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Regression of fetal cerebral abnormalities by primary cytomegalovirus infection following hyperimmunoglobulin therapy

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Objective To assess the effects of maternal and intra-amniotic hyperimmunoglobulin (HIG) infusions among cytomegalovirus (CMV) infected fetuses with ultrasound abnormalities following a primary CMV infection.

Patients and Methods The subjects were fetuses with CMV-associated cerebral and other ultrasound abnormalities. Three mothers were treated with HIG infusions during pregnancy and two were untreated. Fetal ventricle size, organ echodensity and placental thickness were measured by ultrasound before and after HIG infusions. The children were evaluated between 3 and 7 years of age.

Results The ventriculomegaly of all three fetuses of HIG-treated mothers regressed and the ascites, hepatic echodensities, periventricular echodensities, and intestinal echodensities disappeared. Their sensorial, mental and motor development was normal at 4, 4.7, and 7 years of age. In contrast, both infants born of untreated mothers had signs and symptoms of severe CMV cerebropathy.

Conclusion The outcomes of the infants born to HIG-treated mothers support the efficacy of HIG as a treatment for CMV-infected fetuses with ultrasound cerebral abnormalities. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: congenital cytomegalovirus infection; ventriculomegaly; ascites; periventricular; hepatic and intestinal echodensities; CMV-specific immunoglobulins

INTRODUCTION

Cytomegalovirus (CMV) is the most frequent cause of intrauterine infection and associated congenital cerebral damage (Volpe, 2000; Ross and Boppana, 2005). Children with congenital CMV disease often require expensive interventions such as cochlear implantation, speech therapy, and lifelong custodial care (Arvin *et al*., 2004). Prenatal predictive markers of an adverse outcome include placental–fetal abnormalities by ultrasound with positive diagnostic tests for fetal CMV infection (Nigro *et al*., 2005; La Torre *et al*., 2006). Abnormal placental function and morphology are shown by reduced or increased quantity of amniotic fluid and by placentomegaly (Enders *et al*., 2001; La Torre *et al*., 2006). Cerebral ventriculomegaly by ultrasound is associated with a poor postnatal prognosis (Twickler *et al*., 1993; La Torre *et al*., 2006). Other ultrasound abnormalities are intrauterine growth restriction (IUGR), periventricular, hepatic and intestinal echodensities, organomegaly, ascites, hydrops, pyelectasis, microcephaly, calcifications (Abdel-Fattah *et al*., 2005). Unfortunately, these abnormalities are frequently observed after opportunities for intervention have passed (Adler *et al*., 2007). Therefore, the benefit of an effective prenatal intervention would be substantial.

In a previous study, we observed that hyperimmunoglobulin (HIG) treatment of pregnant women with infected fetuses resulted in favorable outcome both at birth and at 2 years of age (Nigro *et al*., 2005). That report did not include our findings concerning the *in utero* resolution of the signs of fetal disease by ultrasound associated with maternal HIG therapy. We now report three cases from our previous study (in all of them ultrasound examinations demonstrated the resolution during pregnancy of fetal ventriculomegaly and other abnormalities after a primary maternal CMV infection and HIG treatment), and two untreated cases of worsening fetal ventriculomegaly, which was followed by severe postnatal CMV disease. One investigator (RLT) performed ultrasound studies for all cases.

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CASE REPORTS

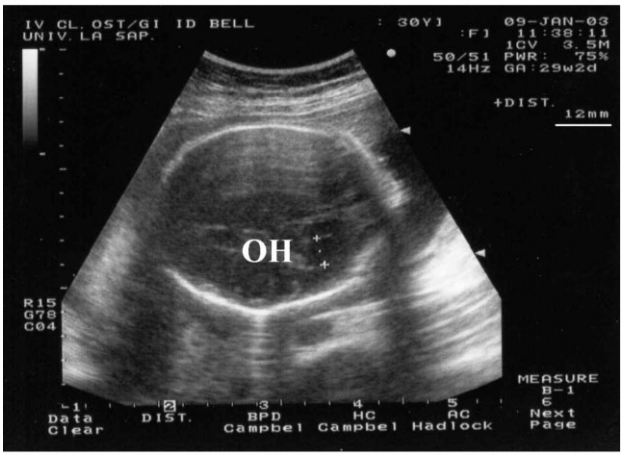
Case 1

A 34-year-old, gravida 2, para 1, seroconverted to CMV between 10 and 21 weeks of gestation. At 25 weeks' gestation CMV-DNA (>1, 000, 000 copies/mL) was detected in the amniotic fluid. At 29 weeks gestation, intravenous HIG (Cytotect Biotest; 200 U/kg) was administered to the mother. Ultrasound and magnetic resonance (MR) evaluations revealed ventriculomegaly (diameter of the occipital horn [OH] of right lateral ventricle: 13 mm; normal <10 mm) and periventricular hyperechodensities. At 33 weeks' gestation periventricular echodensities were absent, ventriculomegaly was slightly reduced (OH: 12 mm) and placenta was enlarged (51 mm). Additional HIG was given both intravenously (200 U/kg) and intra-amniotically (1000 U corresponding to 400 U/kg of fetal body weight). The amount of CMV-DNA in the amniotic fluid before the second infusion of HIG had decreased to 127 780 copies/mL). After 39 weeks of gestation, a CMV-infected but otherwise healthy boy (3200 g) was delivered. Ultrasounds performed before delivery and after birth showed a substantial reduction of ventriculomegaly (OH: 10 mm). A subsequent cerebral MR imaging, and ocular and auditory examinations were normal. The child, who ceased excreting CMV at 3.7 years of age, is now 7 years old with normal mental, visual, auditory and motor development.

Case 2

A 30-year-old woman, gravida 2, para 1, previously CMV seronegative, was referred for a primary CMV infection at 12 weeks gestation with positive CMV IgG and IgM, low CMV IgG avidity, and positive CMV-DNA in her urine. She had mild fever (37.5 °C) from the 7th week of gestation until the 14th, week of gestation associated with pharyngitis, cough, abdominal pain, and deep asthenia. At 21 weeks' gestation, her amniotic fluid contained CMV by culture and CMV DNA by PCR (15 720 copies/mL). At 24 weeks' gestation, HIG was administered (200 U/kg) after an ultrasound revealed slight ventriculomegaly (OH: 10 mm), fetal ascites (6 mm), and an enlarged and hyperechoic liver. Since ascites and ventriculomegaly were further increased (8 and 12 mm, respectively), HIG infusions were also given at 26 and 30 weeks' gestation (Figures 1(a) and 2(a)). At 30 weeks' gestation intra-amniotic HIG (800 U corresponding to 400 U/kg of fetal body weight) was also infused. At 34 weeks' gestation all ultrasound abnormalities were resolved (Figures 1(b) and 2(b)). Placental size, which had increased from 29 mm at 21 weeks' gestation to 72 mm at 29 weeks gestation, decreased to 50 mm at 33 weeks' gestation (Figure 3(a–b)). A CMV-infected, but otherwise healthy, boy (3400 g) was born at 36 weeks. Subsequent cerebral MR, ocular and auditory examinations were normal. At 4.7 years of age, the child, who excreted

(a)



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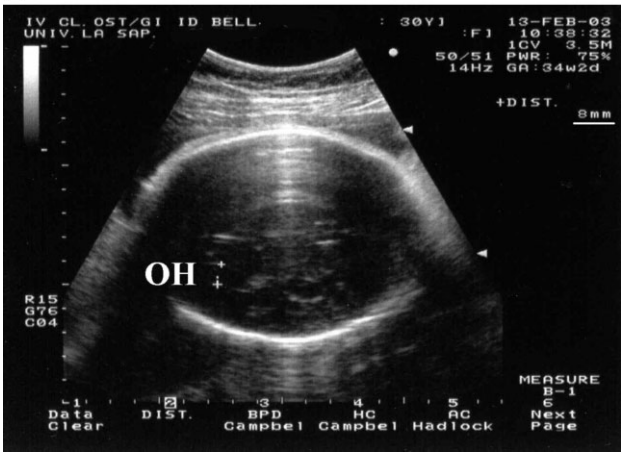

Figure 1—Patient of Case 2, who seroconverted to CMV between 7 and 12 weeks of gestation (WG). She received CMV hyperimmunoglobulin at 24, 26, and 30 weeks' gestation. Shown (a) is the transverse image of the fetal head with enlargement of the occipital horn (OH) at 29 weeks' gestation (12 mm), followed by reduction to 8 mm at 34 weeks' gestation (b)

CMV in the urine until the age of 3 years, had normal mental, sensorial and motor development.

Case 3

A 34-year-old woman, gravida 3, para 2, seroconverted to CMV between 8 and 14 weeks' gestation. At 22 weeks' gestation, CMV-DNA (1540 copies/mL) was detected in the amniotic fluid, and an ultrasound showed intestinal hyperechodensity. HIG (200 U/kg) was infused. At 25weeks' gestation, additional HIG infusion was administered both intravenously (200 U/kg) and intra-amniotically (500 U). There was placentomegaly (45 mm) and rapidly increasing diameters of the lateral ventricles (OH: from 8 to 10 mm within 12 days). Two weeks after HIG infusion, the lateral ventricle size (OH) was reduced to 9 mm and placental size decreased to 37 mm. At 34 weeks gestation, OH was 6 mm but the placenta was again enlarged (50 mm). A CMV-infected boy (2800 g) was born at

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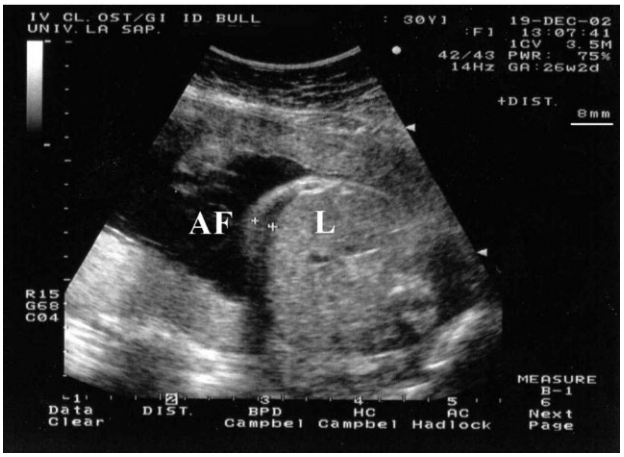
(a)



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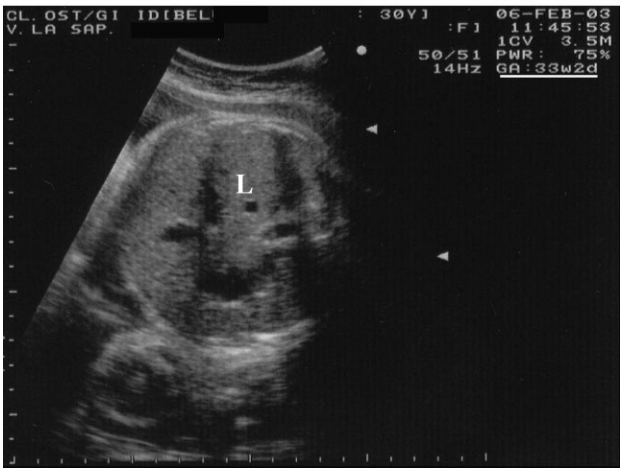


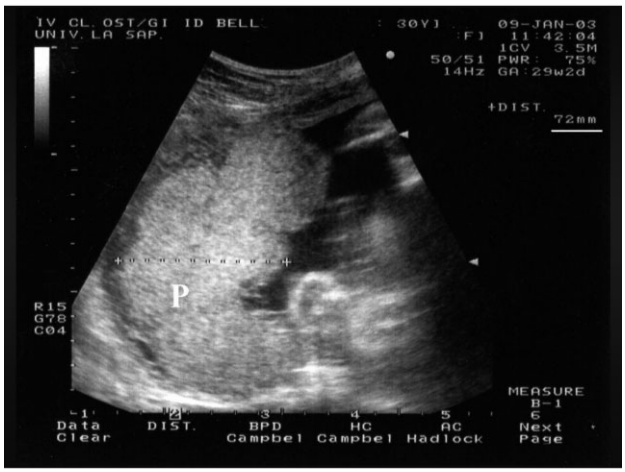
Figure 2—The same subject as in Figure 1. Shown (a) is the transverse image of the fetal abdomen with enlargement of the liver (L) and 8 mm of ascitic fluid (AF) at 26 weeks' gestation, followed by normal liver size and absence of AF at 33 weeks' gestation (b). Liver size was measured by two dimensional measures: transversal and longitudinal from diaphragm to the lower tip

38 weeks' gestation. Cerebral MR, ocular and auditory examinations were normal. At age 3 months, oral ganciclovir was administered for 2 months when the infant developed pneumonia associated with CMV detection in the broncho-alveolar fluid. CMV excretion stopped after 1 month of ganciclovir therapy but resumed at 5 months of age and persisted until 2 years of age. At 4 years of age, the child had normal mental, visual, auditory ABR, and motor development.

Case 4

A 26-year-old, gravida 1, para 0, had CMV IgM antibodies at 9 weeks' gestations. At 19 weeks' gestation, IgG avidity was low and CMV IgM remained positive. At 21 weeks' gestation, the amniotic fluid was positive for CMV by culture and PCR (14 800 DNA copies/mL). At 29 weeks gestation, ventriculomegaly (OH 10 mm) and placentomegaly (41 mm) were detected by ultrasound.

(a)



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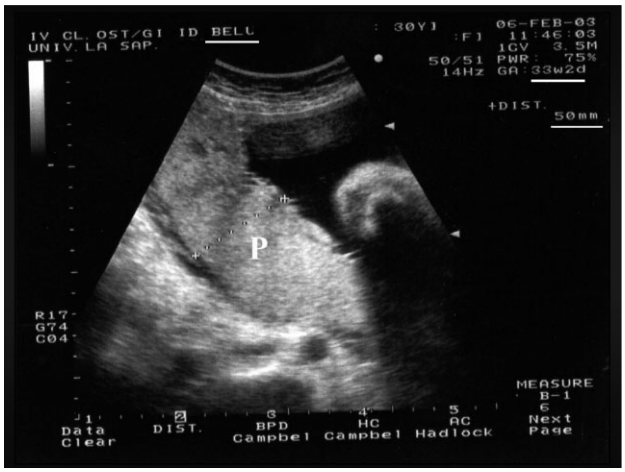

Figure 3—Same subject as in Figure 1. Transverse image of the placental thickness (a: 72 mm at 29 weeks' gestation), which was reduced (b: 50 mm at 33 weeks of gestation)

At 31 weeks' gestation, ventricle size (OH 12 mm) and placental thickness (55 mm) had increased. At 37 weeks' gestation, a boy (3045 g) was delivered who was CMV-infected. He had microcephaly (30.5 cm, <5 percentile), facial dysmorphism, bilateral chorioretinitis and deafness, marked hepatosplenomegaly and anemia. MR showed enlarged and asymmetric lateral ventricles (left OH: 13 mm) with an endoventricular cyst and subcortical atrophy.

Case 5

A 35-year-old woman, gravida 2, para 1, who was previously CMV seronegative developed at 12 weeks' gestation, IgG and IgM antibodies to CMV with very low IgG avidity (4%). At 21 weeks of gestation, ultrasound showed a normal ventricle with an OH diameter of 9 mm (normal <10 mm) but a thickened placenta (41 mm) (Figures 4 and 5(a)). At 35 weeks' gestation, amniotic fluid contained CMV by culture and DNA by PCR (>1, 000, 000 copies/mL), and ventriculomegaly

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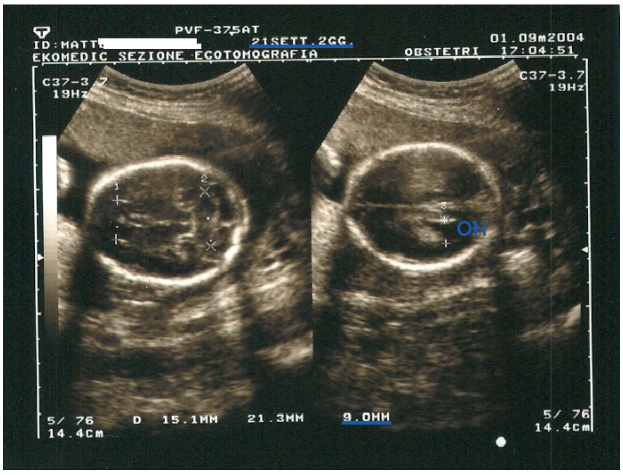
(OH: 12.6 mm) and placentomegaly (74 mm) had developed (Figures 4 and 5(b)). At 36 weeks' gestation, a CMV-infected girl (3350 g) was delivered. Computerized tomography and MR scans of the head revealed ventriculomegaly of both temporal and occipital horns with diffuse periventricular calcifications, cortical and cerebellar atrophy, leukomalacia, bilateral necrosis of the temporal and parietal subcortical white matter.

DISCUSSION

Termination of pregnancy is often offered as the only option when the fetus becomes infected with CMV regardless of whether the disease is detected by ultrasound (Nelson and Demmler, 1997; Volpe, 2000; Ross and Boppana, 2005). In a few pregnant women with fetal CMV infection without ultrasound abnormalities, a low intra-amniotic viral load, and nonprimary maternal infection, oral ganciclovir was reported to be possibly

(a)



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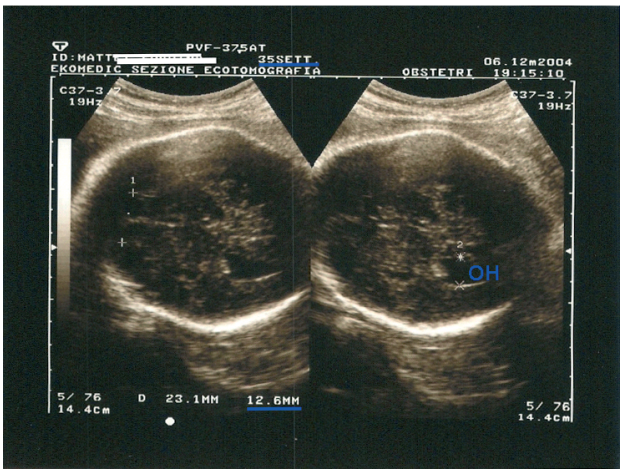
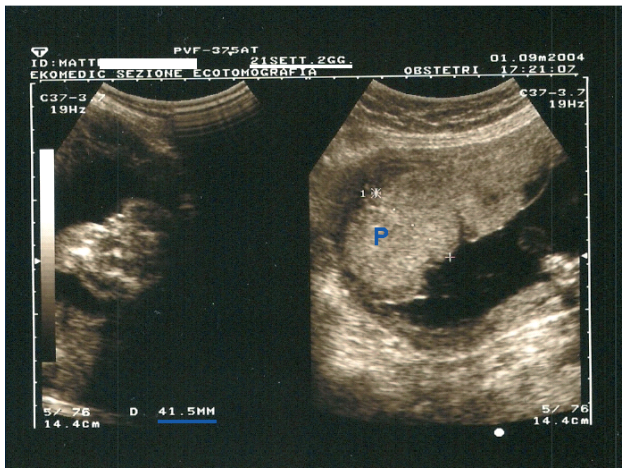


Figure 4—Patient of Case 5, who seroconverted to CMV <12 weeks of gestation and did not receive CMV hyperimmunoglobulins. Transverse image of the fetal head with increasing enlargement of the occipital horn (OH) from 9 mm at 21 WG (a) to 12.6 mm at 35 weeks of gestation (b)

(a)



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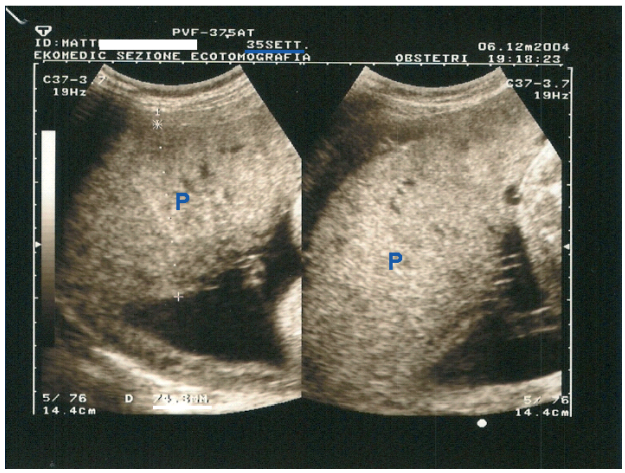

Figure 5—Same subject as Figure 4. Transverse image of the increasing placental (P) enlargement from 41.5 mm at 21 weeks of gestation (a) to 74.8 mm at 35 weeks of gestation (b)

safe and effective (Adler *et al.*, 2007). However, actual efficacy and long-term toxicity of ganciclovir remains to be determined. Recently, it was reported that maternally administered valaciclovir achieved viral inhibitory concentrations in 21 fetuses with CMV disease by ultrasound but their outcome was not statistically different from that of fetuses from untreated mothers (Jacquemard *et al.*, 2007). Infusion of HIG appears to be the only effective drug for the therapy of fetal CMV infection and disease: 14 of 15 pregnant women with placental and/or fetal abnormalities by ultrasound delivered healthy neonates who were also normal at 2 years of age (Nigro *et al.*, 2005). However, this study was not randomized. Thus, an additional clinical trial on fetal CMV immunotherapy was considered necessary before immunotherapy becomes the standard of care (Duff, 2007).

Our report supports the efficacy of HIG, because it shows that initial cerebral and other abnormalities by primary maternal CMV infection disappeared in three fetuses of HIG-treated mothers but not in two fetuses of

Table 1—Outcome among children born to women with CMV-infected amniotic fluid and ultrasonographic evidence of fetal CMV disease after HIG therapy (cases 1–3) or nontherapy (cases 4–5)

Mother–infant pair	Maternal time of seroconversion (weeks gestation)	HIG administered (weeks' gestation)	Ultrasonographic evidence of fetal CMV disease (weeks gestation)	Signs and symptoms at birth
1	10–21	29 IV 33 IV + IA	Ventriculomegaly, periventricular echodensities (29)	None
2	7–12	24 IV 26 IV 30 IV + IA	Ventriculomegaly, ascites, hepatosplenomegaly (24)	None
3	8–19	22 IV 25 IV + IA	Ventriculomegaly, intestinal echodensities (22)	None
4	10	None	Ventriculomegaly (29)	Microcephaly, Ventriculomegaly, endoventricular cyst, subcortical atrophy bilateral chorioretinitis and deafness
5	<12	None	Ventriculomegaly (35)	Periventricular calcifications, cortical and cerebellar atrophy, leukomalacia

CMV, cytomegalovirus; HIG, hyperimmunoglobulin; IV, intravenous HIG administered; IA, intra-amniotic HIG administered.

untreated mothers (Table 1). In each case, CMV seroconversion occurred in the first 4 months of pregnancy, which is the most harmful time for the development of fetal brain abnormalities (Volpe, 2000). In Case 1, periventricular echodensities disappeared soon after the first HIG infusion while ventriculomegaly disappeared just before delivery. A multisystem beneficial effect of HIG was observed in Case 2, in which there was complete disappearance of the fetal ventriculomegaly, hepatomegaly and ascites by 9 weeks after the first HIG infusion. In Case 3, the OH diameter, after a rapid increase from 8 to 10 mm in 2 weeks, decreased to 6 mm after HIG, concomitantly with disappearance of the intestinal echodensities. Moreover, HIG administration was followed by reduction, though not complete, of the placental thickness in all three cases (La Torre *et al*., 2006). In contrast, both untreated cases showed increasing placental thickness and their fetuses developed worsening ventriculomegaly and severe postnatal cerebropathy.

The probable efficacy of HIG in humans is supported by its *in vitro* activity against CMV and by experiments with CMV-infected pregnant guinea pigs in which administration of sera-containing antibody to the whole virus or to the purified glycoprotein B were followed by reduced rates of fetal infection or fetal death, placental inflammation, and pup size (Bratcher *et al*., 1995; Chatterjee *et al*., 2001). In our cases, the regression of fetal CMV ultrasound disease was subsequent to both the neutralizing and immunomodulating activity of HIG (Keller and Stiehm, 2000). Fetal damage may be caused by viral replication and/or inflammatory responses (Dokun *et al*., 2001; Sissons *et al*., 2002). In each treated patient, there also appeared to be a reduction of placental inflammation as suggested by the decreased placental thickness and improved placental function with a better nutrition and oxygenation of the fetus as suggested by the normal size and birth weights of infants with IUGR (La Torre *et al*., 2006; Adler *et al*., 2007).

Each of the treated women in this report received multiple doses of HIG and intra-amniotic infusions. In a previous study the receipt of multiple doses and/or intra-amniotic infusions of HIG was not a predictor of outcome (Nigro *et al*., 2005). However, for fetuses with cerebral manifestations, the optimal number of doses and route of administration may affect the outcome. Thus, the relative importance of intra-amniotic *versus* intravenous administration and the mechanism of action will need to be assessed in future controlled trials.

In summary, these cases support and amplify the existing data about the ability of HIG to induce regression of fetal disease of the brain and other organs and thus prevent the long-term sequelae associated with neonatal CMV disease.

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Approval from Institutional Ethical Committees has been obtained for each HIG-treated patient