sFlt1 and sEng were elevated (18,107 pg/ml and 19.4 pg/ml, respectively). Maternal sera from recurrent infection 7 had elevated sFlt1 (14,816 pg/ml) and sEng (20.3 pg/ml). cmvIL-10 was detected in maternal and cord sera from IUGR cases 2, 3 and 16, in accord with viral replication.

Next, we considered whether HCMV-infected endothelial cells from the umbilical cord could secrete the cytokines detected in utero. For these experiments, HUVEC were infected with VR1814, and sFlt1, PIGF and cmvIL-10 levels were measured in CM. sFlt1 increased modestly, whereas PIGF (Supplemental Fig. 3) and sEng declined (data not shown), in accord with decreased surface expression of Eng in infected HUVEC [29]. cmvIL-10 increased throughout the course of viral replication, reaching the highest level at 6 days (Supplemental Fig. 3).

DISCUSSION

Although recognized as a viral cause of IUGR, congenital HCMV infection is seldom diagnosed in affected newborns without other clinical symptoms [2]. Here we assessed the serological status of women who delivered infants with idiopathic IUGR (group C) and found 5 cases with underlying primary or recurrent infection with placental pathology, including impaired development (Tables 1 and 2). Immunostaining for infected cell proteins revealed that virus replicates in smooth muscle cells of arteries and veins in floating villi and the chorion. HCMV proteins in vesicles of amniotic epithelial cells was taken as evidence of transmission and fetal infection. Nonetheless, accumulation of viral proteins in cytoplasmic vesicles suggested virions were cleared from amniotic fluid and replication was suppressed, which could reduce inflammation.

Considering pathology, large fibrinoids with many avascular villi and edematous villi, which could impair transport functions, and leukocytic infiltration at the basal plate, suggesting inflammation, were the most prominent features in IUGR placentas (Table 2). For primary infection case 16 with transmission, HCMV DNA was detected in the placenta, viral replication was sustained in blood vessels of villi and chorion and cmvIL-10 was present in circulation. For primary infection case 12 with transmission, impaired cytotrophoblast differentiation (cell islands), hypoxia (Tenney-Parker changes) and dilated blood vessels suggested primary infection exacerbated by maternal preeclampsia with elevated sFlt1 and sEng contribute to dysfunction [27, 30]. Extensive edema, also associated with IUGR, was evident in recurrent infection cases 2 and 3. In addition, cord sera contained extremely elevated sFlt1, which inhibits functions of VEGF and PlGF and is associated with hypoxia, as reported for amniotic fluid from untreated (i.e., without HIG therapy) primary congenital HCMV infection [13]. In contrast, placentas 4, 7 and 10 from asymptomatic recurrent infection did not differ from seronegative controls in prevalence of fibrinoids, inflammation, edema or levels of anti-angiogenic factors in sera (data not shown). The results indicate that high-avidity IgG with neutralizing activity has the potential to reduce infection-associated pathology in the placenta. However, understanding the mechanisms by which recurrent infection leads to pathology could benefit from knowing whether reinfection occurred.

In cases of stillbirth associated with congenital HCMV infection, decreased exchange capacity of placentas with progressive fetal thrombotic vasculopathy was identified as the prominent histological abnormality [31-33]. Avascular villi increase the occurrence of IUGR [34] and can lead to major thrombotic events and death in fetuses older than 34 weeks. In cases of acute HCMV infection, arterial and venous thrombosis occurs independently from other risk factors [35]. Albeit infrequently, viral antigens and DNA have been detected in adult human arterial smooth muscle cells [36] and vessels from coronary artery disease [37, 38]. Moreover,

HCMV replicates in smooth muscle cells from human umbilical arteries in vitro [39]. Here we discovered a pattern of viral replication in smooth muscle cells of arteries and veins in cases of primary infection, which indicates stepwise transmission from infected cytotrophoblasts to villous blood vessels, the chorion and fetal circulation [8, 12]. Although transmission occurred in 3 of 5 fetuses from group B asymptomatic recurrent infection, the placental vasculature was not affected. Transmission rates are 30% and 38% when seroconversion occurs in first and second trimester, respectively, and frequently leads to disease [4, 40, 41]. Although transmission is higher (72%) in third trimester, infected babies are usually asymptomatic. Unlike IUGR cases 16 and 12, with primary infection, viral DNA was not detected in placentas from asymptomatic recurrent infection 4, 7 and 10, suggesting transmission may have occurred late in gestation. These findings show that high-avidity, neutralizing IgG reduces HCMV replication and subsequently protects the developing placenta from inflammation and associated damage [9, 10, 12].

Amniotic epithelial cells of fetal membranes represent the first line of defense against intra-amniotic bacteria and respond to pathogens through the function of toll-like receptors (TLRs) [42], key regulators of innate immune defense [43] that inhibit bacterial growth [44]. In spontaneous labor at term and in preterm parturition associated with chorioamnionitis, TLR2 and TLR4 are upregulated in the amniotic epithelium [45]. In primary and recurrent HCMV infection, we observed vesicles containing viral antigens near the apical membranes of epithelial cells, suggesting virion uptake from amniotic fluid (Fig. 5). We recently discovered similar patterns by immunostaining the amnion from two cases of primary maternal infection with HCMV DNA-positive amniotic fluid that confirms virus transmission (unpublished observations). With regard to innate immunity, HCMV gB and gH display determinants recognized by TLR2 with which they directly interact, inhibiting inflammatory cytokine responses to infection in vitro [46]. Whether this pathway protects the amniotic epithelium from virus replication remains to be

determined. Infection with bacterial pathogens could cause additional complications leading to inflammation [10, 11]. Amniotic epithelial cells could also express IgG receptors that could internalize antibody-virion complexes [47]. In this regard, we measured HCMV-specific IgG in amniotic fluid from seropositive mothers and confirmed that amniotic epithelial cells express the neonatal Fc receptor, TLR2 and TLR4 in vitro (unpublished observations), suggesting these molecules could contribute to virion clearance, reduce inflammation and delay the rupture of fetal membranes.

In conclusion, analysis of biopsy specimens revealed crucial information about congenital HCMV infection, involvement of placental blood vessels in transmission and viral proteins in amniotic epithelial cells, providing further evidence of fetal infection. Our findings (two cases) indicate that primary infection impairs placental development and leads to IUGR and virus transmission (Tables 1 and 2). In recurrent infection (eight cases), five babies were asymptomatic, even though virus transmission occurred (three cases) (Table 1, group B). However, three others had IUGR, a placental defect, without virus transmission (Tables 1 and 2, group C). Our detailed analysis shows that high-avidity, HCMV-neutralizing IgG reduces viral replication in the placental-fetal unit but may not preclude transmission [13, 14, 48-50] and suggests antibody treatment merits consideration in the clinical management of primary maternal infection.

Footnotes

Presentation at meetings: A portion of this work was presented at the 4th Congenital

Cytomegalovirus Conference, 2012, San Francisco (a joint meeting with the 14th International

CMV/betaherpesvirus Workshop).

Conflict of interest

The authors have no conflicts of interest.

Current Addresses [8]Dept. of Medicine, University of California San Francisco, San Francisco,

CA

Acknowledgments

References

1. Colugnati FA, Staras SA, Dollard SC, Cannon MJ. Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. BMC Infect Dis **2007**; 7:71.

2. Stagno S, Pass RF, Cloud G, et al. Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome. JAMA **1986**; 256:1904-1908.

3. Pass RF, Fowler KB, Boppana SB, Britt WJ, Stagno S. Congenital cytomegalovirus infection following first trimester maternal infection: symptoms at birth and outcome. J Clin Virol **2006**; 35:216-20.

4. Enders G, Daiminger A, Bader U, Exler S, Enders M. Intrauterine transmission and clinical outcome of 248 pregnancies with primary cytomegalovirus infection in relation to gestational age. J Clin Virol **2011**; 52:244-6.

5. Garcia AG, Fonseca EF, Marques RL, Lobato YY. Placental morphology in cytomegalovirus infection. Placenta **1989**; 10:1-18.

6. Muhlemann K, Miller RK, Metlay L, Menegus MA. Cytomegalovirus infection of the human placenta: an immunocytochemical study. Human Pathol **1992**; 23:1234-37.

7. Fowler KB, Stagno S, Pass RF. Maternal immunity and prevention of congenital cytomegalovirus infection. JAMA **2003**; 289:1008-11.

8. Fisher S, Genbacev O, Maidji E, Pereira L. Human cytomegalovirus infection of placental cytotrophoblasts in vitro and in utero: implications for transmission and pathogenesis. J Virol **2000**; 74:6808-20.

9. Maidji E, McDonagh S, Genbacev O, Tabata T, Pereira L. Maternal antibodies enhance or prevent cytomegalovirus infection in the placenta by neonatal Fc receptor-mediated transcytosis. Am J Pathol **2006**; 168:1210-26.

10. Pereira L, Maidji E, McDonagh S, Genbacev O, Fisher S. Human cytomegalovirus transmission from the uterus to the placenta correlates with the presence of pathogenic bacteria and maternal immunity. J Virol **2003**; 77:13301-14.

11. McDonagh S, Maidji E, Ma W, Chang HT, Fisher S, Pereira L. Viral and bacterial pathogens at the maternal-fetal interface. J Infect Dis **2004**; 190:826-34.

12. McDonagh S, Maidji E, Chang HT, Pereira L. Patterns of human cytomegalovirus infection in term placentas: a preliminary analysis. J Clin Virol **2006**; 35:210-5.

13. Maidji E, Nigro G, Tabata T, et al. Antibody treatment promotes compensation for human cytomegalovirus-induced pathogenesis and a hypoxia-like condition in placentas with congenital infection. Am J Pathol **2010**; 177:1298-310.

14. Nigro G, Adler SP, La Torre R, Best AM. Passive immunization during pregnancy for congenital cytomegalovirus infection. N Engl J Med **2005**; 353:1350-62.

15. Resnik R. Intrauterine growth restriction. Obstet Gynecol **2002**; 99:490-6.

16. Hadlock FP, Harrist RB, Martinez-Poyer J. In utero analysis of fetal growth: a sonographic weight standard. Radiology **1991**; 181:129-33.

17. Navarro D, Paz P, Tugizov S, Topp K, La Vail J, Pereira L. Glycoprotein B of human cytomegalovirus promotes virion penetration into cells, transmission of infection from cell to cell, and fusion of infected cells. Virology **1993**; 197:143-158.

18. Gerna G, Sarasini A, Patrone M, et al. Human cytomegalovirus serum neutralizing antibodies block virus infection of endothelial/epithelial cells, but not fibroblasts, early during primary infection. J Gen Virol **2008**; 89:853-65.

19. Ilic D, Kapidzic M, Genbacev O. Isolation of human placental fibroblasts. Curr Protoc Stem Cell Biol **2008**; Chapter 1:Unit 1C 6.

20. Dondero DV, Pereira L. Monoclonal antibody production. In: Emmons R, Schmidt N, eds. Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections. Washington, D.C.: American Public Health Association, **1990**:101-24.

21. Nozawa N, Fang-Hoover J, Tabata T, Maidji E, Pereira L. Cytomegalovirus-specific, high-avidity IgG with neutralizing activity in maternal circulation enriched in the fetal bloodstream. J Clin Virol **2009**; 46 Suppl 4:S58-63.

22. Bodeus M, Goubau P. Predictive value of maternal-IgG avidity for congenital human cytomegalovirus infection. J Clin Virol **1999**; 12:3-8.

23. Revello MG, Zavattoni M, Baldanti F, Sarasini A, Paolucci S, Gerna G. Diagnostic and prognostic value of human cytomegalovirus load and IgM antibody in blood of congenitally infected newborns. J Clin Virol **1999**; 14:57-66.

24. Daiminger A, Bader U, Enders G. Pre- and periconceptional primary cytomegalovirus infection: risk of vertical transmission and congenital disease. BJOG **2005**; 112:166-72.

25. Revello MG, Genini E, Gorini G, Klersy C, Piralla A, Gerna G. Comparative evaluation of eight commercial human cytomegalovirus IgG avidity assays. J Clin Virol **2010**; 48:255-9.

26. Enders G, Daiminger A, Bader U, Exler S, Schimpf Y, Enders M. The value of CMV IgG avidity and immunoblot for timing the onset of primary CMV infection in pregnancy. J Clin Virol **2013**; 56:102-7.

27. Levine RJ, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med **2006**; 355:992-1005.

28. Yamamoto-Tabata T, McDonagh S, Chang HT, Fisher S, Pereira L. Human cytomegalovirus interleukin-10 downregulates metalloproteinase activity and impairs endothelial cell migration and placental cytotrophoblast invasiveness in vitro. J Virol **2004**; 78:2831-40.

29. Tabata T, Kawakatsu H, Maidji E, et al. Induction of an epithelial integrin alphavbeta6 in human cytomegalovirus-infected endothelial cells leads to activation of transforming growth factor-beta1 and increased collagen production. Am J Pathol **2008**; 172:1127-40.

30. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med **2004**; 350:672-83.

31. Iwasenko JM, Howard J, Arbuckle S, et al. Human cytomegalovirus infection is detected frequently in stillbirths and is associated with fetal thrombotic vasculopathy. J Infect Dis **2011**; 203:1526-33.

32. Syridou G, Spanakis N, Konstantinidou A, et al. Detection of cytomegalovirus, parvovirus B19 and herpes simplex viruses in cases of intrauterine fetal death: association with pathological findings. J Med Virol **2008**; 80:1776-82.

33. Pereira L. Have we overlooked congenital cytomegalovirus infection as a cause of stillbirth? J Infect Dis **2011**; 203:1510-2.

34. Redline RW, Pappin A. Fetal thrombotic vasculopathy: the clinical significance of extensive avascular villi. Hum Pathol **1995**; 26:80-5.

35. Atzmony L, Halutz O, Avidor B, et al. Incidence of cytomegalovirus-associated thrombosis and its risk factors: a case-control study. Thromb Res **2010**; 126:e439-43.

36. Melnick JL, Petrie BL, Dreesman GR, Burek J, McCollum CH, DeBakey ME. Cytomegalovirus antigen within human arterial smooth muscle cells. Lancet **1983**; 2:644-7.

37. Melnick JL, Hu C, Burek J, Adam E, DeBakey ME. Cytomegalovirus DNA in arterial walls of patients with atherosclerosis. J Med Virol **1994**; 42:170-4.

38. Hendrix MG, Daemen M, Bruggeman CA. Cytomegalovirus nucleic acid distribution within the human vascular tree. The American journal of pathology **1991**; 138:563-7.

39. Tumilowicz JJ, Gawlik ME, Powell BB, Trentin JJ. Replication of cytomegalovirus in human arterial smooth muscle cells. J Virol **1985**; 56:839-45.

40. Bodeus M, Kabamba-Mukadi B, Zech F, Hubinont C, Bernard P, Goubau P. Human cytomegalovirus in utero transmission: follow-up of 524 maternal seroconversions. J Clin Virol **2010**; 47:201-2.

41. Revello MG, Fabbri E, Furione M, et al. Role of prenatal diagnosis and counseling in the management of 735 pregnancies complicated by primary human cytomegalovirus infection: a 20-year experience. J Clin Virol **2011**; 50:303-7.

42. Gillaux C, Mehats C, Vaiman D, Cabrol D, Breuiller-Fouche M. Functional screening of TLRs in human amniotic epithelial cells. J Immunol **2011**; 187:2766-74.

43. Akira S. TLR signaling. Curr Top Microbiol Immunol **2006**; 311:1-16.

44. Kjaergaard N, Hein M, Hyttel L, et al. Antibacterial properties of human amnion and chorion in vitro. Eur J Obstet Gynecol Reprod Biol **2001**; 94:224-9.

45. Kim YM, Romero R, Chaiworapongsa T, et al. Toll-like receptor-2 and -4 in the chorioamniotic membranes in spontaneous labor at term and in preterm parturition that are associated with chorioamnionitis. Am J Obstet Gynecol **2004**; 191:1346-55.

46. Boehme KW, Guerrero M, Compton T. Human cytomegalovirus envelope glycoproteins B and H are necessary for TLR2 activation in permissive cells. J Immunol **2006**; 177:7094-102.

47. Stone DL, Suzuki Y, Wood GW. Human amnion as a model for IgG transport. Am J Reprod Immunol Microbiol **1987**; 13:36-43.

48. Adler SP. Editorial commentary: primary maternal cytomegalovirus infection during pregnancy: do we have a treatment option? Clin Infect Dis **2012**; 55:504-6.

49. Visentin S, Manara R, Milanese L, et al. Early primary cytomegalovirus infection in pregnancy: maternal hyperimmunoglobulin therapy improves outcomes among infants at 1 year of age. Clin Infect Dis **2012**; 55:497-503.

50. Nigro G, Adler SP, Parruti G, et al. Immunoglobulin therapy of fetal cytomegalovirus infection occurring in the first half of pregnancy--a case-control study of the outcome in children. J Infect Dis **2012**; 205:215-27.

Figure Legends

Figure 1. Pathology in placentas from cases of IUGR. (A) Necrotic, avascular villi embedded in large fibrinoids, case 16. (B) No large fibrinoids in seronegative control. (C) Edematous villi, case 3. (D) Absence of edematous villi in seronegative control. (E) Leukocytic infiltration in basal plate and decidua, case 16. (F) Increased diameter of villous blood vessel, case 3. (G) Accumulation of cytotrophoblasts (cell islands), case 12. Inset, cytokeratin 7 (CK7). Scale bars A-B = 500 μm, C-G = 50 μm.

Figure 2. Quantification of pathological features in cases of IUGR. (A) Avascular villi embedded in fibrinoids were counted, and distribution among fibrinoids of various sizes (defined as 5-25, 25-50, or >50 villi) determined. Results shown as average number of villi in fibrinoids of each size class per field (10x objective, ~1 mm^2 area per field). At least four biopsies and more than 100 fields were examined per placenta. (B) Edematous villi apparent in IUGR were counted according to severity (3 = most severe, representing a bloated villus with sparse mesenchyme and few visible blood vessels). Average numbers of edematous villi in each class indicated by fill patterns. At least four biopsies and more than 100 fields (10x objective, representing ~1 mm^2) were examined. (C) Leukocytic cell infiltrates in basal plate were counted and the distribution per field presented in a box and whisker format that marks the four quartiles. Median count is indicated by the solid central bar, the second and third quartiles within the boxes below and above, respectively. First quartile is represented by vertical line below the box and fourth (highest) quartile by line above the box. For each placenta, at least four biopsies and between 14 and 37 fields (10x objective, representing ~1 mm^2) were examined.
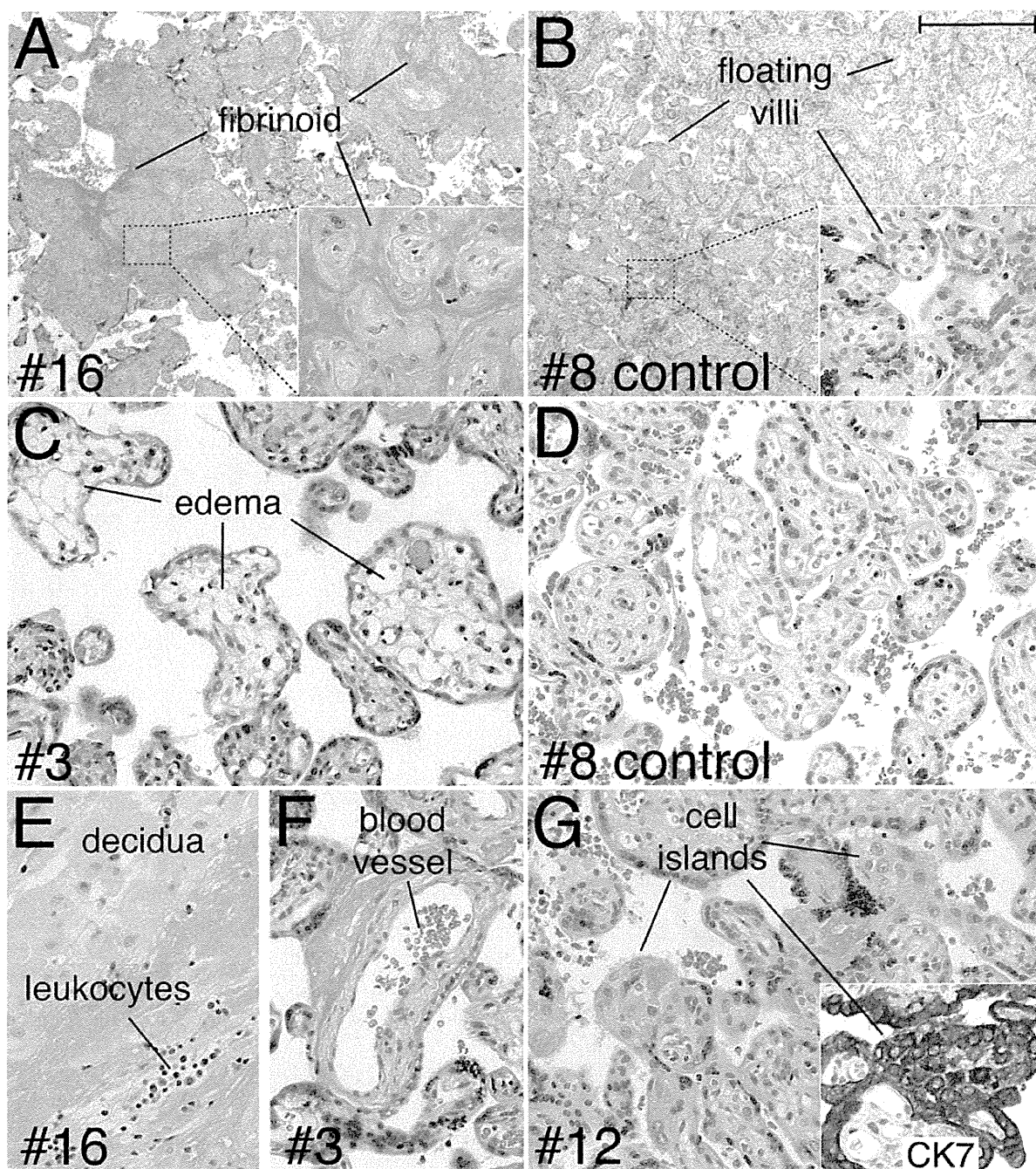
Figure 3. HCMV infected cells in the basal plate of primary and recurrent infections detected by immunohistochemistry. (A) Low power image of a site within the basal plate containing cytotrophoblasts (CTBs) that express HCMV infected cell proteins. (B and inset) High power image of area indicated in A (rectangle) showing infected cell proteins in the cytoplasm of invasive CTBs. (C) Infected cells in a lymphatic vessel in the decidua. Signal was detected in cytoplasm (upper inset) and nuclei (lower inset) of infected cells at the luminal surface. (D) HCMV proteins in cells at the luminal face of an endometrial gland detected in cytoplasm and nuclei. (E) Section adjacent to that in D shows cytokeratin 7 (CK7). Scale bars A = 200 µm, B = 20 µm, C= 50 µm, D-E = 100 µm.
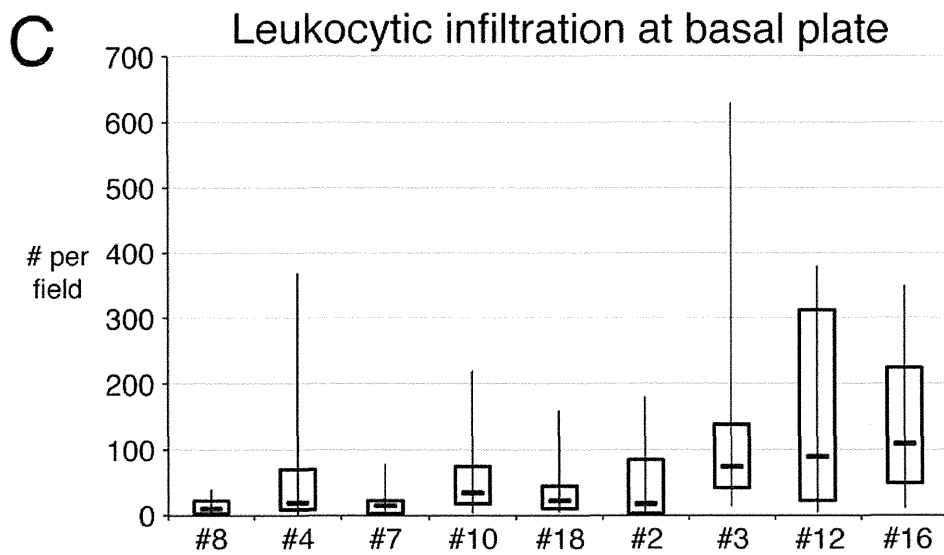
Figure 4. HCMV infected cells in blood vessels of chorion detected by immunohistochemistry in a case of IUGR with primary infection. HCMV proteins (left panels) and smooth muscle (SM) myosin heavy chain (right panels) stained in parallel sections. (A and B) Adjacent sections showing HCMV proteins in smooth muscle cells surrounding an artery in the chorion of placenta of IUGR case 16. (C and D) Adjacent sections showing absence of viral proteins in smooth muscle cells surrounding an artery in the chorionic plate of placenta #3. (E and F) Parallel sections showing HCMV proteins in smooth muscle cells surrounding a vein in the chorionic plate of placenta #16. (G and H) Adjacent sections showing absence of viral proteins in smooth muscle cells surrounding a vein in the chorionic plate of placenta #2. Scale bars in B = 200 µm for A-D. Scale bar in F = 100 µm for E-H.

Figure 5. Immunohistochemical detection of HCMV proteins in epithelial cells of amnion. Viral proteins accumulated in cytoplasmic vesicles of amniotic epithelial cells in placentas from primary and recurrent infections (A-G) but not in a seronegative control (H). (G) Grazing section

across amniotic epithelial cells revealing the highly vesicular nature of the signal. Scale bar in E

= 25 μm for A-C, E, F, and H. Scale bar in D = 25 μm. Scale bar in G = 15 μm.

A Fibrinoid-embedded necrotic villi

Legend:
- ▨ >50 villi/fibrinoid
- ■ 25-50 villi/fibrinoid
- ▥ 5-25 villi/fibrinoid

mean # per field

#8 #4 #7 #10 #18 #2 #3 #12 #16

B Edematous villi

Legend:
- ▨ +3 edema
- ■ +2 edema
- ▥ +1 edema

mean # per field

#8 #4 #7 #10 #18 #2 #3 #12 #16

C Leukocytic infiltration at basal plate

per field

#8 #4 #7 #10 #18 #2 #3 #12 #16

A   HCMV

chorionic artery

#16

B   SM myosin

chorionic artery

#16

C   HCMV

chorionic artery

#3

D   SM myosin

chorionic artery

#3

E   HCMV

chorionic vein

#16

F   SM myosin

chorionic vein

#16

G   HCMV

chorionic vein

#2

H   SM myosin

chorionic vein

#2

IUGR HCMV primary infection   asymptomatic recurrent infection

asymptomatic recurrent infection

grazing section

seronegative control

130

Table 1

Group	PT	WT	GA	Sera	AV[a]	IgG1[b]	Neut[c] HUVEC	Neut HPF	HCMV[d] Immunoblot	HCMV Serostatus
A Controls	8	3310	39	M	Neg	4.76	<1:8	<1:8	Neg	Seronegative
				C	Neg	6.05	<1:8	<1:8	Neg	
	15	3360	37	M	Neg	4.40	<1:8	<1:8	Neg	Seronegative
				C	Neg	4.74	<1:8	<1:8	Neg	
	6	3160	39	M	67.3	2.28	>1:1024	1:128	p150, CM2, gB1, gB2	Long past
				C	73.7	4.76	>1:1024	1:128	p150, CM2, gB1, gB2	
	9	3940	39	M	58.5	3.40	1:512	1:64	p150, CM2, gB1, gB2	Long past
				C	69.3	8.36	1:512	1:64	p150, CM2, gB1, gB2	
B Asymptomatic Infection	1	3220	39	M	49.5	4.58	1:128	1:32	IE1, p150, p65, gB1	Recurrent
				C	62.5	1.14	1:128	<1:64	IE1, p150, p65, gB1	
	11	3810	41	M	NT	NT	NT	NT	NT	Recurrent
				C	59.2	6.16	1:512	NT	IE1, p150, CM2, gB1	
	4	4050	39	M	NT	NT	NT	NT	NT	Recurrent
				C	44.7	5.51	1:512	NT	IE1, p150, p65, gB1, gB2	
	7	4420	39	M	56.1	4.48	>1:1024	1:128	IE1, p150, CM2, p65, gB1, gB2	Recurrent
				C	71.2	6.63	>1:1024	1:256	IE1, p150, CM2, p65, gB1, gB2	
	10	4300	39	M	NT	NT	NT	NT	NT	Recurrent
				C	84.9	1.28	>1:1024	1:128	IE1, p150, CM2, p65, gB1, gB2	
C IUGR	20	2353	37	M	Neg	2.97	<1:8	<1:8	Neg	Seronegative
				C	Neg	6.07	<1:8	<1:8	Neg	
	14	2459	37	M	58.5	3.96	1:1024	1:256	p150, gB1, gB2	Long past
				C	55.1	9.24	1:1024	1:256	p150, gB1, gB2	
	18	2370	38	M	64.3	8.14	1:512	NT	IE1, p150, CM2, p65, gB1	Recurrent
				C	73.5	8.81	1:512	NT	IE1, p150, CM2, p65, gB1	
	2	2822	39	M	63.4	3.39	1:512	>1:12	p150, CM2, p65, gB1, gB2	Recurrent
				C	73.5	6.90	1:512	>1:64	p150, CM2, p65, gB1, gB2	
	3	1847	34	M	64.3	3.68	1:1024	1:32	IE1, p150, CM2, gB1, gB2	Recurrent
				C	67.1	3.82	1:1024	1:32	IE1, p150, CM2, gB1, gB2	
	16	2160	36	M	18.5	7.09	1:512	1:16	IE1, p150, CM2, p65, gB1	Primary
				C	11.1	7.72	1:512	>1:32	IE1, p150, CM2, p65, gB1	
	12	1450	32	M	36	4.54	<1:8	NT	IE1, CM2, p65	Primary
				C	33.5	1.12	<1:8	NT	IE1, CM2, p65	
D Preeclampsia	13	2920	38	M	Neg	3.36	<1:8	<1:8	Neg	Seronegative
				C	Neg	7.37	<1:8	<1:8	Neg	
	17	3442	40	M	Neg	1.47	<1:8	<1:8	Neg	Seronegative
				C	Neg	7.23	<1:8	<1:8	Neg	
	19	3260	38	M	NT	NT	NT	NT	NT	Long past
				C	50.7	7.52	NT	NT	p150, gB1, gB2	

PT patient; WT, weight; GA, gestational age; M, maternal; C, cord; Negative, Neg; NT, not tested.

[a] Percent HCMV-specific avidity (Radim).

[b] IgG1 measured by ELISA (mg/ml).

[c] Neutralization titers ID50 in VR1814-infected HUVEC and HPF.

[d] HCMV Recomblot IgG (Mikrogen).

Table 2

Group	PT	Sera	sFlt (pg/ml)	PlGF (pg/ml)	sEng (pg/ml)	cmv IL-10	HCMV Infection, Hypoxia and Pathology
B Asymptomatic Recurrent Infection	4	M	NT	NT	NT		Infected cell proteins in amniotic epithelium.
		C	608	NT	4.4		
	7	M	14,816	193	20.3		Infected cell proteins in syncytiotrophoblasts and amniotic epithelium. Edema and dilated blood vessels.
		C	895	1.4	3.3		
	10	M	NT	NT	NT		Infected cell proteins in decidual glands and amniotic epithelium. Some leukocytic infiltration.
		C	705	9.4	3.7		
C IUGR with Primary or Recurrent Infection	18	M	524	5.3	5.3		Infected cell proteins in decidual glands.
		C	281	8.0	6.2		
	2	M	2,494	888	5.9	+	Infected cell proteins in interstitial CTBs and decidual glands. Considerable edema, fibrinoid-embedded avascular villi, leukocytic infiltration, T-P changes[a]. HCMV DNA detected by nested PCR.
		C	180,992	572	11.3	+	
	3	M	2,163	376	9.6	+	Considerable edema, variable leukocytic infiltration, few fibrinoid-embedded avascular villi, dilated blood vessels. HCMV DNA detected by nested PCR.
		C	250,306	103	20.4	+	
	16	M	15,157	50.3	3.3	+	HCMV DNA quantified in biopsies (5.0 x 10⁶ genome copies/g). Infected cell proteins in decidual glands, blood vessels in floating villi, arteries and veins in chorion and amniotic epithelium. Many large-size fibrinoid-embedded avascular villi. T-P changes[a], cell islands[b]. Considerable leukocytic infiltration. Infected interstitial CTBs.
		C	16,823	7.8	10.7	+	
	12	M	18,107	68.6	19.4		Infected cell proteins in decidual glands, blood vessels in floating villi and amniotic epithelium. Moderate-size fibrinoid-embedded avascular villi, dilated blood vessels, T-P changes[a], cell islands[b]. Leukocytic infiltration. Infected interstitial CTBs. Preeclampsia.
		C	1,033	10.7	3.3		

M, maternal; C, cord; NT, not tested.

[a] Tenney-Parker (T-P) changes.

[b] Cytotrophoblast (CTB), cell islands.