Morphofunctional Characteristics of Syncytiotrophoblast and Content of Heat Shock Protein 70 in It during Exacerbation of Herpesvirus Infection in Pregnant Women

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Exacerbation of herpesvirus infection during pregnancy is associated with damage to syncytiotrophoblast inflicted by herpesvirus either directly or via TNF-α (due to contact of NK lymphocytes with villus surface). A sharp decrease in the content of heat shock protein with a molecular weight of 70 Da and activation of caspase-3 were noted in placenta homogenate. This leads to disturbances in syncytiotrophoblast cytosol structure and increase in the relative content of apoptotic nuclei.

Key Words: *herpes; syncytiotrophoblast; heat shock protein with a molecular weight of 70 Da (HSP-70); caspase-3*

Heat shock proteins (HSP), a large group of proteins identified at the end of the last century [3-6], mediate organism's response not only to temperature rise, but also to other unfavorable factors, *e.g.* exposure to heavy metals, hormones, oxidants, *etc.* HSP participate in reparation and elimination of abnormally folded or denaturated proteins [1,7]. IL-1α or TNF-α acting on the cell can change HSP structure. Exposure to adverse factors increases the expression of HSP proteins with a molecular weight of 70 Da (HSP-70) protecting the cytosol from damage via prevention of aggregation of partially denaturated proteins [8]. HSP-70 protects cells from the action of lactate dehydrogenase, malate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase.

HSP participate in the regulation of apoptosis. The effect of HSP largely depends on the type of cells and apoptosis induction pathway.

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HSP are constantly synthesized and expressed in most cases at normal temperature. A question arises, whether HSP-70, an integral component of all cell compartments responsible for protein synthesis and enzyme activity, is sensitive to disturbances caused by not only IL and hormones, but also virus particles.

Here we studied the content of HSP-70 in placenta homogenate from women with G_1 herpesvirus infection. In parallel with measurement of HSP-70 content and caspase-3 activity, the structure of syncytiotrophoblast was analyzed at the ultramicroscopic level; particular attention was given to the structure of cytosol and nuclei.

MATERIALS AND METHODS

Placentas obtained after medical abortions (gestation weeks 7-8, 20 women) and after term delivery (gestation weeks 38-40, 30 women) were studied. Twenty pregnant women without herpesvirus infection served as the control. The placenta was obtained within 10-15 min after delivery. The study was performed in Obstetric Department, Hospital of Far-Eastern Research

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Center of Physiology and Pathology of Respiration, Siberian Division of the Russian Academy of Medical Sciences. The studies were performed according to the principles of the Declaration of Helsinki with Amendment (2000) and Regulations for Clinical Practice in the Russian Federation (Order of Ministry of Health Care No. 266, June 19, 2003). The placenta was washed with physiological saline (PS). Placenta fragments were dried on paper filters, weighed, and homogenized; the homogenate was diluted with PS (1 ml PS per 1 g tissue). The homogenate was frozen at -20°C for 24 h and then defrosted and centrifuged at 4000 rpm and 4°C. The supernatant was stored at -20°C in small aliquots until immunoenzyme assay.

The content of HSP-70 (HSP-70 ELISA Kits) and caspase-3 (Bender Medsystems) in the homogenate was measured. Type 1 herpes simplex virus infection was verified by the dynamics of IgG antibody titers in the peripheral blood using standard test systems (Vektor-Best). Analysis was performed on a Stat-Fax2100 microplate reader. The content of natural killers (NC) and HLA DR was analyzed on Coulter Epics XL-MCL flow cytofluorometer (Beckman). Electron microscopy was performed by standard methods with final embedding in araldite. Ultrathin sections were prepared on an LKB microtome and contrasted with uranyl acetate. The sections were examined and photographed using a Tesla microscope.

Apoptosis was detected morphologically (*in situ* end labeling, ISEL) on paraffin sections of the placenta [2]. The content of TNF-α was measured by ELISA using special kits (Citokin).

The data were processed statistically using Student *t* test.

RESULTS

During pregnancy complicated with herpesvirus infection, the major factors affecting the fetoplacental barrier of placental villi were antigen overload and production of TNF-α by immunocompetent cells. The content of TNF-α increased in both peripheral blood and placenta homogenate (Table 1). The content of this IL in the peripheral blood of pregnant women and in placenta homogenate attained maximum at the titer of antibodies to herpesvirus 1:12,800.

Activation of herpesvirus infection is accompanied by an increase in peripheral blood content of NK lymphocytes carrying HLA DR antigen on their surface. The count of NK lymphocytes increased with increasing the aggressiveness of herpesvirus infection (Table 2).

Electron microscopy of this material revealed sites of close contacts of granular lymphocytes located on the side of lacunae with syncytiotrophoblast surface. These lymphocytes had more compact nucleus (compared to lymphocytes not contacting with syncytiotrophoblast) and granular cytoplasm (Fig. 1, *a*, *b*). Spectrophotometry showed that the relative content of HSP-70 in placenta homogenate of these patients sharply decreased, while the content of caspase-3 increased (Table 3).

Electron microscopy of syncytiotrophoblast samples from patients with high titer of antibodies to herpesvirus (1:12,800) and low content of HSP-70 in placenta homogenate revealed disturbances in the structure of cytosol of the fetoplacental barrier. The cytosol in sites of its contact with syncytiotrophoblast nuclei lost its compactness (Fig. 2, *a*). In many cases, the cytosol in the site of its contact with the nucleus disap-

TABLE 1. TNF-α Content in Peripheral Blood and Placenta Homogenate in Herpervirus Infection (pg/ml; *M±m*)

Titer of anti-HSV antibodies	Peripheral blood	Placenta homogenate
Control	12.0±0.7	11.60±0.09
1:1600	25.3±0.9**	21.10±0.08*
1:6400	68.5±1.2**	84.30±0.30**
1:12,800	90.4±0.8**	89.90±0.34**

Note. Here and in Tables 2 and 3: *p<0.01, **p<0.001 compared to the control.

TABLE 2. Content of NK and HLA DR in Peripheral Blood of Pregnant Women in Herpesvirus Infection: Flow Cytofluorometry Data (*M±m)*

Titer of anti-HSV antibodies	NK	HLA DR – CD16 antigens
Control	10.0±0.1	45.00±0.60
1:1600	11.5±0.5*	5.80±0.08**
1:6400	12.8±0.3**	8.10±0.09**
1:12,800	16.8±0.7**	8.4±0.4**

TABLE 3. Content of HSP-70 and Caspase-3 in Placenta Homogenate from Women with Herpesvirus Infection Exacerbation during the Third Trimester (*M±m*)

Titer of anti-HSV antibodies	HSP-70, ng/ml	Caspase-3, ng/ml
Control	56.1±2.6	19.0±1.8
1:1600	34.7±1.9**	26.7±1.8*
1:6400	30.4±2.4*	79.5±3.4**
1:12 800	22.3±2.9*	103.7±3.9**

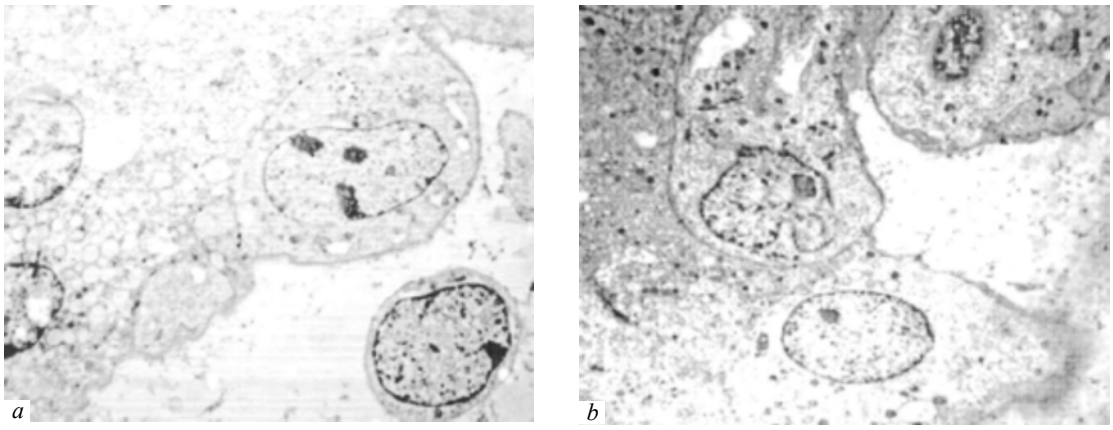

Fig. 1. Syncytiotrophoblast of placental villi contacting with NK lymphocytes during exacerbation of herpesvirus infection, ×800. *a*) lymphocyte contacting with syncytiotrophoblast increases in size, granularity appears in lymphocyte cytosol; *b*) lymphocyte contacting with syncytiotrophoblast changes the structure of trophoblast cells: vacuoles and granularity appear in their cytoplasm.

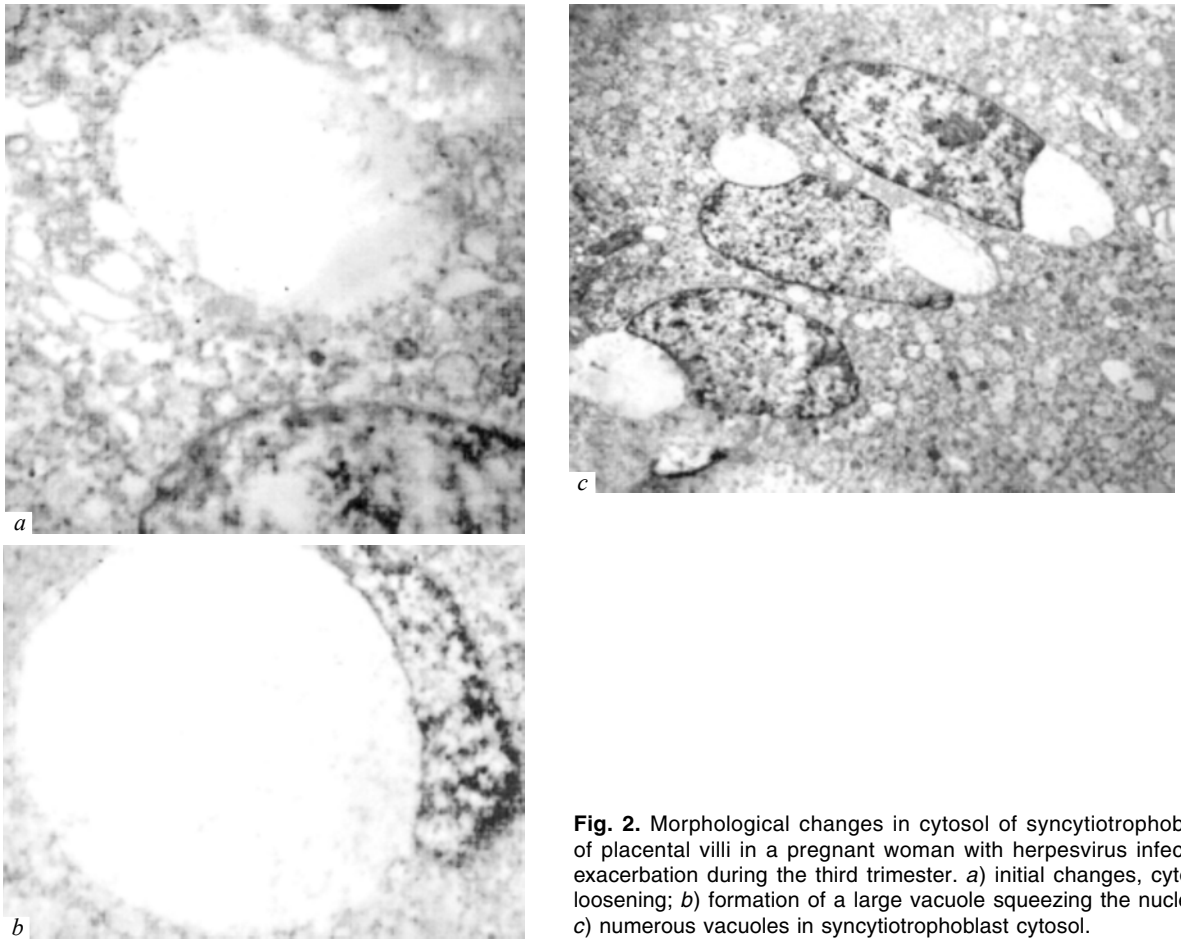

Fig. 2. Morphological changes in cytosol of syncytiotrophoblast of placental villi in a pregnant woman with herpesvirus infection exacerbation during the third trimester. *a*) initial changes, cytosol loosening; *b*) formation of a large vacuole squeezing the nucleus; *c*) numerous vacuoles in syncytiotrophoblast cytosol.

peared and large light vacuoles were formed (Fig. 2, *b*, *c*); these vacuoles squeezed the nucleus and compressed chromatin, which can be considered as initial signs of apoptosis. ISEL analysis showed that the number of apoptotic nuclei in syncytiotrophoblast increased from 1.00±0.02% (control) to 4.00±0.08% (Fig. 3).

Thus, exacerbation of herpesvirus infection during pregnancy induces enhanced production of TNF-α and NK lymphocytes. We observed signs of close contact of NK lymphocytes with syncytiotrophoblast providing the possibility of transmission of TNF-α or herpesvirus antigen into the cytosol. This leads to suppression of HSP-70 production in syncytiotrophoblast and impairs protein synthesis control in the cytosol. Fragments of cytosol loosening and large vacuoles appear and come into contact with syncytiotrophoblast nuclei. Against

Fig. 3. Syncytiotrophoblast of placental villi in a pregnant woman with herpesvirus infection exacerbation during the third trimester: immunohistochemical ISEL method, ×1350. Symplast nuclei in the state of apoptosis.

the background of enhanced caspase-3 production this leads to changes in nuclear chromatin (condensation) and nucleus death. Therefore, high titer of antigens to herpesvirus is associated with the increase in the number of apoptotic nuclei in syncytiotrophoblast.

Our findings suggest that herpesvirus infection suppresses the production of HSP-70 in syncytiotrophoblast and stimulates the synthesis of caspase-3, which determines disturbances in cytosol structure and increase in the number of nuclei entering into apoptosis.

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