Regulatory Mechanisms for Apoptosis in Placental Tissue during Normal Pregnancy and Gestosis-Complicated Pregnancy

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The localization of apoptosis and expression of proapoptotic and antiapoptotic factors by the placental tissue were compared during normal pregnancy and gestosis-complicated pregnancy. The degree of apoptosis did not differ in the third trimester of normal pregnancy and gestosis-complicated pregnancy. Increased expression of Fas, caspase-8, and caspase-3 in placental tissue during normal pregnancy was shown to contribute to the suppression of angiogenesis and growth of placental tissue. No differences were found in the expression of FasL (CD95L), caspase-2, caspase-9, and Mcl-1 by placental cells during normal pregnancy and gestosis-complicated pregnancy. Increased expression of TRAIL by trophoblast cells is a protective mechanism from apoptotic signals of maternal cytotoxic lymphocytes and NK cells during gestosis.

Key Words: *placenta; apoptosis; CD95; CD95L; TRAIL; caspase; Mcl-1*

Apoptosis is a regulated process characterized by the interaction between extracellular molecules, intracellular signal transduction pathways, and resident programs of suicide/survival [6]. Apoptosis contributes to removal of morphologically altered and abnormal cells, which maintains normal function of tissues.

Apoptosis is involved in the development of human placenta. This process plays an important role in abnormalities of placental development and placental dysfunction [12]. Apoptosis is of particular significance during the initial and terminal stages of placental development and function. Apoptotic cells were found in maternal and embryonic parts of the placenta during normal pregnancy. The presence of these cells is associated with stages of placental development, including invasion of trophoblast, transformation of spiral arteries, differentiation of trophoblast [13], and childbirth [15]. The intensity of apoptosis in human placental tissue increases progressively during pregnancy (until delivery) [15]. However, induction and progression of apoptotic processes in the placenta are not directly related to the onset of labor. They are associated with microenvironment of placental cells and concentration of growth factors and other substances [11].

Little is known about the mechanisms of apoptosis in placental tissue during normal pregnancy and gestosis-complicated pregnancy. Here we compared the localization of apoptosis and expression of proapoptotic and antiapoptotic factors by placental tissue during normal pregnancy and gestosis-complicated pregnancy.

MATERIALS AND METHODS

We examined the placentas from 10 women with uncomplicated pregnancy (38-39 weeks) and 10 women with gestosis-complicated pregnancy (38-39 weeks). Fetuses were delivered by cesarean section. Placental

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samples were fixed in 10% neutral formalin for immunohistochemical study. The localization of apoptosis was verified by the TUNNEL method with standard kits (Chemicon). Immunohistochemical study of the expression of Fas (CD95), FasL (CD95L), TRAIL (TNF-related apoptosis-inducing ligand), caspase-2, caspase-3, caspase-8, caspase-9, and Mcl-1 was conducted with serial sections of the placenta.

Immunohistochemical study was performed with mouse monoclonal antibodies against Fas (CD95, 1:50, Novocastra), FasL (CD95L, 1:50, Novocastra), caspase-2 (1:40, Novocastra), caspase-3 (1:50, Novocastra), caspase-8 (1:20, Novocastra), and Mcl-1 (1:50, Novocastra) according to the one-step protocol of antigen unmasking (high-temperature treatment of tissue) in 0.01 M citrate buffer (pH 7.6). The assay with antibodies against caspase-9 (1:30, Novocastra) and TRAIL (1:50, Novocastra) was conducted by means of antigen unmasking (high-temperature treatment of tissue) in 0.01 M EDTA (pH 8.0). A standard kit of biotinylated anti-mouse and anti-rabbit immunoglobulins (Novocastra) was used as secondary antibodies. Visualization was performed using a complex of avidin and biotinylated peroxidase (ABC kit) and was followed by the development of horseradish peroxidase with diaminobenzidine (Novocastra). The area of marker expression in the placental tissue was estimated using a Leica DMR microscope, Leica DC300 digital camera, and computerized system for microscopic image analysis (Leica QWin software). The results were expressed as a percentage of the area of the view filed.

RESULTS

Immunohistochemical study of placental tissue by the TUNNEL method showed that the degree of apoptosis is similar in the third trimester of normal pregnancy and gestosis-complicated pregnancy (area of positive staining 5.29±2.43 and 4.22±1.86%, respectively). It should be emphasized that the localization of apoptosis did not differ during normal pregnancy and gestosis-complicated pregnancy. The positive reaction was mainly found in the villous syncytiotrophoblast (Fig. 1).

Fas (CD95) expression in the placental tissue during gestosis was much lower than during normal pregnancy (Table 1). Fas (CD95) expression during normal pregnancy and gestosis (38-39 weeks) was mainly detected in the villous syncytiotrophoblast and stroma (fibroblasts; Fig. 2).

Immunohistochemical study of placental samples revealed no differences in the expression of FasL (CD95L) by placental cells during normal pregnancy and gestosis (Table 1). At the 38th-39th week of normal pregnancy and gestosis, FasL (CD95L) expression was mainly found in the villous syncytiotrophoblast and stroma (macrophages, fibroblasts, and vascular endothelium; Fig. 2). FasL (CD95L) expression was also revealed near the collagen fibers, which reflects desquamation of these receptors from the cell surface.

The expression of TRAIL in placental tissue during gestosis was much higher than during normal pregnancy (Table 1). At the 38th-39th week of normal pregnancy and gestosis, TRAIL expression was mainly detected in the syncytiotrophoblast (Fig. 2). The expression was lowest in macrophages and endothelial cells.

No differences were found in the expression of caspase-2 by placental cells during normal pregnancy and gestosis (Table 1). Caspase-2 expression occurred mainly in the villous syncytiotrophoblast and stroma (macrophages and fibroblasts; Fig. 2).

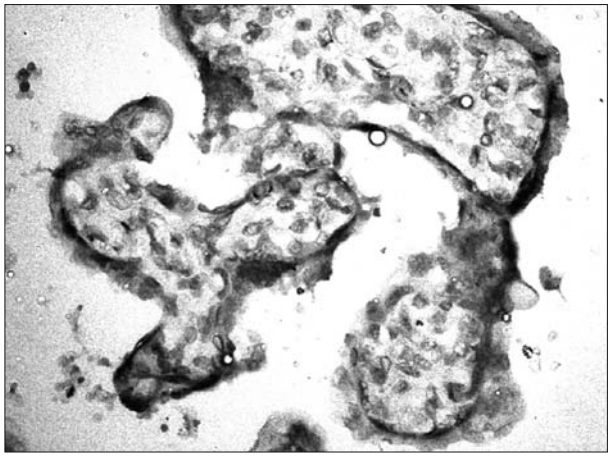

Fig. 1. Localization of apoptosis in placental tissue at the 38th-39th week of normal pregnancy is similar to that during gestosis-complicated pregnancy (×400).

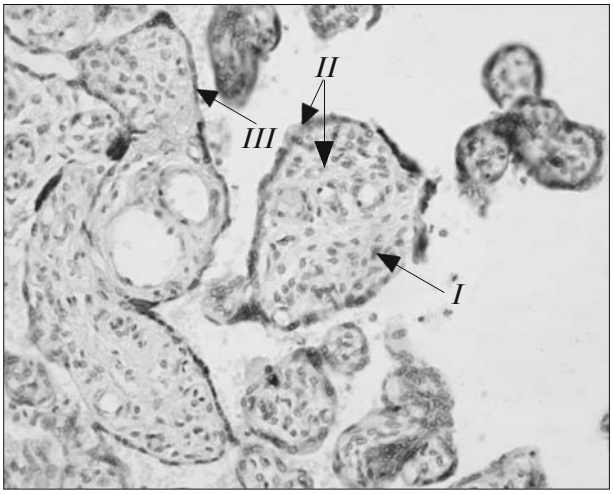

Fig. 2. Localization of regulatory factors for apoptosis in placental tissue at the 38th-39th week of normal pregnancy is similar to that during gestosis-complicated pregnancy (×400). Caspase-8 (*I*); Fas, FasL, caspase-2, caspase-3, and caspase-9 (*II*); TRAIL and Mcl-1 (*III*).

The expression of caspase-3 in placental tissue during gestosis was much lower than during normal pregnancy (Table 1). Caspase-3 expression was mainly found in the syncytiotrophoblast and stroma of villi (macrophages and fibroblasts; Fig. 2).

The expression of caspase-8 in placental tissue during gestosis was much lower than during normal pregnancy (Table 1). Caspase-8 expression was mainly found in the villous stroma (macrophages and fibroblasts; Fig. 2).

No differences were found in the expression of caspase-9 in placental tissue during gestosis and normal pregnancy (Table 1). Caspase-9 expression was mainly detected in the villous stroma (macrophages and fibroblasts; Fig. 2).

No differences were found in the expression of Mcl-1 in the placental tissue during normal pregnancy and gestosis (Table 1). Mcl-1 expression was mainly detected in the syncytiotrophoblast. Mcl-1 expression was minimum in the villous stroma (Fig. 2).

TUNNEL study showed that the localization of apoptosis is similar during gestosis and normal pregnancy (Fig. 1). No differences were found in the localization of study factors in placental tissue (Fig. 2). However, the expression of apoptotic factors was shown to differ under various conditions. TRAIL expression was elevated during gestosis. By contrast, the expression of Fas (CD95), caspase-3, and caspase-8 during gestosis was lower than during normal pregnancy. The expression of caspase-2, caspase-9, and Mcl-1 remained unchanged during gestosis. These data show that gestosis is accompanied by changes in the regulatory mechanisms for apoptosis in the placental tissue. It should be emphasized that apoptosis is strongly regulated by TRAIL.

TRAIL serves as a marker for differentiation and activation of NK cells [10]. The induction of granzyme-independent and Fas-independent apoptosis in target cells by NK cells is mediated by TRAIL. TRAIL exists not only in the membrane form, but also in the soluble form. Soluble molecule of TRAIL binds to surface receptor (DR4 [TRAIL-R1] and DR5 [TRICK2/TRAIL-R2]) on the target cell, which induces cell apoptosis [8]. The third-trimester trophoblast cells express TRAIL to induce apoptosis in activated maternal cytotoxic lymphocytes [9]. Apart from TRAIL, trophoblast cells express decoy receptors (DcR1 [TRID/LIT/TRAIL-R3]). They suppress the apoptotic signal of TRAIL, which prevents cell apoptosis [7]. NK cells of the uterus express KIR2DL4 receptors during normal pregnancy. These receptors bind to HLA-G molecules, which prevents cytotoxicity upon activation of IFN-γ production [2]. The expression of TRAIL by trophoblast cells and macrophages increases significantly under the influence of IFN-γ, which is produced by decidual NK cells. Therefore, gestosis is characterized by activation of NK cells at the site of contact between the placenta and endometrium (without decrease in the cytotoxic potential). This process is accompanied by increased secretion of IFN-γ by cells. This conclusion is derived from increased expression of TRAIL in the syncytiotrophoblast. These data are consistent with the result of our previous studies. We showed that gestosis is characterized by activation of cytotoxic lymphocytes and high content of NK cells in the blood (as compared to normal pregnancy) [3]. Due to the production of IFN-γ, NK cells can activate placental macrophages. Macrophages have a regulatory effect on placental cells and modulate the growth and function of the placenta. Moreover, macrophages produce

TABLE 1. Expression of Factors and Receptors for the Regulation of Apoptosis in Placental Tissue during Normal Pregnancy and Gestosis

Factors and receptors	Area of marker expression in placental tissue, %	
	normal pregnancy, 38-39 weeks	gestosis-complicated pregnancy, 38-39 weeks
Fas (CD95)	13.79±1.33	5.41±0.47*
FasL (CD95L)	3.82±1.46	3.96±0.31
TRAIL	3.14±1.11	16.32±1.32*
Caspase-2	3.82±1.46	1.58±0.36
Caspase-3	8.29±1.31	2.23±0.2*
Caspase-8	6.75±1.71	2.36±0.16*
Caspase-9	6.37±2.01	3.97±1.09
Mcl-1	2.72±0.27	1.45±0.65

Note. *$p<0.01$ compared to normal pregnancy.

interleukin-12 (IL-12) that activates NK cells and induces the early inflammatory response. This assumption is confirmed by the fact that the concentration of IL-12 in blood serum and placental tissue increases significantly during gestosis. These changes are accompanied by an increase in the number of activated NK cells in the peripheral blood [14] and stimulation of their adhesion to endothelial cells [16]. It should be emphasized that activation of placental macrophages, increase in the secretion of IL-12 by these cells, and decrease in the production of IL-10 due to a shift toward the Th1 response and reduction of HLA-G expression by trophoblast cells during gestosis can initiate cytotoxic activity of maternal NK cells and $CD8^+$ lymphocytes.

The expression of Fas (CD95) by trophoblast cells and other cells of the placenta during gestosis is lower than during normal pregnancy. The Fas–FasL interaction probably plays a little role in the induction of apoptosis. These changes illustrate a compensatory increase in the contribution of mechanisms to increase the survival of placental cells during gestosis. This assumption is confirmed by an increase in the secretion of angiogenin [2] and expression of platelet-derived growth factor (PDGF) [1] by placental tissue during gestosis. It may be suggested that these proangiogenic factors contribute to low expression of Fas by placental cells during gestosis (despite the increase in cytotoxicity of maternal lymphocytes and NK cells in relation to trophoblast cells). Fas (CD95) expression by stromal cells of the villi (macrophages, fibroblasts, and vascular endothelium of the villi) at the late stage of pregnancy is probably related to secretory activity of placental macrophages. Due to secretion of cytokines, placental macrophages produce a modulatory effect on the cytokine balance (action on placental cells). Under normal conditions, placental macrophages can produce a variety of factors *e.g.*, thrombospondin-1 (TSP-1) and tumor necrosis factor-α inducing apoptosis in placental cells. Our previous studies showed that the expression of TSP-1 in the placental tissue increases during gestosis [1]. However, Fas expression by placental tissue did not increase under these conditions. These data confirm our hypothesis on the existence of a compensatory protective mechanism from the induction of apoptosis in placental cells (due to the increased production of proangiogenic factors).

Caspase molecules play a central role in the realization of cell apoptosis. They belong to the family of cysteine proteases that cleave cell proteins, thus inducing cascade reactions of apoptosis. Caspase-mediated activation of cascade reactions is observed under the influence of exogenous apoptotic signals on the cell or induction of intracellular mechanisms for apoptosis. Cascade reactions determine activation of caspase-2, caspase-8, caspase-9, and caspase-10. They activate effector caspase-3, caspase-6, and caspase-7 [5]. These caspases destabilize the nuclear membrane and cleave cytoskeletal proteins and other proteins in the cell (including DNA reparation enzymes). These changes result in cell death. We showed that gestosis is characterized by a decrease in the expression of caspase-3 and caspase-8 and low expression of caspase-2 and caspase-9. Our findings confirm the fact that the increase in the production of angiogenin and PDGF has a protective effect. These factors increase viability of placental cells. The expression of caspase-3 and caspase-8 in villus cells is elevated during normal pregnancy. Therefore, these caspases play an important role in the suppression of placental growth at the late stage of its development. TUNNEL study revealed no differences in the degree of apoptosis in placental tissue during normal pregnancy and gestosis. It should be emphasized that apoptosis is mainly observed in the villous syncytiotrophoblast during normal pregnancy and gestosis (Fig. 1). The expression of TRAIL and antiapoptotic protein Mcl-1 was also revealed in this area. These data indicate that gestosis is accompanied by induction of mechanisms protecting trophoblast cells from maternal cytotoxic lymphocytes. It is associated with the production of angiogenic factors and TRAIL, which induces lymphocyte death. By contrast, during normal pregnancy the main goal is the inhibition of placental growth. This is achieved via increased expression of Fas in placental cells and induction of apoptosis via activation of caspase-8 and caspase-3.

We conclude that the increased expression of Fas, caspase-8, and caspase-3 in placental tissue during normal pregnancy contributes to the suppression of angiogenesis and placental growth. By contrast, the increase in TRAIL expression by trophoblast cells is accompanied by the decrease in Fas expression by placental tissue and high-intensity secretion of angiogenin and PDGF. This is a mechanism for the protection from apoptotic signals of maternal cytotoxic lymphocytes and NK cells during gestosis.

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