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**TABLE 1.** Incidence (%) of congenital cytomegalovirus infection at the four participating hospitals, eastern Iowa, 1989-1994

<table>
<thead>
<tr>
<th>Year</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
<th>All sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1989</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td>42</td>
<td>5.0</td>
</tr>
<tr>
<td>1990</td>
<td>0.61</td>
<td>326</td>
<td>0</td>
<td>63</td>
<td>0.26</td>
</tr>
<tr>
<td>1991</td>
<td>1.05</td>
<td>570</td>
<td>0.45</td>
<td>445</td>
<td>0.60</td>
</tr>
<tr>
<td>1992</td>
<td>0.44</td>
<td>682</td>
<td>0</td>
<td>405</td>
<td>0.17</td>
</tr>
<tr>
<td>1993</td>
<td>0.64</td>
<td>468</td>
<td>0</td>
<td>264</td>
<td>0.19</td>
</tr>
<tr>
<td>1994</td>
<td>0.56</td>
<td>180</td>
<td>1.64</td>
<td>122</td>
<td>0</td>
</tr>
</tbody>
</table>

* n, number of infants successfully cultured for cytomegalovirus.

**TABLE 2.** Maternal factors and the risk of delivering an infant with congenital cytomegalovirus infection, eastern Iowa, 1989-1994

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR*</th>
<th>Exact 95% CI*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt;25 years</td>
<td>2.78</td>
<td>1.04–7.99</td>
<td>0.041</td>
</tr>
<tr>
<td>Age (5-year decrements)</td>
<td>1.57</td>
<td>1.08–2.37</td>
<td>0.017</td>
</tr>
<tr>
<td>Non-Caucasian</td>
<td>4.50</td>
<td>0.52–53.88</td>
<td>0.207</td>
</tr>
<tr>
<td>Not married</td>
<td>3.05</td>
<td>1.21–8.20</td>
<td>0.016</td>
</tr>
<tr>
<td>Student</td>
<td>5.01</td>
<td>1.29–23.31</td>
<td>0.017</td>
</tr>
<tr>
<td>No college education</td>
<td>1.25</td>
<td>0.52–3.02</td>
<td>0.728</td>
</tr>
<tr>
<td>Exposure to children</td>
<td>1.20</td>
<td>0.55–2.75</td>
<td>0.767</td>
</tr>
<tr>
<td>Child-care provider</td>
<td>2.12</td>
<td>0.67–6.48</td>
<td>0.223</td>
</tr>
<tr>
<td>Teacher</td>
<td>7.24</td>
<td>0.55–392.91</td>
<td>0.172</td>
</tr>
<tr>
<td>Child-care provider or teacher</td>
<td>2.36</td>
<td>0.84–8.56</td>
<td>0.110</td>
</tr>
<tr>
<td>Physician or nurse</td>
<td>0.24</td>
<td>0.01–2.11</td>
<td>0.316</td>
</tr>
<tr>
<td>Nurse’s aide or nursing student</td>
<td>0.57</td>
<td>0.00–4.54</td>
<td>0.633</td>
</tr>
<tr>
<td>Laboratory technician</td>
<td>0.67</td>
<td>0.00–6.32</td>
<td>0.750</td>
</tr>
<tr>
<td>Physician, nurse, nurse’s aide, nursing student, or laboratory technician</td>
<td>0.14</td>
<td>0.00–0.99</td>
<td>0.049</td>
</tr>
<tr>
<td>Manager or administrator</td>
<td>0.40</td>
<td>0.01–3.49</td>
<td>0.712</td>
</tr>
<tr>
<td>Clerical worker</td>
<td>1.08</td>
<td>0.35–3.01</td>
<td>1.000</td>
</tr>
<tr>
<td>Salesperson</td>
<td>4.93</td>
<td>1.47–18.86</td>
<td>0.008</td>
</tr>
<tr>
<td>Service provider</td>
<td>1.90</td>
<td>0.75–4.80</td>
<td>0.196</td>
</tr>
<tr>
<td>Homemaker</td>
<td>0.58</td>
<td>0.16–1.74</td>
<td>0.427</td>
</tr>
</tbody>
</table>

* OR, odds ratio; CI, confidence interval.
18, and 24 months) and was therefore not considered a potential confounder of the effect of sales.

Analysis of CMV strains

Analysis of CMV strains through the three gene regions identified 27 distinct CMV genotypes. Analysis of the MIE region, the most informative, yielded 15 different genetic polymorphisms among the 33 strains that could be amplified (figure 2). Phylogenetic analysis of the MIE RFLPs revealed that although several MIE genotypes were present, 28 of the 33 (85 percent) typable strains were >76 percent homologous in their HIinfI RFLPs (figure 3). A-sequence analysis was also informative and identified seven pattern groupings; group E (product size, 165–174 base pairs) contained isolates from the largest number of infants (n = 8). However, CMV strains from 10 infants did not yield a-sequence products, a phenomenon observed previously among CMV strains from the same geographic region (13). DNA from the Towne strain of CMV had a genotype distinct from that of any of the CMV strains isolated from congenitally infected infants.

The glycoprotein B genotype for all but one CMV strain could be grouped according to Chou and Dennison's schema (17). The distribution was genotype 1, 49 percent; genotype 2, 11 percent; genotype 3, 34 percent; and genotype 4, none, a distribution similar to that identified among strains in child-care environments in Cedar Rapids–Iowa City (14). One strain, designated group V, had a unique genotype distinct from prior patterns. Another isolate had a glycoprotein B RFLP compatible with a mixture of two CMV strains.

Four groups of CMV isolates containing two infants each had similar CMV genotypes for the three CMV gene regions. To enable further analysis of these strains, the a-sequence PCR products were digested with restriction enzymes MnlI and BssHII. Two pairs of isolates each exhibited different RFLPs for the a-sequence product, indicating distinct strains, whereas two pairs each had indistinguishable RFLPs. Thus, two groups, with two infants in each group, had CMV strains with indistinguishable molecular genotypes, features compatible with point source acquisition. Two additional groups, one with four isolates and one with two isolates, had indistinguishable glycoprotein B and MIE genotypes but lacked a-sequence products, precluding further genetic comparisons.

Analysis of CMV strain clusters

Detailed epidemiologic data were available for one pair of infants who excreted indistinguishable CMV strains. The parents of these infants resided in Cedar Rapids, and both infants were born at hospital 4 in 1991 (on April 25 and December 4). Both infants had older siblings who were toddler-aged (11–30 months) and had concurrently attended the same child-care center in Cedar Rapids during their mothers' pregnancies.

![FIGURE 2. A lane map of the restriction fragment length polymorphisms (RFLPs) of cytomegalovirus products digested with the restriction enzyme HIinfI. Vertical numbers indicate the band size in base pairs of DNA, and lanes 1–14 indicate distinct RFLP patterns. The numbers beneath each lane indicate the numbers(s) of cytomegalovirus strains exhibiting the RFLP pattern. T, Towne CMV strain.](https://academic.oup.com/aje/article-abstract/147/10/940/134896)
Although CMV was not isolated from the siblings, CMV strains had been isolated during child-care studies of other children who attended the center concurrently with the siblings. Comparison of the strains from the congenitally infected infants and two unrelated children in the child-care center revealed indistinguishable genotypes for glycoprotein B, a-RNA, and the HaeIII RFLP of the MIE product (data not shown). The HinfI digest of the MIE product had only a two-band difference, indicating that these CMV strains had very closely related molecular profiles.

Partial epidemiologic information was available for the other pair of infants with indistinguishable CMV isolates. Both sets of parents resided in Cedar Rapids, and the infants were born at hospitals 3 and 4 in 1992 and 1993, respectively. The mother of the infant born in 1993 worked in a child-care center during her pregnancy, but it was not known whether she had contact with the other infant or with children who might have been excreting the same CMV strain. Detailed information was not available on the other infant’s family.

DISCUSSION

This study investigated prospectively the epidemiology of congenital CMV infection and assessed certain occupational factors that might potentially influence the risk of giving birth to a congenitally infected infant. Results indicated that women who were single, students, younger, or employed in sales had an increased risk of delivering congenitally infected infants. Being a teacher or a child-care provider might also have been a risk factor. Conversely, employment in health-care professions appeared to have a protective effect on the potential for delivering an infant with congenital CMV infection. Because information regarding maternal serostatus prior to pregnancy was not known, this study may have assessed factors influencing maternal-fetal transmission of CMV as well as maternal infection.

The observation that the risk of delivering a congenitally infected infant was increased among single women and students may reflect several factors, including young age, susceptibility to CMV (i.e., low rates of CMV seropositivity), or sexual activity.
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Studies in other geographic locations have indicated that young, sexually active women have high rates of primary or recurrent CMV infection (18) and thus have an increased potential for delivering CMV-infected infants. Our prior studies indicated that approximately 60 percent of the women of child-bearing age in Cedar Rapids—Iowa City were susceptible to CMV infection (7, 10). Age less than 25 years and decreasing age in 5-year decrements were associated with an increased risk of delivering a congenitally infected infant, whereas the number of sexual partners during the preceding 24 months was not predictive in the current study.

The association between employment in sales and delivering a congenitally infected infant was unexpected. Although employment in sales, common among young women, could be a confounder for being young or single, this occupation had a strong independent association with giving birth to a congenitally infected infant. Women working in sales did not report higher rates of contact with young children (p = 0.60) or greater exposure to multiple sexual partners (p = 0.26), both of which are potential risk factors for CMV infection. We speculate that employment in sales may have been a marker for socioeconomic status, a factor that may influence the risk of delivering a congenitally infected infant (4).

Univariate analysis suggested that employment in health-care occupations was associated with a reduced risk of delivering a congenitally infected infant and thus appeared to have a protective effect. Prior seroepidemiologic studies have indicated that the risk of primary CMV infection among health-care personnel does not exceed that in the general population (2), although a meta-analysis suggested that a risk might still exist (19). The current study suggests that women in health-care professions may have a reduced risk of delivering congenitally infected offspring. Discrepancies between this and prior studies regarding CMV acquisition among health-care workers may be attributable, in part, to the timing of studies relative to the adoption of standard (universal) precautions.

A reduced risk of congenital infection among the offspring of women who work in health-care occupations could reflect several factors, including enhanced recognition of hygiene, training in standard precautions, or other behaviors that reduce infection risk. Such women are educated about disease transmission and routinely practice hygienic behaviors, such as hand-washing, designed to reduce the transmission of CMV and other pathogens in hospital environments. It is not known, however, whether women with health-care backgrounds are more likely to practice protective hygiene at home or in other settings in which they might be exposed to CMV.

Molecular characterization of the CMVs isolated from the infants indicated that many distinct strains caused congenital infection in eastern Iowa, an observation compatible with abundant sources of CMV. Phylogenetic analysis suggested that CMV strains in eastern Iowa may have common evolutionary origins, at least with respect to the MIE genotype. Molecular analysis also indicated that CMV strains of diverse genotypes can be transmitted vertically and cause congenital infection. Isolates representing three of the four major glycoprotein B genotypes, for example, were identified among these congenitally infected infants. Because viral glycoproteins, especially glycoprotein B, play a major role in eliciting human immune responses to CMV (20), these viral polymorphisms must be considered in the development of effective CMV vaccines.

A major objective of this study was to identify strain patterns that might indicate point source acquisition of CMV. Two pairs of infants excreted CMV strains with indistinguishable molecular profiles, and the epidemiologic and molecular data strongly suggested that a single child-care center was the source of CMV for one pair of these infants. This observation confirmed that child-care centers contribute to the transmission of CMV strains causing congenital infection. However, the diversity of CMV genotypes observed in this study also argues against major point source outbreaks of congenital CMV infection.

In summary, this study indicated that certain occupational or environmental factors influence the risk of delivering infants with congenital CMV infection. This study emphasized the utility of PCR-based methods in tracking potential transmission of CMV within communities. Detecting clusters of CMV strains among congenitally infected infants in eastern Iowa confirmed that some mothers acquired CMV from community sources. This observation has potential implications for CMV seronegative women whose young children attend child-care centers.

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