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H．知的財産権の出願・登録状況

なし

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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Matsuo K, Morioka I, Oda M, Kobayashi Y, Nakamachi Y, Kawano S, Nagasaka M, Koda T, Yokota T, Morikawa S, Miwa A, Shibata A, Minematsu T, Inoue N, Yamada H, Iijima K.	Quantitative evaluation of ventricular dilatation using computed tomography in infants with congenital cytomegalovirus infection.	Brain Dev.	36	10-15	2014

表 1. 先天性感染児の背景

A. 臨床背景

	全感染児 n = 23	症候性 n = 10	無症候性 n = 13
在胎週数 (週)	38 (31–41)	36 * (31–38)	38 (35–41)
出生体重 (g)	2,606 (1,378–3,840)	2,188 * (1,378–3,160)	2,758 (2,060–3,840)
男児 / 女児	6/17	4/6	2/11
精査を施行した日齢 (日)	8 (0–28)	1 ** (0–27)	19 (0–28)

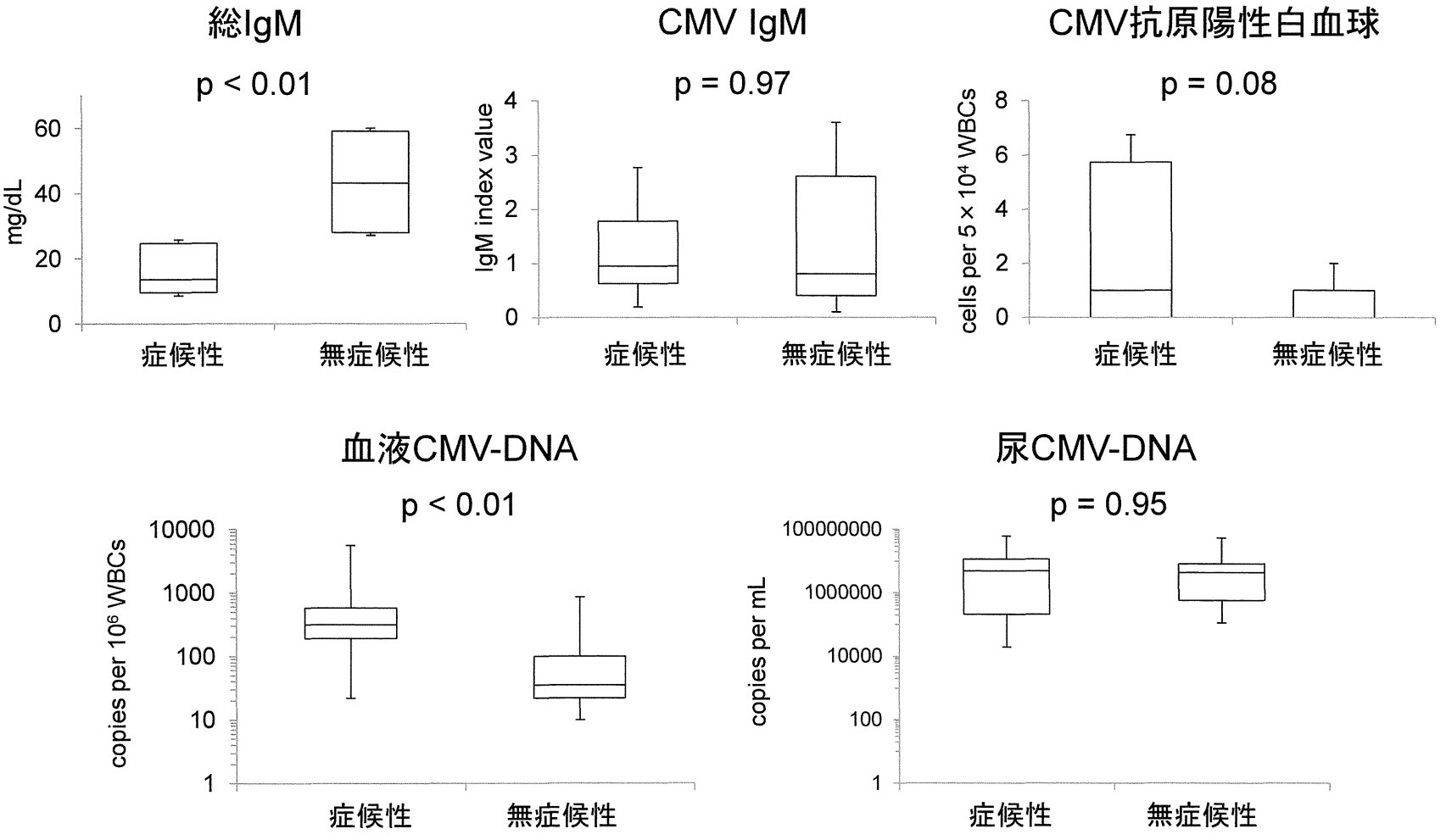
* $p < 0.05$, ** $p < 0.01$ 無症候性と比較して

B. 症候性児

症例	在胎週	出生体重	臨床症状					
#	(週)	(g)	SGA	肝脾腫/肝機能障害	血小板減少	脳画像異常	ABR 異常	網膜脈絡膜炎
1	31	1378		●	●	●	●	
2	31	1824		●	●	●		
3	32	1396	●				●	
4	36	1860	●		●	●	●	●
5	36	2184		●	●	●	●	●
6	36	2192		●	●	●	●	
7	36	2450						●
8	38	2868						●
9	38	2956				●	●	
10	38	3160				●	●	

ABR: 聴性脳幹反応, SGA: Small-for gestational age, ●: 症状ありを示す

図1　症候性児と無症候性児における各検査項目の定量値の比較



研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表レイアウト（参考）

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研究成果の刊行物・別刷

Question

新生児マススクリーニングの対象疾患として，先天性サイトメガロウイルス(CMV)感染を加えることが研究者の間で検討されていますが，どうしてですか？

Answer

先天性 CMV 感染症は，現在新生児マススクリーニングの対象となっているどの疾患よりも高頻度にみられますが，その多くが見逃されています．しかし，早期診断に続く早期治療・早期介入につなげることができれば，予後を改善させることができるからです．

森内浩幸｜長崎大学小児科

CMV：cytomegalovirus

TORCH：toxoplasmosis, other infection, rubella, cytomegalovirus infection, and herpes simplex

＊1
先天性感染は 0.31％に認められ[3]，このうち 23％の感染児が出生時に先天性 CMV 感染として合致するなんらかの臨床症状を呈し，さらに頭部画像検査で脳に異常所見を認めた児を含めると約 30％に異常が検出されている．

＊2
遅発性発症例も含め，仮に感染児の 30％になんらかの健康被害が生じたとすると，年間 1,000 人近く(およそ出生 1,000 人あたり 1 人)となる(❶)．これはダウン症(約 700 人に 1 人)に準じるものであり，先天性障害の原因として大きな重要性をもっている．また，TORCH 症候群全国調査での報告数(年間 50 例)との隔たりは非常に大きく，見逃されている症例が 90％以上にのぼることを示している．

PK：pharmacokinetics

PD：pharmacodynamics

＊3
これらの知見をふまえて，厚生労働科学研究費補助金研究班(古谷野班)は，先天性 CMV 感染児に対する抗ウイルス療法プロトコールを提示した[6]．しかし，副作用(短期的な骨髄抑制に加え，長期的には妊孕性や発癌性の可能性が完全には否定されていない)には十分に注意が必要であり，現時点では保険適用がないことに留意すべきである．

先天性 CMV 感染症の頻度と TORCH 症候群の実態調査

- 先天性 CMV 感染の頻度は国・地域や時代によって異なり，世界的には 0.2～3％と幅がある．先天性感染児の 10～20％が症候性感染とされるが，生下時に無症候性であった感染児でも，その 10～15％になんらかの遅発性障害が生じることが問題である(❶)．そのなかでも重要なものは感音性難聴であり，先天性 CMV 感染に伴う難聴はしばしば新生児聴覚スクリーニングの網をかいくぐってしまう．
- 日本における TORCH 症候群の実態調査が日本小児感染症学会によって実施され，2006～2008 年の 3 年間に 140 例(年間平均 50 人弱)の先天性 CMV 感染症が報告されている[1]．この数は調査の対象となった母子感染のなかで最多であったが，年間 5,000 例以上報告されている米国と比べると著しく少ない[2]．
- ところが一方，最近全国 6 都道県で行われた新生児マススクリーニングのためのパイロット研究において，以前に考えられていたよりも症候性感染の割合は高い可能性がある＊1・＊2．

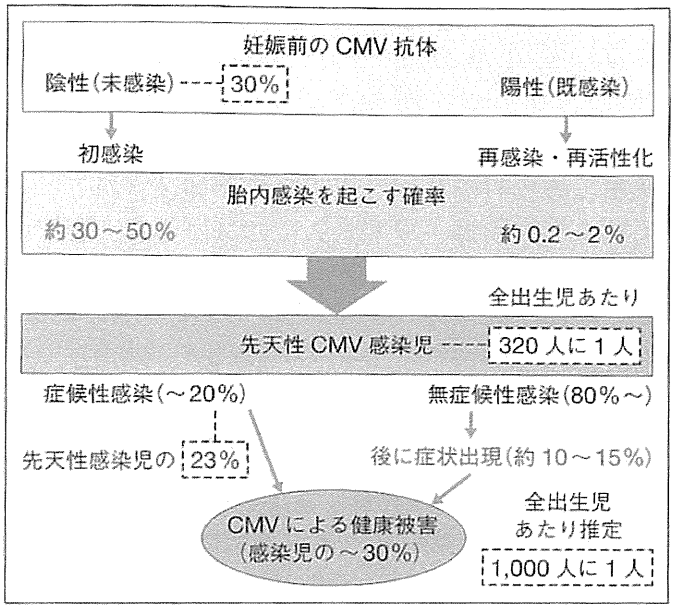
早期の抗ウイルス療法による感染児の予後の改善

- 先天性 CMV 感染児に対するガンシクロビルの長期(6 週間)静注療法により，聴力予後(❷)[4]および精神運動発達(❸)[5]が改善する．
- 一方で，長期にわたる静注療法は患者や家族への負担も大きいため，近年ガンシクロビルに匹敵する PK/PD を示すプロドラッグ製剤バルガンシクロビルの経口投与も行われるようになってきた＊3．

新生児マススクリーニング対象疾患の要件

- 新生児マススクリーニングの対象疾患として満たすべき条件としては，新生児期に診断可能，その疾患の自然歴早期治療により発症予防が可能，スクリーニング時に偽陽性が多すぎず偽陰性がきわめて少ない，集団のなかで一定の発生率があり経済効果が見込める，などがあげられる．
- 症候性先天性 CMV 感染症は約千人に 1 人と推定される＊4．生後 3 週以内

❶ 妊婦の CMV 感染が児にもたらす影響



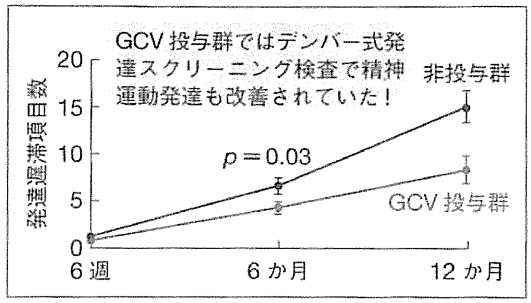
点線の枠で囲った数字は，文献 4)をもとにした日本における実態を示す．

❷ 症候性先天性 CMV 感染新生児へのガンシクロビル治療の聴力的予後への効果

		GCV 投与群 ($n=24$)	非投与群 ($n=19$)
a	改善	4(17%)	0(0%)
b	不変(聴力は正常のまま)	8(33%)	5(26%)
c	不変(聴力障害のレベルが同程度)	7(29%)	1(5%)
d	増悪	5(21%)	13(68%)

(a+b+c)vs(d)：$p=0.002$
(a+b)vs(c+d)：$p=0.133$
(Kimberlin DW, et al. 2003[4]をもとに集計)

❸ 症候性先天性 CMV 感染新生児へのガンシクロビル治療の精神運動発達予後への効果



ガンシクロビル(GCV)投与群と非投与群における生後 6 週，6 か月，12 か月の時点での発達の遅れの程度を，デンバー式発達スクリーニング検査で評価(「言語」を除いた「個人-社会」「微細運動-適応」「粗大運動」の 3 分野について集計)して示してある(mean±SE)．
(Oliver SE, et al. 2009[5])

の尿からのウイルス DNA の検出は高感度で，偽陽性も偽陰性もきわめて少ない*5．

- 先天性 CMV 感染症の疾病負担は非常に大きく*6，早期診断できれば，聴力障害や発達障害の存在を的確にとらえて正しい治療・療育へつなげることができることに加え，早期の抗ウイルス療法が予後を改善することが示されている．
- 最大の問題点は抗ウイルス薬に保険適用がないことであり，現時点ではまだ新生児マススクリーニングは研究段階にとどまっている．

*4
現在，新生児マススクリーニング対象疾患となっている先天代謝異常症のほとんどは数万人から数十万人に 1 人という頻度であり，最多の先天性甲状腺機能低下症でも約 2 千人に 1 人である．

*5
国立感染症研究所の井上直樹博士によって考案された検査法は簡便かつ経済的で，マススクリーニング検査法としての経済性も担保されている[3]

*6
米国ではワクチン開発による社会経済効果が最も大きいものと位置づけられている．

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Intrauterine growth restriction caused by underlying congenital cytomegalovirus infection

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Abstract

Background. Human cytomegalovirus (HCMV) is the major viral etiology of congenital infection and birth defects. Fetal transmission is high (30-40%) in primary maternal infection, and symptomatic babies have permanent neurological, hearing and vision defects. Recurrent infection is infrequently transmitted (2%) and largely asymptomatic. Congenital infection is also associated with intrauterine growth restriction (IUGR).

Methods. To investigate possible underlying HCMV infection in cases of idiopathic IUGR, we studied maternal and cord sera and placentas from 19 pregnancies. Anti-HCMV antibodies, hypoxia-related factors and cmvIL-10 were measured in sera. Placental biopsy specimens were examined for viral DNA, expression of infected cell proteins and pathology.

Results. Among 7 IUGR cases, we identified 2 primary and 3 recurrent HCMV infections. Virus replicated in glandular epithelium and lymphatic endothelium in the decidua, cytotrophoblasts and smooth muscle cells in blood vessels of floating villi and the chorion. Large fibrinoids with avascular villi, edema and inflammation were significantly increased. Detection of viral proteins in the amniotic epithelium indicated transmission in 2 cases of IUGR with primary infection and 3 asymptomatic recurrent infections.

Conclusions. Congenital HCMV infection impairs placental development and functions and should be considered as an underlying cause of IUGR, regardless of virus transmission to the fetus.

INTRODUCTION

Human cytomegalovirus (HCMV) is the most common cause of congenital viral infection and permanent birth defects in the United States and occurs more frequently than other well-known disabilities, including Down syndrome, fetal alcohol syndrome and neural tube defects [1]. Primary maternal infection in the first trimester of pregnancy poses a 30%-40% risk of virus transmission with birth defects that include mental retardation, neuromotor disabilities, intrauterine growth restriction (IUGR) and hearing loss [2-4]. Poor outcome is associated with viral replication, inflammation, edema and fibrinoid development in the placenta [5, 6]. In contrast, immune women have a low risk (0.2-2.0%) of virus transmission, and infected babies are largely asymptomatic [2, 7]. Maternal neutralizing IgG suppresses HCMV replication in the placenta, and viral antigens are sequestered in syncytiotrophoblasts without infection of underlying cytotrophoblasts [8-12]. Recent studies revealed that the placental-fetal unit in congenital infection is hypoxic and that levels of a secreted form of the vascular endothelial growth factor (VEGF) receptor, fms-like tyrosine kinase 1 (sFlt1), are elevated in amniotic fluid [13]. In contrast, treatment of primary maternal infection after seroconversion with hyperimmune globulin enriched for HCMV IgG reduces transmission and improves outcome [14]. Analysis of these placentas revealed infection was suppressed and development of the syncytiotrophoblast surface and numbers of blood vessels in chorionic villi increased [13].

Infants with IUGR, birth weights less than 10th percentile, have a perinatal morbidity and mortality 5 to 30 times that of infants with higher weights [15]. In the present study, we focused on idiopathic IUGR, a manifestation of maternal and fetal disorders, to determine whether underlying congenital HCMV infection was involved. We found serological evidence of primary and recurrent maternal infection, viral replication in blood vessels of floating villi and the chorion and viral proteins in the amniotic epithelium. Development of large fibrinoids with avascular villi,

edema and impaired cytotrophoblast differentiation reduced placental functions, resulting in hypoxia and IUGR.

MATERIALS AND METHODS

Study Groups

Approval for this pilot study was obtained from the Institutional Review Board of Cedars-Sinai Medical Center. Samples included maternal and cord blood and placentas at delivery from 9 uncomplicated deliveries (controls), 7 patients with IUGR, and 3 with preeclampsia. Subjects included only non-smokers without diabetes or chronic hypertension. IUGR was diagnosed based on a pre-delivery clinical estimation of fetal weight, ultrasound evaluation [16] and birth weight below the 10^{th} percentile [15].
http://www.who.int/reproductivehealth/topics/best_practices/weight_percentiles_calculator.xls
Preeclampsia was defined as blood pressure >160/110, proteinuria, symptomatic with headaches and visual disturbance, epigastric tenderness, abnormal laboratory findings or other organ system dysfunction. Mean age of controls was 32.8 ± 6.2 years; pregnancies with IUGR (33.3 ± 4.7 years) and preeclampsia (29.3 ± 5.7 years) were similar.

HCMV Serological Assays

HCMV IgM ELISA (Phoenix Pharmaceuticals), HCMV IgG avidity (Radim) and human IgG1 ELISA (eBioscience) were used to measure values in sera. HCMV IgG RecomBlot kit (Mikrogen) was used to characterize reactivity with viral proteins, including immediate-early 1 (IE1, UL123), p150 (UL32), CM2 (UL44, UL57/p52 DNA-binding proteins), pp65 (UL83), gB1 and gB2 (UL55). Immunoblot profiles of recombinant HCMV protein bands indicated infection was primary (IE1, CM2, p65), recurrent (IE1, p150, CM2, p65, gB1, gB2) or long past (p150,

gB1, gB2). When maternal serum was unavailable (4 cases), determination of HCMV serostatus was based on reactivity of IgG in cord sera.

HCMV Neutralizing IgG Titers

Rapid neutralization assays were performed using the pathogenic clinical strain VR1814 propagated in human umbilical vein endothelial cells (HUVEC, Lonza) [17, 18]. HUVEC and human placental fibroblasts isolated from villous stroma [19] were grown on glass coverslips in 24-well plates. Heat-inactivated sera were mixed with 300-500 PFU for 1 hr before infection. Cells were fixed 30 hr later and reacted with mouse mAb CH160 to HCMV IE1 and IE2 nuclear proteins [20], then goat anti-mouse IgG (Fab) conjugated with fluorescein isothiocyanate (Jackson ImmunoResearch), and IE-positive cells were counted. Neutralizing titer (IC_{50}) was defined as the serum dilution reducing the number of infected cells by 50%.

HCMV DNA Quantification

Biopsy specimens (5 each) were obtained from the placenta and frozen at -80°C. DNA was extracted (approximately 25 mg) using QIAamp DNA mini kit (QIAGEN). Quantitative PCR targeting the HCMV IE1 gene was performed using the Taqman Universal PCR Master Mix kit (Applied Biosystems). Forward (5'- GACTAGTGTGATGCTGGCCAAG) and reverse (5'-GCTACAATAGCCTCTTCCTCATCTG) primers were used with an internal probe (5'-AGCCTGAGGTTATCAGTGTAATGAAGCGCC) labeled at the 5' end with the fluorescent reporter dye FAM and at the 3' end with the quencher dye TAMRA. Assays were performed using the ABI Prism 7900 Sequence Detection System (Applied Biosystems). A six-point standard curve and positive and negative controls were included. The numbers of HCMV IE genome copies were calculated as copies/g tissue. In additional experiments, nested PCR was done as reported [21].

Immunohistochemistry

Biopsy specimens (5 each) were obtained from placentas, fixed in formalin and paraffin embedded. For immunohistochemistry, serial 5 μm-thick tissue sections were deparaffinized using Clear-Rite 3 (Thermo Scientific), and antigen retrieval was performed (described below), followed by blocking with 1-2% normal horse serum in PBS for 30 min to overnight. Sections were incubated with primary antibody overnight at 4°C, washed and processed for color development using Vectastain ABC horseradish peroxidase (HRP) kits (mouse or rabbit). Briefly, slides were incubated with biotinylated secondary antibody for 1 hr, rinsed and incubated with ABC complex (30 min). Slides were developed with a diaminobenzidine (DAB) substrate kit (Abcam) and counterstained with hematoxylin (Sigma). Primary antibodies were as follows: HCMV infected cell proteins (ICP), cocktail of mouse monoclonal antibodies (Millipore MAB8121, containing clones 8B1.2, 1G5.2, 2D4.2), diluted 1/100; for cytokeratin 7, mouse monoclonal antibody (Dako clone OV-TL 12/30), diluted 1/100; for smooth muscle alpha-actin and smooth muscle myosin heavy chain, rabbit monoclonal antibodies (Abcam AB124964 and AB133567) diluted 1/1000 and 1/200, respectively. Antigen retrieval was performed as follows: HCMV ICP, tissue sections were incubated with 0.4% pepsin (Sigma-Aldrich, P-6887) in 0.01 N HCl for 30 min at 37°C then rinsed; cytokeratin 7, smooth muscle alpha-actin and smooth muscle myosin heavy chain, slides were heat treated (~15 min) in 10 mM sodium citrate, pH 6.0, in a 2100-Retriever pressure cooker (Diatome), followed by depressurization and cooling for 2 h. Images were taken on a Nikon TS100 inverted microscope equipped with a Nikon DS-F12 camera controlled by Nikon NIS-Elements F4.

Quantification of secreted cellular proteins and cmvIL-10.

sFlt1, placental growth factor (PlGF) and soluble endoglin (sEng) were measured in sera using ELISA (Quantikine; R&D Systems). For in vitro assays, HUVEC were infected with VR1814 or

mock infected. VEGF-A was depleted for 72 hr before harvesting conditioned medium (CM) at 2, 4 and 6 dpi, then stored at -80ºC. cmvIL-10 was measured by ELISA. cmvIL-10-specific IgG (1 µg/ml; affinity purified, polyclonal) and biotinylated cmvIL-10-specific IgG (0.1 µg/ml; affinity purified, polyclonal) were used for coating and detection, respectively. Protein concentrations were calculated from a standard curve using recombinant cmvIL-10 (R&D Systems).

RESULTS

Serological diagnosis of maternal HCMV infection

All maternal sera lacked HCMV-specific IgM at delivery, which agrees with earlier reports that IgM rapidly declines after primary infection in pregnancy [22-24]. Subjects were grouped by HCMV IgG avidity and profiles of immunoblot reactive proteins as follows: controls (group A), asymptomatic infection (group B), IUGR (group C) and preeclampsia (group D) (Table 1). Neonates with IUGR (group C) had significantly lower birth weights (2,209 ± 446 g) than did controls (group A: 3,443 ± 342 g, $P<0.001$) or those with asymptomatic infection (group B: 3,960 ± 476 g, $P<0.001$) or preeclampsia (group D: 3,207 ± 265 g, $P<0.01$).

Summarized in Table 1, serological status was evaluated based on HCMV IgG avidity (Radim assay) and immunoblot profiles using recombinant HCMV proteins (Supplemental Fig. 1). With regard to IgG avidity [21, 25, 26], infection was judged as long past (>6 months) with avidity above 45% and immunoblot reactivity with HCMV proteins p150, gB1, and gB2. Maternal IgG avidity in recurrent infection (groups B and C) was above 45%, and IgG reacted with proteins IE1, p150, CM2, p65, gB1 and/or gB2. Specific indicators of recurrent infection included IE1 and/or pp65. In primary infections (<90 days after onset), IgG avidity was below 45% and proteins IE1, CM2 and p65 were detected. Additional reactivity with p150 and gB1 indicated late primary infection. HCMV IgG avidity in cord sera was higher than in maternal circulation, as

reported earlier [21], except for IUGR cases 16 and 12 with primary infection and case 14 with past infection, suggesting impaired transport. Supplemental Fig. 1 shows five mothers were seronegative (groups A, C and D) and five had asymptomatic recurrent infection (group B). In IUGR group C, three had recurrent infection (cases 18, 2 and 3) and two had primary infection (cases 16 and 12). Infection was long past in four women (groups A, C and D).

Neutralizing titers agreed with maternal serostatus (Table 1). Twelve seropositive sera had neutralizing activity in HUVEC (ID_{50} 1:512 to 1:1024); lower titers were obtained in placental fibroblasts (ID_{50} 1:16 to 1:256) [18]. Sera from IUGR case 12 lacked neutralizing activity in both cell types, suggesting seroconversion occurred late in gestation. The results indicated that of 7 mothers who delivered babies with IUGR, 3 had recurrent infection and 2 had primary infection that had not been diagnosed during gestation.

Features of pathology in placentas from IUGR cases

Examination of placental pathology revealed that IUGR cases 2, 3, 16 and 12 had evidence of fibrosis, inflammation and hypoxia. These included large fibrinoids containing many necrotic, avascular villi (Fig. 1A) and edematous villi (Fig. 1C) absent in control placenta 8 (Fig. 1B, D). Additional pathology included leukocytic infiltration (Fig. 1E), dilated blood vessels (Fig. 1F) and, in IUGR case 12, clusters of cytokeratin 7-positive cytotrophoblasts (termed cell islands), a pattern suggesting arrested differentiation (Fig. 1G).

We subsequently quantified pathology, including (i) fibrinoids with embedded avascular villi (Fig. 2A), (ii) edematous villi (Fig. 2B), and (iii) leukocytic infiltration (inflammation) in the basal plate (Fig. 2C). Placentas in the control group, including seronegative 8 and recurrent infection 4, 7 and 10, had fewer than 1 fibrinoid per field with 5 avascular villi (Fig. 2A). IUGR with recurrent infection, case 2, had small and large fibrinoids with 25-50 avascular villi,

whereas case 3 had small fibrinoids. In contrast, IUGR cases 12 and 16, with primary infection, had many small and large fibrinoids with 25 to 50 avascular villi. In addition, case 16 had many fibrinoids with 50 or more avascular villi, significantly larger than those in all the other placentas. Edematous villi were abundant in IUGR cases 2 and 3, with recurrent infection, and increased in asymptomatic recurrent infection 4 and 7 (Fig. 2B). Leukocytic infiltration in the basal plate was most evident in IUGR cases 12 and 16 (Fig. 2C). Considerable variability was found in IUGR case 3 and asymptomatic recurrent infection 4 and 10. Together, the results suggest that pathology in the form of large fibrinoids with avascular villi, extensive edema and inflammation, alone or in combination, could significantly reduce perfusion and transport of substances across the placenta, resulting in IUGR.

HCMV replicates in blood vessels of placentas from IUGR with primary infection

Quantitative PCR of frozen biopsy specimens showed that IUGR case 16 with primary HCMV infection contained 5 x 10^6 genome copies/g placenta. Using nested PCR, viral DNA was also detected in one biopsy specimen each from IUGR cases 2 and 3 with recurrent infection (Table 2). Viral DNA was not found in any other placentas or any sera. Next, we investigated HCMV infection using immunohistochemistry to localize infected cell proteins in specialized cell types. In the basal plate, interstitial cytotrophoblasts contained viral antigens (Fig. 3A, B), and endothelial cells in lymphatic vessels contained viral proteins in nuclei and cytoplasmic vesicles (Fig. 3C, insets). Viral proteins were present in a comparable replication pattern in glandular epithelial cells (Fig. 3D, insets) that expressed CK7 (Fig. 3E). In addition, IUGR cases 2, 18, 3, 16 and 12 with congenital infection expressed viral proteins in cells of the basal plate to a variable degree (not shown).

Detailed analysis of placentas from IUGR cases with primary HCMV infection revealed that infected cell proteins were expressed in blood vessels in chorionic (floating) villi and the

chorion. In case 16, virus replicated in smooth muscle (SM) cells in the media (middle layer) of arteries (Fig. 4A) and veins in the chorion (Fig. 4E) that expressed SM myosin (Fig. 4B, F). In contrast, neither arteries nor veins expressing SM myosin (Fig. 4 D, H) in the chorion from IUGR cases 3 and 2 with recurrent infection expressed viral proteins (Fig. 4C, G). For IUGR case 12 with primary infection, blood vessels in some intermediate villi expressed HCMV proteins and SM myosin (Supplemental Fig. 2A, B), whereas other blood vessels lacked viral proteins (Supplemental Fig. 2C, D).

Unexpectedly, evidence of HCMV transmission was found in the amniotic membranes, which are composed of polarized epithelial cells facing the fetus bathed in amniotic fluid (Fig. 5). IUGR cases 12 and 16, with primary infection, contained cytoplasmic vesicles filled with HCMV antigens in accord with fetal infection and virion uptake at the apical membrane (Fig. 5A, B). Amniotic epithelial cells in the membranes of group B placentas 4, 10 and 7, with asymptomatic recurrent infection, also contained cytoplasmic vesicles with virion proteins (Fig. 5C-G), which were clearly visualized in grazing sections (*i.e.,* cross-section of concave surface) (Fig. 5G). Virion proteins were not detected in the amniotic epithelium from a group A seronegative control (Fig. 5H), group B recurrent infection patients 1 and 11, and group C recurrent infection with IUGR cases 2, 18 and 3, indicating these babies were spared (data not shown).

Elevated anti-angiogenic factors and cmvIL-10 in IUGR cases with HCMV infection. We next measured the hypoxia-related factors sFlt1 and sEng, which increase in parallel [27], and cmvIL-10, a viral immunosuppressive cytokine made late in infection [28]. As shown in Table 2, levels of sFlt1 in IUGR cases 2 and 3 were extremely elevated (180,992 pg/ml and 250,306 pg/ml, respectively) and increased in case 16 (16,823 pg/ml). Quantification of sEng showed that IUGR case 3 was highest (20.4 pg/ml), followed by case 2 (11.3 pg/ml) and case 16 (10.7 pg/ml). In maternal sera from case 12, with primary HCMV infection and preeclampsia,