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Histopathological analysis of placentas with congenital cytomegalovirus infection

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ABSTRACT

Introduction: Cytomegalovirus (CMV) infection is the most common cause of congenital viral infections in humans. The unusual structure of the placenta plays a pivotal role in CMV transmission from mothers to fetuses. The aim of this study was to evaluate the histopathological findings of placentas with congenital CMV infections.
Methods: We obtained placental specimens from 35 women who had newborns with congenital CMV infections. Placental specimens, extraplacental membranes, and umbilical cords were stained with hematoxylin and eosin, and subjected to immunohistochemical analysis. We evaluated the localization of CMV-infected cells and other histological parameters.
Results: Thirty (86%) of the 35 placentas tested positive for CMV-infected cell proteins by immunohistochemistry. A majority of CMV-positive cells were present in fibroblasts and endothelial cells in the villi. The number of CMV-infected cells was inversely correlated to gestational age at delivery. The frequency of chronic villitis (65% vs. 11%; $p < 0.01$) and changes of the villi (38% vs. 0%; $p < 0.05$) in the placentas from mothers with symptomatic congenital CMV infections was higher than those observed in samples from mothers with asymptomatic congenital infections. The frequency of changes of the decidua (43% vs. 5%; $p < 0.01$) in the placentas from mothers with non-primary CMV infections was higher than those from mothers with primary infections.
Discussion: Chronic villitis and changes of the villi were associated with symptomatic congenital CMV infections. The changes of the decidua were associated with congenital CMV infections, in mothers with non-primary CMV infections.

1. Introduction

Human cytomegalovirus (CMV) is one of the most common congenital infections worldwide, and can lead to permanent disabilities. The prevalence of congenital CMV infection is 0.2%–2.0% in newborns [1], and 10%–15% of infected newborns have symptomatic CMV infections. Symptomatic infants can have microcephaly, neurological deficiencies, hearing loss, retinopathy, and fetal growth restriction (FGR). In addition, 10%–15% of initially asymptomatic infants will develop long-term sequelae, including sensorineural hearing loss and mental retardation [2].

The placenta is the major route of the mother-to-fetus transmission, in which CMV spreads during maternal viremia [3]. The other route of placental infection is ascending infections from the genital tract. Alternatively, CMV may reactivate from latently infected cells in the pregnant uterus. Circumstantial evidence for ascending CMV infection includes high rates of bacterial vaginosis [4].

CMV infections interfere with robust placental development, central to the maintenance of a healthy pregnancy [5]. In the pregnant uterus, initial infection of the uterine vasculature spreads to extravillous trophoblast [6]. CMV replicates in underlying cytotrophoblast, causing a focal infection that spreads to the villous stroma and fetal capillaries. CMV replication at the uterine-placental interface could impair vascular remodeling and cause fibrosis, occluding blood flow and further reducing the exchange between the maternal and fetal circulation [7]. CMV infection of the placenta and subsequent placental dysfunction can also lead to fetal injuries. Infected placentas are either large and pale or small and fibrotic. Important microscopic findings include prominent

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villous fibrosis and mineralization, plasma cell infiltrates in the villous stroma, and large intranuclear inclusions with or without smaller basophilic cytoplasmic inclusions [8]. In addition, changes of amniotic membranes are observed in symptomatic CMV infections [9].

Placental factors that determine transmission to the fetus and the risk of adverse outcomes are not yet fully understood. The aim of this study was to evaluate the histopathological findings of placentas with congenital CMV infections.

2. Materials and methods

2.1. Participants and diagnosis of congenital infections

Between October 2009 and July 2018, all pregnant women who visited, or were referred to, Kobe University Hospital underwent maternal serological CMV screening. The institutional review board at Kobe University Hospital approved the study, and written informed consent was obtained from all participants. These women underwent initial blood screening for CMV IgG before 22 weeks of gestation. All CMV IgG–positive women were tested for serum CMV IgM levels and IgG avidity index (AI) 2–4 weeks after the initial CMV IgG measurements. Pregnant women who were referred after 22 weeks of gestation received simultaneous CMV IgM, IgG, and IgG avidity testing at their first visits. CMV IgG negative women received an educational intervention that focused on preventing primary infections, followed by repeat measurement of CMV IgG levels at 34–36 weeks of gestation. CMV IgG seroconversion in IgG negative women was regarded as a primary CMV infection during pregnancy. And in this study, women who had an AI < 40% and a positive result for CMV IgM were considered to have primary CMV infections during pregnancy. Non-primary CMV infection was defined as a negative result for CMV IgM or a positive result for CMV IgM with an AI ≥40% in the blood.

Serological tests for CMV-specific IgG (negative: < 230, borderline: 230–240, and positive: > 240) were performed using the Enzygnost assay (Siemens Healthcare Diagnostics, Tokyo, Japan). CMV-specific IgM (negative: < 0.8, borderline: 0.8–1.2, and positive: > 1.2 index) was measured using enzyme immunoassay kits produced by Denka Seiken (Tokyo, Japan). CMV-specific IgG avidity was measured, as described previously (Aisenkai Nichinan Hospital, Miyazaki, Japan) [10].

Urine samples were collected from newborns onto filter paper within 1 week of birth and the presence of CMV-DNA was assessed in the neonatal CMV universal screening. Liquid urine samples were obtained from CMV-positive newborns, and the CMV-DNA copy number was determined using real-time quantitative PCR. In cases of still birth, induced abortion, and neonatal death, amniotic fluid or umbilical cord blood samples were collected. The presence of a congenital CMV infection was confirmed by positive PCR results for CMV-DNA in the liquid urine of the newborns, amniotic fluid, or umbilical cord blood samples. All newborns with positive PCR results for CMV-DNA received a workup to identify the symptoms of congenital CMV infection. Symptomatic congenital CMV infection was diagnosed when newborns with positive PCR results had microcephaly, hepatosplenomegaly/hepatitis, thrombocytopenia, abnormality of brain images, CMV-associated retinopathy, abnormal auditory brain-stem response, or were classified as small-for-gestational age (SGA) [11,12].

2.2. Histochemical staining of the placentas

We examined placental specimens (one central and two to four peripheral sections). Extraplacental membranes from the ruptured edge to the placental margin, and umbilical cords from 35 pregnancies were fixed in 10% formalin (24–48 h), embedded in paraffin, and sectioned for histological and immunohistochemical analysis. Serial sections (5 μm thick) were either stained with hematoxylin and eosin or subjected to immunohistochemical staining.

Immunohistochemical staining for CMV immediate early 1 and 2 proteins (UL123) and DNA-binding protein (UL44) (clones CCH2 + DDG9, Dako, dilution 1:100) was performed in order to identify CMV-positive cells. Immunostaining was performed using a Bond Max autostainer (Leica Microsystems, Wetzlar, Germany) according to the manufacturer's instructions. Purified IgG to cellular proteins were purchased from the following sources: CD31, endothelial cells (mouse monoclonal antibody, clone JC70A, Dako); CD45, lymphocytes and monocytes (mouse monoclonal antibody, clone 2B11 + PD7/26, Dako); CD68, macrophage/dendritic cells (mouse monoclonal antibody, dilution 1:150, Dako); α-SMA, fibroblasts (mouse monoclonal antibody, clone 1A4, Dako); and Cytokeratin, epithelial cells and trophoblast (mouse monoclonal antibody, clone AE1/3, dilutin 1:5, Dako). For double immunolabeling, CD31, CD45, CD68, α-SMA and cytokeratin were visualized using the Bond Polymer Refine Detection kit (Leica) in brown, while CMV was visualized with perma blue/AP (Diagnostic BioSystems, Pleasanton, CA, USA) in blue with a kernechtrot counterstain. In order to confirm that there was no difference in number of CMV-infected cells depending on the antibodies, we stained CMV-infected cells with another anti-CMV antibody (mouse monoclonal antibodies, cocktail of clones 8B1.2, 1G5.2, 2D4.2, Merck Millipore MAB8121), which reacted with immediate early, early, and late antigen preparations in 7 cases.

To examine the alterations of villous basement membrane, periodic acid-methenamine-silver staining (PAM staining) was performed.

2.3. Histological examination

According to the literature [13–17], 10 parameters were analyzed. Histological findings of placental lesions associated with congenital CMV infection were defined as shown in Table 1. We evaluated the number of CMV-positive cells with immunohistochemistry, the presence of cytomegalic cells with intranuclear inclusion with halos, chronic villitis, changes of the villi which was defined as the presence of 5 or more findings from the following: fibrin deposition, calcification, necrosis, hemosiderin deposition, villous congestion, villous edema, avascular villi, and breakdown of villi; delayed villous maturation, chorioamnionitis, funisitis, changes of the decidua (both plasma cell deciduitis and necrosis), changes of the amniotic membranes (multiple findings of irregular, loss of cell-cell junctions, cell fusion, blebbing), hypoxic findings (both increased syncytial knots and villous chorangiosis). The slides were analyzed by one pathologist (M.K.). For each slide the number of positive cells or foci was counted for the whole specimen. For each placental feature, we judged it as positive if at least one slide produced findings.

2.4. Statistical analysis

We compared the clinical and pathological findings of placentas between participants with symptomatic and asymptomatic congenital CMV infections, between symptomatic fetuses who were hyperimmunoglobulin (HIG)-treated and untreated, between mothers with primary CMV infections and non-primary infections, and between those who delivered before 32 weeks and after 32 weeks of gestation.

Between-group differences were analyzed using the Mann–Whitney *U* test, Fisher's exact test, and the Chi-square test. Statistical significance was considered present at *p*-values less than 0.05. All statistical analyses were performed using SAS version 9.1 software (SAS Institute Inc., Cary, NC, USA).

3. Results

Thirty-seven women had newborns with congenital CMV infections during the study period. We obtained placental specimens from 35 of the 37 women. The other 2 women who had newborns with congenital CMV infections produced negative tests for CMV IgM and high AI, and

Table 1
The definition of histological findings of placental lesions associated with congenital cytomegalovirus infection.

Parameter	Definition (findings regarded as positive)
Presence of intranuclear inclusions	(At least one cell)
Number of cytomegalovirus-positive cells	Average number per slide by immunohistochemistry
Chronic villitis	Infiltrates of lymphocytes, histiocytes, plasma cells, and eosinophils (multiple foci of villi, on multiple slides)
Changes of the villi:	
Fibrin deposition	(≥3% of villi)
Calcification	(≥5 small or ≥1 big per slide)
Necrosis	Loss of nuclear staining of the stroma (≥3% of villi)
Hemosiderin deposition	A yellowish brown granular pigment found in macrophage (at least one cell)
Villous congestion	Prominent capillaries in the villi but there is no numerical increase in the number of capillaries (multiple foci of villi)
Villous edema	(Multiple foci of villi)
Avascular villi	Terminal villi with total loss of villous capillaries and bland hyaline fibrosis of villous stroma (≥3 foci of more than 2 terminal villi)
Breakdown of villi	Breakdown of trophoblastic basement membrane (≥30% of villi)
Delayed villous maturation	Diffuse abnormality of distal villi characterized by poor vasculosyncytial membrane formation, prominent villous cytotrophoblalst cellularity, and increased villous stroma (≥30% of villi)
Chorioamnionitis	Redline's criteria (Stage 2 or 3 in maternal inflammatory response)
Funisitis	Redline's criteria (Stage 1 or 2 or 3 in fetal inflammatory response)
Changes of the decidua:	
Plasma cell deciduitis	Presence of mixed plasma cells and lymphocytes in decidua basalis adjacent to maternal floor (multiple foci of decidua)
Necrosis	Loss of nuclear basophilia of decidual stromal cells and vessels in band-like pattern (multiple foci of decidua)
Changes of the amniotic membranes:	
Irregularity	Amnion cells showed columnar change (≥30% of epithelial cells in amniotic membranes)
lost of cell-cell junctions	(≥30% of epithelial cells in amniotic membranes)
Cell fusion	Amnion cells showed pseudostratified columnar (multiple foci in amniotic membranes)
Blebbing	Intracytoplasmic vacuolation (multiple foci in amniotic membranes)
Hypoxic findings:	
Increased syncytial knots	Localized aggregation of syncytiotrophoblastic nuclei in the villi [> 20% (before 34 weeks of gestation) or 30% (after 38 weeks of gestation)]
Villous chorangiosis	Altshuler's criteria (at least 10 vascular profiles per terminal/intermediate chorionic villus in 10 chorionic villi per ×10 objective microscopic field in at least 10 areas of 3 or more random cotyledons)

their placental specimens were not preserved at delivery. Twenty-six newborns had symptomatic congenital CMV infections. HIG fetal therapies were performed on 12 women with symptomatic congenital infections. The institutional review board at Kobe University Hospital approved the study, and written informed consent was obtained from all participants. HIG administration methods consisted of HIG injection into the peritoneal cavities of affected fetuses, and intravenous HIG injections into the maternal blood.

The clinical characteristics of 35 pregnancies with congenital CMV infections are shown in Table 2. Pregnancy outcomes included 28 live births, 3 neonatal deaths, 1 stillbirth at 23 weeks of gestation, and 3 elective pregnancy terminations at 21 weeks of gestation. The birth weight of newborns with symptomatic congenital CMV infections was less than that of newborns with asymptomatic congenital CMV infections ($p < 0.05$). There were no statistical differences in the clinical characteristics of pregnancies in mothers with primary CMV infections and those with non-primary infections.

Thirty (86%) of 35 placentas from pregnancies with congenital CMV infections tested positive for CMV-infected-cell proteins by immunohistochemistry. The median number of CMV-positive cells per specimen was 1.7 (range 0–514). We detected CMV-infected-cell proteins on the villi of the placenta (n = 29), the amniotic membrane (n = 8), the decidua (n = 1), and the chorion (n = 1) (Fig. 1-A, B, C, D).

To determine cell types with CMV infection, double immunohistochemical staining was performed on 5 placentas in which more than 100 CMV-positive cells per one specimen (Fig. 2). The total number of CMV-positive cells in the villi of 5 placentas was 1542, and cell types with CMV infection could be determined in 549 (36%) cells by immunohistochemical staining using specific cell markers. The cell types were as follows: fibroblasts (54%), endothelial cells (31%), macrophage/dendritic cells (8%), lymphocytes/monocytes (4%), and trophoblast (3%).

Double immunohistochemical staining using specific cell markers was also performed on the extraplacental membranes including the amniotic membrane, chorion, and decidua. The total number of CMV-positive cells in the amniotic membrane of 8 placentas was 99, and the localization of CMV-positive cells was as follows: amniotic connective tissue (97%) and amniotic epithelial cells (3%). Fibroblasts of 7/74

Table 2
Clinical characteristics of 35 pregnancies with congenital cytomegalovirus infection.

	All (n = 35)	Congenital cytomegalovirus infection			Maternal cytomegalovirus infection		
		Symptomatic (n = 26)	Asymptomatic (n = 9)	*p value*	Primary (n = 21)	Nonprimary (n = 14)	*p value*
Maternal age, years old	30.2 ± 5.5	30 (19–40)	29 (19–36)	0.85	29 (19–37)	33 (21–40)	0.26
Gravity	2.1 ± 1.3	2 (1–8)	2 (1–4)	0.82	2 (1–4)	2 (1–8)	0.19
Parity	0.6 ± 0.7	0 (0–2)	1 (0–3)	0.58	1 (0–3)	0 (0–2)	0.57
Gestational week at the initial AI measurements	25.4 ± 7.5	25 (16–35)	23 (12–42)	0.68	23 (12–39)	30 (16–42)	0.12
Gestational week at delivery	33.4 ± 5.7	34 (21–40)	38 (21–40)	0.13	36 (21–39)	36 (21–40)	0.67
Birth weight, g	2045.1 ± 901.4	2030 (311–3216)	2786 (410–3320)	$p < 0.05$	2381 (311–3274)	1989 (326–3320)	0.46
Placental weight, g	467.5 ± 161.8	463 (222–950)	496 (150–574)	0.53	496 (150–796)	439 (222–950)	0.50

Data are expressed as the mean ± standard deviation, or median (range).
AI, cytomegalovirus immunogloblin G avidity index.

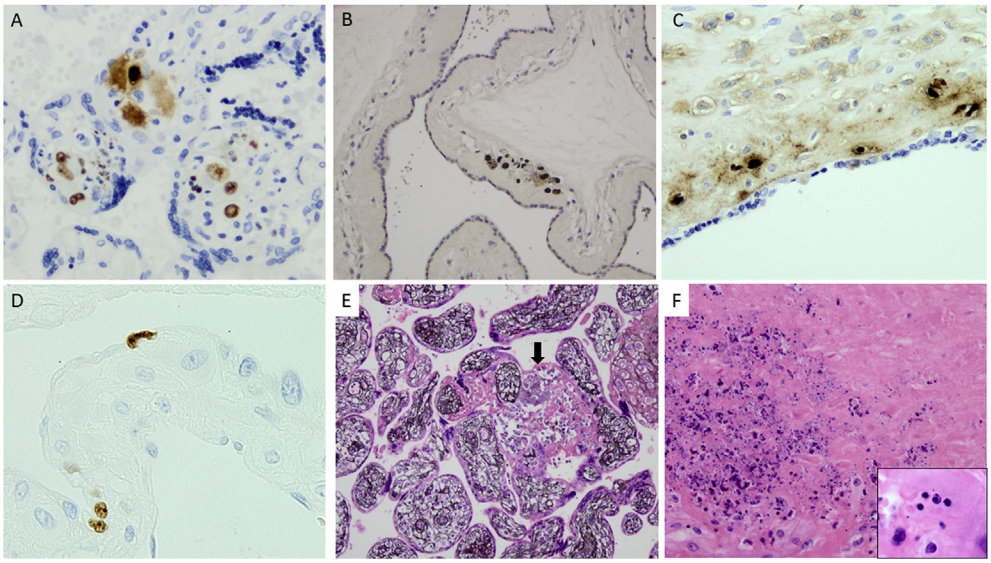

Fig. 1. (A) CMV-infected cells in villi (immunohistolochemical staining). (B) CMV-infected cells in amniotic connective tissue (immunohistological staining). (C) CMV-infected cells in chorion (immunohistological staining). (D) CMV-infected cells in decidua (immunohistological staining). (E) Breakdown of villous basement membrane (arrow). CMV-infected cells, chronic villitis, and fibrin deposition are present (periodic acid-methenamine-silver stain). (F) Plasma cell deciduitis and necrosis in decidua. Inset shows high power view for plasma cell (hematoxylin-eosin).

(9%) in the amniotic connective tissue and fibroblasts of 2/7 (29%) in the chorion was infected. Endometrial glandular epithelial cells (5/5; 100%) were found to be CMV-positive in the decidua.

The number of CMV-infected cells was inversely correlated to gestational age at delivery ($r = -0.68$, $p < 0.001$) (Fig. 3). There were significantly more CMV-infected cells in the 10 placentas obtained before 32 weeks of gestation (median 61.4, range 1–514) than there were in the 25 placentas delivered after 32 weeks of gestation (median 0.85, range 0–24.3; $p < 0.001$). There was no difference in number of CMV-infected cells, regardless of gestational age at delivery, between the two antibodies.

Table 3 shows the results of regression analyses of histological findings of placental lesions associated with congenital CMV infections. The breakdown of the villous basement membrane was detected using PAM staining (Fig. 1-E).

The frequencies of chronic villitis (65% vs. 11%; $p < 0.01$) and changes of the villi (38% vs. 0%; $p < 0.05$) were higher in the placentas from mothers with symptomatic congenital CMV infections than from those with asymptomatic infections. Although not significant, the frequency of chorioamnionitis (35% vs. 0%; $p = 0.07$) and hypoxic findings (35% vs. 0%; $p = 0.07$) were higher in the placentas from mothers with symptomatic CMV infections than from those with asymptomatic infections. Only one placenta from a mother who had asymptomatic congenital CMV infection was found to be normal. The frequency of changes in the decidua (Fig. 1-F) (43% vs. 5%; $p < 0.01$) in placentas from mothers with non-primary CMV infections was higher than those from mothers with primary infections. There were no significant differences in pathological findings between the HIG treated group and the non-treated group for symptomatic congenital CMV infections (Table 4).

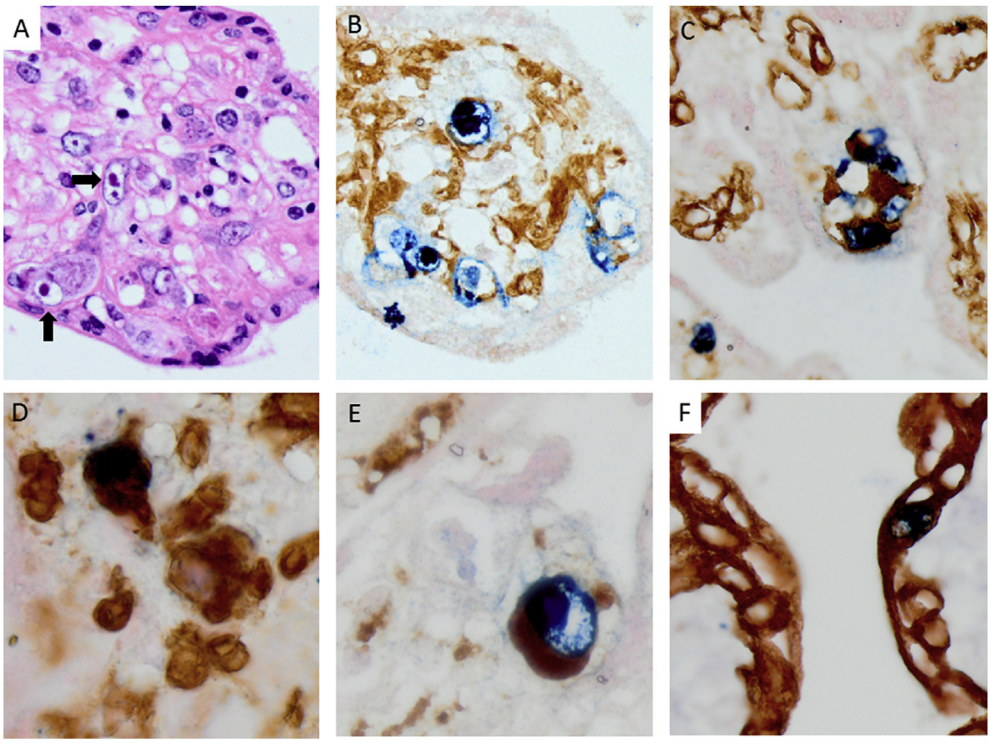

Fig. 2. (A) Cytomegalic cells with intranuclear inclusion with halos (arrows) in villi (hematoxylin-eosin). (B–F) Double immunohistochemical staining of the villi. (B) Serial section of **A**. CMV-infected cells (blue) expressed in α-SMA (brown). (C) CMV-infected cells (blue) expressed in CD31 (brown). (D) CMV-infected cells (blue) expressed in CD45 (brown). (E) CMV-infected cells (blue) expressed in CD68 (brown). (F) CMV-infected cells (blue) expressed in cytokeratin (brown).

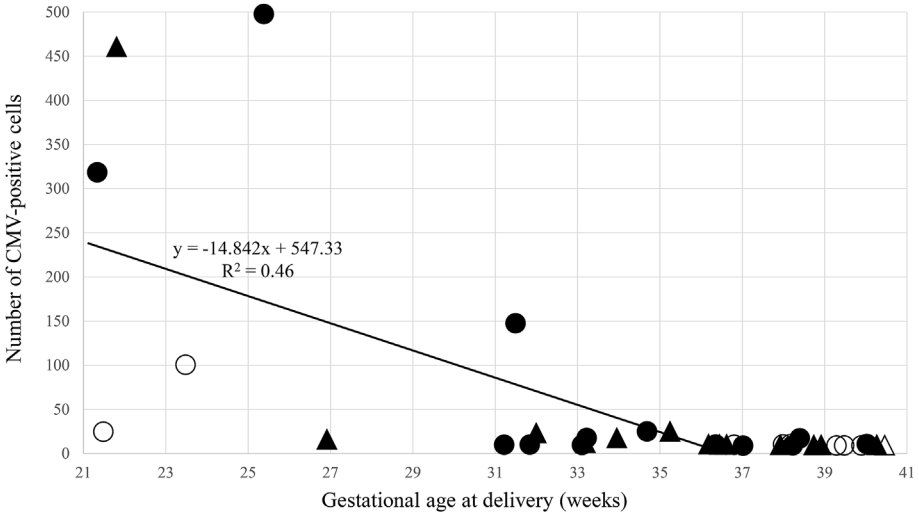

Fig. 3. Correlation between the number of CMV-positive cells and gestational age at delivery. Data from placentas with symptomatic congenital CMV infection and maternal primary infections (n = 13) (closed circles), symptomatic CMV infection and maternal non-primary infection (n = 13) (closed triangles), asymptomatic CMV infection and maternal primary infection (n = 8) (open circles), and asymptomatic CMV infection and maternal non-primary infection (n = 1) (open triangles). A solid line indicates an approximately correlation between the two parameters.

4. Discussion

The present study found that CMV-infected cells were mainly fibroblasts and endothelial cells in the villi; and some were macrophages, lymphocytes, and trophoblast. A few CMV-infected cells were detected in the decidua. The frequencies of chronic villitis and changes of the villi in mothers with symptomatic congenital CMV infections were higher than those in mothers with asymptomatic congenital infections. These results were consistent with previous studies [7,15]. The present study for the first time demonstrated that the frequency of changes of the decidua in mothers with non-primary CMV infections was higher than those in mothers with primary infections; and also, for the first time demonstrated that the number of CMV-infected cells was inversely correlated to gestational age at delivery. Local activation of CMV might be associated with premature delivery. A study of biopsy specimens from early-gestation placentas found that CMV replication proteins were expressed on the epithelia of endometrial glands in the decidua and cytotrophoblast in the floating villi and cell columns [4]. CMV may replicate more readily in early-gestation placentas than late-gestation placentas.

The frequencies of chronic villitis and changes of the villi were higher in the placentas from mothers with symptomatic CMV infections than from those with asymptomatic infections. CMV-infected cells were detected mainly in fibroblasts and endothelial cells in the villi. Endothelial fibrosis, mediated by endothelial-to-mesenchymal transition (EndMT), is involved in inflammatory diseases. TNF-α, IL and TGF-β induce EndMT-mediated endothelial fibrosis [18]. Activation of TGF-β1 contributes to pathological changes and may impair endothelial cell functions in villi that are chronically infected with CMV [19]. CMV replication at the uterine-placental interface could impair vascular remodeling and cause fibrosis, potentially occluding blood flow. These infections may markedly impair placental functions, resulting in hypoxia and fetal symptoms like FGR.

Maternal HIG treatment may suppress CMV replication in the placenta and prevent placental dysfunction [7]. Of those with symptomatic congenital infections, there were no significant differences in the histopathological findings between the HIG treated and the non-treated groups. This result was in agreement with a recent report in which HIG was not able to reduce placental viral load or histological damage [20].

Lymphocytes are normal constituents of the decidua, but plasma cells are not. The presence of plasma cells in the decidua reflects abnormal immune environments, including hematogenous infections (i.e., CMV, herpes simplex virus, syphilis) [17]. Generally, plasma cell deciduitis presents in 5% of all placentas, gradually decreasing as gestational age increases [8]. Laminar decidual necrosis may represent a physiologic change in preparation for parturition, so it may be normal at term but pathologic during the preterm period. In the present study, the frequency of changes of the decidua, including both plasma cell deciduitis and necrosis, was found to be 20%. Furthermore, the frequency of changes in the decidua was higher in placentas from mothers with non-primary CMV infections than from those with primary infections. Recently, we reported that threatened premature delivery was associated with the occurrence of congenital CMV infections in pregnant women with non-primary CMV infections, the pathophysiology of

Table 3
Histopathological findings associated with congenital cytomegalovirus infection.

Factors	All (n = 35)	Congenital cytomegalovirus infection			Maternal cytomegalovirus infection		
		Symptomatic (n = 26)	Asymptomatic (n = 9)	*p value*	Primary (n = 21)	Nonprimary (n = 14)	*p value*
Presence of cytomegalovirus-positive cells	30 (86%)	22 (85%)	8 (89%)	1.00	19 (90%)	11 (79%)	0.37
Number of cytomegalovirus-positive cells, median (range)	1.7 (0–514)	5.6 (0–514)	1.0 (0–98)	0.26	1.7 (0–514)	1.2 (0–468)	0.41
Presence of intranuclear inclusions	19 (54%)	15 (58%)	4 (44%)	0.70	11 (52%)	8 (57%)	0.78
Chronic villitis	18 (51%)	17 (65%)	1 (11%)	$p < 0.01$	10 (48%)	8 (57%)	0.58
Changes of the villi	10 (29%)	10 (38%)	0	$p < 0.05$	5 (24%)	5 (36%)	0.47
Delayed villous maturation	10 (29%)	9 (35%)	1 (11%)	0.23	5 (24%)	5 (36%)	0.47
Chorioamnionitis	9 (26%)	9 (35%)	0	0.07	5 (24%)	4 (29%)	1.00
Funisitis	5 (14%)	4 (15%)	1 (11%)	1.00	3 (14%)	2 (14%)	1.00
Changes of the decidua	7 (20%)	7 (27%)	0	0.15	1 (4.8%)	6 (43%)	$p < 0.01$
Changes of the amniotic membranes	16 (46%)	13 (50%)	3 (33%)	0.46	10 (48%)	6 (43%)	0.78
Hypoxic findings	9 (26%)	9 (35%)	0	0.07	3 (14%)	6 (43%)	0.11

Table 4
Histopathological findings associated with symptomatic congenital cytomegalovirus infection.

	Hyperimmune globulin treatment	Non-treatment	*p value*
	(n = 12)	(n = 14)	
Presence of cytomegalovirus-positive cells	11 (92%)	11 (79%)	0.60
Number of cytomegalovirus-positive cells, median (range)	2.8 (0–144)	9.5 (0–514)	0.61
Presence of intranuclear inclusions	7 (58%)	8 (57%)	1.00
Chronic villitis	9 (75%)	11 (79%)	1.00
Changes of the villi	5 (42%)	5 (36%)	1.00
Delayed villous maturation	5 (42%)	4 (29%)	0.68
Chorioamnionitis	2 (17%)	7 (50%)	0.11
Funisitis	1 (8.3%)	3 (21%)	0.60
Changes of the decidua	1 (8.3%)	5 (36%)	0.39
Changes of the amniotic membranes	7 (58%)	6 (43%)	0.70
Hypoxic findings	4 (33%)	5 (36%)	1.00

which may be closely associated with CMV reactivation during pregnancy [21]. Threatened premature delivery may reflect the inflammatory conditions in which CMV is reactivated; alternatively, it may cause CMV reactivation in pregnant women. Latently infected cells can reactivate CMV as a component of the inflammatory response to pathogenic bacteria within the pregnant uterus [4]. Physical stimulation, bleeding and bacterial infections in the decidua (i.e., the fetomaternal interface) may activate the latent virus in the uterus, and subsequently cause congenital CMV infections of fetuses in pregnant women with non-primary CMV infections.

Conflicts of interest

The authors report no conflict of interest.

Declarations of interest

None.

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