Cytomegalovirus (CMV) reinfections have been associated with damaging congenital infection and adverse outcomes in transplant recipients. To determine the frequency of and risk factors for CMV reinfections, 205 seropositive women were followed up prospectively. The appearance of new antibody specificity against 1 of 4 polymorphic epitopes was considered as evidence of CMV reinfection. Approximately one-third of the study participants (59 [29%] of 205) were noted to have CMV reinfection during follow-up. None of the exposure factors were associated with CMV reinfection. Women with antibodies against at least 1 of the 4 antigens at baseline had a 63% decreased risk of reinfection, suggesting a protective role for strain-specific immunity.

Cytomegalovirus (CMV) is a frequent cause of congenital infection and an important cause of sensorineural hearing loss in children worldwide [1, 2]. Preconceptional immunity against CMV provides only incomplete protection against intrauterine transmission, and adverse outcomes can occur in infected children born to women who were seropositive prior to pregnancy [2–6]. CMV reinfections have also been associated with adverse outcomes in renal transplant recipients [7].

It is not clear whether transplacental transmission of CMV in women with preexisting seroimmunity is secondary to virus reactivation or to infection with a new or different CMV strain (reinfection) during pregnancy. We undertook a prospective study to determine the frequency of CMV reinfections in healthy seropositive women and to understand the various factors associated with such reinfections. Serial serum specimens from the study participants were analyzed for strain-specific immunoglobulin G (IgG) antibodies against the polymorphic determinants on the envelope glycoproteins gH and gB of CMV by means of an enzyme-linked immunosorbent assay (ELISA) method [3, 8].

Methods. Of the 258 CMV IgG-seropositive postpartum women enrolled in the study from February 2000 through June 2004, 205 participants had serum samples from at least 2 visits, and these women constituted the study population. A standardized interview was administered at baseline to obtain demographic characteristics and exposure factors. Standardized prenatal summary information was abstracted onto standard case report forms. The study participants were followed up at 6-month intervals for up to 3 years; at each visit, serum samples were obtained, and a standard questionnaire was administered to obtain an interval history of sexually transmitted infections (STIs), information on sexual partners, and information on child care. Serum specimens obtained at each visit were stored at −20°C until analysis. The study was approved by the institutional review board of the University of Alabama at Birmingham, and informed consent was obtained from the participants prior to study enrollment.

CMV strain–specific antibody responses were determined on the basis of polymorphisms in antibody binding sites within envelope glycoproteins gH and gB, between the 2 prototypical laboratory strains of CMV, AD169 and Towne [8–10]. The detection of new antibody specificities to either epitope (AD169 or Towne) on gH or gB in follow-up serum samples was considered evidence of infection with a new virus strain (reinfection) during the study. One of the 258 women had antibodies to all 4 antigens at enrollment in the study and was excluded from the analysis. To approximate the mean time from study entry until reinfection with a new virus strain, we measured the time from the baseline study visit to the visit during which new antibody specificities were detected.

Recombinant peptides containing antibody-combining sites within the amino terminal regions of the gH and gB genes present in the AD169 and Towne strains of CMV were synthesized and used as antigens to determine strain-specific IgG reactivity, as described elsewhere [3, 8]. Strain-specific antibodies against the polymorphic gH and gB regions of CMV were determined using an ELISA method that has been validated elsewhere [8].
Table 1. Selected Exposure and Demographic Characteristics for Cytomegalovirus (CMV)–Seropositive Women

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) of participants with CMV reinfection (n = 59)</th>
<th>No. (%) of participants without CMV reinfection (n = 146)</th>
<th>Unadjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White race</td>
<td>10 (16.9)</td>
<td>9 (6.2)</td>
<td>3.11 (1.05–9.15)</td>
</tr>
<tr>
<td>Maternal age &lt;19 years</td>
<td>42 (71.2)</td>
<td>103 (70.6)</td>
<td>1.03 (0.51–2.15)</td>
</tr>
<tr>
<td>More than 1 sexual partner</td>
<td>57 (96.6)</td>
<td>143 (98.6)*</td>
<td>0.39 (0.03–5.66)</td>
</tr>
<tr>
<td>Sexually transmitted infection</td>
<td>13 (22.0)</td>
<td>31 (21.2)</td>
<td>1.05 (0.46–2.28)</td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>6 (10.2)</td>
<td>24 (16.4)</td>
<td>0.58 (0.18–1.56)</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>3 (5.1)</td>
<td>6 (4.1)</td>
<td>1.25 (0.20–6.09)</td>
</tr>
<tr>
<td>Herpes</td>
<td>1 (1.7)</td>
<td>3 (2.1)</td>
<td>0.82 (0.01–10.49)</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>3 (5.1)</td>
<td>3 (2.1)</td>
<td>2.55 (0.33–19.64)</td>
</tr>
<tr>
<td>Syphilis</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>...</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>0 (0)</td>
<td>4 (2.7)</td>
<td>0 (0–2.75)</td>
</tr>
<tr>
<td>Direct care of children</td>
<td>41 (69.5)</td>
<td>95 (65.1)</td>
<td>1.22 (0.61–2.50)</td>
</tr>
<tr>
<td>More than 2 children &lt;6 years old living in household</td>
<td>16 (27.1)</td>
<td>34 (23.3)</td>
<td>1.23 (0.57–2.56)</td>
</tr>
</tbody>
</table>

**NOTE.** CMV reinfection was defined as the development of new antibody specificity against the polymorphic CMV gH and/or gB epitopes. CI, confidence interval; OR, odds ratio.

* Data were available for 145 participants.

The demographic and exposure characteristics were compared between women with CMV reinfection and those without CMV reinfection. Statistical significance was determined using the $\chi^2$, Fisher exact, or Wilcoxon rank sum test where appropriate. Univariate odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using the exact method. Multivariate unconditional logistic regression using backward stepwise selection with $P<.10$ was used as a cutoff for retention in the model to assess whether exposure factors were associated with CMV reinfection. All analyses were performed using SAS software (version 9.1; SAS Institute).

**Results.** The demographic characteristics of women with CMV reinfection were not different from those of women without evidence of reinfection. In both groups, the participants were predominantly unmarried, black women and had had 1 previous pregnancy. The mean age of the women in both groups was 18 years, and the study participants had a mean of 11 years of education. None of the study participants tested positive for human immunodeficiency virus (HIV). Twenty-nine percent of the study participants (59 of 205 participants) acquired new antibody specificities against gH or gB epitopes and thus were considered to be reinfected. The mean (± standard deviation) time until the appearance of new strain-specific antibodies was 17.8 ± 10.3 months. The median follow-up duration was 35.4 months (range, 11–50 months) for women with reinfection and 30.6 months (range, 6–58 months) for those without reinfection ($P = .15$). Forty-nine percent of the reinfection group completed 6 study visits, compared with 34% of those without reinfection ($P = .05$). A higher proportion of white women (10 [53%] of 19) than of black women (48 [26%] of 185) had serological evidence of reinfection ($P = .02$).

Baseline exposure characteristics for women with and women without CMV reinfection were similar. The median number of persons living in the household was 5 and 4 for women with and women without reinfection, respectively ($P = .72$). The median age of sexual debut was 15 years in both groups. There were no differences between the 2 groups with regard to the number of lifetime sexual partners (median, 3 partners) or the number of sexual partners in the year prior to study enrollment (median, 1 partner). Approximately one-half of the women in each group had a history of STI. The frequency of gonococcal infection was higher in the group of women without CMV reinfection (25%), compared with the group of women with CMV reinfection (12%; $P = .04$). Sixty-eight percent of the women with reinfection and 60% of those without reinfection were involved in the direct care of young children.

The data on various exposure factors encountered during the study were compared between the group of women identified to have been reinfected with new CMV strains and the group of those without reinfection (Table 1). Only white race was significantly associated with CMV reinfection. To further examine the association between potential risk factors and CMV reinfection, we evaluated exposure factors during the 12-month period prior to the detection of new antibody specificities in women with reinfection and in the year prior to the final study visit for those without reinfection. Again, none of the exposure factors were found to be associated with reinfection (data not shown).

Race, age, new sexual partners, and direct care of children in the year prior to reinfection or in the year prior to the final study visit were entered into a logistic regression model. Race remained the only factor associated with reinfection (adjusted OR, 3.11 [95% CI, 1.1–9.2]).

The association between serological responses to the 4 an-
tigens (AP86, TO86, AP55, and TO55) at baseline and the likelihood of CMV reinfection during the study period was determined. Reactivity to strain-specific epitopes was shown to persist for a mean of 21 months. As illustrated in Figure 1, women with antibodies against 1 or more antigens at baseline were less likely to be reinfected with a new CMV strain during the study period (OR, 0.37 [95% CI, 0.19–0.73]).

Discussion. The results of this prospective study demonstrate that approximately one-third of CMV seropositive women (59 of 205) were infected with a new or different CMV strain during the study period, as evidenced by the appearance of new antibody specificities against the linear polymorphic epitopes on gB and gH of CMV. The study participants were followed up for almost 3 years, and therefore the annualized rate of CMV reinfection was ~10%, a rate similar to the frequency of primary CMV infection in the general population [11]. The ELISA was adapted from a previously described radioimmunoassay to detect strain-specific antibodies against the envelope glycoprotein gH [3] and was validated in a recent study involving 96 seropositive and 51 seronegative individuals [8]. A similar strain-specific ELISA was employed in a recent study of CMV reinfections in renal transplant recipients [7]. However, approximately one-third of CMV-seropositive individuals in the previous study [8] and 46 of 204 women in the present study did not detect serum antibodies against any of the 4 antigens tested using this assay. This finding suggests that these women were infected with viruses containing gH and/or gB epitope variants that were not represented in the ELISA used in this study. Alternatively, our ELISA might lack the sensitivity to detect low levels of strain-specific antibodies in these women. Therefore, it could be argued that our study may have underestimated the actual frequency of CMV reinfections in the population. It is also possible that the appearance of new antibody specificities could be due to reactivation of endogenous virus. However, this is unlikely, because there are no data in the literature in support of this phenomenon and because the stability of CMV hypervariable genes has been shown in vitro in renal transplant recipients [12].

We were unable to identify an association between CMV reinfection and any of the known exposure factors for acquisition of CMV, including STIs, sexual practices, and caring for young children [13]. The demographic and baseline exposure characteristics were similar between the groups of women with and women without reinfection. Although more women without CMV reinfection had a history of gonorrhea at enrollment than did those with CMV reinfection, the number of women with gonorrhea was small, and thus this finding should be interpreted with caution. This study may have underestimated the number of STIs and sexual partners of participants in the population, because this information was obtained through interval questionnaires that relied on participant recall. To minimize recall bias, the study participants were interviewed individually at each visit, using a standardized questionnaire.

Prenatal medical records were reviewed at enrollment for the results of laboratory studies and the dates of STIs. The smaller sample size and the fact that both groups in the study population had similar demographic and exposure characteristics may have led to our inability to identify an association between any of the exposure factors and CMV reinfection.

We did observe that women with a more broadly reactive antibody response at baseline were less likely to be reinfected during the study. Women with strain-specific antibodies to at least 1 antigen at baseline had a 63% decreased risk of CMV reinfection during the study period (OR, 0.37 [95% CI, 0.19–0.73]), compared with participants who had no antibodies against any of the 4 antigens. This reduced risk of reinfection in women with antibodies to at least 1 antigen indicates that individuals infected with multiple CMV strains prior to study entry were less likely to be reinfected and that strain-specific immunity may play an important protective role against infection with new virus strains in seropositive individuals. A recent study of a recombinant CMV gB vaccine suggested that prevention of maternal infection and of intrauterine transmission to offspring of previously nonimmune women could represent a feasible approach [14]. However, other studies have revealed that serum samples from individuals with natural infection produce higher neutralizing antibody titers and higher titers against epithelial cell entry than do serum samples from recipients of Towne or gB/MF59 vaccine [15]. This could be because individuals with natural infection are more likely to develop an antibody response against multiple CMV strains, whereas vaccines may induce antibody responses with only narrow specificity. Therefore, in populations with high maternal seroprevalence, the success of traditional vaccination approaches in
reducing intrauterine CMV transmission and CMV disease in congenitally infected children may be limited.

In the present study, we observed a higher prevalence of reinfection among white women (10 of 20 white women experienced reinfection). However, the number of white participants in our study was small, and therefore this association could be due to sampling bias. In addition, when the exposure factors were examined for white and black women independently, we did not detect differences between women with CMV reinfection and those without reinfection in either racial group. Because the group of women with CMV reinfection was followed up longer than was the group of women without serological evidence of reinfection, it is possible that more women in the group without reinfection could have been observed to acquire new antibody specificities if they had been monitored for a longer duration. However, this is unlikely to have had an effect on the lack of an association between various exposure factors and CMV reinfection, because the demographic and exposure characteristics of the 2 groups were similar.

In summary, the results of this study demonstrate that CMV reinfections are frequent in young, low-income, black seroimmune women. Our findings also suggest that, in addition to exposure, strain-specific immunity and possibly other as-yet undefined factors may play an important role in providing protection from infection with new CMV strains in seroimmune individuals.

References
2. Mussi-Pinhata MM, Yamamoto AY, Moura Brito RM, et al. Birth prev-

3. Boppana SB, Rivera LB, Fowler KB, Mach M, Britt WJ. Intrauterine transmission of cytomegalovirus to infants of women with preconcep-
10. Urban M, Britt W, Mach M. The dominant linear neutralizing anti-
11. Colugnati FA, Staras SA, Dollard SC, Cannon MJ. Incidence of cy-
tomegalovirus infection among the general population and pregnant women in the United States. BMC Infect Dis 2007; 7:71.
13. Fowler KB, Pass RF. Risk factors for congenital cytomegalovirus in-