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Human cytomegalovirus reinfection is associated with intrauterine transmission in a highly cytomegalovirus-immune maternal population

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Abstract

OBJECTIVE: To determine contribution of reinfection with new strains of cytomegalovirus in cytomegalovirus seromimmune women to incidence of congenital cytomegalovirus infection.

STUDY DESIGN: In 7848 women studied prospectively for congenital cytomegalovirus infection from a population with near universal cytomegalovirus seroimmunity, sera from 40 mothers of congenitally infected infants and 109 mothers of uninfected newborns were analyzed for strain-specific anticytomegalovirus antibodies.

RESULTS: All women were cytomegalovirus seroimmune at first prenatal visit. Reactivity for 2 cytomegalovirus strains was found in 14 of 40 study mothers and in 17 of 109 control mothers at first prenatal visit ($P = .009$). Seven of 40 (17.5%) study women and 5 of 109 (4.6%) controls ($P = .002$) acquired antibodies reactive with new cytomegalovirus strains during pregnancy. Evidence of infection with more than 1 strain of cytomegalovirus before or during current pregnancy occurred in 21 of 40 study mothers and 22 of 109 controls ($P < .0001$).

CONCLUSION: Maternal reinfection by new strains of cytomegalovirus is a major source of congenital infection in this population.

Keywords

congenital cytomegalovirus infection; cytomegalovirus reinfection; maternal reinfection with cytomegalovirus

Human cytomegalovirus (CMV) is the most common viral infection transmitted to the developing fetus with rates of infection ranging from 0.2-2.0% of live births.[1,2] Importantly, congenital CMV infection is a major cause of sensorineural hearing loss in infants and children.[3–8] Studies of prophylactic vaccines have suggested that prevention of transmission

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to offspring of previously non-immune women could be effective.[9] However, findings from studies in maternal population with high CMV seroprevalence have demonstrated that intrauterine infection and disease occurs not infrequently in the offspring of women with existing immunity, so called nonprimary infections.[2,7,8,10–12] Thus, preconceptional immunity against CMV provides only partial protection against congenital infection[12] and in maternal populations with high CMV seroprevalence, most congenital CMV infections follow nonprimary maternal infections.[10,12–14] Studies from Brazil, the Ivory Coast, India, as well as urban African American populations in the United States, have demonstrated a direct relationship between maternal CMV seroprevalence and the incidence of congenital CMV infection.[7,8,11,15–17] Proposed mechanisms for nonprimary maternal infections include reactivation of an existing persistent infection or reinfection with new strain of CMV. Only inferential evidence supports the first mechanism; however, this mechanism is consistent with lifelong persistence of CMV infection. Thus, reactivations from latency or a chronic infection could result in recurrent infections in previously infected women. Alternatively, reinfections with new strains of CMV have been documented in immunocompetent and immunocompromised patients.[18–20] Mechanisms leading to intrauterine CMV transmission and congenital infection remain undefined in maternal populations in the developing world with seroprevalences approaching 100%. Because infection with more than 1 CMV strain in immunocompetent pregnant women can lead to fetal damage, reinfection could contribute significantly to the natural history of congenital CMV infections.[21]

In the current study, we analyzed serum samples obtained at the initiation of prenatal care and at delivery from women prospectively enrolled in a study of congenital CMV infections in a highly seroimmune maternal population.[17,22] Women delivering congenitally infected infants and control women delivering uninfected infants from the same population were studied for CMV strain-specific serological responses to determine the contribution of maternal reinfection during pregnancy to congenital CMV infection in this population with near universal preconceptional CMV seroimmunity.

Materials and Methods

Study population and design

Forty mothers of infants with congenital CMV infection and 109 mothers of uninfected infants were enrolled in the study. These subjects were selected from 7848 mothers of 8047 infants born at 2 maternity hospitals in the municipality of Ribeirão Preto, Brazil, whose infants were screened (85% all live births) for congenital CMV infection (1.1% rate of congenital CMV infection).[8,17] Among 84 mothers of 87 infants (3 twins) who were identified with congenital CMV infection, 58 (69%) were residents and received prenatal care in Ribeirão Preto. Of these 58 women, 40 (74%) had prenatal serum specimens stored in a central repository and represented the study population. The control mothers were selected from women delivering uninfected infants at the same hospital, residents of Ribeirão Preto, matched for gestational age of their newborn infants, and had prenatal serum specimen stored in the central repository. The study and control population were derived from a maternal population with an overall CMV seroprevalence of 96%, thus it was not unexpected that all the women in this study were seropositive for CMV at entry into the

study. The study protocol was approved by the Research Ethics Committee of the University Hospital (processes no. 4782/2002 and 9145/2003).

Diagnosis of congenital infection was based on the detection of CMV DNA in saliva and/or urine samples by polymerase chain reaction (PCR) and confirmed by virus isolation from 2 urine and/or saliva samples collected before 3 weeks of life.[23,24] Infants with clinical findings, including petechiae, purpura, jaundice with direct bilirubin >2 mg/dL, hepatosplenomegaly, microcephaly, and chorioretinitis within the first 15 days of life were classified as having a symptomatic congenital CMV infection.[24]

Determination of maternal CMV serostatus

Sequential serum specimens (first prenatal visit and at delivery) from mothers were assayed for anti-CMV IgG antibodies by a conventional ELISA and anti-CMV IgG avidity indicies were determined in all prenatal serum specimens (VIDAS CMV IgG Avidity, Biomérieux, France).[25–27] An IgG avidity index of >80% is strongly suggestive of an infection that occurred at least 12 weeks earlier; however, the original data indicated that an avidity index of as low as 73% excluded 93% of CMV infections of <12 weeks' duration.[27]

Maternal CMV strain-specific serologic responses

Sequentially obtained maternal samples were tested for CMV strain-specific serologic responses based on the polymorphism within an antibody binding site on glycoprotein H (gH) between 2 prototypic laboratory strains of CMV, AD169 (gH-AP86) and Towne (gH-TO86), and a second polymorphic site for antibody reactivity on glycoprotein B (gB) that has been defined on AD169 (gB-AD54) and Towne (gB-TO54) virus strains.[21,28] Both antibody binding sites are defined by a linear sequence of amino acids.[21,29] As there is no known linkage between serologic reactivity against linear epitopes on gH and gB, 7 different patterns of antibody reactivities are possible for each study participant, including lack of recognition of the gH or gB-specific serologic determinants (Figure). Reactivity for both polymorphic antigenic sites on gH or gB indicated exposure to >1 strain of virus. The detection of new antibody reactivity to either epitope on gH or gB in delivery serum samples of seropositive women was considered as seroconversion and infection with a new virus strain (reinfection) during pregnancy.

CMV strain-specific ELISA

This assay is described in a recent report and uses recombinant peptides encoding the AD169 gH or the Towne gH and by the AD169 gB or the Towne gB antigens.[28,30] The N-terminal region of gH was expressed as beta-galactosidase fusion protein in *Escherichia coli.*[21,29] A 106 amino acid fragment from the aminoterminal region of gB was his-tagged by cloning into pET21a(+) (EMD, Gibbstown, NJ) vector, expressed in *E. coli,* and purified using TALON Super flow columns (Clonetech, Mountain View, CA). A positive control used the highly conserved and dominant antigenic domain (AD-1) from gB cloned into both vectors.[31–34] Reactivity against empty vectors expressing fusion protein alone or unrelated proteins of mouse origin were used as negative controls. A positive result was defined as 3 standard deviations (SD) above the OD value obtained from serum from a CMV seronegative individual.

Sequence analysis of viruses recovered from infected infants

CMV DNA was extracted from peripheral blood, saliva, and urine from infected infants as described.[23,35] Viral DNA was amplified (Fusion; New England Biolabs, Beverly, MA) using primers to amplify a 460 base pair (bp) product from the 5′ end of the UL75 orf (gH) (nucleotides 110,603-111,063) or a 300-bp product from the UL55 orf (gB) (nucleotides 84,117-84,423, AD169). Gel-purified amplimers were sequenced directly or in some cases cloned into the pCRBlunt vector (Invitrogen, Carlsbad, CA) and propagated in *E. coli.* Approximately 10-12 clones were selected and plasmid DNA sequenced. Nucleotide sequences were analyzed using Vector NTi software (Invitrogen).

Statistical analysis

Statistical analysis was performed using the EPI INFO software program, v. 6.4 (Center for Disease Prevention and Control). The proportion of strain-specific serologic responses to different epitopes in study and control groups were compared using χ^2 test or Fisher's exact test.

Results

Mothers of infected and uninfected infants did not differ in age (median, 20 vs 22 years), years of formal education (median, 8 years vs 9 years), exposure to children <2 years of age (14/40 vs 23/109), age of sexual debut (median, 15 vs 16 years), or number of sexual partners (median, 2). When exposure to young children was extended to include children ≤3 years, significantly more mothers of infected infants cared for young children (23/40 vs 37/109; $P = .01$).

The median gestational age at which the prenatal sample was obtained for study and control women was 13 weeks (range, 4–27 weeks). The median interval between prenatal and delivery serum specimens was 24 weeks (range, 8–32 weeks) in both groups. Serum from the first prenatal visit from all 40 mothers of infected offspring and 109 control mothers contained CMV IgG antibodies, a finding consistent with the CMV seroprevalence of this population.[8,17] Anti-CMV IgG antibodies of high avidity index could be demonstrated in serum specimens from women in the study group (median, 96%; range, 74–100%) and the control group (median, 94%; range, 76–100%).

CMV strain-specific antibody responses to gH and gB epitopes in the serum samples obtained during pregnancy

The strain-specific response to each CMV epitope on gH, gB, and combinations of reactivity at first prenatal visit and at delivery of mothers of infected infants and control mothers are shown in Table 1. Reactivity to at least 1 CMV polymorphic site on gH or gB was observed in the serum specimens obtained during pregnancy in all but 1 of the 40 women who delivered congenitally infected infants (97.5%) but in only 84 of 109 (77%) mothers of non-infected infants ($P = .003$).

Analysis of prenatal sera revealed that infection with 2 or more CMV strains was more frequent in mothers of infected infants than in controls (35% vs 15.6%; $P = .009$; Table 2).

Similarly, reinfection during pregnancy as evidenced by acquisition of antibody reactivity at delivery was more frequent in mothers of infected infants (7/40; 17.5%) as compared with control mothers (5/109; 4.6%; $P = .02$; Table 2). Because the median interval of observation in these women was 24 weeks, these rates represented an annualized rate of reinfection of 35% and 9% in the study and control groups, respectively. When the results from prenatal and delivery serum were combined, a higher proportion of mothers of infected infants had evidence of infection with >1 CMV strain in the past or in the current pregnancy than controls (52.5% vs 20%; $P < .0001$; Table 2). All infected infants of mothers with serologic evidence of reinfection during pregnancy were asymptomatic at birth. Among the infants born to 21 mothers with serologic evidence of infection with more than 1 CMV strain before pregnancy, 1 infant (1/21; 5%) had symptomatic congenital CMV infection.[24]

Sequence analysis of viruses from infants with congenital CMV infection

CMV DNA from blood, saliva, or urine collected from infected infants during the perinatal period was analyzed for the polymorphic regions of gH and gB by nucleotide sequencing of the respective viral genes (UL75, UL55). Of the 7 infants born to seroimmune women with evidence of reinfection by a new CMV strain during pregnancy, viral DNAs isolated from 6 (6/7; 86%) infants were shown to contain sequences encoding antigenic determinant detected by antibody reactivity that followed seroconversion during pregnancy (Table 3). In a single case (infant 7), sequence analysis of plasmids from 10 different colonies derived from the cloned PCR products resembled the sequence of AD169 gB (data not shown). Thus, seroconversion in the mother of this infant during pregnancy following reinfection with a virus encoding Towne like gB sequences was not associated with transmission of this new virus to the offspring (Table 3). Alternatively, it was also possible that in this limited sampling we failed to isolate an amplimer from a virus encoding a Towne-like gB.

Comment

Women from this region of Brazil with evidence of infection with multiple CMV strains, including women acquiring new virus strains during pregnancy, were more likely to deliver congenitally infected infants than women who lacked serologic evidence of infection with multiple CMV strains. These findings provided support for the hypothesis that reinfections with new virus strains were responsible for a significant number of congenital CMV infections in offspring of women from this highly seroimmune population. It has been argued that congenital CMV infections after nonprimary maternal infections results from reactivation of existing persistent infections (recurrent maternal infection). Although this is a possible explanation for congenital infections after nonprimary maternal infections, our findings that seroconversion to a new virus-encoded determinant was observed in 17.5% of women delivering infected infants as compared with only 4.6% in control mothers of uninfected infants from the same populations argued against recurrent maternal infection as the sole cause of congenital CMV infections in this population. Furthermore, 52.5% of women who delivered congenitally infected infants exhibited evidence of infection with multiple strains of CMV as compared with only 20% of women in the control group suggesting that maternal infection after exposure to new strains of virus was a risk factor for the delivery of a congenitally infected infant. Although CMV-specific serologic responses

have not been used conventionally to identify reinfection with a new strain of CMV, the finding of new antibody specificities in sequential blood samples from seropositive mothers was taken as evidence of an infection with a new virus strain (serotype), consistent with observations in other virus infections.[36–42] Alternatively, new antibody specificites in sequential serum specimens in these women could be explained by mutations in the coding sequence of CMVs persisting in the host, leading to production of new antibody specificities. However, there is little evidence for instability of the sequence encoding these specific CMV glycoproteins even after prolonged in vitro virus passage. Stanton et al,[43] have reported the stability of CMV hypervariable genes over time in vivo during the course of a persistent infection in renal transplant recipients, a finding arguing against genome instability as an explanation for expression of new antigenic determinants on CMV in seropositive individuals. A recent analysis of the coding sequences of several CMV genes indicated extensive variation between viral strains and suggested that a large number of CMV strains circulate within human populations.[44]

Considering the assays used in this study identified only women who generated antibody responses against linear peptides expressed by the laboratory CMV strains AD169 and Towne gH and gB, the frequency of reinfection is almost certainly higher. A number of CMV genes have been shown to exhibit considerable DNA sequence variability, but our studies have suggested that only a very limited number of these changes have resulted in differences in amino acid sequences that induce viral strain-specific antibody responses. Thus, we are limited in our capacity to distinguish between specific strains of CMV within the multitude of genetically unique strains that circulate in the human population by serologic assays such as described in this report. Yet, even with this limitation in our assays, the annualized reinfection rate in women transmitting virus to their offspring was 35%, a rate approximately 5-7 times higher than the maternal seroconversion rates in populations of women with lower CMV seroprevalence but similar to rates of primary CMV infections (approximately 13%) observed in mothers of young children (<3 years of ages) excreting CMV and in day care.[45–47] When these results are viewed together, the incidence of congenital infection associated with maternal reinfection in this Brazilian population reflected the phenomena of increasing incidence of congenital CMV infection with increasing maternal seroprevalence of CMV. Frequent exposure of these populations to CMV could also be expected to limit the protective activity of vaccine-induced immunity. Thus, caution must be applied to generalized estimates of vaccine efficacy and results from vaccine trials may be interpretable only in terms of the seroprevalence of a specific population.

In our study, 1 mother of an infected infant and 22 control mothers with preconceptional immunity did not have reactivity against AD169- specific gH or gB antigens at the first prenatal visit and failed to produce antibodies against these antigenic sites during pregnancy. This finding raised the possibility that additional polymorphic antibody sites are present on these 2 CMV envelope glycoproteins and that identification of these epitopes could increase the sensitivity of our assays for detection of reinfection with new strains of virus. A recent report demonstrated that serological reactivity to the AD169 and Towne gH linear antibody-binding sites in CMV seropositive blood donors was 48% and 16% respectively, and 19% had no reactivity to either epitope.[48] Increasing age in this population was correlated with

increasing seroreactivity for both linear epitopes, perhaps secondary to increasing exposure to serologically distinct CMV strains through reinfection.[48] As other CMV glycoproteins can also be targets of antibody responses, polymorphic sites for antibody reactivity on other envelope glycoproteins such as gN, a glycoprotein that exhibits considerably more sequence variation than either gH or gB, could be useful in this assay.[49–51]

It is well established that previous immunity induced after primary CMV infection does not protect against infection with different strains of the virus.[18,52] We have previously demonstrated that maternal CMV reinfection can lead to fetal damage and symptomatic infection.[53] Ishibashi et al[48] demonstrated an increased frequency of adverse outcomes in transplant recipients with serologic responses consistent with reinfection with different CMV strains, a finding similar to those reported by Grundy and Chou.[19,20] Congenital CMV infections after nonprimary maternal infections can lead to symptomatic congenital CMV infection and long-term sequeale.[54,55] In fact, recent evidence suggested that the incidence of hearing loss in infants infected after nonprimary maternal infection was similar to the incidence of hearing loss in infected infants born to women with primary infection.[56] Thus, the consequences of reinfection with a new and immunologically unrecognized strain of CMV could be similar to those after primary infection in immunologically naive women. Although such a mechanism is attractive, based on the failure of immune responses such as antiviral antibodies to protect against infection and disease in viral infection such as influenza and other respiratory viruses, the pathogenesis of congenital CMV disease is complex and likely multifactorial.

Exposure to young children is a well-established risk factor for acquisition of CMV and our findings suggested that exposure to young children represented a risk for reinfection by a new strain of CMV in women with seroimmunity to CMV. Reinfections with new strains of virus have been reported in children attending group child care facilities and in individuals attending STD clinics.[18,52–57] Although mechanisms responsible for acquisition of new strains of CMV are unknown, strain-specific virus neutralizing antibodies have been suggested as an explanation for infection in previously infected host after exposure to new strains of virus.[58,59] Studies in women with primary CMV infections during pregnancy have demonstrated an association between virus transmission and levels of virus neutralizing antibodies, suggesting a threshold of seroimmunity could be required to limit intrauterine transmission in seroimmune women reinfected with a new strain of virus during pregnancy.[60]

In conclusion, results from this study demonstrated that reinfection with a new CMV strain is a risk factor for delivery of a congenitally infected infant. In this study, infection with a new strain of CMV is not an infrequent event in women in this region of Brazil. The increased rates of congenital CMV infections in highly seroimmune populations may be associated with exposure to multiple viruses leading to maternal reinfection. Strain-specific immune responses during primary CMV infection could be a major challenge for vaccine development for preventing congenital CMV infections in such populations.

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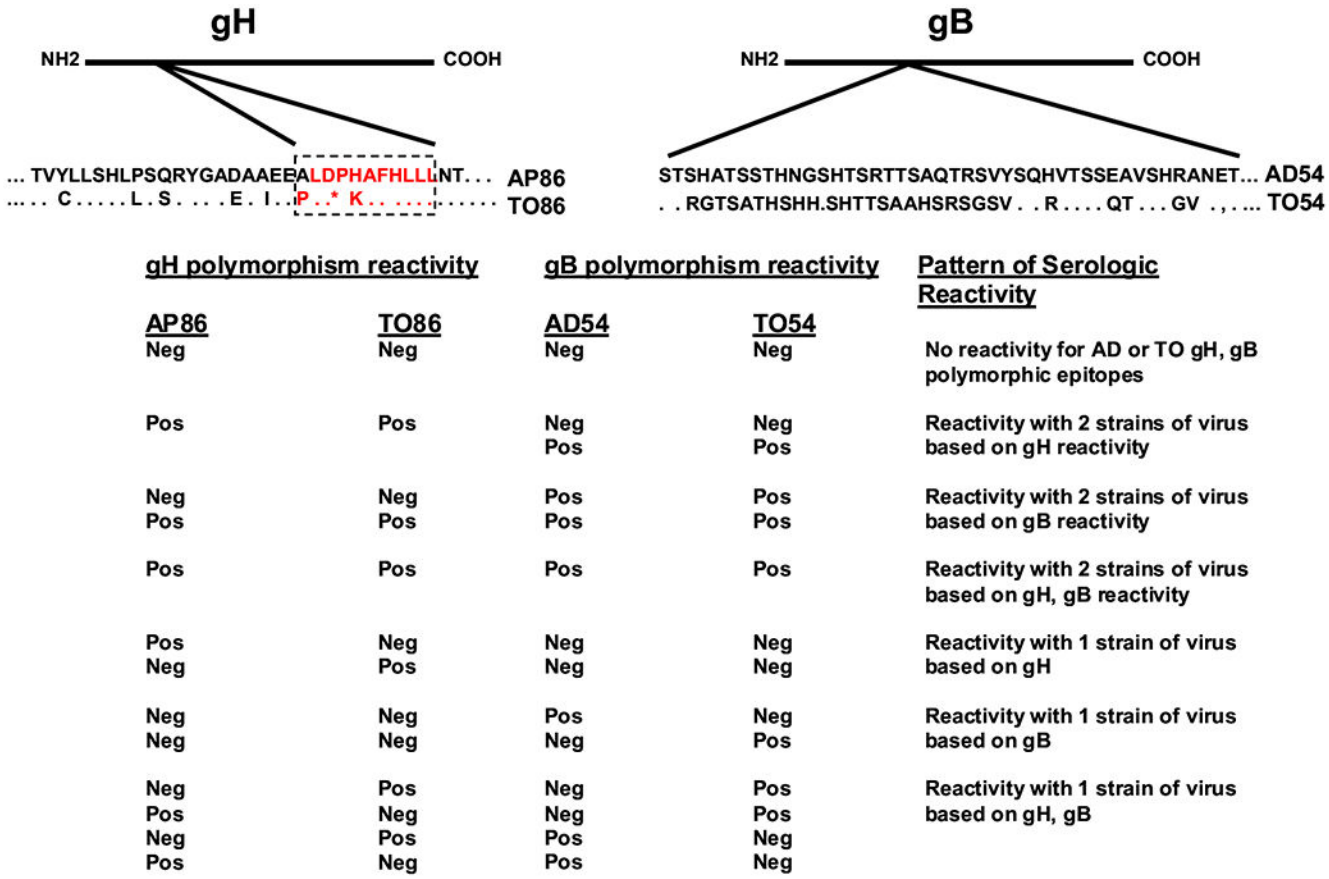

gH polymorphism reactivity		gB polymorphism reactivity		Pattern of Serologic Reactivity
AP86	TO86	AD54	TO54	
Neg	Neg	Neg	Neg	No reactivity for AD or TO gH, gB polymorphic epitopes
Pos	Pos	Neg Pos	Neg Pos	Reactivity with 2 strains of virus based on gH reactivity
Neg Pos	Neg Pos	Pos Pos	Pos Pos	Reactivity with 2 strains of virus based on gB reactivity
Pos	Pos	Pos	Pos	Reactivity with 2 strains of virus based on gH, gB reactivity
Pos Neg	Neg Pos	Neg Neg	Neg Neg	Reactivity with 1 strain of virus based on gH
Neg Neg	Neg Neg	Pos Neg	Neg Pos	Reactivity with 1 strain of virus based on gB
Neg Pos Neg Pos	Pos Neg Pos Neg	Neg Neg Pos Pos	Pos Pos Neg Neg	Reactivity with 1 strain of virus based on gH, gB

FIGURE. Patterns of reactivity for polymorphic linear epitopes on CMV glycoproteins gH and gB

Schematic representation of primary amino acid sequence of CMV strain-specific antibody-binding sites present on envelope gH and gB. Possible patterns of antibody reactivity shown on far left with the interpretation of reactivity for number of viral strains that have infected a single individual.

TABLE 1

Maternal CMV strain-specific serologic responses

Study population (n = 40)				**Control population (n = 109)**			
Prenatal serum							
gH reactivity [a]		**gB reactivity** [a]		**gH reactivity** [a]		**gB reactivity** [a]	
AP86,TO86	**Negative**	**AD54,TO54**	**Negative**	**AP86, TO86**	**Negative**	**AP86,TO86**	**Negative**
34 (85%)	6 (15%)	29 (72.5%)	11 (27.5%)	71 (65%)	38 (35%)	71 (65%)	38 (35%)
Acquisition of new serotypic reactivity during pregnancy [b]							
gH reactivity [c]		**gB reactivity** [c]		**gH reactivity** [c]		**gB reactivity**	
AP86↔TO86	**Neg→pos**	**AD54↔TO54**	**Neg→pos**	**AP86↔TO86**	**Neg→pos**	**AD54↔TO54**	**Neg→pos**
2 (5%)	3 (7.5%)	2 (5.0%)	2 (5.0%)	1 (1.4%)	2 (5.3%)	0 (0%)	2 (3.1%)
Total 4 (10.0%)[3]		Total 3 (7.5%)[3]		Total 3 (2.7%)		Total 2 (1.8%)	

CMV, cytomegalovirus; *gB*, glycoprotein B; *gH*, glycoprotein H; *neg*, negative; *pos*, positive.

[a]Reactivity of CMV antibody positive serum specimens in ELISA-based assay for linear antibody binding sites on gH or gB as described in Materials and Methods. Reactivity shown is number of positive specimens with percentage of total number in parentheses;

[b]Seroconversion during pregnancy represents acquisition of reactivity in delivery serum specimen against previously unrecognized antibody- binding site on gH or gB antigens that was not present in prenatal specimen, including detection of antibody reactivity against either antigen in delivery serum specimens from women with non-reactive prenatal serum specimens. Results are shown as number with acquisition of antibody reactive with gH or gB linear epitopes with percentage of responders in parentheses;

[c]Total represents the number of women in population who exhibited acquisition of antibody reactivity against previously unrecognized antibody-binding sites on gH or gB antigens in their delivery serum when compared to the reactivity of their prenatal serum. Two women in the study population developed antibodies to new serotypes of both gH and gB, thus the total number of women seroconverting was 4 for gH and 3 for gB.

TABLE 2

Infection with multiple CMV strains in mothers according to serologic responses to 2 polymorphic determinants

Variable	Mothers of infected infants, n (%) (n = 40)	Mothers of uninfected infants, n (%) (n = 109)	*P* value
Antibody reactivity against ≥2 CMV strains at first prenatal visit[a]	14 (35.0)	17 (15.6)	.009
Seroconversion to new CMV strain during pregnancy	7 (17.5)	5 (4.6)	.02
Infection with ≥2 CMV strains before and/or seroconversion during pregnancy	21 (52.5)	22 (20.2)	< .0001

CMV, cytomegalovirus; *gB*, glycoprotein B; *gH*, glycoprotein H.

[a]Antibody reactivity determined as described in Materials and Methods against polymorphic linear epitopes on gH and gB.

TABLE 3

Predicted amino acid sequence of viruses isolated from congenitally infected infants

Infant	Seroconversion	Source of Viral DNA	Sequence of amplified viral DNA
1	AD169 gH[a]	Blood	YLLSHLPSQRYGADAASE***ALDPHAFHLLL*** (**AD169 gH**)
1	AD169 gH	Saliva	YLLSHLPSQRYGADAASE***ALDPHAFHLLL*** (**AD169 gH**)
2	AD169 gH	Blood	YLLSHLPSQRYGADAASE***ALDPHAFHLLL*** (**AD169 gH**)
2	AD169 gH	Saliva	YLLSHLPSQRYGADAASE***ALDPHAFHLLL*** (**AD169 gH**)
3	AD169 gH	Urine	YLLSHLPSQRYGADAASE***ALDPHAFHLLL*** (**AD169 gH**)
3	AD169 gH	Saliva	YLLSHLPSQRYGADAASE***ALDPHAFHLLL*** (**AD169 gH**)
4	AD169 gH	Urine	YLLSHLPSQRYGADAASE***ALDPHAFHLLL*** (**AD169 gH**)
4	AD169 gH	Blood	YLLSHLPSQRYGADAASE***ALDPHAFHLLL*** (**AD169 gH**)
5	AD169 gH	Blood	YLLSHLPSQRYGADAASE***ALDPHAFHLLL*** (**AD169 gH**)
5	AD169 gH	Saliva	YLLSHLPSQRYGADAASE***ALDPHAFHLLL*** (**AD169 gH**)
6	TogB[b]	Urine	HGTSATHSHHSSHTTSAAHSRSGSVSSQRVTSSEAVSHRANET (**Towne-like gB**)
7	TogB	Saliva	HATSSTHNGSHTSRTTSAQTRSVYSQHVTSSEAVSHRANE (**AD169 gB**)

gB, glycoprotein B; *gH*, glycoprotein H.

[a]Viral DNA amplified from sample obtained from congenitally infected offspring of women undergoing seroconversion during pregnancy as detected by acquisition of antibody reactivity for AP86 epitope of AD169 gH. AP86 epitope listed in ***bold italics.*** DNA sequence obtained directly from amplified PCR product;

[b]Viral DNA from infants 6 and 7 were amplified and PCR products cloned into plasmid pCRBlunt. Plasmids from 10-12 colonies were isolated and sequenced. A mixture of viral gB genotypes were identified. In the case of patient 6, the epitope associated with seroconversion during pregnancy in this mother, Towne-like gB (TO54), was demonstrated. In the case of infant 7, all sequenced plasmids expressed AD169-like gB (A54).