REVIEWS

Cytokines and Placental Macrophages in Regulation of Birth Activity

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The article reviews present notions on functional activity of cytokines of the fetoplacental complex. Particular emphasis is placed on the role of these molecules in the regulation of gestation processes and in pregnancy incompetence. The mechanism of the involvement of placental macrophages and their products in gestation and delivery is discussed.

Key Words: *placental macrophages; cytokines; birth activity; T helper cells*

Pregnancy incompetence is a topical medical and social problem. At the same time, preterm interruption of pregnancy serves as a model of maternal-fetal relationships impaired by various endo- and exogenous factors. To understand the causes of miscarriage and to design therapeutic approaches, it is necessary to know, which factors lead to preterm maternal rejection of the fetus, and where and when the signal inducing placental disorders and uterine contractions is formed. In this context, it is important to evaluate the mechanisms of cell-cell interactions in the fetoplacental complex and their interrelation with other body cells.

Cytokines mediate cell-cell interactions under physiological and pathological conditions. The cytokine system plays an important role in various processes, including cell growth, differentiation, and cooperation, neuroimmunoendocrine interrelation, hemopoiesis, and angiogenesis [4]. Recent studies showed that cytokines are also involved in birth activity. R. Romero *et al.* [48-50] studied changes in the content of inflammatory cytokines during preterm labor induced by infectious agents. Further *in vivo* [56] and *in vitro* [31, 57,62] studies revealed a correlation between birth activity during term or preterm labor and increased production of some cytokines in the fetoplacental complex. N. Tetruashvili *et al.* [4] summarized current views about the role of cytokines in gestational processes and in induction of spontaneous abortion and proposed a nomenclature system for these substances based on their biological effects. The major properties of cytokines, including inducible pattern of their production and reception, cascade effects, local functioning (under normal conditions), and interrelation between components of the cytokine system, allow us to hypothesize that this system is involved in the regulation of birth activity.

New experimental data modify the notions about the role of the cytokine system in the regulation of birth activity. It was previously believed that increased production of cytokines in the uterine is the sign of preterm labor caused by infection [14,47-49]. However, recent studies showed that this phenomenon is typical of spontaneous labor independently on the cause and gestational period [30,56]. These data suggest that the mechanisms of preterm and term labors are similar.

Cells and tissues of the fetoplacental complex are the source of biologically active substances, including cytokines [5,38]. Cytokines play a role in reproductive processes during pregnancy and maintain normal development of the fetus [5,35]. It was reported that the

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contents of interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor (TNF) changes in labor [6,29,30, 69,70].

The uterine origin of these regulatory molecules is confirmed by the ability of fetoplacental tissues to produce the above mentioned cytokines. There is a great body of data indicating that the content of TNF-α in the amniotic [30] and cervicovaginal [55] fluids increases during preterm and term labors, respectively. It was also shown that activity of IL-1, IL-6, and IL-8 in the amniotic fluid increased during preterm and term labors [29,31,32]. It should be emphasized that the increase in IL-1 activity in the third trimester of pregnancy is primarily related to changes in IL-1α content, while during labor this elevation is due to a rise of IL-1β concentration [38]. R. Romero *et al.* [48-51] reported that activity of inflammatory cytokines increases during preterm labor due to intraamniotic infection. High concentrations of IL-1, IL-6, and IL-8 were found in the cervicovaginal fluid of women with spontaneous term or preterm labor [56,57,61]. Accumulation of TNF-α, IL-1β, IL-6, and IL-8 in the lower uterine segment revealed in coming to term labor suggests that these cytokines are involved in birth activity [69,70].

In vitro experiments showed that gestational tissues produce those cytokines, whose concentrations correlate with birth activity. It was shown that IL-1α and IL-6 are released from cultured placental explants and fetal (extraembryonic) membranes, and their enhanced production is associated with the onset of labor [31,32]. These tissues are also the source of IL-8 [33]. It was reported that cultured placental explants produce IL-1 (primarily IL-1β), and this production after spontaneous labor is higher than before spontaneous onset of labor [62]. TNF-α is *in vitro* produced by choriodecidual and placental explants [30]. Cytokine production by gestational tissues was confirmed by polymerase chain reaction (PCR). These studies demonstrated not only the presence of TNF-α, IL-1β, IL-6, and IL-8 mRNA, but also the increase in their content after the onset of labor [7,17]. Hence, molecules involved in the initiation and/or regulation of birth activity are produced by the fetoplacental complex.

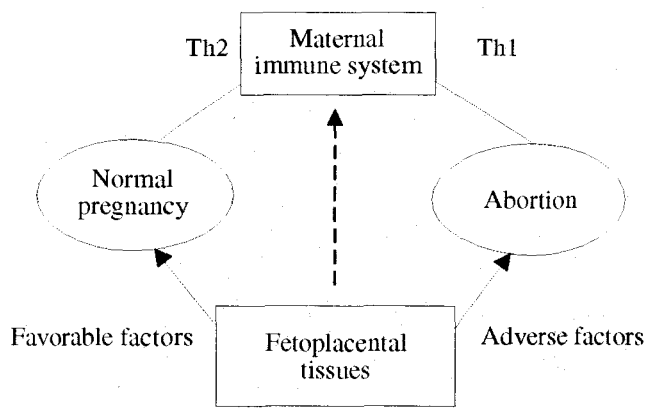

Fig. 1. Two hypothetic pathways of cytokine-mediated regulation of pregnancy.

It remains unclear whether the increase in the content of some inflammatory cytokines in the uterus is the cause or the consequence of uterine contractions. The majority of authors believe that cytokines of the fetoplacental complex can trigger some reactions inducing uterine contractions. This is confirmed by the fact that the content of cytokines stimulating production of prostaglandins (PG) E_2 and $F_{2\alpha}$ playing a key role in initiating birth activity increases during labor [11,23,38].

Thus, the intrauterine origin of cytokines during pregnancy is beyond doubt. The question arises: which cell source provides normal levels of cytokines in the uterus during pregnancy and their changes in preterm and term labors? It was believed that decidual cells are the main producers of some cytokines. At present, attention is focused on the placenta, the organ with abundant cytokine network [5,38]. Some authors reported that placental cells can maintain the necessary concentration of cytokines in the uterus. Various cells of chorionic villi, including trophoblast cells (syncytiotrophoblast and cytotrophoblast), mesenchymal cells, and resident macrophages, synthesize cytokines.

Until now, fetoplacental macrophages (FPM) and their functions received little attention. It is clear that among various cell populations of placental tissues, FPM play a role in the regulation of reproductive processes. In respect to induction of birth activity during preterm and term labors, FPM are of special interest due to their peculiar properties.

Placental macrophages (Kashchenko—Hofbauer cells) belong to the mononuclear phagocyte system presented by various cell populations of the same origin in many organs and systems. The majority of immunocompetent cells in the placenta are placental macrophages [12,42]. Some authors reported that macrophages constitute 40% of neutrophilic cells in chorionic villi [22].

Macrophages are pluripotent cells possessing a variety of biological functions. In the fetoplacental complex, mononuclear phagocytes are responsible for removal of cell detritus, antimicrobial protection, and fetal protection from the maternal immune response [25]. They are also involved in the realization and modulation of the immune response and protect the fetus from infections by providing nonspecific immune reactions and producing regulatory signals for specific immune reactions. On the one hand, FPM are effector cells contacting with infectious agents and other products entering the body (the mother—placenta—fetus system). On the other hand, FPM activated

by this contact produce many soluble signal molecules modulating function of adjacent cells.

The content of cytokines (TNF-α, IL-1β, IL-6, and IL-8) changes with the onset of spontaneous labor. It is assumed that these macrophage-derived cytokines stimulate the synthesis of PG and, therefore, initiate uterine contractions [36,37,49-51]. Moreover, macrophages also produce PGE_2 and $PGF_{2\alpha}$ affecting the myometrium [43,64] and secrete transforming growth factor-α (TGF-α) playing a role in embryonic morphogenesis [65] and regulating functions of the myometrium and trophoblast [26,40].

Recent *in vitro* experiments confirmed the involvement of placental cells in birth activity: secretion of TNF-α by macrophages and production of IL-1β and IL-6 by placental endotheliocytes increase during labor [58].

These data indicate that placental cell, including macrophages, contribute to the accumulation of cytokines in the fetoplacental complex during spontaneous labor.

In parallel with studies of the local regulation of birth activity by paracrine secretion of cytokines in the uterus, considerable attention is now focused on the effects of systemic immune response on the outcome of pregnancy. The hypothesis is based on the existence of two types of the immune response mediated by Th1 and Th2 helper cells. Th1 cells produce interferon-γ, TNF, IL-2, and IL-12 and induce the cell-mediated immune response, while Th2 cells secrete IL-4, IL-5, IL-6, IL-9, and IL-10 and induce the humoral immune response [39]. Th1 and Th2 immune reactions are induced by various infectious agents and inhibit each other. Previous studies showed that the type of the immune response probably determines the outcome of pregnancy [45]. It is believed that Th2 immune response predominates during normal pregnancy, while the shift from the humoral to cell immune response (Th2→Th1) causes spontaneous abortion [24,46]. Various infectious agents inducing Th1-type immune response are the most probable exogenous factor contributing to this shift. There are data that Th1-type immune response caused by infection increases the incidence of spontaneous abortion in mice [28]. It is believed that cytokines typical of Th1 immune response directly damage the placenta or activate cytotoxic cells [45].

Thus, we assume that the cytokine-mediated regulation of pregnancy is performed at two levels (Fig. 1). Local regulation is realized via production of regulatory molecules by fetoplacental tissues (placental cells). Systemic regulation involves maternal immune cells. Probably, these levels of regulation are interrelated and interact with each other. This assumption is confirmed by the existence of endogenous modulatory signals determining the type of the systemic response. The deficiency or excess of immunoregulatory molecules, in particular soluble factors produced by placental cells, cytokines (including IL-10 and IL-12), and $TGF-\beta_2$, changed the type of the immune response [39,45]. Local excess of some cytokines during activation of T cells can determine the shift of the immune response [45]. These immunomodulators are probably produced by B cells, dendrite cells, and macrophages possessing the antigen-presenting activity and, therefore, initiating Th1 and Th1 immune responses by production of certain cytokines.

So, FPM attract much recent attention. These antigen-presenting cells serve as a link between the local and systemic levels of cytokine-mediated regulation. FPM secrete IL-10, IL-12, and TGF-β shifting Th1/Th2 immune response.

The data suggest that FPM activated by intraamniotic infection secrete cytokines. Some cytokines accumulating in the fetoplacental complex contribute to their local excess, while others shift the systemic immune response from Th2 type characteristic of normal pregnancy to Th1 type adversely affecting the outcome of pregnancy [45,46]. These changes can result in preterm labor.

Our previous studies showed that cultured FPM obtained after spontaneous abortion produce much more TNF-α, IL-1α and IL-1β (by 20, 8, and 5 times,

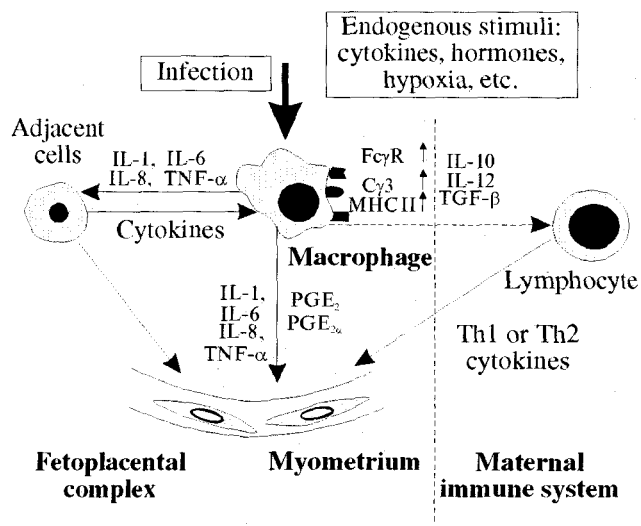

Fig. 2. Role of placental macrophages in initiation of birth activity. Exogenous or endogenous signal activates macrophage. This stimulates expression of surface cell membrane antigens (MHC II, FcγR, and CR3) and changes cytokine production. Cytokines secreted by an activated macrophage directly affect myometrium or modulate production of biologically active molecules by other cells of the fetoplacental complex (decidual cells and trophoblasts) and/or maternal immune system, which directly or indirectly cause uterine contractions. FcγR: receptors for Fc fragment of IgG; CR3: receptors for C3bi complement component; MHC II: major histocompatibility complex class II antigen; PG: prostaglandins; IL: interleukins; TNF: tumor necrosis factor; TGF: transforming growth factor.

respectively) than placental macrophages of the same gestational age (the second trimester of pregnancy) in the absence birth activity [3].

Probably, term labors are realized by the same mechanisms triggered by endogenous (cytokines, hormones, O_2 concentration, *etc.*) instead of exogenous (infection) signals. It is known that morphological and functional characteristics of FPM are very sensitive to activators and inhibitors [64]. Therefore, modulation of the production of cytokines or other signal molecules by tissues and cells of the fetoplacental complex can affect functions of macrophages.

Recent data indicate that local O_2 concentration is an important regulatory factor. Local hypoxia considerably changes production of cytokines and other factors by peripheral mononuclear cells [41], tissue macrophages [63], placental tissue [8,27], and FPM [67]. *In vitro* studies of secretory activity and other functions of placental cells under hypoxic conditions seem to be promising for elucidating the mechanisms of many obstetrical pathologies characterized by hypoxia of placental tissues and changes in the production of cytokines by placental cells [15].

Figure 2 illustrates possible role of FPM in regulatory processes causing uterine contractions and rejection of the fetus during preterm and term labors.

Recent studies indicate that spontaneous labors irrespective on the gestational age are controlled by the same humoral mechanisms, in which cytokines play a key role. Changes in the secretion of some cytokines accompanying birth activity prompted investigation of peculiarities of cytokine production by tissues and cells of the fetoplacental complex and identification of the cell source modulating this cytokine response.

These investigations are hindered by some peculiarities of the studied object. The placenta and membranes responsible for the maintenance and normal development of the fetus are temporal structures, which can be studied only after labor. The models based on tissue and cell cultures are promising for studies of fetoplacental cells [1-3,31-33,58,62].

In vivo and *in vitro* studies undoubtedly proved the involvement of immunocompetent cells in reproductive processes. The role of cytokines, which were believed to be responsible for the communication of immune cells, is now revised. It is obvious that the present nomenclature for growth factors, cytokines, and hormones is rather conventional and does not reflect adequately their pleiotropic properties. This primarily concerns local effects of these factors. For example, in reproductive biology TGF-β received the name "growth factor", while in immunology it is termed "cytokine". Evidently, all borderlines between various medical and biological sciences will disappear with refining the understanding of biological processes.

FPM deserve more attention than has been accorded them in the past. It was believed that these cells are tissue macrophages and their functions and properties are similar to those of mononuclear phagocytes. Specific localization of FPM was not considered. Recent experiments indicate that FPM play an important role in reproductive processes due to functions typical of immunocompetent cells. C. Vince and P. Johnson [64] reported that uteroplacental macrophages are multipotent cells with a wide range of biological effects. Our experiments [2,3] and published data confirm the key role of FPM in the regulation of birth activity. Here we considered only one aspect of FPM functioning: their involvement in the cytokine-mediated regulation of labor. However, this problem requires further detailed analysis. It is hoped that the present line of investigation combining immunology, reproductive biology, and cell biology will elucidate cell and molecular mechanisms underlying birth activity and solve the problem of incompetent pregnancy.

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