

CLINICAL RESEARCH

Circulating Immune Complexes in Pregnancy Complicated by Chronic Cytomegalovirus Infection

Michael T. Lucenko, PhD, ScD*; Irina A. Andrievskaya, PhD, ScD

*Far Eastern Scientific Center of Physiology and Pathology of Respiration,
Siberian Branch of Russian Academy of Medical Sciences
Blagoveshchensk, Russian Federation*

Abstract

Chronic cytomegalovirus (CMV) infection in pregnant women is accompanied by the accumulation of large amounts of the circulating immune complexes (CIC) in the peripheral blood. On reaching the placental villous syncytiotrophoblast, the CIC induce a concentration of lysosomes of the syncytiotrophoblast cytosol on the outer membrane of the fetoplacental barrier. Contact between the circulating CIC and the lysosomes on the outer syncytiotrophoblast membrane triggers the release and expression, on its surface, of acidic phosphomonoesterase, which leads to lipid peroxidation and damage to the outer syncytiotrophoblast membrane itself.

Keywords: *chronic cytomegalovirus infection, pregnancy, lysosomes, syncytiotrophoblast, circulating immune complexes.*

Introduction

The CIC formation is a normal immune response phenomenon. Antibodies are produced in the organism for the neutralization and elimination of the antigens; however, a small amount of CIC possessing different structural and biological properties is constantly present in the blood [1].

In the peripheral blood of healthy pregnant women, generally, a slight increase in the CIC level is observed; however, this level dramatically increases in different diseases [2]. Therefore, an increased CIC level was observed in the peripheral blood of pregnant women with exacerbation of the herpes viral infection [3,4], which was accompanied by damage to the fetoplacental barrier following the penetration of CIC into the syncytiotrophoblast cytosol [5].

The aim of our study was to identify the mechanisms causing the damage to the placental syncytiotrophoblast during activation of the CMV infection.

Material and Methods

In all, 75 pregnant women between 18 and 35 years of age, during the I, II, and III trimesters of gestation with

exacerbation of the chronic CMV infection and into the period of convalescence (study group) and 30 healthy pregnant women (control group) were examined. Investigations were conducted in the Clinic Hospital's Maternity Ward of the Far Eastern Scientific Center of Physiology and Pathology of Respiration SB RAMS.

CMV infection was diagnosed in a comprehensive study of the peripheral blood to check for the presence of IgM or a four-fold or more increase in the IgG antibody titer in paired serum in the dynamics after 10 days, an avidity index reading of more than 50%, and the presence of CMV DNA. Simultaneously, a morphological study of the placentas of the same pregnant women was performed after childbirth.

The studies were conducted in line with the requirements of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" update 2000 and the Rules of Clinical Practice in the Russian Federation, approved by Order #266 of the Ministry of the Russian Federation of June 19, 2003.

The verification of CMV, the definition of type-specific antibody, and the avidity index were determined by ELISA on the spectrophotometer "Stat Fax 2100" (USA) using the sets of CC "Vector-Best" (Novosibirsk) and by PCR on the machine DT-96 using sets of "DNA-Technology" (Moscow).

The CIC were detected spectrophotometrically using 35% polyethylene glycol. Pieces of placenta were fixed in 10% neutral-buffered formalin and embedded in paraffin. The presence of the lysosomes in the syncytiotrophoblast was revealed by staining with Fast Green FCF, according to V.E. Pigarevsky [6]. Only 1-2 micron thick sections were used. Reaction to acid phosphatase

*Corresponding author: Prof. Michael T. Lucenko, PhD, ScD, Academician of RAMS, Head of Far Eastern Scientific Center of Physiology and Pathology of Respiration SB of RAMS, 95A, Gorky st., 675000, Blagoveshchensk, Russian Federation.
Phone: 8 -416-2-52-2922. E-mail: Lucencomt@mail.ru

was histochemically performed, according to Gomori, at pH 6.2 [7]. The histochemical reaction to determine the lipid peroxidation activity was performed according to Winkler-Schulze [7]. Lysosome density was determined using the BioVision software (Version-2, WestMedica GmbH, Pixera – model PVC 100C; USA).

Specialized software was used for the automated processing of medical data (copyright holder - the Far Eastern Scientific Center of Physiology and Pathology of Respiration SB RAMS, Blagoveshchensk). For data with normal distribution, inter-group comparisons were performed using Student's t-test and F-test. The Mann-Whitney (U-test) was used to compare the differences between the two independent groups (for nonparametric data). *P* value less than 0.05 was considered significant.

Results

An “antigen-antibody” complex is formed with moderate excess of the antigen. Under physiological conditions, the immune complexes are rapidly phagocytosed and, almost always, eliminated by the liver. In chronic infections the CIC level significantly increases and creates the optimal conditions for the activation of the inflammatory process.

Our study has shown that with the increase in the IgG antibody titer in response to the CMV, the CIC content in the peripheral blood also progressively increases. Thus, by the end of the pregnancy, the CIC levels were as follows: 0.172 ± 0.007 AU (antibody titer 1:200) and 0.193 ± 0.007 AU (antibody titer 1:400). If the IgG antibody titer reached up to 1:1600, then the CIC level in the peripheral blood of pregnant women in the third trimester increased up to 0.218 ± 0.009 AU (Table 1).

Table 1.

CIC levels (AU) in the peripheral blood of pregnant women with exacerbation of the chronic CMV.

Gestational age	IgG antibody titer		
	1:200	1:400	1:1600
First trimester			
CMV infection	$0.160 \pm 0.006^*$	$0.189 \pm 0.007^*$	$0.205 \pm 0.005^*$
Control	0.079 ± 0.004	0.079 ± 0.005	0.079 ± 0.004
Second trimester			
CMV infection	$0.169 \pm 0.009^*$	$0.180 \pm 0.006^*$	$0.191 \pm 0.004^*$
Control	0.085 ± 0.005	0.085 ± 0.005	0.085 ± 0.005
Third trimester			
CMV infection	$0.172 \pm 0.007^*$	$0.193 \pm 0.007^*$	$0.218 \pm 0.009^*$
Control	0.098 ± 0.003	0.098 ± 0.003	0.098 ± 0.003

**P* < 0.05 vs control

Under such circumstances, damage to the fetoplacental barrier was noted. Expression of the CIC on the outer syncytiotrophoblast membrane led to serious violations of the morpho-functional state of the part of the syncytiotrophoblast, which was in direct contact with the lacunar blood. Aggregation of a large number of lysosomes was observed on the inner syncytiotrophoblast membrane (Fig. 1a, b). This explains the emergence of a high acid phosphatase activity at pH 6.2 (Fig. 2).

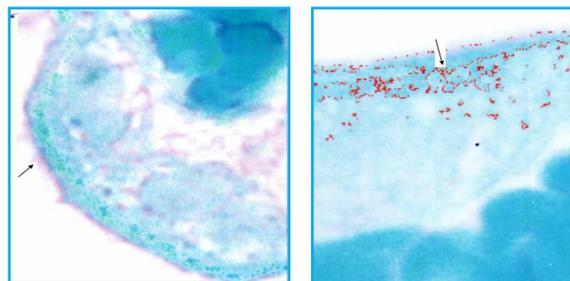


Figure 1.

Placental villous syncytiotrophoblast of pregnant woman underwent the exacerbation of the chronic CMV infection at the third trimester of gestation: (a) IgG antibody titer – 1:400; (b) IgG antibody titer – 1:800. Lysosome clusters (arrow) on the inner syncytiotrophoblast membrane. Fast Green staining. Computer processing done using the BioVision program. ($\times 1500$).

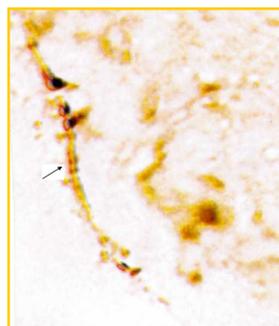


Figure 2.

Placental villous syncytiotrophoblast of pregnant woman underwent the exacerbation of the chronic CMV infection at the third trimester of gestation (IgG antibody titer – 1:1600). High acid phosphatase activity (pH 6.2) in the area of the lysosome clusters (arrow). The reaction according to Gomori ($\times 1000$).

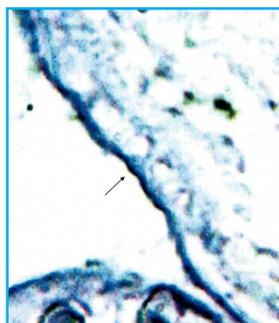


Figure 3.

Placental villous syncytiotrophoblast of pregnant woman underwent the exacerbation of the chronic CMV infection at the third trimester of gestation (IgG antibody titer – 1:1600). The intensive reaction on the peroxide fatty acids on the outer syncytiotrophoblast membrane (arrow). The reaction according to Winkler-Schulze ($\times 1000$).

Moreover, on the outer syncytiotrophoblast membrane, an intensive reaction on the peroxide fatty acids was observed that had been manifested as an increase in the permeability of the outer membrane (Fig. 3). Thus, the distance between the pores was found to increase up to 1.0 nm, which under normal circumstances did not exceed 0.5 nm.

The initial stage of the histochemical reaction is known to be characterized by penetration of both the substrate and the capture agent into the tissue [8]; however, in this case, other conditions were noted for this process. In the first stage, the substrate gets separated from the capture agent (lysosomes). The CIC induce an increase in membrane permeability, which, in turn, facilitates their capture by the lysosomes.

According to our research [8], the high titer of the IgG antibodies to CMV (1:1600) was accompanied by an increase in the size of the pores of the outer syncytiotrophoblast membrane from 0.5 nm to 1.0 nm and more. This created the conditions under which contact between the CIC and lysosomes was realized, by the advent of the high active hydrolytic reaction to acid phosphatase at pH 6.2 (Fig. 2).

Conclusion

Thus, an exacerbation of the CMV infection during pregnancy becomes a strong damaging factor for the fetoplacental barrier. The CIC attached on the syncytiotrophoblast (on the side of the lacunar blood) cause the accumulation of a large number of lysosomes on the inner portion of the membrane. This condition facilitates high acid phosphatase activity, an increase in the activity of the hydrolytic processes in the membrane and development of the lipid peroxidation reaction. The syncytiotrophoblast membrane thus becomes more permeable to the coarser structures.

References

1. Dolgykh VT. Fundamentals of immunopathology. Nizhny Novgorod, 1998. [Book in Russian].
2. Gabekova KA. The role of fixed placental immune complexes in the pathogenesis of preeclampsia. Abstract of PhD Thesis. St. Petersburg, 1999. [Abstract in Russian].
3. Solovieva AS. Cellular and humoral immune indices in pregnant patients with herpes virus infection. *Bull Physiol Pathol Resp* 2005; 20:7-11. [Article in Russian].
4. Solovieva AS, Lucenko MT. Circulating immune complexes in pregnant women under herpes virus infection. *Far Eastern Med J* 2008; 1:54-56. [Article in Russian].

5. Lucenko MT. Morphofunctional characteristics of the fetoplacental barrier in herpes virus infection. *Bulletin of the Far Eastern Branch of RAS* 2004; 3:155-166. [Article in Russian].

6. Pigarevsky VE. Granular leukocytes and their properties. M.: "Medicine"; 1978. [Book in Russian].

7. Pierce E. Histochemistry. Translated from English into Russian. M: 1962.

8. Lucenko MT. A method for evaluating the permeability of the placental villous syncytiotrophoblast membrane using a metachromasia in obstetric patients undergoing third-trimester exacerbation of herpes virus infection with antibody titers 1:12800. Patent of the Russian Federation, # 2463604; 10.10.2012.