Published in final edited form as:

J Clin Virol. 2009 December ; 46(Suppl 4): S58–S63. doi:10.1016/j.jcv.2009.10.004.

Cytomegalovirus-specific, high-avidity IgG with neutralizing activity in maternal circulation enriched in the fetal bloodstream

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Abstract

Background—Cytomegalovirus (CMV) is the major cause of congenital infection and disease leading to permanent birth defects. In about 35–40% of pregnant women with primary CMV infection, virus crosses the placenta, resulting in the birth of congenitally infected babies. In contrast, this happens in only 1–3% of seropositive women with strong CMV-specific humoral immunity. Whether CMV reaches the fetus and disseminates depends on the level of high-avidity antibodies in the maternal circulation and the passive immunity of the fetus.

Objectives and study design—To identify CMV infection in uncomplicated deliveries based on detection of viral DNA in placental biopsy specimens at term. To quantify CMV-specific IgG avidity, neutralizing titer, IgG1 concentration, and characterize the immunoblot profiles for CMV proteins in paired samples of placental and cord blood sera.

Results—In accord with earlier reports, CMV DNA was detected in 39% (11/28) of placentas with mean- to high-avidity CMV-specific IgG. In seropositive women, the concentration of antiviral antibodies, specifically IgG1, increased in the fetal bloodstream, and CMV neutralizing titers in maternal and fetal blood were comparable.

Conclusions—CMV-specific, high-avidity neutralizing antibodies from maternal circulation are transcytosed to the fetal bloodstream, contribute to suppression of viral replication in the placenta and could prevent congenital disease.

Keywords

congenital CMV infection; passive immunity; neonatal Fc receptor; high-avidity IgG; neutralizing titer

1. Introduction

Human cytomegalovirus (CMV) is the leading cause of congenital viral infection in the United States. Birth defects from congenital CMV disease are as frequent as those from Down syndrome, fetal alcohol syndrome and neural tube defects.¹ Epidemiological studies report

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Conflict of interest

The authors have no conflicts of interest.

sharp ethnic/racial disparities in congenital disease, with the highest force of infection in non-Hispanic blacks (5.7%) and Mexican Americans (5.1%).^{1, 2} Each year thousands of pregnant women become infected with CMV, and most are unaware of congenital CMV disease.³ In 35–40% of these primary infected case, virus will be transmitted to the fetus, a quarter of whom will have permanent birth defects: hearing loss, mental retardation, cerebral palsy, seizures and chorioretinitis.^{4–7} Some babies will die before birth or in early childhood.

Symptoms of viral infection in the fetus and the newborn are more severe when primary maternal infection occurs in early gestation⁸ or near the time of conception.^{9, 10} Prenatal diagnosis by CMV isolation or detection of viral DNA in amniotic fluid and maternal blood indicates a 4- to 6-week window between primary infection, transplacental transmission and fetal infection.^{9–11} Recurrent infections are mostly asymptomatic when mothers have CMV-specific, high-avidity immunoglobulin G (IgG).^{12, 13} Although universal screening of pregnant women for CMV antibodies is a feasible approach to identify those at risk for transmission,¹⁴ large-scale testing has not been implemented.^{10, 15, 16} In infected neonates, virus can be found in serum,^{17, 18} urine^{19, 20} and saliva.²¹

Passive immunity of the fetus to pathogens depends on transcytosis of maternal IgG facilitated by the neonatal Fc receptor in syncytiotrophoblasts of the placenta and progressively increases in the last trimester of gestation.^{22, 23} Studies from our group have shown that the extent of CMV replication in the placenta depends on the degree of maternal immunity.^{24–26} In early gestation, the neonatal Fc receptor transcytoses IgG and immune complexes of virions across the syncytiotrophoblasts and CMV glycoprotein B (gB) accumulates in caveolae.²⁷ With low neutralizing titers, CMV replication proteins are detected in cytotrophoblast progenitor cells in the placenta; high levels of CMV DNA are present, and fetal blood vessels contain neutrophils with viral replication proteins.²⁶ With moderate neutralizing titers, occasional focal infection is found in villous cytotrophoblasts. With high neutralizing titers, little if any viral replication occurs in the placenta although viral DNA and virion gB are present in syncytiotrophoblasts and fetal macrophages in the villous core.

In the study reported here, we examined placental biopsy specimens from uncomplicated deliveries at term for viral DNA and analyzed CMV-specific antibodies in sera from fetal (cord) blood and from maternal blood in the placental intervillous space to quantify antiviral antibodies. We found that CMV-specific, high-avidity IgG with neutralizing activity was elevated in the fetal bloodstream as compared with the maternal circulation and that viral DNA in the placenta was reduced in the presence of high-avidity IgG.

2. Methods

2.1. Biopsy samples

Biopsy samples were obtained from non-selected population of 54 pregnancies with uncomplicated deliveries at Moffitt Hospital, University of California–San Francisco, with approval of the institutional review board. According to the requirement of the Committee on Human Research approval for this research program, no identifiers about maternal age, race or ethnicity were obtained. The population of the greater metropolitan area of San Francisco estimated by the American Community Survey of the U.S. Census Bureau (2005–2007) includes caucasians (56.3%), African Americans (7.6%), Asians (33.2%) and other races (5.9%).

Immediately after delivery, blood was collected from the umbilical cord (fetal) and the intervillous blood space (maternal). Table 1 lists seropositive serum samples from placentas that contained CMV DNA (Group A), or did not (Group B). Paired sera from maternal and fetal blood samples (n=54) were subjected to the following analyses: IgG1 subclass (n=41),

CMV-specific IgG (n=41), CMV-specific IgG avidity (n=52), neutralization titers (n=48) and immunoblot assay (n=54). From each placenta (n=54), five biopsy specimens were excised, one at the center and four at the periphery, and flash frozen. CMV DNA was detected by PCR from each biopsy specimen (n=270).

2.2. Polymerase chain reaction (PCR)

DNA was extracted from 10–25 mg of biopsy specimens using the QIAamp DNA mini kit (Qiagen) and stored at –20°C. DNA from human foreskin fibroblasts infected with the clinical CMV strain VR1814²⁸ was used as a positive control. DNA from uninfected cells and water were used as negative controls. The presence of CMV DNA was analyzed by nested-PCR using primers for immediate-early (IE) genes.²⁹ Briefly, 25 µl of reaction mixture contained 1x REDTaqBuffer (Sigma-Aldrich), 0.2 mM dNTP mixture, 0.2 µM of each primer, 0.5 Unit REDTaq DNA polymerase (Sigma-Aldrich), and 5 µl of extracted DNA. Cycle conditions were one cycle of 2 min at 94°C followed by 40 cycles of 45s at 94°C, 45s at 50°C and 1 min at 72°C, and final incubation for 2 min at 72°C. First PCR product (2 µl) were subjected to 2nd PCR. Cycle conditions for 2nd PCR were same as 1st PCR except for applying 30 cycles of reaction. PCR products were analyzed by gel electrophoresis. CMV gB genotype was determined by nested PCR using the AmpliTaq Gold DNA polymerase (Applied Biosystems), followed by sequencing as described previously.²⁹ An outer primer pair—gBout and gBout2³⁰—and an inner primer set²⁹ were used.

2.3. IgG subclass analysis

The concentration of each IgG subclass in maternal and fetal was quantified using the human IgG subclass ELISA kit (Invitrogen). IgG1 concentration was quantified using the human IgG1 subclass profile kit (Invitrogen).

2.4. CMV IgG ELISA

CMV-specific IgG was measured using the CMV ELISA Quantitation Kit (GenWay).

2.5. CMV-specific IgG avidity assay

CMV-specific IgG avidity was analyzed using an enzyme immunoassay (CMV IgG avidity, Radim) following the manufacturer's instructions. Briefly, sera were diluted 1:300 and applied to the CMV antigen-coated plate in duplicate. After the first incubation and washing, replicate wells were incubated with buffers with or without urea. Bound antibody was detected with horseradish peroxidase-conjugated anti-human IgG, followed by color development with tetramethylbenzidine. The ratio between the optical densities of the wells in buffer with or without urea was calculated and expressed as percent avidity.

2.6. Neutralization assay

CMV neutralization assays were performed as described with modifications.³¹ Briefly, human foreskin fibroblasts were seeded in 24-well plates with cover glass slides (2×10^4 cells/well). Sera were heat-inactivated (56°C) for 30 min. Two hundred fifty microliters of diluted sera (1:8–1:1,024) in Dulbecco's modified Eagle's medium (DMEM) was incubated in duplicate with an equal amount of VR1814 virus stock (10^3 PFU per well) for 1 h at 37°C. The virus–serum mixture was added to the confluent monolayer of human foreskin fibroblasts and incubated for 2 h at 37°C. Cells were washed with phosphate-buffered saline, cultured with DMEM containing 10% fetal bovine serum for 24 h, and fixed with 70% methanol. Cover slips were stained with mouse monoclonal antibody CH160 against IE1 and -2 proteins.³² IE1&2-positive nuclei were visualized using fluorescein isothiocyanate-conjugated anti-mouse IgG (Jackson ImmunoResearch) and examined using a Nikon Eclipse 50i microscope. The serum dilution that produced 50% inhibition of virus infectivity was reported as the neutralizing titer.

2.7. Immunoblot assay

CMV-specific antibodies were characterized using the *recomBlot* CMV IgG kit (Mikrogen). Purified recombinant CMV proteins (IE1, p150, CM2, p65, gB1 and gB2) were separated by sodium dodecyl sulfate—polyacrylamide gel electrophoresis and immobilized on nitrocellulose membrane strips. The strips were incubated with diluted serum (1:100), and specific bands were visualized by horseradish peroxidase-conjugated anti-human IgG followed by substrate solution. Recent infection, long-past (remote) infection, and reactivation/recurrent infection were assessed according to the manufacturer's instructions.

2.7. Statistical analysis

The statistical significance of the data was analyzed by one-tailed, paired Student's *t*-test. *P* values of < 0.01 were considered significant.

3. Results

3.1. IgG1 transcytosed from mother to fetus across the placenta

In the first set of experiments, the concentration of different IgG subclasses in maternal and fetal circulation was measured in 8 paired serum samples and the mean ratio of fetal/maternal IgG subclass was calculated (Fig. 1A). The mean ratio of fetal to maternal IgG1 concentration was significantly higher than that of IgG2 ($P = 0.0031$), IgG3 ($P = 0.0025$), and IgG4 ($P = 0.0032$) (Fig. 1A). The mean ratio of fetal to maternal IgG subclasses concentration were as follows: IgG1 (1.56) > IgG4 (1.21) > IgG2 (1.17) > IgG3 (1.13). We quantified the IgG1 concentration in 41 paired maternal and fetal serum samples. As anticipated, IgG1 concentration in fetal sera was significantly higher than that in maternal sera and the mean ratio was 2.18 (range, 0.33–5.74). Next, we compared the placental IgG1 transfer (fetal/maternal IgG1 concentration ratio) to the maternal IgG1 concentration by scatter diagrams.³³ The fetal/maternal IgG1 concentration ratio was reciprocally correlated to the maternal IgG1 concentration (Fig. 1B). These results confirm that IgG1 in maternal circulation is transcytosed across the placenta and significantly elevated in the fetal bloodstream.

3.2. Increased CMV-specific, high-avidity neutralizing antibodies in the fetus

We determined the presence of CMV-specific IgG and measured the avidity in 52 paired samples. CMV IgG ELISA indicated that 26 pairs were seronegative or from recent seroconversions and the avidity was below the cutoff value ($\leq 2\%$) (data not shown). In 26 paired sera that contained CMV-specific IgG, the mean avidity in fetal sera (61.6%) was significantly higher than that in maternal sera (57.5%, $P < 0.01$) although the differences were small (Table 1). The scatter plot of relationship between maternal and fetal IgG avidity showed positive correlations in individual mother-infant pairs (Fig. 2). Neutralization assays for 26 paired sera that contained CMV-specific IgG confirmed that the presence of high-avidity IgG paralleled that of neutralizing activity (1:8 to 1:256). Together these results indicate that the increased CMV-specific, high-avidity IgG in fetal circulation had virus neutralizing activity.

3.3. CMV proteins recognized by circulating IgG in recurrent maternal infection

Immunoreactivity to CMV proteins in 54 paired sera was characterized using immunoblot (*recomBlot*) assays to identify major viral antigens recognized by circulating IgG. Figure 3 shows representative staining profiles for CMV proteins of paired serum samples with mean to high-avidity IgG (Fig. 3, sera 113, 144, 125). Negative or weak reactions were obtained with sera lacking CMV-specific IgG by a commercial assay (Fig. 3, sera 128, 134, 142). Eleven maternal serum samples (20%) with high avidity IgG reacted with p150, gB1 and gB2, a profile that indicates long-past (remote) infection. Twelve paired serum samples (22%) reacted with IE1, p150, gB1 and gB2, suggesting recurrent infection during gestation. Although profiles of

paired serum samples were comparable, a broader profile of viral proteins was occasionally recognized in fetal sera (Table 1, Group A, placentas 57, 127; Group B, placenta 120). Profiles of 5 paired serum samples (9%) with high-avidity IgG, neutralizing titers and weak (low) reactions with viral peptides CM2, p65, gB1 or gB2, a profile that suggested infection occurred several months before conception (Table 1, placentas 124, 158, 146, 135, 131). Three paired serum samples (6%) were weakly reactive with IE1, p65, and p150, lacked IgG avidity and neutralizing antibodies, suggesting that these mothers may have acquired a primary infection only recently (data not shown). Twenty-three paired serum samples (43%) lacked CMV-specific IgG and showed negative reactions by immunoblot assays (data not shown).

3.4. High-avidity IgG associated with reduced levels of CMV DNA in the placenta

Placentas from CMV-specific IgG seropositive women (Table 1, n=28) and those lacking CMV-specific IgG measured with a commercial assay (n=26) (data not shown) were examined for the presence of CMV DNA. Five biopsy specimens from each placenta were evaluated using PCR. Viral DNA was detected in 50% (13/26) of placentas lacking CMV-specific IgG, avidity, and with weak or negative immunoblot reactions indicating very recent primary infection. In contrast, CMV DNA was found in 39% (11/28) of placentas with mean to high-avidity IgG in maternal and/or fetal sera. To determine whether reactivation of endogenous strains or possible reinfection with a second CMV strain had occurred, CMV gB genotypes were analyzed in 10 placentas. Of these, we detected gB3 in 70% (7/10), gB2 in 10% (1/10) and coinfections with gB2 and gB3 in 20% (2/10). Interestingly, one coinfection was a recent primary infection lacking detectable CMV-specific IgG (data not shown) and another was recurrent infection or reactivation (Table 1, placenta 104). These results showed that simultaneous infection with two strains could occur in women who seroconvert or undergo recurrent infection.

4. Discussion

We analyzed (i) paired samples of fetal cord blood and maternal blood from the placenta from uncomplicated deliveries at term for CMV-specific IgG and (ii) five biopsy specimens from each placenta for viral DNA. Our results indicate that significantly increased IgG1 levels, CMV-specific neutralizing antibodies with high avidity that recognize gB constitute passive immunity acquired by the fetus. Development of these high-avidity antibodies suppresses viral replication in the placenta²⁶ and precludes symptomatic fetal infection.^{7, 34, 35} Passive immunity escalates in the third trimester of pregnancy²² mediated by the neonatal Fc receptor²³ that mediates transcytosis of CMV-specific IgG across syncytiotrophoblasts to the fetal compartment.²⁷ The CMV-specific IgG avidity test is one of the most reliable procedures to identify the timing of primary maternal infection since avidity increases progressively as the immune response matures.^{31, 34} Low-avidity IgG is present in acute or recent primary maternal infection and persists for approximately 5 months in immunocompetent persons.³⁶ High-avidity IgG indicates long-past or remote infection. In addition, the combination of PCR to detect viral DNA, gB genotyping to determine simultaneous infection with multiple strains, and immunoblot analysis of proteins recognized by maternal IgG provides a more comprehensive picture of ongoing infection at the uterine-placental interface. Results of the present study support our earlier finding that two CMV strains can be detected in primary maternal infection.^{25, 26} It should be mentioned that we did not anticipate finding CMV DNA in placentas from women judged to be seronegative for CMV-specific IgG using a commercial assay. We cannot exclude the possibility that nested-PCR analysis could give some false positive results. Nonetheless, results of this study agree with other published reports that CMV DNA is detected in many placentas from uneventful pregnancies.^{24, 26, 27, 29} Thus, the sensitivity of present commercial ELISA assays for CMV IgG could be less sensitive. Possible reasons include the use of laboratory strains to prepare viral antigen, extraction procedures,

and inclusion of buffers designed to reduce non-specific binding. Current efforts focus on evaluating these samples for additional evidence of infection including the presence of CMV-specific IgM.

These results extend those of earlier studies showing that CMV frequently reactivates in seropositive women and that a high level of maternal neutralizing antibodies reduces viral replication in the placenta and limits possible transmission to the fetus.^{25, 26} Circulating maternal IgG1 with high avidity and virus-neutralizing activity, transcytosed by the neonatal Fc receptor across the placenta, increases in concentration in the fetal bloodstream. CMV gB is a major target for neutralizing antibodies,^{37–39} and IgG1 reacts with both linear epitopes and domains on the full-length gB complex.⁴⁰ Thus, protective, antiviral IgG can cross the placenta and reach higher levels in the fetal bloodstream.

Our results revealed that IgG avidity and passive immunity to CMV in the fetus exceeds that in the circulation of immune women with uncomplicated deliveries. Specifically, the results provide a strong rationale for large-scale clinical studies to evaluate prenatal treatment of women diagnosed with primary infection during pregnancy with CMV-specific hyperimmune globulin to suppress virus replication in the developing placenta.⁴¹ A combination of immunization of susceptible woman of childbearing age and passive immunization in management of maternal gestational infection could ultimately prevent congenital CMV disease.

Abbreviations

CMV	cytomegalovirus
DMEM	Dulbecco's modified Eagle's medium
ELISA	enzyme-linked immunosorbent assay
gB	glycoprotein B
IE	immediate early
PCR	polymerase chain reaction

Acknowledgments

We thank Jean Perry for assistance in obtaining placentas and cord blood and Mary McKenney for editing the manuscript. This work was supported by grants AI46657-09 and AI073752-01 from the National Institutes of Health and by grant 02821-7 from the Thrasher Research Fund (L.P.). N.N. was supported by Japan Herpesvirus Infections Forum, Scholarship Awards in Herpesvirus Infections Research.

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Fig. 1.

IgG1 subclass is preferentially transcytosed from mother to fetus across the placenta. (Panel A) The ratio of each IgG subclass concentration in fetal (cord) blood to that in maternal (placental) blood (8 paired samples). The mean ratio for IgG1 was significantly higher than that for other subclasses. Asterisk indicates a significantly higher IgG1 concentration in fetal blood ($P < 0.01$; Student's *t*-test). (Panel B) Relationship between fetal/maternal IgG1 concentration ratios and maternal IgG1 levels ($n = 41$). Line represents linear regression ($Y = -0.0004x + 3.733$, $R^2 = 0.416$).

Fig. 2.

CMV-specific, high-avidity IgG is preferentially transcytosed across the placenta. CMV-specific IgG avidity was analyzed in paired maternal and fetal sera. Scatter diagrams shows CMV-specific IgG avidity in paired serum samples (n= 26) that were CMV-specific IgG positive (cutoff value $\leq 2\%$). Line represents linear regression ($Y = 0.864x + 11.971$, $R^2 = 0.642$).

Fig. 3.

IgG reactivity to CMV proteins in paired serum samples. CMV protein profiles of placental (p) and cord blood (c) using the *recomBlot* kit (Mikrogen). Representative protein profiles obtained with high-avidity IgG are seen in samples 113, 144 and 125; those without reactivity are 128, 134 and 142. Asterisks indicate reactivity to p65 (134) and IE (142 c) in sera lacking detectable IgG avidity.

Table 1

Serological analysis of CMV-seropositive women with uncomplicated deliveries.

Group	Placenta	Sera	Avidity (%) ^a	Neut (<1:8 = neg)	IgG1 Conc. (µg/ml)	recomBlot CMV IgG ^b	CMV DNA ^c , gB genotype
A	123	PB	49.0	1:16	2625	p150, gB1, gB2	1/5
		CB	55.0	1:16	7750	p150, gB1, gB2	
	117	PB	52.0	1:8	4825	p150, gB1, gB2 ^{low}	1/5
		CB	70.8	1:16	8500	p150, gB1, gB2 ^{low}	Type 3
	104	PB	71.7	1:64	5000	IE1 ^{low} , p150, gB1, gB2	2/5
		CB	67.7	1:64	10,125	IE1 ^{low} , p150, gB1, gB2	Type 2 & Type 3
	107	PB	53.5	1:32	4250	IE1 ^{low} , p150, CM2, p65, gB1 ^{low}	2/5
		CB	67.2	1:32	6500	IE1 ^{low} , p150, CM2, p65, gB1 ^{low}	Type 3
	115	PB	NT	NT	3700	ND	1/5
		CB	63.0	NT	4763	IE1, p150, p65, gB1, gB2	
	152	PB	75.6	1:128	6000	p150, gB1, gB2	1/5
		CB	73.9	1:128	3850	p150, gB1, gB2	
	113	PB	52.1	1:32	NT	IE1 ^{low} , p150, CM2, p65, gB1, gB2	5/5
		CB	54.0	1:64	NT	IE1 ^{low} , p150, CM2, p65, gB1, gB2	
	124	PB	55.5	1:16	2438	p150, CM2 ^{low} , gB1 ^{low} , gB2 ^{low}	2/5
B		CB	44.8	1:16	NT	p150, CM2 ^{low} , gB1 ^{low} , gB2 ^{low}	Type3
	125	PB	64.3	1:64	2500	IE1 ^{low} , p150, gB1	3/5
		CB	66.9	1:64	9750	IE1 ^{low} , p150, gB1	Type 3
	57	PB	72.8	1:8	NT	IE1, p150, CM2, p65, gB1,	2/5
		CB	65.5	1:16	NT	IE1, p150, CM2, p65, gB1, gB2 ^{low}	Type 2
	127	PB	66.5	1:32	2875	p150, CM2 ^{low}	3/5
		CB	69.7	1:32	9000	IE1, p150, CM2, p65, gB1	Type 3
	158	PB	61.0	1:64	5625	p150, CM2 ^{low} , gB1 ^{low}	Neg
		CB	65.0	1:64	11,375	p150, CM2 ^{low} , gB1 ^{low}	
	140	PB	40.6	1:16	4500	p150 ^{low} , gB1, gB2 ^{low}	Neg
		CB	51.3	1:16	8500	p150 ^{low} , gB1, gB2 ^{low}	
	165	PB	48.8	NT	NT	p150, gB1, gB2	Neg
		CB	47.1	NT	NT	p150, gB1, gB2	

Group	Placenta	Sera	Avidity (%) ^a	Neut (<1:8 = neg)	IgG1 Conc. (µg/ml)	recomBlot CMV IgG ^b	CMV DNA ^c gB genotype
	133	PB	50.0	1:32	3875	p150, gB1, gB2	Neg
		CB	52.0	1:32	5375	p150, gB1, gB2	
	98	PB	45.1	1:16	NT	p150, gB1, gB2	Neg
		CB	NT	1:16	NT	IE1, p150, gB1, gB2	
	146	PB	58.3	1:64	3000	p150, CM2 ^{low} , p65 ^{low} , gB1	Neg
		CB	66.8	1:64	3875	p150, CM2 ^{low} , p65 ^{low} , gB1	
	150	PB	44.2	1:64	4875	p150, CM2, gB1, gB2	Neg
		CB	49.0	1:64	1625	p150, CM2, gB1, gB2	
	147	PB	35.0	1:16	6000	p150, gB1, gB2	Neg
		CB	32.6	1:16	7500	p150, gB1, gB2	
	129	PB	54.9	1:32	NT	IE1, p150, CM2, gB1, p65 ^{low} , gB2	Neg
		CB	57.4	1:32	NT	IE1, p150, CM2, gB1, p65 ^{low} , gB2	
	120	PB	56.0	1:64	1250	p150, gB1, gB2	Neg
		CB	59.8	1:64	3950	IE1, p150, gB1, gB2	
	135	PB	49.6	1:32	NT	p150, p65 ^{low} , gB1 ^{low} , gB2 ^{low}	Neg
		CB	71.3	1:32	NT	p150, p65 ^{low} , gB1 ^{low} , gB2 ^{low}	
	130	PB	68.0	1:64	5283	IE1 ^{low} , p150, CM2, p65, gB1, gB2	Neg
		CB	67.0	1:64	7125	IE1 ^{low} , p150, CM2, p65, gB1, gB2	
	139	PB	84.5	1:256	NT	IE1, p150, CM2, p65 ^{low} , gB1, gB2	Neg
		CB	88.3	1:256	NT	IE1, p150, CM2, p65 ^{low} , gB1, gB2	
	144	PB	60.0	1:64	3450	IE1, p150, CM2 ^{low} , p65 ^{low} , gB1, gB2	Neg
		CB	67.5	1:64	9500	IE1, p150, CM2 ^{low} , p65 ^{low} , gB1, gB2	
	106	PB	49.5	1:8	4000	p150, gB1, gB2	Neg
		CB	46.8	1:8	9375	p150, gB1, gB2	
	131	PB	69.3	1:16	1750	p150, CM2 ^{low} , gB1	Neg
		CB	83.9	1:16	3538	p150, CM2 ^{low} , gB1	
	157	PB	51.2	1:64	1113	IE1, p150, p65 ^{low} , gB1, gB2	Neg
		CB	60.2	1:64	4250	IE1, p150, p65 ^{low} , gB1, gB2	

Group A, CMV DNA-positive; Group B, CMV DNA-negative. Abbreviations: PB, placental blood; CB, cord blood; Neut, neutralizing; NT, not tested; ND, not determined; Neg, negative.

^aCMV-specific IgG avidity was quantified by ELISA (Radim).

^b *recomBlot* antigen (open reading frame/protein): IE1 (UL123/IE1); p150 (UL32/pp150); CM2 (UL44, UL57/p52); p65 (UL83/pp65); gB1 and gB2 (UL55/gB).

^c Five biopsy specimens from each placenta were tested by PCR for the CMV IE genes.