

# Placental Enlargement in Women with Primary Maternal Cytomegalovirus Infection Is Associated with Fetal and Neonatal Disease

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(See the editorial commentary by Schleiss on pages 1001–3)

**Background.** Serological testing for primary maternal cytomegalovirus (CMV) infection during pregnancy is not routine, but ultrasound studies are routine. Therefore, we evaluated placental thickening in women with primary CMV infection during pregnancy.

**Methods.** The study included 92 women with primary CMV infection during pregnancy and 73 CMV-seropositive pregnant women without primary CMV infection. Neonatal CMV transmission was determined by CMV culture of urine samples. Thirty-two women were treated with CMV hyperimmune globulin to either prevent or treat intrauterine CMV infection. Maximal placental thickness was measured by longitudinal (nonoblique) scanning with the ultrasound beam perpendicular to the chorial dish. Programmed placental ultrasound evaluations were performed from 16 to 36 weeks of gestation.

**Results.** At each measurement between 16 and 36 weeks of gestation, women with primary CMV infection who had a fetus or newborn with CMV disease had placentas that were significantly thicker than those of women with primary CMV infection who did not have a diseased fetus or newborn ( $P < .0001$ ); the latter group, in turn, had placentas that were significantly thicker than those of seropositive control subjects ( $P < .0001$ ). For both women with and women without diseased fetuses or newborns, receipt of hyperimmune globulin after primary CMV infection was associated with statistically significant reductions in placental thickness ( $P < .001$ ). Placental vertical thickness values, which are predictive of primary maternal infection, were observed at each measurement from 16 to 36 weeks of gestation, and cutoff values ranged from 22 mm to 35 mm, with the best sensitivity and specificity at 28 and 32 weeks of gestation.

**Conclusions.** Primary maternal CMV infection and fetal or neonatal disease are associated with sonographically thickened placentas, which respond to administration of hyperimmune globulin. These observations suggest that many of the manifestations of fetal and neonatal disease are caused by placental insufficiency.

Cytomegalovirus (CMV) is the most common congenital infection and causes mental retardation and sensorineural defects [1, 2]. Neonates with CMV infection represent 0.5%–2% of all live births, and 10% of these have signs or symptoms of disease at birth. The majority of symptomatic congenital CMV infections follow a primary maternal infection during pregnancy and have

a poor prognosis [3]. Fetal lesions including ventriculomegaly, ascites, and growth restriction caused by prenatal CMV infection are frequently observed on ultrasound, although they are often observed too late for interventional decisions to be made. CMV hyperimmune globulin (HIG) may be able to reduce fetal morbidity caused by CMV infection and prevent maternal-fetal transmission of CMV [4]. Thus, prenatal diagnosis of maternal CMV infection is important.

Because CMV infects the placenta before it infects the fetus, we prospectively investigated placental size following maternal primary CMV infection to determine if placental size (as measured by maximal placental thickness) indicates infection of the fetus.

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## METHODS

**Patients.** Eighty-four Italian women who had placental thickness measurements taken between 16 and 36 weeks of gestation were part of a previously published prospective study reporting the apparent effect of treatment with HIG in preventing and treating congenital CMV infection among women with primary CMV infection during pregnancy [4]. The diagnosis of primary CMV infection was made by screening asymptomatic women with serological testing (measuring CMV IgG, IgM, and IgG avidity) of serial serum samples obtained during pregnancy. Also included in the study were 8 women with primary CMV infection during pregnancy who were evaluated after enrollment in the study closed, as well as 73 pregnant women who were identified during the study by serological screening and who were seropositive for CMV at 6 weeks of gestation. These women were found not to have recent or active CMV infection (including reinfection or a reactivated infection); none had an increase in the concentration of IgG to CMV, none had CMV IgM or DNA in maternal blood, and all had high-avidity IgG antibodies to CMV. As described elsewhere [4], 32 women with primary CMV infection received HIG at various times during the pregnancy either to prevent fetal infection or to treat fetal disease.

Fetal infection was determined by the presence of CMV DNA and/or culture of CMV in the amniotic fluid, and neonatal infection was determined by CMV DNA detection in blood and urine samples and by culture of CMV from urine [4]. None of the 73 seropositive women had newborns with CMV infection.

Smokers and women with twin pregnancies, diabetes, hypertension in pregnancy, and fetal malformations or chromosomal abnormalities were excluded from this study. All women had test results that were negative for HIV infection, syphilis, toxoplasmosis, and rubella. Signed consent was obtained from the study subjects.

**Laboratory methods.** The laboratory methods used in this study, including PCR for CMV DNA and serological testing, have been described elsewhere [4, 5].

**Ultrasound examination.** We assessed placental thickness from 16 weeks of gestation to 36 weeks of gestation. Ultrasound was performed using Aloka Color Doppler SSD-2000 (Aloka) with convex sound at 3.5 MHz and with the woman lying supine; it included basic parameters, such as presentation, biometry, morphology, fetal growth, amniotic fluid index, and maternal-fetal doppler velocimetry related to the gestational age. Placental studies included localization, placental echo structure, and placental maximum vertical thickness as assessed in a bidimensional scan [6].

For each subject, we recorded the maximum vertical thickness of the placenta. The placental thickness was measured by

longitudinal (nonoblique) scanning, with the ultrasound beam perpendicular to the chorial dish. The measurements avoided areas of amniochorial detachment or those induced by uterine contractility or fibrotic areas [6]. In real time, we repeated the process until 3 measurements of vertical thickness were performed; each sweep required ~15 sec. To obtain a mean value, we considered only evaluations that provided the clearest view of the placenta.

All ultrasound studies were performed by the same person (R.L.T.), who was unaware of serological testing results but was, of course, aware of fetal ultrasound abnormalities and of which women were receiving HIG.

**Fetal and neonatal disease.** We previously observed that, in mothers with primary CMV infection during pregnancy, fetal disease was an independent predictor of disease due to CMV infection both at birth and at >2 years of age, and we observed that the resolution of fetal disease was associated with maternal HIG administration [4]. In the current analysis, involving 20 women with diseased fetuses or neonates, we observed only fetal disease in the live-born fetuses of 7 women (6 of whom who received HIG) and in 4 fetuses who were aborted; we observed neonatal disease without fetal disease for 3 subjects and both fetal and neonatal disease for 6 subjects. Fetal disease was defined as 1 or more of the following: ventriculomegaly, microcephaly, intrauterine growth restriction, ascites, organomegaly detectable by ultrasound, pyelectasis, megaloureter, and periventricular or hepatic and intestinal echodensities. Manifestations of neonatal disease included small size for gestational age, microcephaly, thrombocytopenia, periventricular calcifications, agyria, cerebral and cerebellar atrophy, hemiparesis, chorioretinitis, microphthalmia, and liver disease.

**Statistical methods.** Group mean values at baseline were compared across groups using analysis of variance, and proportions were compared using a  $\chi^2$  test. The trends in placental thickness between groups and across time were analyzed using a repeated-measures mixed model (analysis of variance). The model details are shown in the Results section. Tukey's Honestly Significant Difference procedure was used to adjust for multiple comparisons. Because placental thickness was strongly skewed, log-transformed data were used in analyses. Statistical significance was assessed at  $\alpha < .05$ . All analyses were performed using either SAS or JMP software (version 9.1 and version 5.1, respectively; SAS Institute).

## RESULTS

Initially, 92 women with primary CMV infection during pregnancy were divided into those who transmitted CMV infection to the fetus (47 women) and those who did not (45 women); these 2 groups were compared with a group of 73 seropositive women without CMV infection during pregnancy (table 1).

**Table 1. Characteristics of pregnant women, by type of cytomegalovirus (CMV) infection, presence of CMV transmission, and presence of disease in fetuses or neonates.**

Variable	Patients with primary CMV infection, by transmission of CMV infection			Patients with primary CMV infection, by presence of disease in fetuses or neonates			CMV-seropositive control subjects (n = 73)
	Transmission (n = 47)	No transmission (n = 45)	P	Fetal or neonatal disease (n = 20)	No fetal or neonatal disease (n = 72)	P	
Maternal age, mean years ± SD	28.4 ± 5.3	28.1 ± 4.8	<.001 <sup>a</sup>	28.6 ± 6.1	28.1 ± 4.8	.01 <sup>a</sup>	31.9 ± 4.6
Gestational age at time of maternal primary infection, mean weeks ± SD	14.3 ± 6.9	13.9 ± 6.9	.74	11.6 ± 4.5	14.8 ± 7.3	.016	NA
Women with first pregnancy, %	49	56	.67	40	56	.38	47

**NOTE.** NA, not applicable.

<sup>a</sup> Statistically significant difference versus the CMV-seropositive control group.

Women with primary infection (including both those who transmitted infection to the fetus and those who did not) were a mean of 4 years younger than the seropositive women ( $P < .0001$ ). Among women with primary CMV infection, we compared women who had fetuses or neonates with disease with women whose fetuses or neonates did not have disease (table 1). The 20 mothers with primary CMV infection whose fetuses and neonates had disease and the 72 mothers of healthy fetuses and neonates were, on average, younger than seropositive women ( $P = .01$ ). Women who had fetuses or neonates with disease were infected with CMV earlier in pregnancy than women whose fetuses or neonates were uninfected or did not have disease ( $P = .02$ ). The percentage of women with a first pregnancy was similar in all groups ( $P = .67$ ) (table 1). Because there were statistically significant differences between the groups with respect to maternal age and gestational age at the time of maternal infection, these variables were included as possible confounders in all subsequent analyses.

Women were assessed for maximal placental thickness at 4-week intervals during the second half of pregnancy. A total of 32 women with primary CMV infection received HIG at various times during the pregnancy. A total of 91 (55%) of the women had  $\geq 3$  measurements; the frequency of placental measurements by gestational age and the number of women receiving HIG are listed in table 2.

To determine whether there was a difference in maximal placental thickness among groups and across the duration of the pregnancy, the following factors were included in a repeated-measures mixed-model analysis of the log-transformed thickness: initial patient group (CMV transmission group, nontransmission group, or CMV-seropositive control group), gestational age at the time of maternal seroconversion, receipt of HIG, maternal age at conception, and gestational week when placental thickness was measured. The repeated measures on each subject were taken into account by allowing

for an unstructured covariance between the measurements of placental thickness. In this model, the 3 initial groups (CMV transmission group, nontransmission group, and CMV-seropositive control group) were significantly different ( $P < .0001$ ), and there was a significant increase in maximal placental thickness for each measurement between 16 and 36 weeks of gestation ( $P < .0001$ ). Gestational age at the time of maternal infection was a significant predictor of placental thickness ( $P = .0028$ ), but maternal age was not ( $P = .418$ ).

Next, we considered placental thickness among mothers with fetuses or neonates with disease to determine whether CMV transmission was more strongly related to placental thickness than was fetal and neonatal disease. After the presence or absence of fetal or neonatal disease was added to the multivariate model, we found that the placental thickness of the seropositive mothers, compared with that of the mothers with primary infection, was significantly lower at each measurement ( $P < .0001$ ), and the placental thickness of women with fetuses or neonates with disease was larger at each measurement than the placental thickness of mothers with fetuses or neonates without disease ( $P < .0001$ ). After adjustments were made for these differences, we found that, among women with primary infection and fetuses or neonates without disease, the placental thickness of women who transmitted CMV did not significantly differ from that of women who did not transmit CMV ( $P = .1731$ ). Again, maternal age was not a statistically significant predictor of placental thickness ( $P = .3670$ ). In the final reduced multivariate model, the only significant independent predictors of placental thickness were patient group ( $P < .001$ ), receipt of HIG ( $P < .001$ ), and time of measurement of placental thickness. Gestational age at maternal seroconversion was not significant ( $P = .1106$ ) after adjustment for these factors.

The mean predicted values for placental thickness for each group and each measurement are shown in table 3 and figure 1. The 2 case groups had significantly different placental thick-

**Table 2. Pregnant women with placental thickness measurements, by type of cytomegalovirus (CMV) infection, presence of CMV transmission, and presence of disease in fetuses or neonates.**

Gestational week	No. of patients with primary CMV infection with placental thickness measurement (no. of patients who received HIG)				No. of CMV-seropositive control subjects with placental thickness measurement
	With transmission of CMV infection	Without transmission of CMV infection	With disease in fetuses or neonates	Without disease in fetuses or neonates	
16	15	16 (2)	7	24 (2)	29
20	27	30 (4)	13	44 (4)	36
24	24 (4)	29 (6)	10 (3)	43 (7)	42
28	25 (8)	32 (7)	12 (5)	45 (10)	31
32	21 (5)	33 (7)	6 (1)	48 (11)	31
36	20 (7)	17 (5)	5 (2)	32 (10)	22

**NOTE.** HIG, CMV hyperimmune globulin.

nesses, compared with the CMV-seropositive control group, at each of the 6 monthly measurements ( $P < .001$ ). Mothers of both diseased and nondiseased fetuses and neonates received HIG at a mean of 24 weeks of gestation. Mothers receiving HIG had significantly larger placentas at week 20 ( $P = .0057$ ) and at week 28 ( $P = .0081$ ), but they had significantly smaller placentas ( $P = .0010$ ) at week 36 (figure 1).

We performed a receiver operator characteristic curve analysis for all placental thicknesses throughout the gestational ages evaluated (table 4). In the initial analysis, primary maternal CMV infection was the variable to be predicted by placental thickness, whereas placental measurements were independent variables. From 16 to 36 weeks of gestation, cutoff values ranged from 22 mm to 35 mm, with the best sensitivity and specificity at 28 and 32 weeks (table 4). In a second analysis, we included only women with primary infection, and fetal or neonatal disease was the variable to be predicted by placental thickness. From 16 to 36 weeks of gestation, cutoff values ranged from 32 mm to 61 mm, with the best sensitivity and specificity again at 28 and 32 weeks (table 4). Although this analysis was limited by the low number of available measurements, the negative predictive values were excellent ( $>0.9$ ) from 16 to 36 weeks of gestation, with narrow 95% CIs (table 4). Finally, we observed that, for women with an increased placental thickness (defined in table 4 as the cutoff values between CMV-seropositive women and women with primary infection) at any time between 16 and 32 weeks, the increased placental thickness persisted in 82 (93%) of 88 women.

## DISCUSSION

In the United States alone, it is estimated that 40,000 women annually acquire an asymptomatic primary CMV infection during pregnancy, and as a result, 8000 newborns will develop serious neurologic handicaps [3]. In most countries, screening for maternal CMV infection during pregnancy is not per-

formed, but ultrasound evaluations are usually routine. Therefore, suspecting primary maternal CMV infection on the basis of the findings of an ultrasound examination may lead to a diagnosis of CMV infection. We have previously reported on the clinical manifestations, abnormal laboratory findings, and fetal ultrasound abnormalities associated with primary maternal infection during pregnancy [4, 7].

Although CMV infection of the placenta precedes virus transmission to the fetus, this is, to our knowledge, the first report of placental thickening associated with primary maternal CMV infection. In our study, placental weights were not measured, but assuming that the density of a thickened placenta remains unchanged, primary maternal CMV infection should be associated with increased placental weight at birth. A previous study of 27 placentas of newborns or fetuses with congenital infection whose mothers had CMV infection of unknown status observed a tendency towards increased placental weight [8]. This study also observed placental inflammation consisting of diffuse vascular inflammation, villitis, necrosis, and calcifications [8].

Preliminary histological analysis of biopsy specimens from placentas in this study showed differences between untreated and HIG-treated women (E. Maidji and L. Pereira, unpublished observations). All infected placentas had damage to villi that float in maternal blood, suggesting CMV pathogenesis, as reported by others [8]. Numerous syncytial knots were found; these resembled defects observed in placentas with hypoxia and intrauterine growth restriction. Many very large fibrinoids encased floating villi, replacing trophoblasts and fetal blood vessels. Leukocytic infiltration, calcification, and necrosis—hallmarks of CMV infection—were evident. Differences were found in placentas from HIG-treated women (notably, small villi with fetal blood vessels developed across the surface). Necrosis was absent in placentas from individuals in the HIG-treated group, who were treated early after primary maternal infection. These

**Table 3. Maximal placenta thickness, by week of gestation.**

Patient group, receipt of HIG	Maximal placenta thickness, least squares mean, mm (95% CI)					
	16 weeks	20 weeks	24 weeks	28 weeks	32 weeks	36 weeks
Women with primary CMV infection with fetuses or neonates with disease <sup>a</sup>						
No	29.9 (25.8–34.8)	38.2 (34.0–42.8)	45.5 (40.67–0.94)	49.2 (43.1–56.1)	57.0 (48.5–67.0)	64.9 (52.6–80.2)
Yes	ND	ND	49.8 (42.9–57.8)	59.5 (50.7–70.0)	55.4 (43.3–71.2)	55.2 (41.0–74.3)
Women with primary CMV infection with fetuses or neonates without disease <sup>b</sup>						
No	23.2 (21.3–25.1)	29.1 (27.4–31.0)	32.8 (31.0–34.6)	37.0 (35.0–39.0)	39.6 (37.2–42.2)	43.6 (40.4–47.0)
Yes	20.0 (15.3–26.2)	36.0 (30.4–42.5)	34.7 (31.6–38.2)	40.7 (37.0–44.8)	37.5 (34.3–41.1)	35.5 (31.8–39.7)
CMV-seropositive control subjects <sup>c</sup>						
No	18.3 (17.0–19.8)	22.3 (20.9–23.7)	25.1 (23.8–26.4)	26.9 (25.3–28.5)	27.7 (26.0–29.5)	28.6 (26.5–30.9)

**NOTE.** CMV, cytomegalovirus; HIG, CMV hyperimmune globulin; ND, not done.

<sup>a</sup> Includes 20 patients.

<sup>b</sup> Includes 72 patients.

<sup>c</sup> Includes 73 patients.

initial observations suggest that placental enlargement could result from fibrinoid deposition and small vascularized villi that form to compensate for hypoxia in utero. Reduced placental size after treatment could be caused by suppressed viral infection and decreased inflammation.

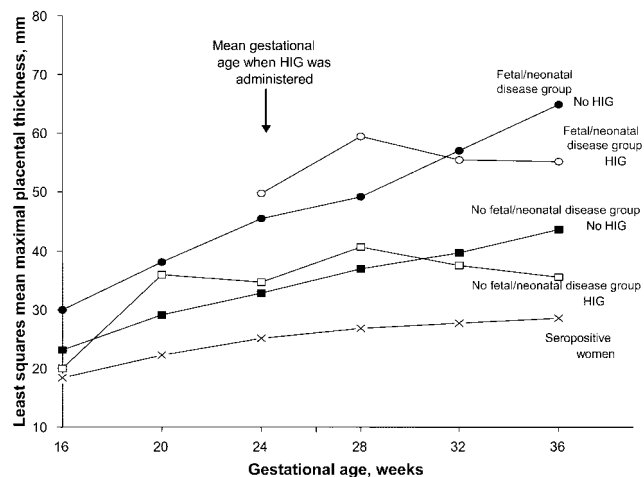
Sonographically thickened placentas have been previously associated with increased fetal and perinatal mortality, abnormally low and high birth weights, fetal hydrops, maternal diabetes, chromosomal abnormalities, maternal and fetal anemia, fetal heart failure, and congenital nephrotic syndrome [9–15]. Thus, a thickened placenta is a nonspecific marker of fetal disease.

Our data have 2 important implications. Recording the placental thickness and comparing it to reference values should be helpful in the prenatal diagnosis of maternal CMV infection. For women lacking another obvious cause for placentomegaly, the diagnosis of a recent CMV infection is possible with a blood sample that can be accurately tested for IgM antibodies to CMV, the avidity and quantity of IgG antibodies to CMV, and CMV DNA. If a maternal infection is confirmed or if there are ultrasound findings of fetal involvement or growth restriction, amniocentesis may be appropriate. After diagnosis of fetal or maternal infection, possible available interventions include not only termination of the pregnancy but also the use of HIG (which appears to be effective to either prevent maternal-fetal CMV transmission or treat CMV disease in utero), prompt neonatal diagnosis and evaluation, and possible treatment with ganciclovir [4, 16].

A limitation of our data is that the maternal infections that we studied all occurred in the first or second trimester of pregnancy, with a mean gestational age at maternal infection of 11–14 weeks. It is unknown whether maternal infections occurring later in pregnancy are also associated with an increase in placental thickness. Another possible limitation is that our com-

parison group was composed of CMV-seropositive women with inactive CMV infections. However, we presume that the placental thickness for these women would be the same as that for seronegative women, because the values that we observed for seropositive women were nearly identical to published values for normal, uncomplicated pregnancies [15].

A final possible limitation is that selection bias could have been introduced, because not all women had placental measurements at each gestational week in the last half of pregnancy.



**Figure 1.** Maximal placental thickness, by week of gestation, for women who received cytomegalovirus hyperimmune globulin (HIG) and had fetuses or neonates with disease (fetal/neonatal disease group), women who did not receive HIG and were in the fetal/neonatal disease group, women who received HIG and were not in the fetal/neonatal disease group, and women who did not receive HIG and were not in the fetal/neonatal disease group, compared with women who were seropositive for cytomegalovirus. Statistically significant differences and *P* values are listed in the Results section.

**Table 4. Cutoff values for maximal placental thickness, by week of gestation.**

Comparison group, week of gestation	Cutoff value, mm	Area under ROC	Sensitivity (95% CI); no. of women	Specificity (95% CI); no. of women	PPV (95% CI); no. of women	NPV (95% CI); no. of women
CMV-seropositive control subjects vs. patients with primary CMV infection						
16	≥22	0.76	0.66 (0.46–0.82); 29	0.79 (0.6–0.92); 29	0.76 (0.55–0.91); 25	0.70 (0.51–0.84); 33
20	≥25	0.82	0.79 (0.66–0.89); 53	0.72 (0.55–0.86); 36	0.80 (0.66–0.90); 52	0.70 (0.53–0.84); 37
24	≥28	0.85	0.79 (0.64–0.90); 43	0.81 (0.66–0.91); 42	0.81 (0.66–0.91); 42	0.79 (0.64–0.90); 43
28	≥30	0.93	0.88 (0.74–0.96); 42	0.90 (0.74–0.98); 31	0.93 (0.80–0.98); 40	0.85 (0.68–0.95); 33
32	≥34	0.93	0.81 (0.66–0.91); 42	0.94 (0.79–0.99); 31	0.94 (0.81–0.99); 36	0.78 (0.62–0.90); 37
36	≥35	0.88	0.80 (0.59–0.93); 25	0.86 (0.65–0.97); 22	0.87 (0.66–0.97); 23	0.79 (0.58–0.93); 24
Women with primary CMV infection with fetuses or neonates without disease vs. women with primary CMV infection with fetuses or neonates with disease						
16	≥32	0.83	0.86 (0.42–1.00); 7	0.86 (0.65–0.97); 22	0.67 (0.30–0.92); 9	0.95 (0.75–1.00); 20
20	≥40	0.84	0.69 (0.39–0.91); 13	0.88 (0.73–0.96); 40	0.64 (0.35–0.87); 14	0.90 (0.76–0.97); 39
24	≥40	0.90	0.86 (0.42–1.00); 7	0.81 (0.64–0.92); 36	0.46 (0.19–0.75); 13	0.97 (0.83–1.00); 30
28	≥51	0.77	0.71 (0.29–0.96); 7	0.89 (0.73–0.97); 35	0.55 (0.21–0.86); 9	0.94 (0.80–0.99); 33
32	≥54	0.86	0.80 (0.28–1.00); 5	0.92 (0.78–0.98); 37	0.57 (0.18–0.90); 7	0.97 (0.85–1.00); 35
36	≥61	0.94	1.00 (0.29–1.00); 3	0.82 (0.69–0.95); 22	0.43 (0.10–0.82); 7	1.00 (0.82–1.00); 18

**NOTE.** NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic curve.

This type of bias is very unlikely for 2 reasons. First, the differences that we observed in placental size for each group occurred at each gestational week from week 16 to week 36. Second, we observed that, among 88 women with multiple measurements, placental thickness persisted in 82 (93%) of subjects. Thus, placental enlargement was consistent throughout the last half of pregnancy, regardless of the number of women who were sampled at each time point.

The second implication of our data is that the 2-fold increase in placental size that we observed for mothers with primary infection and the nearly 3-fold increase in placental size for those with fetuses or neonates with disease, plus the partial reduction in placental size after treatment with HIG, provide further evidence to support the hypothesis that treatment with HIG resolved fetal disease by neutralizing virus and, thus, reducing placental inflammation and insufficiency; this would then lead to improved fetal nutrition and oxygenation. This is plausible, because most manifestations of congenital CMV infection resolve during the first weeks of life with improved nutrition and oxygenation. Thus, the placenta is likely to be one site of action of HIG, and many of the manifestations of congenital CMV infection at birth, including intrauterine growth restriction, may be due to placental insufficiency.

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