In Vitro Secretion of Tumor Necrosis Factor-α and Interleukin-1 by Placental Macrophages in Various Outcomes of Pregnancy

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Production of tumor necrosis factor-α and interleukin-1β by placental cells obtained after term and premature labors with or without labor activity increases with an increase in the gestational period. The secretory activity of macrophages significantly increases during spontaneous premature labor in the second trimester of pregnancy (abortion) and decreases during normal term labor. The use of phorbol myristate acetate and lipopolysaccharide for stimulating functional activity of macrophages revealed the differences in cell responses in case of the presence or absence of spontaneous labor activity.

Key Words: *placental macrophages; culture; cytokines; premature labor; term labor*

The development and outcome of pregnancy depend on numerous interrelations between the fetus and environment. Various complications of pregnancy can cause premature labor.

The fetus and maternal organism interact at the cell level via direct cell-cell contact or soluble molecules released by cells. It is now generally accepted that cytokines playing the major role in the immune response are involved in the regulation of embryonic development. Some cytokines produced by the fetoplacental complex are involved not only in implantation (as an immune process), but also regulate embryonic growth and differentiation and initiate and control labor [5,7,10,11].

The placenta producing a wide range of biologically active molecules is of considerable interest in this aspect. Various elements of the placental cytokine network are involved in the regulation of normal fetal development and in realization of mechanisms of pregnancy interruption and term labors [2,8].

Placental macrophages attract much attention as the most probable natural producers of cytokines involved in the reproduction, such as tumor necrosis factor-α (TNF-α), interleukin-1, colony-stimulating factors, transforming growth factor-β, and interferons α and γ [3-5,13,14]. Exogenous (infections) and endogenous (cytokines, hormones, etc.) factors are assumed to activate macrophages, modulate secretion of macrophageal cytokines, and, therefore, affect the outcome of pregnancy [3]. Some cytokines produced by macrophages directly or indirectly affect secretion of tissue prostaglandin E_2 fetoplacental complex, a potent stimulator of myometrium contractile activity. Moreover, macrophages also can produce prostaglandin E_2 [9].

The contribution of various cell types to cytokine production is difficult to assess *in vivo*, but cultural methods allow us to isolate homogenous population of placental macrophages and to study their properties *in vitro*.

Here we analyzed secretory activity of macrophages isolated from placentas of various gestational ages in the case of presence or absence of spontaneous labor activity.

MATERIALS AND METHODS

Human placentas of various gestational ages obtained after spontaneous or induced labor with normal labor activity or after delivery by cesarean section (D. O. Ott

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Institute of Obstetrics and Gynecology, St. Petersburg Hospital No. 19) were studied. Placentas (n=30) were taken at 21-26 (trimester 2), 31-34 (trimester 3), and 38-42 (term labor) weeks gestation and divided into 6 groups depending on gestational age and form of delivery. The method of isolating and culturing of macrophageal macrophages was described previously [1]. The cells were placed into a 48-well plate (1.5×10^6 cells/well). Nonadherent cells were removed 2 h later. The content of adherent cells was 20-63% (average 37±12%).

After overnight incubation, the medium was changed, and the cells were incubated for 5 h in the absence or presence of 5 ng/ml phorbol myristate acetate (PMA, Sigma) or 1 μg/ml lipopolysaccharide (LPS, Sigma). The culture medium was collected, frozen, and stored at -40°C. The cells were 3 times washed with phosphate buffer (Flow), NaOH (0.2 ml, 0.1 N) was added to wells, and the content of protein was determined [6].

The concentration of cytokines in the conditioned medium was measured by test systems for TNF-α (Biomar Diagnostic Systems), interleukin-1α and interleukin-1β (Tsitokin, St. Petersburg). The content of cytokine was calculated per 1 mg cell protein in the well. The stimulation coefficient was calculated as the ratio of cytokine secretion in the presence and absence of the stimulator.

RESULTS

TNF-α production was minimum in macrophages from placentas of the early gestational age in the absence of labor activity (Table 1). However, there was a 20-fold increase in secretion of this cytokine by placental macrophages of the same gestational age obtained after spontaneous abortion. Spontaneous TNF-α production in other groups was similar (20 ng/mg cell protein).

The addition of PMA and LPS into the culture medium stimulated cytokine secretion by cells except for cultures obtained in the 2nd trimester of pregnancy with labor activity. The initial level of TNF-α secretion in these cultures was extremely high, and PMA had no effect, while LPS even inhibited this process. In other groups, these stimulators induced an approximately 2.5-fold increase in secretory activity of cells, except term labor without labor activity, when LPS induced a 4-fold increase in TNF-α secretion by macrophages.

Secretion of interleukin-1β in the 2nd trimester of pregnancy was similar to that of TNF-α. Interleukin-1β secretion was minimum in the absence of labor activity and increased by 8 times in cells obtained after spontaneous abortion (Table 2). In cultures obtained after term labor with normal labor activity, interleukin-1β production decreased 2-fold (Table 2). Stimulators

TABLE 1. TNF-α Production by Cultured Placental Macrophages ($M\pm m$)

Groups	Without stimulators, ng/mg cell protein	Stimulation coefficient	
		PMA	LPS
Trimester II, without labor activity	9.88±1.81	4.09	2.19
with labor activity	242.12±39.79	1.17	0.67
Trimester III, without labor activity	20.06±6.89	2.07	2.56
with labor activity	22.76±4.11	2.21	—
Term labor without labor activity	21.95±2.77	2.43	4.21
with labor activity	18.99±3.84	2.94	2.32

Note. Here and in Tables 2 and 3: dash, data are absent.

TABLE 2. Interleukin-1β Production by Cultured Placental Macrophages ($M\pm m$)

Groups	Without stimulators, ng/mg cell protein	Stimulation coefficient	
		PMA	LPS
Trimester II, without labor activity	10.00±1.35	1.73	1.32
with labor activity	86.38±6.97	1.05	0.83
Trimester III, without labor activity	23.12±2.58	1.98	1.10
with labor activity	--	--	--
Term labor without labor activity	30.32±5.83	1.08	1.26
with labor activity	15.44±1.61	1.15	1.09

TABLE 3. Interleukin-1α Production by Cultured Placental Macrophages (*M±m*)

Groups	Without stimulators, ng/mg cell protein	Stimulation coefficient	
		PMA	LPS
Trimester II, without labor activity	40.66±4.54	1.00	0.87
with labor activity	204.64±12.32	0.75	0.83
Trimester III, without labor activity	17.93±2.12	0.98	1.09
with labor activity	--	--	--
Term labor without labor activity	30.45±6.14	1.25	0.99
with labor activity	13.32±4.40	1.05	1.09

considerably increased production of interleukin-1β in cells during the 2nd trimester of pregnancy without labor activity and had no effect (PMA) or decreased (LPS) secretion of the cytokine during the 2nd trimester of pregnancy with normal labor activity.

Spontaneous labor activity was accompanied by an increase in interleukin-1α secretion by cells of the early gestational age (Table 3). PMA and LPS had no significant effects on interleukin-1α production except for this parameter in cells obtained during the 2nd trimester of pregnancy with labor activity (both stimulators decreased interleukin-1α secretion).

Our findings agree with the data on increased contents of some cytokines in the amniotic fluid after premature labor [8,11,13]. Therefore, we assume that the rise in interleukin-1 and TNF-α levels was due to the increase in secretory activities of placental macrophages. By contrast to other authors reporting LPS-induced stimulation of interleukin-1α production by placental explants [5], we did not observe such stimulatory effects of LPS on cultured placental macrophages. This probably indicates the contribution of other placental cells in *in vivo* interleukin-1α production. This also might be due to *in vivo* prestimulation of cells with LPS (for example, during bacterial infections), which decreases the efficiency of this stimulator *in vitro*.

PMA and LPS were used in *in vitro* experiments to reveal some differences in cell responses to exogenous factors. These differences were most pronounced during the 2nd trimester of pregnancy: the stimulation coefficient in the group without labor activity was higher than in the group with labor activity. Hence, exogenous factors (infection) activating placental macrophages can cause spontaneous abortion during these gestational periods. After term labor without labor activity, the coefficient of LPS-induced stimulation was higher for TNF-α and interleukin-1β, while PMA affected only interleukin-1α production.

The data agree with our previous *in vitro* experiments demonstrated enhanced expression of class II MHC antigens on the surface of placental macrophages and their high phagocytic activity in groups with increased cytokine production. This indicates activation of placental macrophages in spontaneous abortion.

Low activation of cells obtained after term labor with spontaneous labor activity is surprising, because normal term labor are known to be accompanied by increased production of some cytokines, particularly interleukin-1 and TNF-α, by the fetoplacental complex [5,11,12]. It can be hypothesized that amniotic fluid contains cytokines released by other gestational tissues. However, this problem requires further investigation.

REFERENCES

1. O. V. Pavlov, S. A. Sel'kov, A. V. Selyutin, and M. S. Shamugiya, *Byull. Eksp. Biol. Med.*, **125**, No. 5, 579-582 (1998).
2. S. V. Shirshev, *Usp. Sovrem. Biol.*, **114**, No. 2, 223-239 (1994).
3. J. Hunt, *J. Reprod. Immunol.*, **16**, 1-17 (1989).
4. J. Hunt, M. Scares, M.-G. Lei, *et al.*, *J. Immunol.*, **143**, 1606-1613 (1989).
5. N. Laham, S. Brennecke, K. Bendtzen, and G. Rice, *J. Endocrinol.*, **150**, 515-522 (1996).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265-275 (1951).
7. J. Martal, N. Chene, S. Camous, *et al.*, *Reprod. Fertil. Dev.*, **9**, 355-380 (1997).
8. M. D. Mitchell, M. S. Trautman, and D. J. Dudley, *Placenta*, **14**, 249-275 (1993).
9. E. Norwitz, P. Starkey, *et al.*, *J. Endocrinol.*, **131**, 327-334 (1991).
10. R. Romero, D. Brody, E. Oyarzun, *et al.*, *Am. J. Obstet. Gynecol.*, **160**, 1117-1123 (1989).
11. A. Steinborn, M. Kuhnert, and E. Halberstadt, *J. Perinat. Med.*, **24**, 381-390 (1996).
12. T. Taniguchi, N. Matsuzaki, T. Kameda, *et al.*, *Am. J. Obstet. Gynecol.*, **165**, 131-137 (1991).
13. V. Toder, H. Carp, and D. Strassburger, *Isr. J. Med. Sci.*, **30**, 922-927 (1994).
14. G. Vince and P. Johnson, *Placenta*, **17**, 191-199 (1996).