Placental Transfer of Naturally Acquired, Maternal Cytomegalovirus Antibodies in Term and Preterm Neonates

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Maternal antibodies may protect the fetus and neonate against severe forms of CMV-caused disease, therefore this study investigated the efficiency of the placental transfer of naturally acquired, maternal total anti-cytomegalovirus (CMV) IgG and neutralizing antibodies at different gestational ages. The study was conducted on 182 healthy CMV-seropositive Brazilian mothers and their 196 infants who were not infected congenitally with CMV, as determined by CMV detection in urine. The study groups were composed of 44 infants aged 28–30 weeks; 51 infants aged 31–33 weeks; 62 infants aged 34–36 weeks, and 39 infants of gestational age ≥37 weeks. Quantitative detection of total CMV IgG was carried out using EIA and virus neutralizing titers were determined by a microneutralization assay in sera from mothers and infants. CMV IgG levels and neutralizing titers of the infants correlated with maternal levels (r = 0.873 and r = 0.841, respectively). The efficiency of placental transfer of these antibodies was enhanced significantly as gestation progressed until 34–36 weeks, when values similar to those of full-term infants (90–100%) were found. Transfer ratios were significantly higher for neutralizing compared to total CMV IgG antibodies at gestational age 31–33 weeks (100% vs. 84%, respectively) and at gestational age 28–30 weeks (75% vs. 60%, respectively). We conclude that placental transfer of naturally acquired maternal CMV neutralizing and total CMV IgG antibodies are similarly efficient above 34 weeks of gestational age. At less than 34 weeks of gestational age, transfer of neutralizing antibodies may be favored and these antibodies reach the neonatal serum of 99% of these premature infants. J. Med. Virol. 69:232–239, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: maternally-acquired immunity; neutralizing antibodies; IgG antibodies

INTRODUCTION

Cytomegalovirus (CMV) is the most common agent of human congenital and perinatal infection [Alford et al., 1990]. Congenital and perinatal infection rates are usually higher in developing countries than in developed countries, due to elevated CMV seropositivity and easy dissemination of the infection. In Ribeirão Preto, São Paulo, Brazil, maternal CMV seropositivity is estimated to be 95%, with incidences of 2.6% for congenital CMV infection and 38.2% for perinatal infection [Yamamoto et al., 1999a,b]. Prematurity is a frequent finding associated with symptomatic, congenital CMV infection [Boppana et al., 1992; Yamamoto et al., 2001]. In addition, perinatal or postnatal CMV infection as a consequence of exposure to genital secretion, raw mother's milk or multiple blood transfusions can also cause severe disease in premature infants [Ballard et al., 1979; Yeager et al., 1981, 1983; Vochem et al., 1998].

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Maternal antibodies do not prevent CMV transmission during the prenatal or perinatal periods, although passively acquired antibodies can prevent or attenuate fetal disease. There is some evidence that antibodies against CMV protect those premature newborns infected as a result of an infusion of CMV-containing blood [Yeager et al., 1981; Snydman et al., 1995]. In addition, it has been shown recently that passively acquired virus-neutralizing antibodies prevent fetal infection and limit fetal damage after maternal guinea pig CMV infection during pregnancy [Chatterjee et al., 2001].

The immunoglobulin G isotypes (IgG) are the only antibodies transported across the placenta. Such transport is active and depends on the presence of cell receptors [Kameda et al., 1991; Simister and Story, 1997]. A correlation has been found between gestational age and IgG concentration at birth [Toivanen et al., 1968]. Similarly, there is a positive correlation between the levels of specific antibodies in the newborn infant and the mother [Toivanen et al., 1968]. Recent studies evaluating the transfer pattern of different specific antibody types from the mother to the neonate, however, have demonstrated that efficiency of placental transfer of different antibody types may vary according to antibody specificity [Gonçalves et al., 1999] and in different populations [Wesumperuma et al., 1999]. Such differences have been attributed to variation in the production of IgG subclasses [Costa-Carvalho et al., 1996] in response to specific antigens and in the affinity of these IgG subclasses for Fc receptors [Simister and Story, 1997]. Various data suggest that the influence of gestational age on the efficiency of antibody transport may vary according to the antigen tested and to the characteristics of the antibody against a given antigen. In this regard, viral antibody concentrations in preterm infants are lower than [Linder et al., 1988; Gonçalves et al., 1999; Ozbek et al., 1999] or similar to [Linder et al., 1997; Wesumperuma et al., 1999] those in term infants.

The placental transfer of antibodies against CMV at different gestational ages has not yet been addressed. Reynolds et al. [1978] found that neutralizing antibodies acquired from the mother do not protect full-term infants against perinatal CMV infection. Gotlieb-Stematsky et al. [1983] detected significantly higher titers of CMV antibody in the cord blood of term infants than in maternal blood. The aim of the present study was to investigate the quantitative and qualitative nature of anti-CMV IgG antibodies transferred to newborns from mothers in a population with naturally acquired elevated seropositivity to CMV. We measured the total anti-CMV antibody concentrations and the virus-neutralizing activity of these antibodies in vitro in infants of different gestational ages, and analyzed the influence of gestational age and specific maternal anti-CMV antibody levels on the efficiency of placental antibody transfer. We attempt to disclose which group(s) of preterm infants receive lower neutralizing antibody levels and therefore may be more susceptible to CMV disease.

MATERIALS AND METHODS

A total of 214 pairs of mothers and their respective newborns, seen at the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo (UHFMRP-USP), Ribeirão Preto, São Paulo, Brazil, were selected shortly after birth from May 6, 1998 to February 28, 1999. The study (number 769/98) was approved by the Ethics Committee of UHFMRP-USP and informed consent was obtained from all mothers. These mother–infant pairs belonged to a cohort prospectively enrolled at delivery to determine the clinical presentation and frequency of congenital CMV infection in infants from this population [Yamamoto et al., 2001].

Only mothers who did not harbor chronic or acute infections, such as human immunodeficiency virus infection, syphilis, Chagas’ disease or hepatitis B, identified during routine serological tests on admission for delivery, were included in the study. The newborn infants included in the study consisted of the first 39 term infants (gestational age of 37 weeks or more) and all preterm infants (gestational age <37 weeks) born subsequently during the study period, independent of delivery type. Only infants who did not receive blood transfusion or blood derivatives during fetal development, immediately after birth, or before collection of blood and urine samples for analysis of CMV infection status were included.

After selection, the mothers were tested for anti-CMV antibodies and all infants were evaluated for congenital CMV infection by virus detection in the urine (CMV was cultured and detected in urine by PCR for three genomic targets). CMV seropositivity was confirmed in 203 mothers (94.8%); congenital CMV infection was diagnosed in six infants (2.9%). The remaining 197 infants were CMV-uninfected (negative CMV viruria and negative PCR for three genomic targets in urine). Congenital syphilis was diagnosed in one infant (0.5%). Eleven mother–child pairs in which the mothers were seronegative for CMV, and seven pairs in which the neonates were infected congenitally with CMV (six pairs) or had congenital syphilis (one pair) were excluded from the study. The study was concluded in a total of 196 mother–child pairs divided into four groups, according to complete gestational age period.

Group 1: 44 mother–child pairs, with gestational age between 28 and 30 weeks;
Group 2: 51 mother–child pairs, with gestational age between 31 and 33 weeks;
Group 3: 62 mother–child pairs, with gestational age between 34 and 36 weeks, and
Group 4: 39 mother–child pairs, with gestational age between 37 and 41 weeks.

Information on maternal and gestational background was obtained by interviewing the mother and consulting ultrasound exams in their medical records. All infants underwent an anthropometrical evaluation and a
complete physical examination. Gestational age was estimated from the reported date of last menstruation period and somatic evaluation according to the method of Capurro et al. [1978] and Ballard et al. [1991] in the case of extremely premature infants. Infant intrauterine growth was classified, by comparing gestational age and weight using the reference curve of Bataglia and Lubchenco [1967], into two categories according to the presence or absence of intrauterine growth retardation: adequate for gestational age and small for gestational age, respectively.

**Quantitative Measurement of Total Anti-CMV IgG Antibody**

A maternal blood sample obtained on admission for delivery and 1 ml of infant venous peripheral blood sample collected at a median age of 1 day (range 1–5 days) were stored at −70°C until the anti-CMV antibody detection tests were carried out simultaneously on stored samples. Anti-CMV IgG antibody concentrations were measured using an automated, commercially available, indirect enzyme immunoassay (Cobas Core IgG EIA, Roche, Mannheim, Germany) [Steinmann and Weigel, 1994] according to the manufacturer's instructions. This assay detects IgG antibodies against CMV antigens derived from cell cultures. Known concentrations of IgG antibodies (0, 1, 2.5, 5, 10, and 20 U/ml) provided by the manufacturer and calibrated against a reference serum (1988) from the Paul-Ehrlich Institute (Langen, Germany) were used to construct a standard curve to quantify anti-CMV IgG antibody concentrations in the blood samples.

**Neutralization Assay**

The microneutralization assay was carried out as described previously [Gonzol et al., 1986]. Briefly, heat-inactivated (56°C, 30 min) serum was added to culture medium (minimal essential medium plus 7.5% fetal calf serum) in duplicate wells of 96-well, flat-bottomed culture plates (Falcon, Lincoln Park, NJ) and serial two-fold dilutions were made. Sera with high anti-CMV IgG antibody concentrations were tested at dilutions of 1:32 to 1:4,096, and sera with lower anti-CMV IgG antibody concentrations were tested at dilutions of 1:8 to 1:1,024. Some sera, in which the neutralizing antibody (NA) titer was not reached, were retested at higher dilutions. Control wells for virus replication received culture medium. To each well, 0.06 ml of virus suspension containing 7.5 × 10^3 plaque-forming units of HCMV (Towne strain) and 0.005 ml of guinea pig complement (Bio-Whittaker, Walkersville, MD) were added. After incubation for 1 hr at 37°C in a CO2 incubator, 0.15 ml of cell suspension (MRC-5, normal human fetal lung fibroblast cells; purchased from Aging Cell Repository, Coriell Institute for Medical Research, Camden, NJ) between passages 23–26, 1.5 × 10^4 cells/well) was added and the plates were incubated for an additional 2–3 days. NA titers were read as reciprocals of the highest dilution of serum that inhibited the viral cytopathic effect (CPE) (<10% CPE) compared to 100% CPE in virus control wells. A reference human serum pool with known neutralizing titer and a neutralization-negative human serum pool were included in each microneutralization assay as positive and negative controls, respectively. Positive neutralizing titers were considered to be ≥1:8.

**CMV Detection**

To exclude congenital CMV infection, a sterile urine sample was obtained from all newborn infants at a median age of 1 day (range 1–5 days) for virus isolation by human fibroblast culture and by a multiplex polymerase chain reaction (PCR), using the primer pairs MIE gene (amplification of a 435-bp sequence of CMV DNA that codes for a portion of the major immediate-early antigen), and gB (296 bp) and gH (215 bp), which amplify parts of the glycoprotein B and glycoprotein H genes, respectively [Yamamoto et al., 1998]. Urine samples with at least two amplified CMV genomic fragments were considered positive. Cell cultures were inoculated immediately after a sample aliquot was stored at −70°C until PCR detection of genomic CMV fractions.

**Statistical Analysis**

Data were analyzed using the EPI INFO 6.04 and Statistical Analysis System (SAS Institute Inc., Cary, NC, version 6.12) software packages.

The sample size of each group was calculated assuming that the mean anti-CMV antibody concentration for the group of children at 37–41 weeks gestational age was 95% of that of the mothers. The objective was to demonstrate a 30% difference in mean neonatal antibody concentration between groups. Using an α error of 5%, 80% test power and a 95% confidence interval, the sample size ranged from 34–49 participants in each group.

Analysis of variance (ANOVA) was used to compare birth weight and gestational age between the four study groups. Previous number of births was compared by the Kruskal-Wallis test. The χ2 test was used to compare the categorical variables in the four groups, and the χ2 test for linear trends was used to compare the proportion of women receiving prenatal care.

Antibody levels are shown as geometric mean and range levels. The placental transfer ratios of anti-CMV IgG antibodies and neutralizing titers were calculated by dividing the antibody concentration for the infant by that of the respective mother. Correlation, between maternal and infant antibody levels and between gestational age and infant antibody levels, was calculated using Spearman’s correlation coefficient.

Except for total CMV IgG transfer ratio, untransformed antibody data differed from normality. Logarithmic transformation of maternal and infant total
CMV IgG antibody levels restored normality; however, no transformation that was carried out on maternal and infant neutralizing titers or on CMV neutralizing transfer ratios was successful in restoring normality. Thus, parametric and nonparametric tests were used when appropriate. A two-sided paired Student’s t-test was used to compare the differences between the logarithms of maternal and infant total CMV IgG antibody levels in each study group and for the combined data for the four groups. For comparisons of maternal and infant CMV neutralizing titers, the nonparametric two-sided sign test was used.

Multiple regression analysis was used to assess the effect of maternal anti-CMV IgG antibody concentration, gestational age, and adequacy of intrauterine growth on logged values of total CMV IgG concentrations in the infants. In this analysis, gestational age was divided into four categories (according to study groups) and adequacy of intrauterine growth into two categories (adequate for gestational age and small for gestational age).

Comparisons of placental transfer ratios of CMV total IgG and CMV neutralizing antibodies between groups at the same gestational age interval were carried out using the nonparametric one-sided sign test (Snedecor and Cochran, 1989).

RESULTS

The demographic characteristics of mothers and infants included in the study are shown in Table I. Birth weight and gestational age differed significantly between groups. In addition, there were differences in the percentage of mothers receiving prenatal care, with a significant trend toward increasing proportions from Group 1 to Group 4.

Total Anti-CMV IgG and CMV Neutralizing IgG Antibodies in Mothers and Infants

As shown in Table II, infant levels of both total CMV IgG and CMV neutralizing antibodies were significantly lower than the respective maternal levels. In addition, total IgG and neutralizing antibody levels in infants correlated positively and significantly with maternal levels of the respective antibody (Table II). Regarding gestational age, there was a weaker correlation with both infant total anti-CMV IgG concentrations \((r = 0.185, P < 0.01)\) and CMV neutralizing titers \((r = 0.340, P < 0.01)\); however, a significant interaction was found between gestational age and maternal concentration of total CMV IgG antibodies \((P = 0.03)\) on infant antibody concentrations. In contrast, intrauterine growth retardation did not influence infant antibody concentrations \((P = 0.46)\). Furthermore, total CMV IgG concentrations and CMV neutralizing titers were correlated significantly in both mothers \((r = 0.501, P < 0.01)\) and infants \((r = 0.568, P < 0.01)\). Maternal neutralizing titers also correlated with total anti-CMV IgG concentrations in infants \((r = 0.525, P < 0.01)\) as did neonatal neutralizing titers with total anti-CMV IgG concentrations in mothers \((r = 0.414, P < 0.01)\).

Total Anti-CMV IgG and CMV Neutralizing IgG Antibodies in the Four Study Groups

Table III shows the CMV antibody levels of mothers and infants in the four groups. Maternal total anti-CMV IgG antibody concentrations were similar. Although there was a trend of increasing maternal neutralizing antibody titers with increased gestational age, these differences were not significant. All mothers had positive neutralizing titers \((≥ 1:16)\). In only one of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 (28–30)</th>
<th>2 (31–33)</th>
<th>3 (34–36)</th>
<th>4 (37–41)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>44</td>
<td>51</td>
<td>62</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Mean (SD) age of mother (years)</td>
<td>27 (6.2)</td>
<td>26 (7.4)</td>
<td>25 (7.6)</td>
<td>24 (5.8)</td>
<td>0.31a</td>
</tr>
<tr>
<td>Median (range) previous number of births</td>
<td>1 (0–4)</td>
<td>1 (0–9)</td>
<td>1 (0–4)</td>
<td>1 (0–5)</td>
<td>0.85b</td>
</tr>
<tr>
<td>Prenatal care (%)</td>
<td>34 (79.1)</td>
<td>46 (86.8)</td>
<td>58 (90.6)</td>
<td>37 (94.9)</td>
<td>&lt;0.01c</td>
</tr>
<tr>
<td>Mean (SD) gestational age (weeks)</td>
<td>29.4 (0.8)</td>
<td>32.5 (0.7)</td>
<td>35.0 (0.7)</td>
<td>38.8 (1.1)</td>
<td>&lt;0.01a</td>
</tr>
<tr>
<td>Mean (SD) birth weight (g)</td>
<td>993 (231)</td>
<td>1585 (325)</td>
<td>2129 (480)</td>
<td>2971 (558)</td>
<td>&lt;0.01a</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.22d</td>
</tr>
<tr>
<td>Female (%)</td>
<td>24 (54.5)</td>
<td>20 (39.2)</td>
<td>34 (54.8)</td>
<td>23 (59)</td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>20 (45.5)</td>
<td>31 (60.8)</td>
<td>28 (45.2)</td>
<td>16 (41)</td>
<td></td>
</tr>
<tr>
<td>Intrauterine growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.38e</td>
</tr>
<tr>
<td>SGA (%)</td>
<td>4 (9)</td>
<td>5 (10)</td>
<td>12 (19)</td>
<td>4 (10)</td>
<td></td>
</tr>
<tr>
<td>AGA (%)</td>
<td>40 (91)</td>
<td>46 (90)</td>
<td>50 (81)</td>
<td>35 (90)</td>
<td></td>
</tr>
</tbody>
</table>

* n, number of cases; SGA, small for gestational age; AGA, adequate for gestational age.
* ANOVA.
* Kruskal-Wallis test.
* \(\chi^2\) for linear trend.
* \(\chi^2\) test.
* \(\chi^2\) test (with combination of data from groups 3 and 4).
196 (0.5%) newborns, no positive neutralizing titer (≥1:8) was found (a preterm infant of 29 weeks gestational age). The lowest levels of both CMV antibodies were detected in infants of the youngest gestational age group. Although total anti-CMV IgG antibody concentrations in the infants did not approach maternal concentrations until 37–41 weeks of gestational age, CMV neutralizing titers for infants aged 34–36 weeks and older were found to be similar to maternal levels.

**Placental Transfer of Total Anti-CMV IgG and CMV Neutralizing IgG Antibodies**

The infant/mother CMV IgG antibody ratios increased significantly from Group 1 to Group 3 (median transfer ratios of 60, 84, and 91%, respectively). The efficiency of antibody transfer from mother to infant increased progressively with gestational age until 34–36 weeks, when the value for infants of gestational age greater than 37 weeks was reached, i.e., 90–100% of maternal anti-CMV IgG antibodies were transferred to the infant. Improvement in the efficiency of neutralizing antibody transfer from mothers to infants with gestational age was also observed (median transfer ratios of 75, 100, 100, and 100% in Groups 1, 2, 3, and 4, respectively). Compared to IgG CMV antibodies, however, higher neutralizing antibody transfer ratios were found in the two youngest gestational age groups (Fig. 1), indicating a favored transfer of neutralizing antibodies for those under 34 weeks of gestational age.

**DISCUSSION**

The present study was carried out while taking into consideration that information on the placental transfer of anti-CMV IgG antibodies is scanty. In addition to investigating the quantitative nature of passively acquired neonatal anti-CMV IgG antibodies, we evaluated the qualitative characteristics of these antibodies with regard to their viral neutralizing activity in vitro. In this study, the inclusion of women of low socioeconomic background from a population with an elevated incidence of CMV seropositivity allowed us to evaluate the placental transfer of CMV IgG antibodies under conditions favoring recurrence of the infection and antigenic challenge with different virus strains, which may boost the synthesis of specific antibodies by the mother [Stagno et al., 1985]. In addition, total anti-CMV IgG and neutralizing CMV antibody concentrations from mothers of infants of the different gestational age groups studied did not differ, permitting comparison of placental antibody transfer between these groups.

Overall, the placental transfer of CMV antibodies was found at all gestational ages studied, i.e., 28–41 weeks. A significant positive correlation was observed between maternal and infant antibody levels, as reported by other investigators, for the transfer of antibodies against different agents [Christensen et al., 1984; Linder et al., 1998; Ozbek et al., 1999]; however, differing from the strong correlation reported for total IgG concentrations [Toivanen et al., 1968], infant anti-CMV antibody levels and gestational age were only weakly correlated. Even so, an interaction between the effects of maternal anti-CMV antibody levels and gestational age on total CMV IgG antibody concentrations in the newborn infants was found, i.e., the higher the maternal antibody levels, the higher those seen in the infant. This effect became more pronounced with progressive gestational age.

In this respect, the efficiency of total anti-CMV IgG antibody transfer increased significantly as gestation progressed from 28 to 34–36 weeks, as demonstrated by the considerable enhancement in the infant/mother total anti-CMV IgG placental transfer ratios (Table III, Fig. 1). Furthermore, considering the quantity of these antibodies that effectively reached the neonatal serum in the different gestational age groups and the correlation with maternal levels, the present data provide evidence for the existence of transport mechanisms that mature progressively during gestation with a reduction in rate of increase of antibody transfer from a gestational age of 34–36 weeks to term, when neonatal total CMV IgG antibody levels are similar to those found in mothers.

Placental transfer of CMV neutralizing antibodies becomes similarly enhanced with gestational age (Table III, Fig. 1). Our results indicate that the passage
of neutralizing IgG antibodies is more effective earlier than that of total CMV IgG antibodies, however, because a significantly higher ratio of placental transfer of neutralizing antibodies was found in newborns of 28–30 (75% vs. 60%) and of 31–33 weeks of gestational age (100% vs. 84%). In addition, a non-significant trend, which would probably be significant if the sample size were larger, was found in newborns of 34–36 weeks of gestational age (100% vs. 91%). Although the amount of total CMV IgG that effectively reached the neonatal serum was similar to maternal levels only at 37–41 weeks, comparable neonatal and maternal levels of neutralizing antibody were found at 34–36 weeks of gestational age.

Few studies have measured the placental transfer of specific antibodies to preterm infants. With few exceptions [Linder et al., 1998; Wesumperuma et al., 1999], the specific antibody levels detected in preterm infants were significantly lower than those in term infants [Christensen et al., 1984; Linder et al., 1998; Gonçalves et al., 1999; Ozbek et al., 1999; Costa-Carvalho et al., 1999], as also seen in the present study. Ozbek et al. [1999], studying passive immunity against measles at different gestational age intervals, demonstrated a higher transfer ratio than the total CMV IgG ratio found here (82% vs. 57%, respectively) for less than 32 weeks of gestational age, whereas similar ratios were found for gestational ages above 32 weeks (approximately 85%). Regarding neutralizing antibodies, Linder et al. [1999] have shown diminished placental transfer of neutralizing antibodies to rubella in preterm infants when compared to term infants; no significant difference

### Table III. Placental Transfer of Total IgG and Neutralizing IgG Antibodies Against CMV According to Gestational Age

<table>
<thead>
<tr>
<th>Group 1 (28–30 wk)</th>
<th>Group 2 (31–33 wk)</th>
<th>Group 3 (34–36 wk)</th>
<th>Group 4 (37–41 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total IgG antibody against CMV (U/ml)</strong></td>
<td><strong>Neutralizing IgG antibody against CMV (U/ml)</strong></td>
<td><strong>Total IgG antibody against CMV (U/ml)</strong></td>
<td><strong>Neutralizing IgG antibody against CMV (U/ml)</strong></td>
</tr>
<tr>
<td>Mothers</td>
<td>Newborns</td>
<td>Mothers</td>
<td>Newborns</td>
</tr>
<tr>
<td>Geometric mean level (range)</td>
<td>Geometric mean level (range)</td>
<td>Geometric mean level (range)</td>
<td>Geometric mean level (range)</td>
</tr>
<tr>
<td>3.24 (1.1–10.0)</td>
<td>1.78 (0.2–7.6)</td>
<td>3.44 (1.1–17.3)</td>
<td>2.55 (0.5–13.2)</td>
</tr>
<tr>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
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</table>

*P* values from paired Student’s *t*-test for comparison between logarithms of maternal and infant total CMV IgG concentrations, and from two-sided sign test for comparison between maternal and infant neutralizing titers.

![Fig. 1. Comparison of placental transfer ratios (neonatal/maternal) of IgG antibodies against CMV, and of CMV neutralizing antibodies, for the four groups. (*P*-values were obtained by the one-sided sign test)](image)
was found for hemagglutination inhibition antibodies with respect to placent al transfer in preterm and full-term infants. In general, these findings seem to disclose the heterogeneity of placent al maturation of the transport process for antibodies with different specificities. Different characteristics of antibody production against diverse agents in different populations and the influence of maternal, premature infant and placental characteristics so far, have not been identified.

On the whole, in the absence of chronic maternal infections or hyperimmunoglobulinemia, the characteristics of specific antibodies with respect to their IgG subclass determine the effectiveness of their placent al transfer [Einhorn et al., 1987]. Antibodies against viral proteins, which are predominantly of the IgG1 subclass [Gupta et al., 1996] are transferred more efficiently than those against encapsulated bacteria, where IgG2 antibodies predominate [Costa-Carvalho et al., 1996]. With respect to CMV, subclasses IgG1 and IgG3 are the most frequent and active immunoglobulins found in healthy seropositive individuals [Weber et al., 1993]; IgG1 represents 96% of the anti-CMV IgG subclass, followed by IgG3 at 3% [Gilljam and Wahren, 1989; Gupta et al., 1996]. In the present study, the progressive improvement in transferal of maternal CMV IgG to infants may reflect the facilitated transport of this immunoglobulin subclass with the progression of gestation.

With respect to neutralizing antibodies, the variation in transfer ratios was greater than that for total CMV antibodies, suggesting preferential transfer of these antibodies before 34 weeks of gestation. Lower transfer ratios for total IgG antibodies than neutralizing antibodies in younger gestational age groups might be explained by a lower sensitivity of the enzyme immunoassay. It is possible that the antigen or other components of this assay did not react as strongly when lower concentrations of antibody were present, although the neutralization assay still detected antibody activity. Considering that placent al transport of maternal IgG depends on expression and affinity of various and heterogeneous types of Fc receptors on placent al tissues [Saji et al., 1999], it may be postulated that mechanisms of placent al transport of neutralizing antibodies, which belong mostly to IgG1 and IgG3 subclasses [Gupta et al., 1996], mature early. This may lead to preferential passage of these antibodies in younger gestational age groups even at relatively low concentrations.

In summary, the placent al transfer of naturally acquired maternal total and neutralizing CMV antibodies is equally efficient above 34–36 weeks of gestational age and at term, with transfer ratios close to 100%. The transfer of neutralizing antibodies may be favored under 34 weeks of gestational age, and such antibodies effectively reach the neonatal serum of the vast majority (99%) of young preterm infants. Although antibody requirements for adequate protection against CMV infection remain to be determined, given that the CMV antibody concentrations received by both term and preterm infants is a function of the interaction between the maternal antibody concentration and gestational age, we suggest that an increase in circulating antibody levels in the mother might result in higher anti-CMV antibody concentrations, even in newborn infants of low gestational age. This finding provides useful information for the study of measures that lead to an increase in maternal antibody levels, either through active or passive immunization.

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