ORIGINAL ARTICLE

HUMAN DECIDUAL CELLS ACTIVITY IN WOMEN WITH SPONTANEOUS ABORTIONS OF PROBABLE CMV AETIOLOGY DURING THE FIRST TRIMESTER OF GESTATION. AN IMMUNOHISTOCHEMICAL STUDY WITH CMV-ASSOCIATED ANTIGEN

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Summary: Aim: To determine the expression of CMV-associated antigen in the human decidual endometrial stromal cells in spontaneous abortions with no evidence of maternal relapse during the first trimester of gestation. Experimental design: We examined 15 placentas resulting from intrauterine fetal death after spontaneous abortion during the 8th, 10th, and 12th week of gestation respectively, and in which CMV reactivation was ruled out from serological evaluation of the pregnant women at admission, versus equal controls after voluntary abortion following well-documented maternal viral recurrence. In addition, a panel of monoclonal antibodies for the identification of leukocytes (CD45/LCA), B-lymphocytes (CD20/L-26), and T-lymphocytes (CD45RO/UCHL1), was performed. All women received hormonal medication to support gestation, in the cases of spontaneous abortions. Results: Immunohistochemical examination using a specific antibody against cytomegalovirus showed large multinucleated infected cells with intranuclear inclusions, located primarily in the decidual stroma within a lymphoplasmacytic infiltrate in the cases of spontaneous abortions. No evidence of infection was observed in the chorionic villi. In the cases of voluntary abortions same findings were observed in the relevant areas, and a strong evidence of infection was observed in the chorionic villi. Conclusion: This study demonstrates 1) that the decidual endometrial stromal cells can express the CMV-associated antigen prior to serological manifestation of the viral replication, 2) the expression of the antigen is higher in cases of hormonal administration to support gestation. In these cases a mild mononuclear infiltrate of UCHL1 (T marker) positive cells, accompanies the CMV-associated antigen positive cells.

Key words: *CMV-associated antigen; Human decidual cells; Estradiol; Immunohistochemistry*

Introduction

Human cytomegalovirus (CMV) infection, which usually has a benign course in immunocompetent individuals, can have catastrophic consequences during pregnancy (6). Primary CMV infection during gestation poses a 30 to 40% risk of intrauterine transmission and clinical disease (36,37). Reactivated infection is associated with at least a 10-fold-lower rate of transmission. Congenital CMV infection is a relatively common occurrence, as approximately 1 to 4% of newborns in the United States and Europe are infected with CMV (6), and transmission could be higher in developing countries (9). Many infected infants show no clinical manifestations of the congenital CMV syndrome. Symptomatic infants often succumb in the neonatal period (12%), and most survivors have permanent debilitating sequelae, including mental retardation, vision loss, and sensorineural deafness. Since CMV establishes latent infections in granulocyte-dendritic progenitors (17,24,35), the fetus may also become infected after reactivation of maternal infection, a scenario that is usually associated with less severe clinical disease in the offspring (14,36). CMV seroconversion rates and restriction endonuclease analyses of virus strains indicate that heterosexual activity (7,8,13,19) and contact with young children (21,29) are the major modes of virus dissemination in women of childbearing age.

Despite the morbidity and mortality associated with prenatal CMV infection, little is known about how the virus infects the conceptus. Approximately 15% of women with primary infections during early pregnancy abort spontaneously (16). In this case the placenta, but not the fetus, shows evidence of infection, which suggests that placental

involvement is important in its own right and precedes virus transmission to the fetus (4,20,26). Later in pregnancy CMV infection causes premature delivery and, in 25% of affected infants, intrauterine growth retardation (22), outcomes that are often associated with placental pathology. Numerous reports indicate that placentas from these births also contain viral proteins (26,27), suggesting that placental infection and virus transmission to the infant are related causally.

To the best of our knowledge no previous report exists on Medline database up to now, concerning CMV antigen expression by human endometrial decidual cells.

In our series we focused on CMV signs of infection in cases of spontaneous abortions with no maternal re-involvement, compared to controls with well-established CMV affection of the pregnant women. We concluded that decidual stromal endometrial cells express the CMV-associated antigen prior to the serological manifestation of the virus. Our results are in harmony with what we know about the virus, that is, placental involvement precedes fetal involvement (12). We also confirmed that the increase in CMV infection observed in pregnancy is caused by reactivation of the virus induced by hormones (23).

Materials and methods

Samples representing 15 placentas from fetuses after spontaneous (involuntary) abortion occurring in pregnant women once infected by CMV but with no serological evidence of recurrence at admission, administrated with estradiol (300–600 mg per os until the 12th gestational week), and 15 placentas from fetuses after voluntary abortion with well-documented CMV maternal affection, were obtained at 8th, 10th and 12th week of gestation. Placentas were cut as thick as 3mm, then fixed in 10% neutral buffered formaldehyde at 4^{o} C for 24 hours and processed for routine paraffin embedding. Paraffin blocks were available in all cases, and three-μm thick tissue sections were stained routinely with hematoxylin – eosin, PAS and Giemsa, and subsequently, using immunohistochemistry. Microscopically, a diagnosis of CMV infection was made on the presence of cytomegalic inclusion bodies. Immunoperoxidase method was performed as follows: sections were deparaffinized in 70% alcohol and endogenous peroxidase was blocked with 3% H_2O_2 in methanol. Sections were preincubated in 20% serum of the species from which the secondary antibody was raised and the primary antibody was applied. After overnight incubation at room temperature, the secondary biotinylated antibody was applied for 30 minutes. Staining was visualized using the Vector Elite System (Vector Laboratories, Burlingame, CA) with diaminobenzidine as the chromogen. Sections were counterstained in dilute hematoxylin. The primary antibodies used were as follows: cytomegalovirus (CMV), mouse monoclonal antibody (Dako), (CD45/LCA) leukocyte common antigen, mouse monoclonal antibody (Dako), (CD20/L-26) B-lymphocytes, mouse monoclonal antibody (Dako), and (CD45RO/UCHL1) T-lymphocytes, mouse monoclonal antibody (Dako).

Analysis of CMV positive decidual cells: For each sample, the CMV positive decidual population was assessed by enumeration of labeled cells in each tissue compartment for a minimum of five random fields per section viewed at 40-fold magnification through a grid. Cell number was calculated per 1 mm^2 of tissue section. The counted areas were selected from random placental tissue sections, taking into account that the ratio of the area of the decidual stroma according to the area of the chorionic villi was representative of the entire field. Areas with obvious necrosis or hemorrhages were excluded. Statistical analysis was undertaken using the ANOVA test.

Results

Five microscopic fields of the placentas were evaluated in each case without knowledge of the clinical data. The sections were examined independently by two observers, and positive cellular staining for each antibody was manifested as fine red cytoplasmic granularity and/or surface membrane expression.

8th week of gestation: The immunohistochemical study of the placentas, during this period for the detection of CMV positive cells, in cases of spontaneous abortions, showed small clusters or scattered, large-sized CMV positive decidual cells in all settings examined (Fig 1, 2), with percentages varying from 3.2 to 3.9 (mean values, 3.61 ± 0.16). In the neighboring decidual stroma a slight cell infiltration was observed, consisting of rounded mononuclear cells of approximately 10μm in diameter with an eccentric kidney-shaped nucleus and expressing a CD45/LCA and CD45RO/UCHL1 phenotype. In one of our cases a dense polymorphonuclear infiltrate was observed, surrounding large-sized cells with an intense nuclear membrane positivity to the CMV antigen (fig 3). The microscopic examination (H-E, and PAS) of these cells showed an abundant pale cytoplasm, a nucleus with marginated chromatin, and a prominent inclusion-like eosinophilic nucleolus. The immunohistochemical study of the placentas, in the cases of voluntary abortions, showed a smaller number of large-sized CMV positive decidual cells in all settings examined, with percentages varying from 3.1 to 3.7 (mean values, 3.42 ± 0.17). No inflammatory infiltrates or necrosis were noted in the neighboring decidual stroma.

10th week of gestation: During this period, in cases of spontaneous abortions, the immunohistochemical examination for the identification of CMV decidual cells, showed a higher number of positive cells, in comparison with those found at 8th week of gestation, with percentages varying from 4.9 to 5.6 (mean values, 5.27 ± 0.19). The number of inflammatory infiltrates in the decidual stroma, expressing the phenotype CD45/LCA and CD45RO/UCHL1 was minimal. The immunohistochemical study of the placentas, in the cases of voluntary abortions, showed a relatively equal

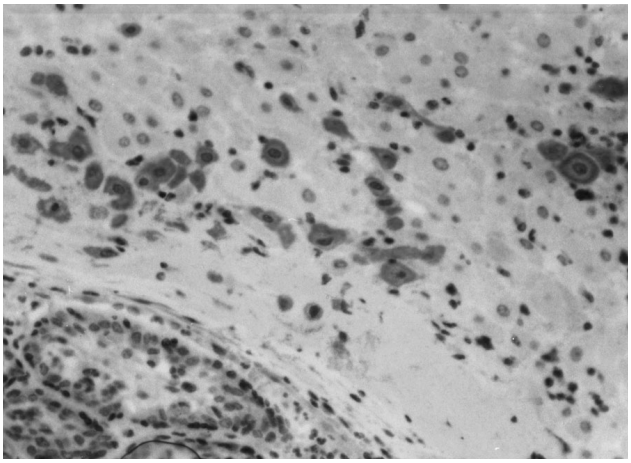

Fig. 1: Aggregates of large-sized CMV positive decidual cells. Immunostaining with CMV antibody, APAAP technique, magnification x 200.

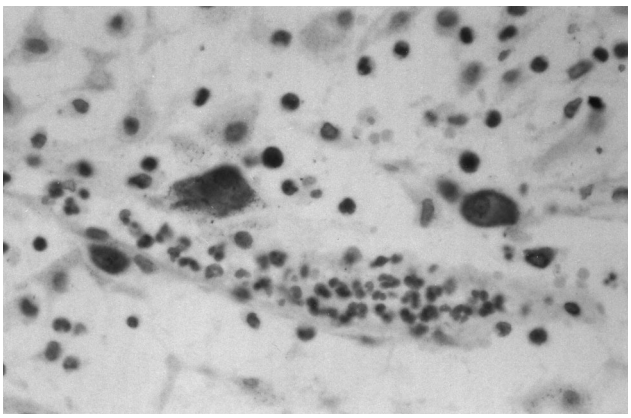

Fig. 3: CMV positive decidual cells against a background of polymorphonuclear infiltrate. Immunostaining with CMV antibody, APAAP technique, magnification x 400.

number of large-sized CMV positive decidual cells, in comparison with those found at 8th week of gestation, with percentages varying from 3.2 to 3.9 (mean values, 3.43 ± 0.18). No inflammatory infiltrates or necrosis were noted in the neighboring decidual stroma.

12th week of gestation: During this time, an even higher number of CMV positive decidual cells was found, compared to that at 10th week, with percentages varying from 4.8 to 5.7 (mean values, 5.34 ± 0.23). Respectively, the number of CMV positive decidual cells in the cases of voluntary abortions, was more or less the same as that at 8th and 10th weeks, with percentages varying from 3.2 to 3.7 (mean values, 3.41 ± 0.17). No differences in the immune reactions were noted in the neighboring decidual stroma in cases of spontaneous abortions as well as in cases of voluntary ones, in comparison to 8th and 10th gestational weeks.

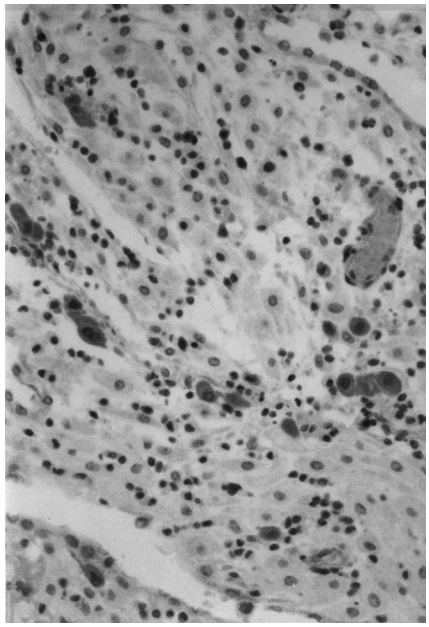

Fig. 2: Isolated large-sized CMV positive decidual cells. Immunostaining with CMV antibody, APAAP technique, magnification x 200.

A statistically significant difference was found between CMV positive cells at 8th, 10th, and 12th gestational week after spontaneous abortions ($p<0.0001$). No significant difference was observed between CMV positive cells at 8th, 10th, and 12th gestational week after voluntary abortions ($p=0.95$).

Discussion

Approximately 15% of all clinically recognized human pregnancies end in early spontaneous abortion (SAB) (11). Infectious disease is a significant problem in maternal health. An estimated 5% of maternal deaths reported to the Centers for Disease Control and Prevention are associated with recurrent spontaneous abortion (5). Of these deaths, approximately one half are caused by infection (32). Bacterial, viral, parasitic, fungal, and zoonotic infections have all been associated with recurrent abortion. The association of infection with recurrent abortion is among the most controversial and poorly explored of potential etiologies for reproductive loss.

Embryo is a semi-allograft of a different nature, considering that it contains proteins coming from the father, and unfamiliar to the mother. There is a common sense, that circulating blocking factors protect embryos from maternal lymphocytes which may cause miscarriage due to reaction against paternal antigens. Such factors can be progesterone and chorionic gonadotropin; their levels in blood serum are low, in cases of habitual abortions (11,18). Mixed lymphocyte culture has been applied to detect circulating blocking

factors. However, the above hypothesis was not confirmed by well-developed perspective studies. In not miscarrying women no circulating blocking factors were detected in serum, to slow down mixed lymphocyte culture. Recently, it was implied, that mixed lymphocyte culture results are representative of habitual abortion consequences rather than the causes of their appearance.

Clinical observations indicate that subclinical infection with CMV and genital CMV excretions are most common during pregnancy (10,25,38,41). The increase in productive CMV infection observed during pregnancy can be caused by increased initial acquisition or by progressive reactivation of latent virus due to an altered immune state or increase in one or more pregnancy hormones.

The latency of human CMV has yet to be demonstrated experimentally. Except for one initial report of studies based on a small series of patients (10), it has not been possible to activate CMV from the leukocytes of large numbers of blood donors who were seropositive for antibodies to CMV (2). The state of the viral genome and the cells in which the virus is present during latency is unknown. Some studies suggest that there may be a low level of replication that is not detected by standard techniques (1,34). Other data favor a nonreplicating state (31,39,40).

The mechanism by which estradiol cares modulate the state of CMV in endometrial cells is unknown. Zerbini et al. (42) have demonstrated that in human embryo tissue culture acidic medium inhibits the production of CMV viral progeny, allowing only the expression of the immediate early and early antigens of the virus (3). It is possible that hormones enable the expression of the late viral genome functions, i.e., late proteins which are inhibited by the low pH. There are other systems in which steroid hormones play a role in modulation of viral replication. Murine mammary tumor virus expression is regulated in vitro by glucocorticoids (33), probably through an interaction of the glucocorticoid receptor complex with a sequence within the long-terminal repeat (15,30). In the case of HSV-2, the addition of hydrocortisone to Raji cells persistently infected with the virus caused a marked increase in virus production (28).

Our results 1) indicate that CMV antigen investigation in human decidual cells can be applied as a routine process for the determination of viral recurrence, and 2) confirm that CMV expression in decidual endometrial stromal cells is induced by estradiol control. Studies of CMV expression in decidual cells would provide new insight into its biological functions.

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