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Short Communication

Immunological Mechanisms of Trophoblast Invasion and Placental Development Violation at the Cytomegalovirus Infection

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- Chronic cytomegalovirus infection
- Trophoblast invasion
- Humoral and cell-mediated immunity
- Threat of abortion

Abstract

Objective: To establish immunological patterns of trophoblast invasion and placental development violation during chronic CMV-infection exacerbation at 7-8 weeks of gestation.

Methods: A total of 30 pregnant women at 7-8 weeks of gestation were examined, including 15 CMV-seropositive with CMV-infection exacerbation and 15 CMV-seronegative. All investigations were carried out at a single Institution (Far Eastern Scientific Center of Physiology and Pathology of Respiration (FESC PPR)) during one year time. Non-specific immunoglobulins (IG) of M and G class, high-avid antibodies of G class to CMV, interleukin (IL) 4, interferon γ (IFNγ), tumor necrosis factor α (TNFα) were determined via enzyme-linked immunosorbent assay, circulatory immune complexes (CIC) - by spectrophotometric method. Gangliosides in blood smears were revealed via histochemical method in accordance with Bruckner, herpetic bodies - by M.T. Lucenko method. The intensity of histochemical response was evaluated using digital microscope Meiji Techno according to a Scion program. Lymphocyte expression on the surface area of villous chorion was studied by means of electron microscopy.

Results: During the exacerbation of chronic cytomegalovirus infection at 7-8 weeks of gestation established were reliable 2.2-fold increase of non-specific IgM ($p < 0,001$), 2,6-fold increase of CIC ($p < 0.001$), the increase in the activity of reaction products to gangliosides in macrophage membranes and in herpetic bodies content in blood. TNFα content increased in blood -6,4-fold ($p < 0,001$), in chorion homogenate - 10,0-fold ($p < 0,001$), indices for IFNγ increased in blood - 3,6-fold ($p < 0,001$), in chorion homogenate - 5,0-fold ($p < 0,001$). IL-4 content decreased in blood -1,3-fold ($p < 0,001$), in chorion homogenate -1,4-fold ($p < 0,001$).

Conclusion: Chronic CMV-infection exacerbation at 7-8 weeks of gestation causes the violation of system and local intercellular interrelations between B- and T-cells, leads to antibody production imbalance, increased CIC and herpetic bodies formation, Th1- type immune response development, local inflammation in uteroplacental zone what becomes a cause of violation of trophoblast invasion and uteroplacental circulation formation and of threatened miscarriage.

INTRODUCTION

As is generally known, trophoblast invasion and placentation are under the immunological control of pregnant woman organism. Herewith, the control over the balance maintenance between the anti- and angiogenic factors as well as pro- and antiapoptotic factors in placental tissue is provided by the immune system cells indirectly through various cytokine activation [1] that determines the development of placenta. An important factor, ensuring the resistance to the developing embryo during its invasion, is the restriction of decidual cell cytolytic and apoptogenic activity through equivalent interaction of T-cell link and Th-2 type immune response formation [2].

However, there is a wide range of pregnancy complications that are manifested at early stages as the violation of blastocyst implantation and spontaneous abortion [3]. They may be caused by the infectious-inflammatory diseases of women including those caused by the exacerbation of chronic cytomegalovirus infection (CMV) [4,5]. It should also be noted that the forms of pregnancy complications will be defined both by immune response focus and recurrence period.

The research of placental morphogenesis in the aspect of relations with indices of humoral and cell mediated immunity both at the system and local level will enable to prove possible causes of trophoblast invasion and placentation violations for early diagnosis and threatened miscarriage prevention in women with the exacerbation of chronic CMV- infection at 7-8 weeks of gestation.

METHODS

Design research

It was conducted a prospective case-control study.

Compliance criteria

Study inclusion criteria: Inclusion criteria were exacerbation of CMV-infection at 7-8 weeks of gestation with the clinical features of acute respiratory viral infection, persistent clinical remission of herpesvirus infection.

Study exclusion criteria: Exclusion criteria were primary CMV-infection, exacerbation of other inflammatory diseases of extragenital pathology, presence of sexually transmitted infections.

The primary CMV-infection was clinically diagnosed by presence in peripheral blood of class M antibodies to CMV (immunoglobulin, Ig), low-avidIgG (avidity index < 65%), as well as DNA-CMV detected by Polymerase Chain Reaction (PCR) technique in blood or urine; chronic CMV-infection exacerbation was diagnosed by presence of IgM to CMV , high-avid IgG (avidity index > 65%), as well as DNA-CMV in buccal epithelium scrapings and in the neck of the womb mucosa.

Conditions for the research

All studies (clinical, biochemical, histochemical, electron microscope) were conducted at a single institution (FESC PPR, Blagoveshchensk). No specific factors that might affect the external generalizability of conclusions are detected.

Research outcomes

As a primary evaluated index considered were the amount of circulatory immune complexes (CIC), their expression on the surface area of the trophoblast cells, indices of humoral (immunoglobulines M and G) and cell-mediated immunity (TNFα, IL-1β,TFNγ, IL-4) both in blood and in villous chorion homogenate.

In addition, the clinical signs of threatened miscarriage were evaluated.

Research duration

Material sampling lasted in the course of a year. Samples of blood serum, urine, buccal epithelium and of the cervical canal content were investigated simultaneously following biomaterial sampling. Villous chorion was collected immediately after medical abortion.

Description of medical intervention

Blood samples were drawn out of ulnar vein at 7-8 weeks to perform PCR, enzyme-linked immunosorbent assay and histochemical analysis as well. Samples of urine, buccal epithelium and cervical canal content were collected for PCR-analysis. Villous chorion was taken for morphological investigations.

Methods for outcomes registration

To perform PCR blood samples from the patients under survey were drawn into standard vacuum test-tubes with coagulant in amount of 5 ml. Blood samples not containing coagulant were used for serologic tests. The extraction of mononuclear blood cells for PCR was performed using fikkoll-urograffin solution, density 1,077 g/ml (NPO "DNA-technology", Russia). Serologic tests were conducted in paired sera with 10-14 days interval. Early shift of urine for PCR-analysis was taken into the sterile container of 60 ml volume. Samples of buccal epithelium and of cervical canal content were collected into standard plastic test-tubes containing 0,5 ml of saline solution, using sterile cotton swab.

For homogenate preparation villous chorion was taken within 10-15 minutes after medical abortion and processed according to the method [6]. The supernatant liquid was dispensed into small aliquots and stored at -20°C prior to the IFA.

Type-specific antibodies to CMV and HSV-1,2of class M and G(immunoglobulin, Ig), non-specific IgM and IgG, high-avid antibodies to CMV of G-class (avidity), interleukin (IL) 4, interferon γ (IFNγ), tumor necrosis factor (TNFα) were determined by means of standard test-systems for enzyme-linked immunosorbent assay (CJSC "Vector-Best", Russia). CIC were detected by spectrophotometric method using polyethylenglycol (3,5%) [7].

Gangliosides in blood smears were evaluated by Bruckner method.

Blood smears were studied using the computer connected digital microscope Meiji Techno (Japan) according to a Scion program (USA).

For electronic microscopy samples of villous chorion were fixed in 2,5% glutaraldehyde in Cacodylate buffer (0,1M) and embedded in araldit-Epon-812.

The ethical review

The whole study was conducted with regard to the requirements of World Medical Association Declaration of Helsinki (2008) and the Rules of Clinical Practice in the Russian Federation adopted by Order of the Ministry of Health of the Russian Federation dated June 19, 2003 N266. The study was conducted within the framework of the SRW 059 and can be considered not contrary to the fundamentals of medical ethics. No further recommendations were given by the Ethics Commission at the FSFSI "Far Eastern Scientific Center of Physiology and Pathology of Respiration". The written informed consent was obtained from all pregnant women.

Statistical methods

Statistical analysis and processing of obtained data were performed using comprehensive statistical software package v.6.0 developed by Statistica (StatSoft Inc., USA). Non-paired parametric Student's T-test was used to determine the difference reliability. Nonparametric Kolmogorov - Smirnov and Mann-Whitney criteria were used to determine the difference reliability in case of non-Gauss distributions. The critical level of significance was considered to be 5% ($p < 0,05$). The obtained data are presented as arithmetic mean and standard error of arithmetic mean (M±m).

RESULTS

Survey participants

The main group consisted of 15 CMV-seropositive women at 7-8 weeks of gestation with the exacerbation of chronic CMV-infection. Control group included 15 CMV-seronegative women at the same stage of pregnancy. Mean age of pregnant women in the main group was 22,8±0,3 years and there was no statistically significant difference regarding mean age of pregnant women in control group - 23,9±0,4 years (p >0,05).

Key findings of the research

In the course of the study it was ascertained the alteration in indices of the system nonspecific humoral immune response in CMV-seropositive pregnant women in the main group that manifested in 2,2-fold increase of total IgM (p <0,001) and 1,4-fold decrease of total IgG (p <0,001) compared to the control group (Table 1). The revealed changes in levels of nonspecific immunoglobulins of M and G class were consistent with the 2,6-fold (p <0,001) increase of CIC content in blood as opposed to the control group.

Electron microscopic investigation of villous chorion from women with the exacerbation of CMV-infection at 7-8 weeks of gestation has shown the elevated expression of immune complexes on the surface area of trophoblast cell (Figure 1A) that is presented as lymphocyte groups with the products of active CMV-replication (Figure 1B).

At the same time it was detected an increased intensity of histochemical response to gangliosides in macrophage membrane which was observed in blood smears of CMV-seropositive pregnant women in the main group (Figure 1A). Frequently in blood smears of such a type it was observed the fusion of negatively charged macrophages into conglomerates, so called herpetic bodies (Figure 1B) what also increased the chance of infection and early inflammatory response development, violating processes of trophoblast invasion.

On studying cytokine profile of peripheric blood and villous chorion from pregnant women detected were the following patterns which are represented in Table (2). Compared to the control group, in CMV-seropositive pregnant women of the main group the increase of proinflammatory cytokines of Th-1 type in blood took place: TNFα - 6,4-fold (p <0,001), THFγ -3,6-fold (p <0,001). With that the regulatory IL-4 level was 1,3-fold lower (p <0,001) than the analogous index in the control group.

Integral calculation of the ratio between the indices for cytokines of Th-1 and Th-2 type has shown that the difference between TNFα and IL-4 was higher than that between IFNγ and IL-4 - 8,7-fold (p <0,001) and 4,8-fold (p <0,001), respectively, what was indicative of the primary role of TNFα in the development of early inflammatory processes in uteroplacental zone during the exacerbation of chronic CMV-infection at 7-8 weeks of gestation.

With that it should be noted that cytokine profile of villous chorion homogenate from CMV-seropositive pregnant women in the main group was characterized by the unidirectional alterations in pro- and anti-inflammatory markers content but they were more expressed as opposed to the system immune response indices (Table 2). As compared to the control group indices for THFα increased 10,0-fold (p <0,001) and for IFNγ - 5,0-fold (p <0,001). At that time IL-4 values were reliably1,4-fold lower (p <0,01) compared to the values of the control group.

On subsequent integral calculating, TNFα/IL-4 ratio in the main group increased 15,5-fold (p <0,001) and IFNγ/IL-4 - 7,5-fold (p <0,001) as compared to the control group what was indicative of the initiation of local inflammatory response, altering the processes of trophoblast invasion and placental development.

Table 1: Indices of humoral immune response in women with chronic CMV-infection exacerbation at 7-8 weeks of gestation.

Indices	main group	control group
IgMtotal,mg/ml	2,70±0,41*	1,22±0,06
IgGtotal,mg/ml	14,00±0,41*	10,10±0,09
CIC, ODU	0,21±0,02*	0,08±0,02

Note.*- difference reliability compared to the controls at p<0,001

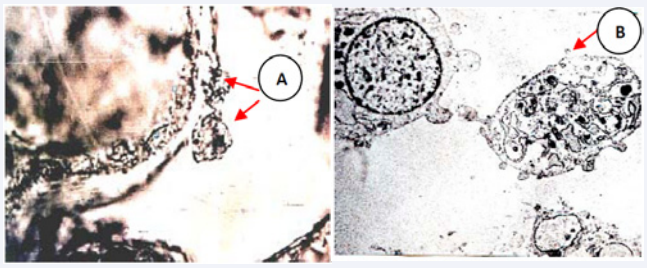

Figure 1 Villous chorion at 7-8 weeks of gestation. Chronic cytomegalovirus infection during exacerbation at the gestation of 7-8 weeks. Electron microscopy. A: lymphocyte expression on the surface area of trophoblast. Magnification 7000x. B: lymphocytes with the products of active CMV-replication. Magnification 20000x.

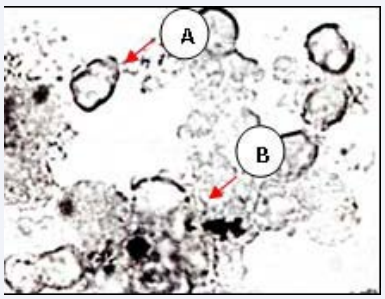

Figure 2 Peripheric blood. Chronic cytomegalovirus infection during exacerbation at the gestation of 7-8 weeks. A. Active reaction to gangliosides in macrophage membrane. B: macrophage fusion and herpetic bodies forming. Histochemical test by Bruckner. Magnification 10 x 100.

Additional research results

At the time of the study all pregnant women in the main group had clinical signs of threatened miscarriage manifesting as stretching cramping and pain in lower abdomen and bloody discharge. In all patients under survey medical abortion was

Table 2: Indices of system and local cell-mediated immune response in women with chronic cytomegalovirus infection during exacerbation at 7-8 weeks of gestation.

Indices	Main group		Control group	
	blood	chorion	blood	chorion
TNFα,pg/ml	95,23±1,11*	101,30±3,24*	14,86±0,16	9,40±0,77
IFNγ,pg/ml	420,30±7,11*	357,21±5,33*	117,13±0,31	68,62±2,23
IL-4,pg/ml	15,50±0,71*	9,22±0,62*	20,50±0,10	13,22±0,73
TNFα/IL-4	6,10±0,08*	10,98±0,10*	0,70±0,10	0,71±0,08
IFNγ/IL-4	27,10±0,10*	38,74±0,12*	5,70±0,21	5,19±0,09

Note.*- difference reliability compared to the controls at p<0,001

performed at 7-8 weeks of gestation due to spontaneous abortion.

DISCUSSION

Abstract of the key findings of the research

In the course of the present study it was performed the analysis of the main indices for non-specific humoral and cell-mediated immunity both at the system (blood from pregnant women) and local (villous chorion) levels during the exacerbation of chronic CMV-infection at 7-8 weeks of gestation. Defined is the role of CMV-infection in the forming of antigen- and cytokine mediated damages to utero-placental zone that determines the trophoblast invasion violation, uteroplacental circulation formation and the development of threat of abortion.

Discussion of the key findings of the research

The study of nonspecific humoral and cell-mediated immune response indicators during the exacerbation of chronic CMV-infection at 7-8 weeks of gestation was chosen in view of known influence of CIC and certain cytokines on the trophoblast migration activity what defines its invasion and subsequent setting of uteroplacental circulation [8,9].

As for CIC produced with the participation of nonspecific immunoglobulins of M and G class there are data about their pathogenic effect on tissues and vascular walls [10] via the activation of proteolytic and oxidative processes what alters the surface properties and permeability of cellular membranes [11]. Considering the enhanced expression of CIC on the surface area of the trophoblast at the exacerbation of chronic CMV-infection one can speak about the development of its activity destabilizing processes, what also changes the ability to migrate. Similarly, one can estimate the noticeable increase in intensity of histochemical reaction to gangliosides in blood macrophage membrane that initiates processes of herpetic bodies formation [5] and their circulation in uteroplacental zone enhancing risk for infection and early inflammatory response development.

It is also to note that the enhancement of the activity of immunopathologic reactions, mediated by Th-1 type cytokines (TNFα, INFγ), induces the structural disarrangement in cellular elements of the trophoblast and vascular endothelium and becomes a cause of threatened miscarriage [12,13]. With regard to TNFα there are data that the increase in its production by placental macrophages above the norm may lead to thromboses and ischemic necroses in the structures of fetoplacental tissues [14] and incompetent secretion of cytokines having the restricting action against the inflammatory reactions, as it is presented in our study with IL-4, aggravates the heaviness of pathological condition.

At the same time it should be noted that the primary CMV-infection in pregnant women, as shown by recent studies [15-17], is followed by the intensified production of type-specific antibodies - immunoglobulins of M class, having embriotoxic effect, and low-avidity immunoglobulins of G class. Herewith, the risk of damage to placenta increases due to viremia and DNA-emia that alters the character of the local Th1/Th2 immune response, poses a threat of embryonic lesions and abortion.

Hence, the local immunosuppression must take place in placenta, ensuring the decrease of cytotoxic potential of immunocompetent cells as possible implementers of miscarriage, but it does not take place at the CMV-infection.

CONCLUSION

During the exacerbation of chronic CMV-infection at 7-8 weeks of gestation in primary (IgM) and secondary (IgG) immune response there form the alterations what causes the violation of systemic and local intercellular interrelations between B- and T-cells, leads to the imbalance in antibodies production, increased forming of CIC and herpetic bodies, development of Th-1 type immune response, infection and local inflammation in the uteroplacental zone. The latter violates the trophoblast invasion and uteroplacental circulation formation and leads to a threatened abortion.

SOURCE OF FINANCING

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