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Cell types infected in human cytomegalovirus placentitis identified by immunohistochemical double staining

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Abstract. Chronic villitis is almost always present in intrauterine infection with human cytomegalovirus (HCMV). The inflammatory response to this virus has been described in detail. However, little is known about the types of placental cells that may be infected by HCMV and six cases of HCMV placentitis were thus investigated to identify the vulnerable cell types. Immunohistochemical double staining analyses were performed using antibodies to HCMV immediate early antigens and to specific cellular marker proteins. Fixed connective tissue cells could be demonstrated to be the predominantly infected cell type in each placental tissue. Endothelial cells and macrophages were also found to be infected in all six cases, whereas evidence of trophoblast infection was obtained in four cases. It is concluded that release of infectious virus by connective tissue cells, macrophages and endothelial cells may play a critical role in transplacental transmission of HCMV. The findings further suggest that the cytopathic effect of HCMV infection on these cells might be involved in the pathogenesis of intrauterine HCMV disease.

Key words: Cytomegalovirus – Congenital cytomegalic inclusion disease – Placenta – Chorionic villi – Immunohistochemistry

Introduction

Transmission of human cytomegalovirus (HCMV) from mother to child is the most common cause of congenital viral infection (Weller 1971; Alford et al. 1981). This infection may be asymptomatic, but may also result in abortion (Sever 1980), stillbirth or congenital sequelae (Weller and Hanshaw 1964; McCracken et al. 1969; Berenberg and Nankervis 1970). Thrombocytopenia, hepatosplenomegaly, microcephaly, mental retardation or auditory deficit are the main symptoms of the syndrome (Weller and Hanshaw 1964; McCracken et al. 1969; Berenberg and Nankervis 1970) and chronic inflammation of the placenta is almost always found in these cases (Garcia et al. 1989). Fetuses are at particularly high risk of developing severe sequelae when the mother undergoes a primary infection during pregnancy but in cases where acute HCMV infection of the mother is due to reactivation of latent virus or reinfection, the child rarely suffers from serious disease (Stagno et al. 1982). It is generally believed that the virus spreads hematogenously from the mother to the fetus (Benirschke and Kaufmann 1990b), but the detailed mechanisms of transmission through the placenta are still unknown at the cellular level. The major histopathological feature of HCMV infection of the placenta is chronic lymphoplasmocytic villitis (Blanc 1981; Garcia et al. 1989), but focal necrosis and vasculitis may also occur (Garcia et al. 1989). Factors important in pathogenesis may include a direct cytopathic effect of the virus, inflammatory reaction or immunoreaction both in fetal organs and in the placenta itself. Twin studies emphasize the importance of the fetal immunoreaction in the abolition of HCMV infection (Vogler et al. 1986; Ahlfors et al. 1988). Several reports detail the inflammatory reaction in HCMV villitis (Garcia et al. 1989; Schwartz et al. 1992), but little is known about the role of placental cells in viral transmission or in pathogenesis. In earlier work, identification of placental cell types that appeared to be infected was made solely on the basis of morphological criteria or the localization of the cells in the organ (Garcia et al. 1989). As cells may be altered due to infection with HCMV, no accurate determination of the types of specific cells infected was obtained. In a recent report, a subpopulation of placental cells infected with HCMV was identified as being macrophages by using a monoclonal antibody against a cell-specific marker (Schwartz et al. 1992). Other authors failed to demonstrate infected macrophages, but identified infected endothelial cells using

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antibodies against von Willebrand factor (Mühlemann et al. 1992). However, the majority of infected cells lacked those markers. This study was designed to identify the placental cells infected by HCMV during acute placentitis. Immunohistochemical double staining was performed using monoclonal antibodies against HCMV-specific antigens and a variety of cell-specific determinants. The putative role of these cells in the transmission of the virus from mother to child is discussed.

Materials and methods

Formalin-fixed paraffin-embedded tissue sections from cases of HCMV placentitis were included in this study. Diagnosis was made by the detection of characteristic inclusion body cells, so-called owl eye cells, using light microscopy. Chronic villitis, as diagnosed by haematoxylin-eosin staining, was present in each case. The gestational age of the placentas ranged from 20 weeks to 39 weeks (Table 1).

Monoclonal antibodies (mAb) directed against HCMV proteins characteristic for each stage of replication were used in this study. MAb E13 (Biosoft, Paris, France), also known as M-810 (Chemicon, Temecula, Calif., USA), was directed against the nonstructural immediate early proteins (IEA; Mazeron et al. 1992). MAb CCH2 (generously provided by B. Zweygberg Wirgart and L. Grillner, Stockholm, Sweden) was directed against the nonstructural early DNA-binding protein of 52 kDa (EA; Plachter et al. 1992). MAb XP1 (NCNL 7325, Behring, Marburg, Germany) was directed against the late tegument protein pp150 (LA; Jahn et al. 1990).

To identify the types of placental cells infected by HCMV, a set of monoclonal and polyclonal antibodies was used (Table 2). Polyclonal antibody (pAb) anti-vimentin was directed against mesenchymal cells. MAbs HAM56 (Ortho-Diagnostics, Neckargemünd, Germany) and KP1 (Dako, Hamburg, Germany) were used as macrophage markers. MAb anti-factor VIII (Dako) was directed against factor-VIII-related antigen on endothelial cells. MAb anti-leucocyte common antigen (LCA) (Dako) was used as a pan-lymphocyte marker. MAb anti-actin (Ortho-Diagnostics) was used as a marker for smooth muscle cells. PAbs anti-human chorionic gonadotropin (β-subunit; Dako) and anti-placental alkaline phosphatase (Dako) were specific for trophoblast cells.

Immunohistochemical staining was performed using the streptavidin-biotin-peroxidase technique with diaminobenzidine (DAB; Sigma, St. Louis, Mo.) as a chromogen. Briefly, tissue sections were deparaffinized and endogenous peroxidase was blocked by incubation with 0.6% hydrogen peroxide in methanol for 20 min at room temperature (RT). After rehydration of the tissue sections by graded ethanol and predigestion with 0.1% protease (Merck, Darmstadt, Germany) in phosphate-buffered saline (PBS) for 5 min at RT, rabbit nonimmune serum was added for 20 min to prevent nonspecific binding of antibodies. Tissue sections were then incubated with the different HCMV mAbs for 90 min at RT. Following that, slides were incubated with biotinylated rabbit anti-mouse-Ig antibody (Dako) for 60 min at RT and, subsequently, incubated with streptavidin-biotin-peroxidase complexes (Dako) for 20 min at RT. The slides were rinsed in PBS for 5 min after each incubation step, except after the incubation with nonimmune serum. Staining was performed using 0.6% DAB in PBS. After counterstaining with haematoxylin (Merck) and mounting in Kaiser's glycerol gelatine (Merck), the slides were read in a Polyvar light microscope (Cambridge Instruments, Nussloch, Germany).

Table 1. Gestational age, histopathological findings in placental tissue and clinical outcome in the six cases of intrauterine human cytomegalovirus (HCMV) infection

Case number	Gestation age (weeks)	Histopathological findings	Clinical outcome
1	39	Lymphoplasmocytic villitis, dysmaturity	Congenital HCMV syndrome, petechiae, jaundice, hepatosplenomegaly, microcephaly
2	20	Lymphoplasmocytic villitis, focal necrosis, vasculitis	Abortion
3	36	Lymphoplasmocytic HCMV villitis and toxoplasmosis, focal necrosis	Stillbirth
4	30	HCMV villitis, vasculitis	Fetal death sub partu
5	35	HCMV villitis	Stillbirth
6	20	Lymphocytic villitis, polymorphonuclear infiltrates, vasculitis	Abortion

Table 2. Specificity of antibodies used in this study

Antibody	Species	Specificity	Supplier
E13	Mouse monoclonal	Human cytomegalovirus (HCMV) immediate early antigen	Biosoft, Paris, France
CCH2	Mouse monoclonal	HCMV early protein p52	Dako, Hamburg, Germany
XP1	Mouse monoclonal	HCMV late protein pp150	Behringwerke, Marburg, Germany
Vimentin	Rabbit polyclonal	Cells of mesenchymal origin	Dako
Factor VIII	Mouse monoclonal	Endothelial cells	Dako
Actin	Mouse monoclonal	Smooth muscle cells	Ortho, Neckargemünd, Germany
KP1	Mouse monoclonal	Macrophages	Dako
HAM56	Mouse monoclonal	Macrophages	Ortho
HCGβ	Rabbit polyclonal	Trophoblast	Dako
Placental alkaline phosphatase	Rabbit polyclonal	Trophoblast	Dako
Leucocyte common antigen	Mouse monoclonal	Lymphocytes	Dako

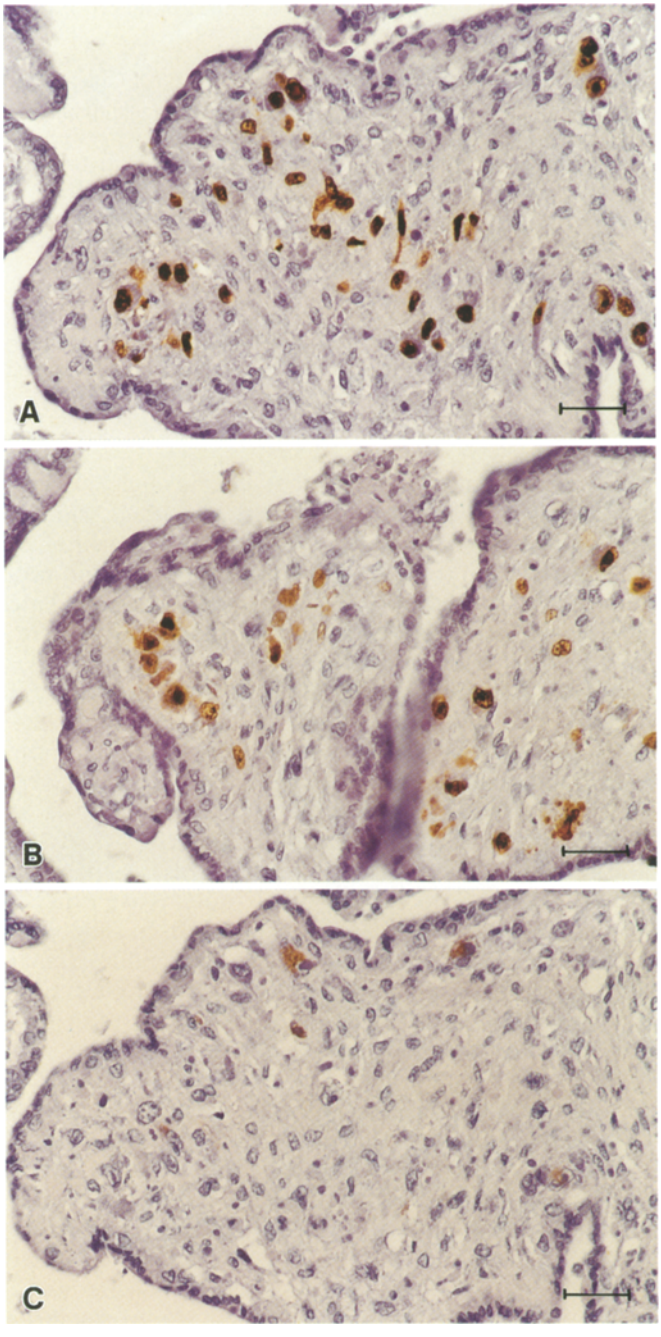

Fig. 1A–C. Detection of viral antigens in tissue sections of a human cytomegalovirus (HCMV)-infected placenta from the 36th week of gestation using an immunoperoxidase method with diaminobenzidine as a chromogen. The immunostaining results in a brown colour. Counterstaining was done with haematoxylin. **A** Staining of immediate early 1 and 2 antigens in morphologically unaltered and cytomegalic cells using monoclonal antibody (mAb) E13. **B** Staining of the DNA binding protein p52 predominantly in cytomegalic cells with or without inclusion bodies using mAb CCH2. **C** Staining of the tegument protein pp150 in cytomegalic cells with inclusion bodies using mAb XP1. The number of cells that were positive for nonstructural viral proteins (**A, B**) by far exceeded the number of late stage infected cells with characteristic inclusion bodies (**C**). Original magnification in each panel ×400, *bar* = 100 μm

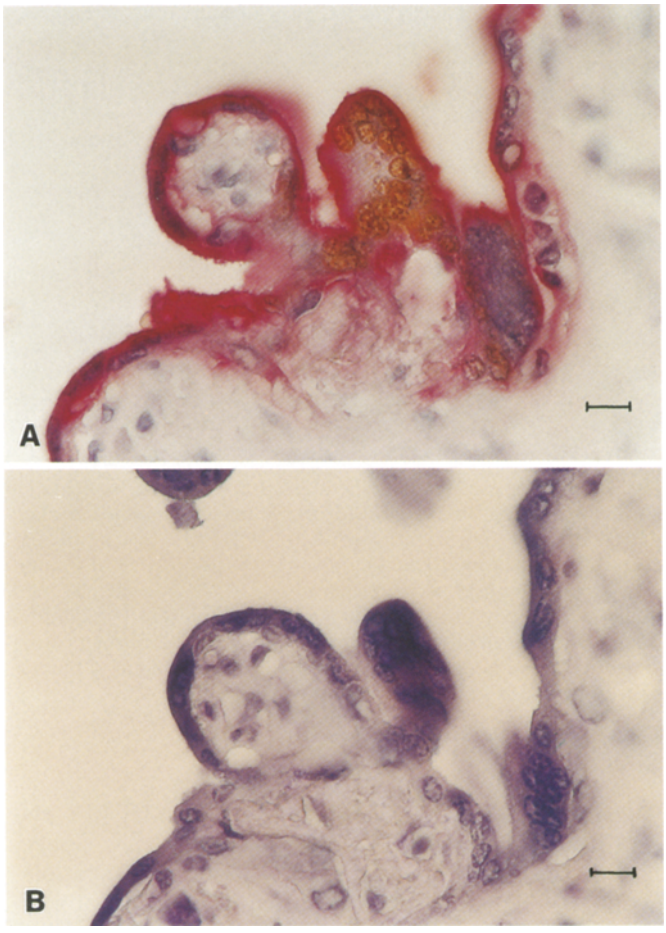

Fig. 2A, B. Immunohistochemical detection of viral antigens in the syncytiotrophoblast of a placenta from the 36th week of gestation. **A** Immunohistochemical double staining of HCMV immediate early antigen (IEA; immuno-peroxidase technique, brown nuclei) and placental alkaline phosphatase (immuno-alkaline-phosphatase technique, red cytoplasm) demonstrates infection of the morphologically unaltered syncytiotrophoblast. Counterstaining was done with haematoxylin. **B** No late viral antigen could be detected by immunoperoxidase staining for the structural viral protein pp150. Counterstaining was done with haematoxylin. Original magnification ×800, *bar* = 30 μm

Brown staining of the nuclei (E13, CCH2) or the cytoplasm (CCH2, XP1), respectively, was considered specific for HCMV-infected cells.

An indirect immunostaining method combining the streptavidin-biotin-peroxidase and streptavidin-biotin-alkaline-phosphatase techniques was used to detect HCMV-IEA and the respective cell marker simultaneously. In a first step, HCMV-IEA was detected using mAb E13 as described above. After DAB staining of this antigen, the respective cell markers were detected in the same way with the following modifications (TRIS buffer was used for all washing steps). Biotinylated swine antibody to rabbit Ig was used as a second antibody if the cell-specific antibody was polyclonal. Streptavidin-biotin-alkaline-phosphatase complex was used instead of peroxidase complex. Staining was performed with a fast red (Sigma) solution. If the cell-specific antibody was polyclonal, swine nonimmune serum was used instead of rabbit nonimmune serum for the whole double staining procedure. Predigestion was done with 0.4% pepsin (Sigma) at 37° C for 30 min when anti-factor VIII was the cell-specific antibody. After the second staining, slides were counterstained and mounted as described above. Red cytoplasmic staining was considered specific. Controls included

Table 3. Cell types infected in six cases of human cytomegalovirus (HCMV) placentitis, identified by immunohistochemical cell marker binding

Case number	IEA-positive cells (mean of 7 specimens)	Percentage of HCMV IEA-positive cells							Leucocyte common antigen
		Vimentin	Actin	Factor VIII	HAM 56	KP1	HCGβ	AP	
1	20	68	0	16	17	0	8	6	0
2	60	71	0	4	5	4	6	1	0
3	600	71	<1	9	1	2	<1	<1	<1
4	30	73	0	7	16	11	0	0	0
5	6000	71	<1	3	2	2	0	0	0
6	200	74	<1	6	2	1	<1	0	0

HCMV immediate early antigen (IEA)-positive cells/tissue section are given as absolute numbers. IEA-positive cells, double stained by specific cell markers, are given as percentage of HCMV IEA-positive cells

HCMV-infected placental tissue stained with β-Gal monoclonal antibody as a negative control for the primary antibodies, normal placental tissue as a negative control for HCMV placentitis and HCMV infected lung tissue as a positive control for HCMV staining. All cell markers were extensively tested in normal placental tissue before they were used in HCMV placentitis. In addition, double staining of normal placental tissue was performed, using vimentin antibody and HAM56 antibody, to determine, if macrophages were also stained by vimentin.

Results

The distribution of HCMV-infected cells in HCMV placentitis was investigated by immunohistochemical detection of various viral antigens. Tissue sections from placental tissue of six cases of symptomatic fetal or congenital HCMV infection were included in the study. MAb E13 has previously been shown to be directed against IEA of HCMV, encoded by immediate early regions 1 and 2 (Mazeron et al. 1992). These antigens are found very early after HCMV infection of culture cells. In all tissue sections studied, a characteristic nuclear staining pattern was found with mAb E13. Viral IEA was detected not only in cytomegalic cells but also in a number of morphologically unaltered cells (Fig. 1A). Another mAb, CCH2, has been demonstrated to be directed against EA of HCMV (Plachter et al. 1992). The initiation of the synthesis of this protein requires the presence of viral IEA. It is expressed at early and, more abundantly, at late stages after infection with HCMV. In tissue sections, mAb CCH2 showed nuclear, as well as cytoplasmic, staining (Fig. 1B). In most cases CCH2 predominantly stained cytomegalic cells. Only a few of the cells stained by CCH2 showed no morphological signs of HCMV infection. MAb XP1 was directed against LA (Jahn et al. 1990) and stained the cytoplasm of characteristically altered cytomegalic cells in tissue sections exclusively (Fig. 1C). It can be used as an indicator of de novo synthesis of viral particles and permissiveness of cells for HCMV replication.

HCMV-antigen-positive cells were heterogeneously distributed within the tissue. Specifically stained cells were found to be located either in foci or as single cells. In addition, regions without any HCMV-specific staining were found in the same sections. The number of infected cells varied from 20/section to 6000/section (Table 3) in the tissues from different cases. No clear correlations could be found between gestational age, clinical symptoms, histopathological findings and detection of HCMV IEA. All three HCMV antigens were found predominantly in stromal cells of immature intermediate villi, mature intermediate villi or terminal villi. Less frequently they were detected in the endothelial layer of capillaries and sinusoids. This included cells with and without morphological alterations and nuclear inclusions. In contrast, neither cytomegalic alterations nor nuclear inclusions could be seen in the trophoblast layer. HCMV-IEA was detected in the syncytiotrophoblast of four specimens. Characteristically, a few clusters of 10–40 syncytial nuclei were stained in these cases using mAb E13 (Fig. 2A). Early or late viral proteins were never detected in the trophoblast layer (Fig. 2B). In summary, HCMV caused an infection of the placental stromal layer as well as of cells in the blood vessels of intermediate villi and terminal villi in all six cases investigated; marked differences were found in the number but not in the tissue distribution of infected cells.

An immunohistochemical double staining technique was employed to determine the different types of placental cells infected by HCMV. In all cases, the characteristic brown nuclear staining pattern with the HCMV-specific monoclonal antibody was easy to distinguish from the red cytoplasmic staining of cell markers. In order to verify the specificity of cell-specific antisera, tissue sections from normal placental tissue were investigated. MAb anti-factor VIII labelling resulted in a strictly cytoplasmic staining of endothelial cells of arteries, veins, sinusoids and capillaries. No staining was detectable in trophoblast cells or stromal cells. MAbs HAM56 and KP1, specific for macrophages, showed cytoplasmic staining in cells within the stromal layer. No specific binding was found in endothelial cells and trophoblast cells of normal placental tissue. Labelling of actin resulted in cytoplasmic staining of vascular smooth muscle layers. PAb anti-vimentin stained stromal cells and endothelial cells. No staining of trophoblast cells was observed. Anti-HCGβ antibody and anti-alkaline-phosphatase antibody showed cytoplasmic staining of trophoblast cells. No other cells were stained by either antibody. MAb anti-LCA stained the cytoplasm of lymphocytes. Macrophages or granulocytes were very rarely

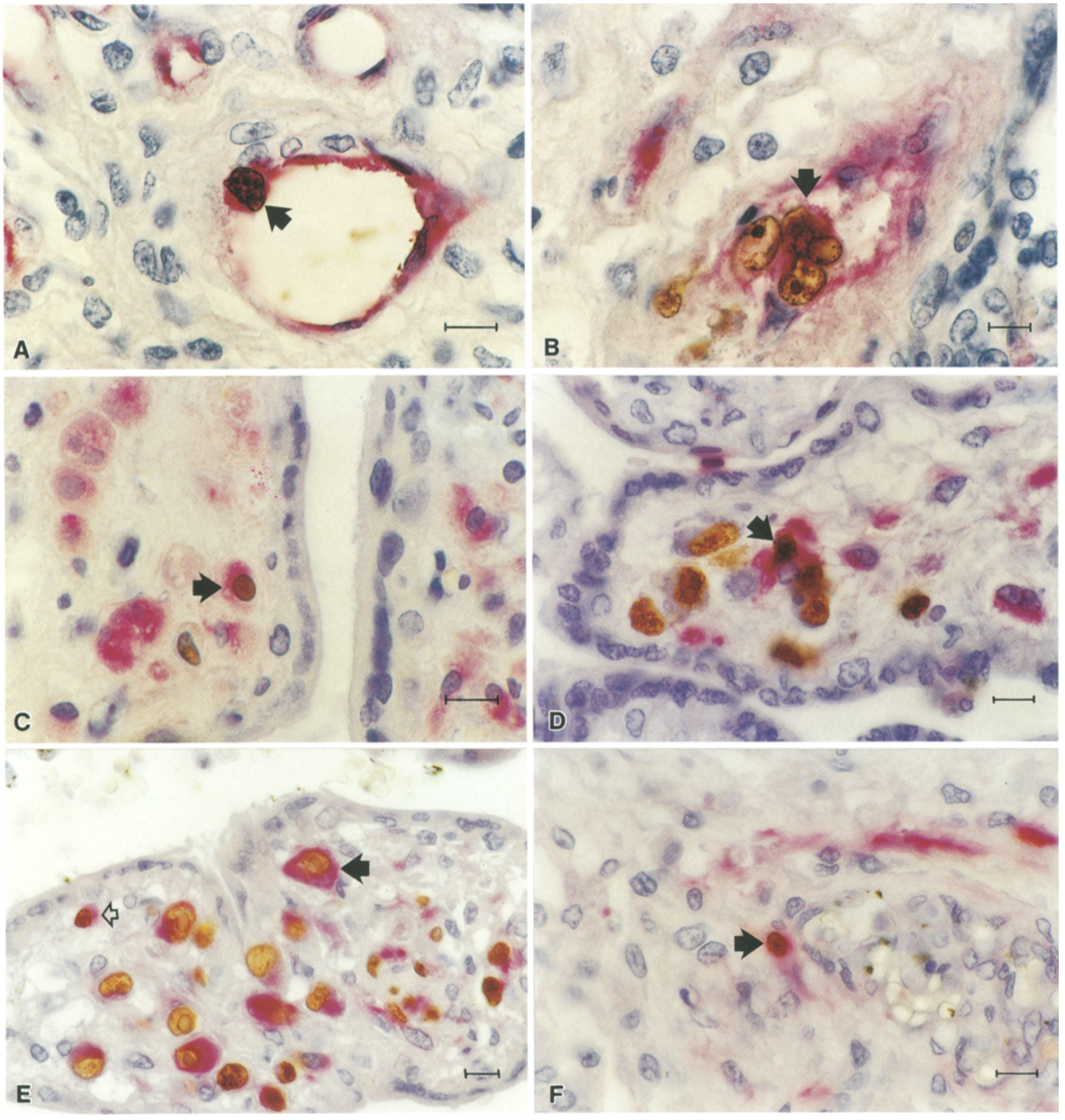

Fig. 3A–F. Identification of HCMV-infected cell types in HCMV placentitis by immunohistochemical double staining. Detection of HCMV immediate early antigen (IEA) by immuno-peroxidase technique yielded brown nuclear staining. Detection of specific cell markers by immuno-alkaline-phosphatase technique yielded red cytoplasmatic staining. Counterstaining was performed with haematoxylin. **A** Binding of endothelial cell marker anti-factor VIII in an HCMV-infected cell of a placenta from the 39th week of gestation (case no. 2, original magnification ×1250). **B** Anti-factor VIII binding in cytomegalic HCMV-infected cells of a placenta from the 36th week of gestation (case no. 3, original magnification ×1000). **C** Binding of macrophage marker HAM56 in a morphologically unaltered HCMV infected cell of a placenta from the 35th week of gestation (case nr. 5, original magnification ×1250). **D** Binding of macrophage marker KP1 in a morphologically unaltered HCMV infected cell of a placenta from the 35th week of gestation (case no. 5, original magnification ×1000). The majority of infected cells were unstained by this marker. **E** Binding of mesenchymal cell marker anti-vimentin in morphologically unaltered (*open arrow*) as well as cytomegalic (*filled arrow*) HCMV-infected cells of a placenta from the 20th week of gestation (case no. 5, original magnification ×800). **F** Binding of anti-actin in an unaltered HCMV-infected cell of a placenta from the 36th week of gestation (case no. 3, original magnification ×1000). *Bar* in each panel = 30 μm

stained by this mAb in the formalin-fixed tissue sections investigated.

Using the anti-factor VIII antibody, 3–16% of HCMV-positive cells were identified as being endothelial cells (Table 3) including endothelial cells with normal morphology as well as rounded cells (Fig. 3A) or cytomegalic cells (Fig. 3B). The cells were predominantly located in venules, capillaries and sinusoids. Some HCMV-positive cytomegalic cells, double stained by factor VIII antibody, were found in the lumen of vessels without contact with the vessel wall. Frequently, infected cells not expressing factor VIII were detected around capillaries. Infected smooth muscle cells, identified by binding of mAb anti-actin (Fig. 3F), were always below 1% of HCMV-infected cells. Using mAbs HAM56 and KP1, 0–17% of HCMV-infected cells were identified as being of macrophage origin (Table 3). Cells that stained positively with these markers usually showed normal morphology (Fig. 3C, D). Cytomegalic cells with or without inclusion bodies were rarely found. They were predominantly located in the stromal layer and, at low frequency, in the vascular lumen. MAb anti-LCA, which intensively stained the lymphocytic infiltrations, could only be detected in one single HCMV-infected cell, which resembled a macrophagal cell rather than a lymphocyte. Overall, about 70% of HCMV-positive cells were double stained with anti-vimentin antibody in all six cases of HCMV placentitis. These mesenchymal cells included spindle-shaped stromal cells, cells with rounded morphology, and cytomegalic cells with or without inclusion bodies (Fig. 3E). They were distributed predominantly throughout the stromal layer of mature intermediate villi, immature intermediate villi and terminal villi. Due to the lack of cell markers other than vimentin, the majority of these cells seemed to be fixed connective tissue cells such as small reticulum cells, large reticulum cells, fibroblasts, or undifferentiated mesenchymal cells. Few of these infected vimentin-positive cells were found to be located in vessel walls suggestive of their endothelial origin. Antibodies against HCGβ or placental alkaline phosphatase were detectable in the cytoplasm around clusters of HCMV-positive nuclei in the syncytiotrophoblast layer, confirming the trophoblast origin of these infected syncytia, which never showed signs of morphological alteration (Fig. 2A). No staining by trophoblast markers occurred in cytomegalic cells.

In summary, the vast majority of HCMV-infected cells were demonstrated to be mesenchymal, carrying the intermediate filament vimentin. Most of them seemed to be fixed connective tissue cells. A minor portion of HCMV-infected cells were shown to be of endothelial origin, indicated by their binding of factor VIII antibody. Macrophages were also shown to be infected by HCMV, as indicated by HAM56 binding. However, their relative frequency did not exceed 17% of the total of HCMV-infected cells.

Discussion

Intrauterine HCMV infection is a well-known cause of fetal and congenital disease. Chronic villitis is almost always present and the inflammatory reaction has been investigated in detail by morphological means (Altshuler and Russell 1975; Garcia et al. 1989). However, only a small percentage of infected cells have been identified as macrophages or endothelial cells by cell marker binding (Mühlemann et al. 1992; Schwartz et al. 1992). As reliable information about the cell types infected in HCMV placentitis is lacking, little is known about the mechanisms of transplacental virus transmission and about the role of direct cytopathic effects of HCMV on placental cells in the development of disease. We have examined six cases of HCMV placentitis employing an immunohistochemical double staining technique. This method allows for the identification of infected cells by simultaneous detection of HCMV-specific antigen expression and cell-specific markers. For HCMV antigen detection, the mAb E13 was used. This mAb has been previously shown to be directed against proteins encoded by immediate early regions 1 and 2 of HCMV (Mazeron et al. 1992). These proteins are synthesized by 1 h after infection and remain detectable until cell death (Stenberg et al. 1989). Therefore, cells in all stages of infection are detected by this antibody. The different cell markers used in this investigation for immunohistochemical double staining were specific for trophoblast, lymphocytes, macrophages, endothelial cells, smooth muscle, and mesenchymal cells, respectively. As determined by their location, mesenchymal cells detected by an anti-vimentin antibody included endothelial cells. However, macrophages did not seem to carry detectable amounts of vimentin, as vimentin antibody and HAM56 stained distinct cell populations in double staining experiments. Therefore, it can be concluded that the majority of vimentin-positive cells were fixed connective tissue cells.

The distribution of HCMV-infected cells in the six placentas examined in this study represents a multifocal infection of villous stroma rather than vessels, trophoblast being a rare site of infection. This pattern is consistent with previous studies that focused on the distribution of HCMV-infected cells using the detection of characteristic inclusion bodies (Benirschke et al. 1974; Garcia et al. 1989; Schwartz et al. 1992) as well as the expression of HCMV-specific antigens as criteria for infection (Garcia et al. 1989; Mühlemann et al. 1992). The number of HCMV-infected cells indicated by the presence of viral nonstructural proteins by far exceeded the number of owl eye cells, which are characteristic for the late stage of HCMV infection. Therefore using morphological methods alone will result in the vast majority of infected cells being missed, a fact also reported by Mühlemann et al. (1992). However, immunohistochemical staining of HCMV-IEA allows for detection of infected cells in all stages of viral infection.

Consistent with previous reports (Mühlemann et al. 1992; Schwartz et al. 1992), endothelial cells and macrophages were identified as a minor fraction of infected cells in HCMV placentitis. In addition, since some of these cells were cytomegalic with inclusion bodies, both cell types appear to be permissive for HCMV replication. Both endothelial cells (Lathey et al. 1990; Waldman et al. 1991) and macrophages (Ibanez et al. 1991) have been shown to be permissive in cell culture systems. The major fraction of infected cells was identified as fixed

connective tissue cells, since they were exclusively stained with pAb anti-vimentin, but lacked other cell markers. This is consistent with the observation that fibroblasts are highly permissive for HCMV in cell culture. The appearance of many vimentin-positive owl eye cells might reflect a high permissivity of fixed connective tissue cells in placental villi. In contrast, infected syncytiotrophoblast, indicated by the presence of HCMV-IEA, showed no signs of permissive infection. Neither morphological alterations nor late viral proteins were detected in this cell layer. We note that authors who have previously reported trophoblast to be permissively infected by HCMV in vivo (Mühlemann et al. 1992) or in vitro (Amirhessami-Aghili et al. 1987) have failed to present any data suggestive of virus production in these cells. Consistent with that, Rosenthal et al. (1981) were not able to permissively infect trophoblast in vitro. About 20% of HCMV-antigen-positive cells remained unidentified by the cell-specific antisera. These cells might have gone undetected because of a low expression of cell-specific markers, undetectable in formalin-fixed tissues with the methods used. Particularly, loss of cell-specific proteins might occur in HCMV-infected cells, as has been demonstrated for factor VIII in endothelial cells (Bruggeman et al. 1988). This might lead to an under-representation of certain endothelial cells, consistent with the finding of infected cells in vessel walls lacking factor VIII staining. The observation of fixed connective tissue cells, macrophages and endothelial cells being permissively infected by HCMV in the human placenta has implications concerning the mechanisms of viral transmission from mother to fetus as well as concerning the role of virus-induced cytopathic effects in the pathogenesis of fetal and congenital HCMV disease. Little doubt can be raised that HCMV is haematogenously transmitted from mother to the fetus via the placenta (Becroft 1981; Benirschke and Kaufmann 1990b). Different possible modes of viral transmission are shown in Fig. 4. The role of the syncytiotrophoblast layer for HCMV transmission remains unclear. In this study, using specific cell marker binding, evidence is provided that HCMV can enter the trophoblast and translation of viral proteins is initiated. However, due to the lack of early and late viral proteins, it is not clear whether, by infection of these cells, HCMV can also be released to the villous stroma. Production of small amounts of structural viral proteins, undetectable with the methods used, cannot be excluded. Alternatively, the virus could be carried to the villous stroma by infected maternal blood cells. Maternofetal transfusion of white blood cells has been demonstrated by several authors (El-Alfi and Hathout 1969; Walknowska et al. 1969; Moszkowski et al. 1971). In addition, this mechanism has recently been suggested for the transmission of the human immunodeficiency virus (Peuchmaur et al. 1991). However, further analyses are required to investigate whether this also applies for HCMV infection.

Once the virus has entered the villous stroma, several routes of viral transmission are possible. Connective tissue cells seem to be very susceptible to permissive HCMV infection. Therefore, large amounts of infectious virus particles are expected to be produced in lytically infected connective tissue cells and spread to reach endothelial capillary cells. Productive infection of these cells would result in release of infectious HCMV into the fetal circulation. In support of this, HCMV-infected cytomegalic cells of endothelial origin were recently shown to circulate in the peripheral blood (Grefte et al. 1993). An alternative mechanism of viral spread throughout the villous stroma to the fetal capillaries involves infection of macrophages. Macrophages are able to migrate from the trophoblast layer to the villous capillaries. In this study, it could be shown that tissue macrophages in the placenta are infected by HCMV. Additional analyses on a larger number of placentas will be necessary to resolve the question of whether macrophages can serve as a vehicle to transport HCMV from placental stroma to the fetal circulation.

Infection of the placenta by HCMV may also result in an impairment of the physiological functions of this organ during pregnancy. As detailed in this study, HCMV can be found in fixed connective tissue cells, macrophages and endothelial cells of placental villi. Fibroblasts are known to be important for the architecture of the villous tissue (Benirschke and Kaufmann 1990a). Macrophages were reported to be involved in the immunological balance (Hunt et al. 1984; Hunt 1989) as well as in trophic functions (Castellucci and Kaufmann 1982) and in tissue remodelling in the placenta (Nathan 1987). Endothelial cells are important for the proper perfusion of the placenta and HCMV infection is known to result in endarteritis obliterans (Garcia et al. 1989). The abortion or intrauterine growth retardation observed during intrauterine HCMV infection

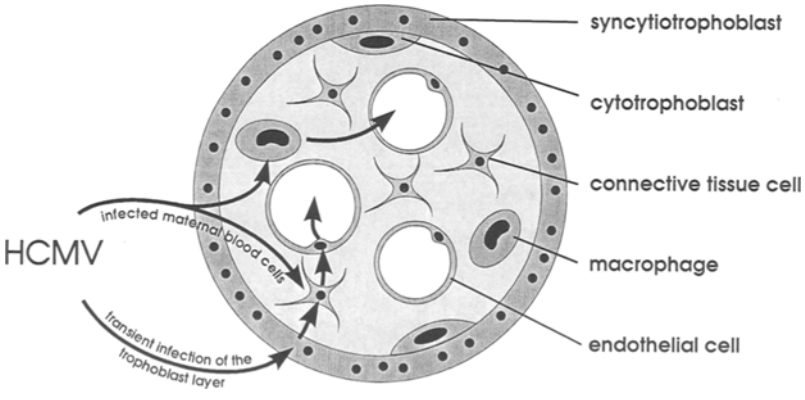

Fig. 4. Schematic representation of hypothetical mechanisms of transplacental HCMV transmission. HCMV is haematogenously transmitted from mother to fetus. In a first step, the syncytiotrophoblast might be passed by productive infection of the syncytiotrophoblast or by maternofetal transfusion of infected maternal leucocytes. Both mechanisms are hypothetical. Once released to the villous stroma, HCMV can be spread in the stroma by permissive infection of fixed connective tissue cells and macrophages. By permissive infection of endothelial cells HCMV can enter the fetal circulation. Recirculation of infected macrophages might be an alternative route

might therefore be attributable, at least in part, to infection of functionally important cells within the placenta.

In summary, this investigation of six cases of HCMV placentitis by immunohistochemical double staining demonstrated that trophoblast cells, fixed connective tissue cells, endothelial cells and macrophages are infected in HCMV placentitis.

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