Cytomegalovirus Viruria and DNAemia in Healthy Seropositive Women

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Viruria and DNAemia patterns were investigated in 205 seroimmune women enrolled in a prospective cytomegalovirus (CMV) reinfection study. CMV DNA was detected at least once in urine and blood specimens from 83% and 52% of patients, respectively. At baseline, 39% of patients had viruria, and 24% had DNAemia. Intermittent viruria and viremia was observed throughout the study. There were no differences in baseline CMV positivity by polymerase chain reaction or in longitudinal DNAemia and viruria between the women with and without serological evidence of reinfection. In young seropositive women, CMV DNAemia and viruria are common, which suggests that naturally acquired immunity to CMV does not alter shedding patterns.

Cytomegalovirus (CMV) is a frequent cause of congenital infection and an important opportunistic pathogen in immunocompromised individuals. The virologic characteristics of primary CMV infection have been described in a small number of healthy individuals. CMV shedding in urine, saliva, and vaginal secretions and CMV DNA (DNAemia) in peripheral blood, as assessed by qualitative polymerase chain reaction (PCR), have been observed in most individuals after CMV seroconversion. However, the DNAemia became undetectable within a few months after primary infection when patients were followed up for at least 1 year [1, 2]. CMV is shed in the urine for ≥6 months after seroconversion; thereafter, viruria becomes intermittent. However, the virologic characteristics of CMV infection in seroimmune women (ie, nonprimary infection), especially in those with frequent CMV reinfections, are not known.

Most sequelae associated with congenital CMV infection are thought to result from primary maternal CMV infection during pregnancy. Early reports by Ahlfors et al [3, 4] suggested that congenitally infected children born to women with preexisting CMV immunity are also at significant risk of adverse neurodevelopmental sequelae. More recent studies have confirmed these observations and shown that congenital CMV infection after nonprimary maternal infection contributes significantly to CMV-associated morbidity [5–7]. Therefore, vaccine strategies aimed at prevention of primary maternal infection to reduce the morbidity associated with congenital CMV infection will be of limited value, especially in highly seropositive populations. Although the mechanisms and the pathogenesis of intrauterine transmission and severe fetal infection in the presence of preexisting maternal immunity are unknown, an analysis of CMV strain–specific antibody responses revealed an association between intrauterine transmission of CMV and reinfection with new or different virus strains in seroimmune women [8, 9]. Knowledge of the virologic characteristics in women seroimmune to CMV infection is important not only for a better understanding of the natural history and pathogenesis of this chronic viral infection but also for designing strategies to prevent or reduce sequelae associated with congenital CMV infection. In the present study, we examined viruria and peripheral blood DNAemia in a cohort of seropositive women enrolled in a prospective study of CMV reinfection.

Methods. The study population consisted of 205 healthy CMV-seropositive women who participated in a longitudinal study of CMV reinfection. Women were recruited from the postpartum ward at the University of Alabama Hospital (Birmingham) and were derived from a predominantly urban, low-income, black population. The mean age of the study women was 18 years, and the majority of women were unmarried and had 1 previous pregnancy [10]. Study participants were followed up at 6-month intervals with a goal follow-up period of 3 years. At each study visit, urine and blood samples were obtained. The first urine and/or blood specimen was obtained from the study women at a mean (± standard deviation) of 81 ± 48.7 days after delivery. The study specimens consisted

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of 814 urine and 800 peripheral blood samples. Approximately one-third (59 [29%] of 205) of study participants were noted to have CMV reinfection on the basis of the appearance of strain-specific antibody responses during follow-up [10]. Informed consent was obtained from all study participants, and the study was conducted in accordance with the guidelines of the Institutional Review Board for Human Use of the University of Alabama at Birmingham.

Urine and peripheral blood specimens were processed within 24 h after collection, and DNA was extracted using a commercial spin column kit (Qiagen). Each extraction run included a negative control. The presence and the amount of CMV DNA was assessed using a real-time PCR assay with an ABI 7500 Sequence Detection System (Applied Biosystems) and Absolute Low ROX QPCR mix (ABGene), as described elsewhere [11]. Each PCR run included plasmid standards incorporating the target regions of CMV gB and IE-2 to generate standard curves. CMV burden in whole blood was expressed as CMV genomic equivalents (ge) per milliliter. The sensitivity of the assay was determined using 10-fold serial dilution of known quantities of the AD169 strain DNA to be ~250 ge per 1 mL of blood [11].

Results. The study women were followed up for a median duration of 30.3 months (range, 6–58 months), and the median number of follow-up visits was 5 (range, 2–7 visits). The median number of study visits during which urine and blood specimens were positive by PCR was 2 (range, 1–5 visits) and 1 (range, 1–5 visits), respectively. Figure 1A shows the proportion of patients with urine and blood samples positive by PCR at each study visit. An analysis of serial specimens collected from the participants during the study showed that most study women (171 [83%] of 205) had at least 1 CMV-positive urine specimen, and approximately half (105 [52%] of 204) had at least 1 CMV-positive blood sample (Table 1). At study entry, 59 (39%) of 150 and 36 (24%) of 150 participants had CMV-positive urine and blood specimens by PCR, respectively. During subsequent visits (visits 2–6), results of PCR of blood and urine specimens were positive for 11%–19% and 39%–45% of participants, respectively (Figure 1A). Among women who completed at least 2 years of follow-up (5 study visits), the frequency of positive PCR results during visits 1–5 was 10%–43% and 49%–69% for blood and urine specimens, respectively. The median peak viral load was 3.9 × 10^7 ge/mL (range, 4.4 × 10^7 to 8.8 × 10^7 ge/mL) in urine samples and 5.2 × 10^3 ge/mL (range, 1.0 × 10^3 to 2.3 × 10^8 ge/mL) in blood samples.

Of the 205 participants, 59 (29%) of 205 had serological evidence of reinfection during the course of the study [10]. In the group with reinfection, 49 (83%) of 59 and 32 (54%) of 59 had CMV-positive urine and blood samples, respectively, by PCR at ≥1 visit; these findings were similar to those observed in women without reinfection. An analysis of specimens collected during the visit immediately before reinfection showed that approximately one-fifth of blood specimens were positive by PCR, and one-third of urine specimens were positive by PCR. The rate of blood PCR positivity steadily decreased to ~10% for the subsequent visits, and viruria was present in a

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Table 1. Cytomegalovirus Polymerase Chain Reaction (PCR) Positivity and Visit Distribution in Blood and Urine Compartments of 205 Healthy, Seropositive Women

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Blood</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR positivity</td>
<td>105 (61.5%)</td>
<td>171 (83.4%)</td>
</tr>
<tr>
<td>No. of positive visits, mean ± SD</td>
<td>1.4 ± 0.7</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td>Positive at &gt;1 visit</td>
<td>34/105 (32.3%)</td>
<td>96/171 (66.1%)</td>
</tr>
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</table>

**NOTE.** Data are no. (%) of participants, unless otherwise indicated. SD, standard deviation.

* 204 women.
higher proportion of patient during the remainder of the follow-up period (Figure 1B). Virologic characteristics in the group of 146 women without reinfection were similar to those in the 59 women with documented reinfection (data not shown).

Discussion. The virologic characteristics of primary CMV infection in healthy adults have been examined and have typically been described as several weeks of viremia with virus shedding in the urine for months intermittently [1, 2]. In the present study of young seropositive women, a similar pattern of intermittent virus shedding and DNAemia was seen. The majority of our study population had detectable CMV DNA in their urine at least once during the study, and approximately half of the women had detectable CMV DNA in peripheral blood at 1 of the study visits. No differences in the presence of CMV DNA in blood or urine samples were found between the group of women with and without serologic evidence of reinfection. These findings demonstrate that, in healthy young women known to be at increased risk of delivering a congenitally infected infant, CMV can be frequently detected in urine and blood.

Although intrauterine CMV transmission can occur even in the presence of preconceptional immunity, most of the newborn disease and CMV-related sequelae were thought to occur in infected infants born to mothers with primary CMV infection. Therefore, strategies to prevent morbidity associated with congenital CMV infection have been focused on prevention of primary maternal infection. However, the accumulation of data demonstrates that CMV-specific immunity resulting from naturally acquired infection does not prevent reinfection with new or different virus strains, and such reinfections have been associated with intrauterine transmission in seropositive women and with symptomatic congenital infection [6,8-10,12]. In addition, similar rates of hearing loss were observed in children with congenital CMV infection born to women with primary CMV infection and in those born to seroimmune mothers [6,7]. It has been suggested that CMV vaccines, although unlikely to prevent reinfection, could change the natural history of infection, resulting in shorter duration of viremia or viral shedding [1]. In the present study, 59 of the 205 seropositive women had serological evidence of reinfection with a new virus strain. There were no differences in baseline CMV positivity by PCR, timing of DNAemia and viruria, and peak viral load between study women with and without reinfection. At study entry, CMV DNAemia and viruria were present in approximately one-fifth and one-third of all study women, respectively. In addition, 8 (21%) of 38 study women had CMV DNA in peripheral blood samples obtained during the visit immediately before the documentation of reinfection. This was followed by a peak of 36% (15 of 42 women) during the visit at which serological evidence of reinfection was captured. Subsequently, there was a decrease in DNAemia; however, intermittent CMV shedding in urine samples was observed throughout the study. This pattern of viral shedding is very similar to that in reports of virologic findings in primary CMV infections in populations with similar demographic characteristics [1]. Although it is not known whether intermittent DNAemia and viruria in the study population represent reactivation of previously acquired CMV or reinfection with new virus strains, our findings suggest that preexisting immunity to CMV neither prevents reinfection nor shortens the duration of viremia or viruria. This information could have important implications for CMV vaccine development and, in addition, provides baseline virologic data for an evaluation of interventional strategies to prevent or reduce morbidity associated with congenital CMV infection in highly seropositive populations.

Previous studies in populations with demographic characteristics similar to those of the current study participants showed that acquisition of CMV infection (primary infection or reinfection) occurs frequently and that these young women are at increased risk of delivering a congenitally infected infant [1, 10, 13]. A recent study showed that in this young, urban population, caring for young children and recent onset of sexual activity greatly increased the risk of having a child with congenital CMV infection [14]. In our recent study of CMV reinfection, we were unable to identify an association between exposures and the acquisition of a new strain of CMV [10]. However, more than two-thirds of the study population was involved in the direct care of young children, and the majority of women had multiple sexual partners—factors known to be associated with acquisition of CMV infection [14]. Our findings reveal that, in this population of young, low-income, black women, CMV circulates frequently, resulting in the high rate of viremia and viruria. Although the mechanisms for the increased risk of delivering an infant with congenital CMV infection are not known, the high rate of virus shedding could be an explanation for the higher prevalence of congenital CMV infection in this population. Our findings reinforce the suggestion for an urgent need for public health interventions in young, low-income, black populations because of the disproportionately higher burden of congenital CMV infection and disabilities associated with it [15]. However, the persistent virus shedding in seropositive women reveals the challenges in designing prevention strategies.

Because the study population is known to have an increased risk of primary CMV infection and reinfection, the results of this study may not be applicable to the general population of women. Another possible limitation of our study is that the study participants were followed up at 6-month intervals. Although CMV shedding in the urine has been shown to last at least 6 months in the majority of women with primary CMV infection, DNAemia is expected to last for a much shorter duration;
therefore, the number of women with viremia could have been underestimated. Because not all study participants completed 3 years of follow-up, the overall frequency of reinfection could have been underestimated in our study. The number of PCR-positive specimens in women who completed at least 2 years of follow-up was similar to that in the overall study group, arguing against the possibility that variable follow-up may have influenced the virologic findings.

In summary, this study of the virologic characteristics in young, seropositive women shows that viremia and viruria are common and that naturally acquired immunity to CMV, whether recently acquired or not, does not appear to alter shedding patterns, even when CMV infection due to a new virus strain has occurred.

References