Spontaneous and LPS-Induced Secretion of Cytokines by Villous Chorion Tissue

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Secretion of IL-1α, IL-1β, IL-6, IL-10, and TNF-α cytokines by villous chorion cultures (7-14 weeks) during normal pregnancy and in spontaneous abortions was studied. Secretion of IL-1α and IL-6 increased 4.5 and 7.3 times in miscarriages, while secretion of IL-1β, IL-10, and TNF-α decreased. LPS stimulated the production of IL-1α and IL-6 in samples obtained during surgical abortion. LPS stimulated IL-1α and TNF-α secretion in miscarriages, while the level of IL-6 production decreased significantly. It is hypothesized that increased production of IL-1α and IL-6 and attenuation of the antiinflammatory effect of IL-10 play an important role in the pathogenesis of miscarriages at early stages of gestation. The results suggest that cytokine regulation of the fetus rejection is different at early and late stages of gestation.

Key Words: *villous chorion; normal pregnancy; spontaneous abortion; cytokines; lipopolysaccharide stimulation*

The regulatory mechanisms responsible for pregnancy prolongation and fetus rejection in normal labor and spontaneous abortions remain little studied. Cytokines play an important role among the factors regulating gestation processes throughout pregnancy from implantation to delivery. Despite obvious progress in studies of the gestation effects of cytokines, it is still impossible to outline a more or less integral and logical scheme describing the regulatory effects of cytokines in the processes of pregnancy development and fetus rejection, because available experimental data are ambiguous and sometimes even contradictory. This is explained by the specific object of the study and difficulties in obtaining reliable information not distorted by researcher's manipulations, use of different experimental approaches, and limitations in studies of human biological materials.

The placenta is one of the few available objects of studies; it is obtained as a result of natural or artificial rejection of the fetus. At present the majority of scientists consider that the events most important for gestation take place within the intrauterine compartment, and the production of factors involved in initiation and regulation of labor activity is realized by tissues of the fetoplacental complex. Hence, studies of placental cells and tissues provide valuable information on the regulatory processes in the pregnant uterus *in vivo*.

Late terms of gestation attract special interest of scientists: term and preterm deliveries, studies of the material obtained in artificial delivery. However, studies of early terms of pregnancy are also important, because they will help us to understand not only the regularities of one of the critical stages of gestation development, during which the placental structure forms and which largely determines the outcome of pregnancy, but will show ontogenetic changes in the regulation of gestation processes and possible causes of spontaneous abortions at the early stage (early miscarriage).

We previously studied secretion of proinflammatory cytokines in forming placental tissues in normal

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and pathological gestation [1]. Here we more precisely evaluated some facts obtained previously for IL-1β, IL-6, TNF-α, and investigated a wider spectrum of cytokines, including antiinflammatory cytokine IL-10 and the α-form of IL-1. In addition, studies of secretory activity under conditions of stimulation by bacterial endotoxin gave us additional information on the cytokine regulation at the early stage of gestation in different outcomes of pregnancy.

MATERIALS AND METHODS

Villous chorion tissue was obtained at weeks 7-14 of gestation in women aged 18-36 years. Two experimental groups were formed. Group 1 included cases of artificially aborted normal pregnancy (n=12). Group 2 included spontaneous abortions (n=9) and pregnancies with symptoms of threatened miscarriages, which were artificially aborted (n=3).

Villous chorionic tissue explants were put into wells of a 4-well plate (Nunc) with 1 ml DMSI/F-12 medium supplemented with 10% EFC (Bioclear) and cultured in an incubator (Sanyo) for 24 h at 37°C and 5% CO_2 without stimulant or with 1 μg/ml LPS (Sigma). Supernatant was collected after incubation and cytokines were measured in culture medium by enzyme immunoassay using commercial kits (Protein Contour and Cytokine). The explants were dried on filter paper and weighed on analytical scales. The data were presented as medians (secreted cytokine content/mg tissue). The results were statistically processed using Mann—Whitney's test.

RESULTS

The data on spontaneous secretory activity in organotypical cultures of villous chorion are presented in Table 1.

Spontaneous abortion was associated with a significant increase in IL-1α and IL-6 secretion and decreased levels of IL-1β, IL-10, and TNF-α in villous chorion tissue cultures in comparison with specimens of the same gestation period obtained as a result of induced abortion.

LPS stimulation increased production of IL-1α and IL-6 in cultures of placental tissue obtained after induced abortion. In cultures of tissues obtained as a result of miscarriage addition of LPS increased IL-1α secretion. In group 2 the production of IL-6 did not increase like in group 1, but decreased. In addition, TNF-α secretion increased under the effect of LPS in group 2 (Table 1).

Stimulation coefficients (Table 2) more demonstratively show the differences in the secretory response of tissue in normal course of pregnancy (group 1) and its abnormalities (group 2).

Miscarriage was associated with increased secretion of IL-1α. Similarly as in our previous study [1], we observed a significant (7.5 times) increase in IL-6 production in early spontaneous abortion. However, we previously failed to detect differences in the secretion of IL-1β and TNF-α, while present findings indicate a significant decrease in the production of these proinflammatory cytokines (and antiinflammatory IL-10) in miscarriage.

Together with IL-1, IL-8, and TNF-α, IL-6 is most often mentioned in connection of fetus rejection, timely and premature. Some authors consider IL-6 as a labor activity marker, because spontaneous labor activity is associated with increased level of this cytokine in the amniotic fluid [2], cervicovaginal secretion [14], and gestation tissues [5,13]. However, other scientists failed to detect unambiguous relationship between labor activity and IL-6 production [3,7,11].

Taking into account significant increase in the production of IL-6 by the placenta and its capacity

TABLE 1. Spontaneous and LPS-Induced Cytokine Production by Villous Chorion Tissue (pg/mg; $M \pm m$)

Group	IL-1α	IL-1β	IL-6	IL-10	TNF-α
Spontaneous production					
1	0.05	1.28	40.3	1.54	8.0
	(0.00-2.81)	(0.41-4.60)	(4.3-1738.9)	(0.38-2.44)	(0.38-14.49)
2	0.23**	0.70*	296.5**	0.75*	6.3**
	(0.00-10.47)	(0.23-1.50)	(2.3-2971.6)	(0.08-2.12)	(1.08-9.95)
LPS-induced production					
1	0.89+	1.14	203.8+	1.79	9.15
	(0.19-10.80)	(0.55-5.72)	(37.8-4000.2)	(0.86-3.41)	(6.18-27.48)
2	2.89+	0.72	252.0+	1.22	12.0+
	(0.53-7.72)	(0.32-1.97)	(45.5-830.2)	(0.47-1.76)	(5.90-23.37)

Note. Range of values is shown in parentheses. *p<0.01, **p<0.05 compared to group 1; +p<0.01 compared to spontaneous secretion.

to activate NK cells and prostaglandin production by gestation tissues we can hypothesize that IL-6 is involved in spontaneous rejection of the fetus during the 1st trimester of pregnancy.

Similarly as IL-6, IL-1α is a mediator of inflammatory reaction and can also activate NK cells and stimulate prostaglandin production (initiate the mechanisms which are believed to play a key role in preterm rejection of the fetus). The content of IL-1α in the maternal plasma and amniotic fluid was not changed in term labor, though some authors, similarly as we did, noted a relationship between increased production of the cytokine by placental tissue and labor activity [8]. Our results attest to possible abortogenic effect of IL-1α not only at late terms of gestation, but also in miscarriages at earlier stages.

In contrast to IL-1α, the content of IL-1β in supernatants of villous chorion cultures decreased in early spontaneous abortion. The role of this cytokine in gestation processes was studied not once; the majority of scientists detected correlations between labor initiation and increased IL-1β level in biological fluids [10,14] and gestation tissues [15].

Our data on the role of IL-1β seem to contradict these facts. However, this report concerns late period of gestation. It is quite possible that the expression of IL-1β varies in the course of gestation, as was revealed in animal experiments. Presumably, both IL-1 forms participate in labor activity regulation at late stages and only IL-1α participates in these processes during the 1st trimester.

The level of TNF-α secretion, a marker of preterm and full-term delivery [14,15], did not increase in association with early miscarriages in our study; moreover, it decreased. Our findings are more in line with the findings of the authors who did not observe increased in TNF-α level in maternal blood [3] and in gestation tissues [5] during labor. Presumably, disagreement between the effects observed in our study (cytokine response) and previous results can be due to the phenomenon of ontogenetic regulation of cytokine expression, when the same factors are differently expressed at different stages of gestation and/or cause different, sometimes opposite effects.

Decreased level of IL-10 production in miscarriage, observed in our study, is in line with the hypothesis on suppression of Th2 response in abortion. The capacity of placental tissues to produce IL-10 during the 1st trimester of pregnancy was regarded by some authors as an evidence of suppression of proinflammatory cytokines, unfavorable for gestation [6,9]. The production of IL-10 appreciably decreased before labor [6] and in pre-eclampsia [9], which also confirms the regulatory role of this cytokine in pregnancy prolongation.

Study of the effect of bacterial endotoxin on placental cells and tissues *in vitro* help to simulate the effects caused by *in vivo* infection leading to loss of the fetus and evaluate the secretory potential of cells and tissues obtained in different outcomes of pregnancy.

In our study addition of LPS to villous chorion cultures stimulated production of IL-1α, but not IL-1β, in both groups. It was IL-1α that was intensively secreted by non-stimulated cultures in spontaneous abortion, which indicates the predominance of IL-1α in the regulation of gestation processes at early terms of gestation. The absence of secretory response of IL-1β to LPS stimulation in both groups can indicate that this component of the placental cytokine regulatory network is not yet completely formed at this stage of gestation. These hypotheses were confirmed by experiments, in which the production of IL-1β was stimulated by hypoxic exposure of villous chorion cultures only after week 9 of gestation. Moreover, changes in TNF-α secretion under the effect of an external stimulus were different: the cytokine secretion by the tissue decreased at weeks 8-9 and increased at 11 weeks [4].

In normal gestation LPS stimulation of chorionic villi led to increase of IL-6 production, while LPS addition to pathological tissue led to inhibition of cytokine secretion. It seems that activation of IL-6-producing cells in spontaneous rejection of the fetus completely exhausts their secretory potential, and additional stimulation has a suppressive effect, as we observed *in vitro* during long-term or high-concentration LPS treatment.

LPS did not modify the production of IL-10, one of the key cytokines providing the normal course of pregnancy. IL-10 suppresses LPS-induced production of TNF-α by the negative feedback mechanism. Presumably, increased production of TNF-α under the effect of LPS in pathological

TABLE 2. Coefficients of LPS Stimulation of the Villous Chorion Secretory Activity

Group	IL-1α	IL-1β	IL-6	IL-10	TNF-α
1	17.8	0.89	5.06	1.16	1.14
2	12.6	1.03	0.85	1.62	1.90

pregnancy in our study was caused by decreased production of IL-10 by the chorionic villi.

On the whole, our results give us an picture of the production of some cytokines by chorionic villi at early terms of gestation and its changes in spontaneous abortions. These changes in the cytokine production can reflect the pathological shifts in the regulatory mechanisms of gestation processes and can help a develop proper treatment strategy for patients with threatened miscarriage. In addition, our findings indicate differentiated production of various cytokines by the placenta, depending on the gestation period, which is particularly demonstrative in stimulation by exogenous factors, such as LPS.

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