Morphofunctional Characteristics of Fetoplacental Barrier of Placental Villi during Pregnancy Complicated by Herpes-Virus Infection

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Analysis of placenta homogenate from pregnant women having an episode of herpes-virus infection during the third trimester revealed suppression of Bcl-2 protein activity and increase in caspase 3 concentration. Activity of Bcl-2 protein decreases with increasing the titer of antibodies to herpes virus. Increased caspase-3 activity increases the risk of DNA damage in syncytiotrophoblast nuclei.

Key Words: *herpes; syncytiotrophoblast; Bcl-2 protein; caspase-3*

Placenta is responsible for feto-maternal exchange and performs gas exchange, trophic, endocrine, excretory, and defense functions. The transport of proteins, carbohydrates, and lipids from the maternal to fetal blood is a result of complex processes of enzymatic cleavage and synthesis. The placenta contains enzymes necessary for this metabolism [2,4-6]. Exposure to pathological factors induces changes in the placenta and impairs the barrier function of the syncytiotrophoblast, which can led to metabolic disturbances in the cytosol of the fetoplacental barrier and promote the entry of toxins, viruses, circulating immune complexes, and interleukins into the fetal blood. Special attention during pregnancy should be paid to relapses of persistent infections, *e.g.* herpes-virus infection. Herpes simplex virus (HSV) impairs the immune status of pregnant women, activates the production of TNF-α, IL-8, and IL-4, increases serotonin content in the peripheral blood, and simultaneously suppresses activity of IFN-α and IFN-γ [1,5].

Here we evaluated morphofunctional state of the fetoplacental barrier of placental villi during pregnancy complicated by relapse of herpes-virus infection. Special attention was given to the effect of HSV on caspase-3 activity and content of Bcl-2 responsible for viability of syncytiotrophoblast nuclei [2,6-9].

MATERIALS AND METHODS

We analyzed peripheral blood and placentas obtained after term delivery at 38-40 weeks' gestation. The control group comprised 20 women with uncomplicated course of pregnancy. A total of 60 women with type I herpes-virus infection of different severity (antibody titers 1:1600, 1:6400, and 1:12,800) were examined (20 women in each subgroup). The examinations were performed in Obstetric Department (Hospital of Far-Eastern Research Center of Physiology and Pathology of Respiration). 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. 226, June 19, 2003).

The placenta was obtained within 10-15 min after delivery. Its surface on the side of decidua basalis was washed with a large volume of physiological saline (PS). The fetal part of the placenta (villous chorion) was cut off with a scalpel in the form of plates (plate area 2-3 cm^2 and thickness <1 mm). Tissue fragments

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were placed in chemical glasses with 200 ml PS and washed from blood cells on a magnetic stirrer for 15 min. Before extraction, the washed fragments were slightly dried on paper filters and weighed. The tissue was then crushed in a porcelain mortar to homogenous cream-like mass. The homogenate was diluted with PS (the volume of PS was equal to the initial weight of the tissue sample). The suspension was placed in Falcon plastic tubes and frozen at -20°C for 1 day. The homogenate was then defrosted and ultracentrifuged at 4000 rpm and 4°C. The supernatant was stored at -20°C in small aliquots until immunoenzyme assay.

Bcl-2 protein and caspase-3 were measured in the homogenate of the placenta using Bender MedSystems kits (Austria). The presence of HSV-1 was verified and aggressiveness of the infection was evaluated 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. Electron microscopy was performed by standard methods with final embedding in araldite. Ultrathin sections were prepared on an ULB microtome and contrasted with uranyl acetate. The sections were examined and photographed using Tesla microscope.

Apoptosis was evaluated morphologically (*in situ* end labeling, ISEL) on paraffin sections of the placenta [3]. The number of apoptotic syncytiotrophoblast nuclei was determined in 100 terminal villi of the placenta in each case (2000 nuclei were analyzed). The content of TNF-α was measured by ELISA using special kits (Citokin company).

For light microscopy, the material was fixed in 0.5% paraformaldehyde in 80% ethanol and embedded in paraffin. Bcl-2 protein was detected immynohistochemically using monoclonal antibodies (clone 124, Dako Cytomation). The data were processed statistically using Exel software.

RESULTS

During pregnancy complicated with HSV-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 in the placenta homogenate at anti-HSV antibody titers of 1:1600, 1:6400, and 1:12,800 was 21.10±0.08, 84.3±0.3, and 89.98±0.34 ng/ml, respectively (compared to 11.60±0.09 ng/ml in the control; $p<0.001$).

The content of caspase-3 in the placenta homogenate sharply increased under these conditions: 26.70±0.08, 79.5±0.4, and 103.7±0.8 ng/ml at titers of 1:1600, 1:6400, 1:12,800, respectively (compared to 19.0±0.8 ng/ml in the control; $p<0.001$).

In parallel, we evaluated the dynamics of the content of Bcl-2 protein, an antiapoptotic factor in cells, in these homogenates. The content of Bcl-2 protein increased with increasing anti-HSV antibody titers to 1:1600 (to 9.24±0.09 pg/ml *vs*. 3.80±0.02 pg/ml in the control), attained the maximum at anti-HSV antibody titer of 1:6400 (42.5±0.6 pg/ml), and then sharply decreased with increasing anti-HSV antibody titer to 1:12,800 (28.14±0.70 pg/ml, Table 1).

The antiapoptotic function of syncytiotrophoblast started to decrease, which manifested in increased number of apoptotic nuclei in the fetoplacental barrier (4.00±0.02% *vs*. 0-1.50±0.01% in the control). Thus, the antiapoptotic function of syncytiotrophoblast depends on the concentration of antigen in pregnant woman during the relapse of HSV infection.

Hence, at the initial stages of infection, the protective properties of syncytiotrophoblast can limit the apoptosis (the content of Bcl-2 protein increases almost 11-fold compared to the control), while in severe HSV infection (antibody titer 1:12,800) the synthesis

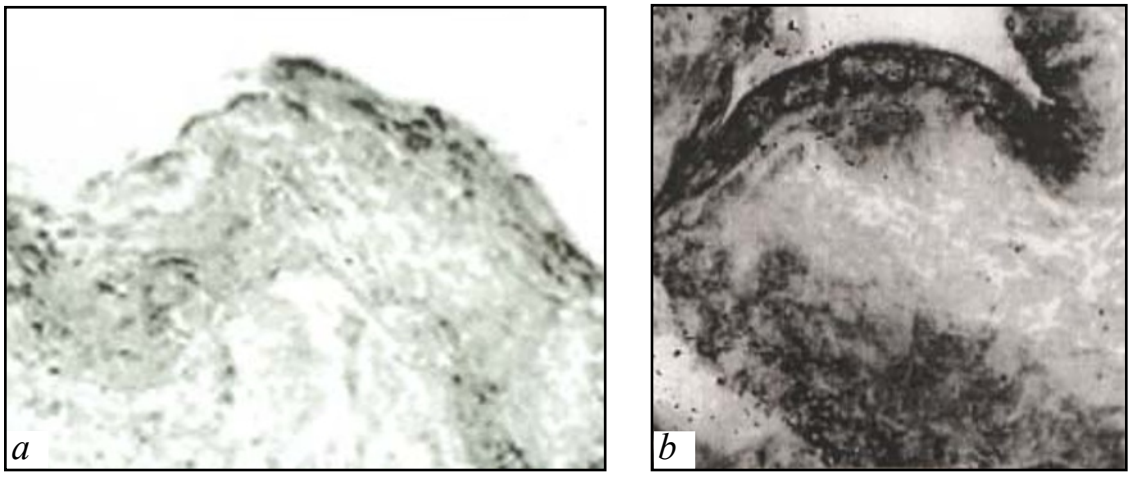

Fig. 1. Immunohistochemical staining of Bcl-2 protein in placental villus syncytiotrophoblast from pregnant woman with relapse of herpesvirus infection during the third trimester (*a*) and in the control (*b*); ×400.

TABLE 1. Dynamics of Caspase-3 and Bcl-2 Protein in Homogenate of Placenta in Third Trimester after Relapse of Herpes Virus Infection of Different Intensity

Proteins and enzymes of villus syncytiotrophoblast	Control	Titer of anti-HSV antibodies		
		1:1600	1:6400	1:12 800
Caspase-3, ng/ml	19.00±0.09	26.70±0.05*	79.5±0.4*	103.7±0.8*
Bcl-2 protein, pg/ml	3.80±0.02	9.24±0.09*	42.5±0.3*	28.14±0.79*

Note. *p<0.001 compared to the control.

of Bcl-2 is suppressed (Fig. 1), which creates the conditions for preterm development of apoptosis of nuclei in syncytiotrophoblast of the fetoplacental barrier.

Thus, HSV infection suppresses activity of Bcl-2 protein in syncytiotrophoblast of placental villi and promotes the increase in caspase-3 activity, which stimulates apoptosis in syncytiotrophoblast of placental villi.

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