REVIEWS

Modern Concept on the Role of Phagocytes in the Pathogenesis of Complications during Pregnancy

G. T. Sukhikh, V. G. Safronova*, and L. V. Van'ko

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Here we review modern concept of the role of phagocytes, the key cell component of natural immunity, in the course of pregnancy and in the pathogenesis of its complications. Phagocytes contribute to the development, maintenance, and favorable outcome of pregnancy. These cells play a role in the pathogenesis of various pregnancy complications, including fetal growth retardation, late gestosis, and intrauterine infections. The understanding of the pathophysiological mechanisms that occur in the mother—placenta—fetus system would allow us to improve diagnostic procedures, perform pathogenetically substantiated therapy, and decrease the incidence of obstetrical complications.

Key Words: *pregnancy; complication; natural immunity; phagocytes*

NORMAL PREGNANCY

Normal pregnancy is accompanied by considerable changes in the maternal immune system. There are two concepts regarding immunological relationships between the mother and fetus. According to the classical concept "fetus as allotransplant", an important role in maternal-fetal relationships is played by adaptive immune mechanisms, which are similar to those observed between the recipient and genetically foreign transplant [54]. Immune interactions between the mother and fetus are weak due to inability of fetal cells to present antigens to maternal lymphocytes or suppressed functional activity of maternal lymphocytes. This concept was developed in many studies [4]. It was hypothesized that the immunity in pregnancy is realized via the humoral, rather than cellular pathway. Th2 cytokines suppress the Th1 response thus improving fetus survival, but inhibiting the reaction to some pathogens [99], *i.e.*, pregnancy is an immunosuppressive state. However, many considerable changes in the maternal immune system cannot be explained. It should be emphasized that many comparative studies of immune reactions in pregnancy and transplantation often ignored the peculiar features of pregnancy. Pregnancy is developed in a highly specialized organ (uterus). The influence of pregnancy on the maternal organism increases with time. Many hormonal, lipid, and cytokine products indistinguishable from extrauterine compounds may enter the maternal venous system and, therefore, directly regulate various maternal systems [72]. Maternal-fetal immune interactions proceed in the decidua and blood.

An alternative concept was recently developed. According to this concept, suppression of the specific immune response in the maternal organism is accompanied and, probably, compensated by activation of nonspecific innate immune reactions [33,72,91,104]. This concept suggests dysregulation of the innate and specific immunity in the maternal organism, when monocytes, but no lymphocytes, play a central role in immunological adaptation of the mother [33,72,91]. T cells may be of greater importance in implantation,

Research Center for Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, Moscow; *Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino. ***Address for correspondence:*** safronova@icb.psn.ru. Safronova V. G.

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when their relative number in the decidua is high, but not in immunological maintenance of pregnancy [91]. Particular attention is given to systemic activation of innate immune components during pregnancy. For instance, monocyte count increases, phagocytosis and secretion of monocyte chemotactic peptide 1 and interleukin-12 (IL-12) in response to endotoxin become more intensive, expression of surface CD14, CD11b, and CD64 and the concentration of intracellular reactive oxygen species (ROS), plasma neopterin, and tumor necrosis factor-α (TNF-α) also increase. Changes in the granulocyte system include an increase in their count, intensive phagocytosis, enhanced expression of surface CD14, CD11b, and CD64, and high concentrations of intracellular ROS, alkaline phosphatase, plasma lactoferrin, and elastase [72]. Activity of natural killer cells playing an important role in implantation is suppressed [45]. Large granular endometrial lymphocytes play a role in the implantation and maintenance of pregnancy. During implantation these cells constitute 70-80% endometrial leukocytes [91]. Pregnancy is accompanied by an increase in the count of phagocytizing monocytes in the blood, intensification of surface expression of FcγRI (CD64) and FcγRII (CD32) on monocytes, and activation of FcγR-mediated reactions. However, surface expression of MHC class II molecules remains unchanged [33]. These cells are highly potent in absorbing IgG-opsonized human erythrocytes.

Human uteroplacental tissues display complex cytokine activity. Leukocytes and non-leukocytic cells produce various cytokines [71,91,104]. Cytokines play an important role in modulation of the immune response to infections and in the development and maintenance of pregnancy. Th2 cytokines are necessary for successful implantation and pregnancy maintenance, while the presence of Th1 cytokines is associated with termination of pregnancy and infertility. However, the Th1 response determines the outcome of acute infections [104]. Cytokine activity should be regulated locally to prevent pathological changes, since disturbances in the Th1 response contribute to immunological reproductive disorders in women [91]. Neutrophils that *in vitro* and *in vivo* produce interferon-γ determine immune reactivity in the female reproductive system [104].

The course of chronic inflammatory diseases changes during pregnancy, which indicates that the activity of phagocytes playing an important role in natural immunity undergoes considerable variations [25,26]. Pregnancy suppresses the development of rheumatoid arthritis due to inhibition of neutrophil activity [25]. Attenuation of the respiratory burst in polymorphonuclear leukocytes (PMNL) during pregnancy probably determines better state of patients with rheumatoid arthritis [29]. Biochemically, functional inactivation of neutrophils is related to decreased content of fatty acids in cells [26], inhibition of phospholipase A_2, and suppression of leukotriene B_4 release [25].

Published data do not allow drawing conclusions about changes in the state of granulocytes and neutrophils during pregnancy. Some authors believe that blood leukocytes and granulocytes are stimulated during late pregnancy [4,13], while others reported that functional activity of neutrophils decreases in this period [25,26,29]. Discrepant experimental results and different approaches to evaluating the role of innate and adaptive immunity in the development and course of pregnancy give reasons for wide discussions. During pregnancy the natural immune system performs one of the major immune functions: it ensures organism survival [72,91]. Studies of the role of the natural immune system and its specific components (*e.g.*, phagocytes) would elucidate the mechanisms of immunological adaptation in the maternal organism, formation of the fetal immune system, and immune interactions between the mother and fetus. Deviations from the normal and impaired control over these processes in regulatory and intracellular signal systems may cause clinical problems in pregnant women. Here we review the role of phagocytes in the pathogenesis of pregnancy complications.

COMPLICATED PREGNANCY

Habitual abortion

Immune disturbances in women with repeated spontaneous abortions of unknown etiology are widely studied. Habitual abortions are associated with increased Th1 activity, while predominance of Th2 activity is typical of normal pregnancy [48]. Pregnancy-specific glycoprotein affecting IL-10 secretion acts as a local factor modulating the inflammatory Th1 response in the endometrium of women with habitual abortions of unknown etiology. Expression of genes for glycoprotein in the endometrium during the preimplantation period correlates with the risk of pregnancy loss in women with habitual abortions of unknown etiology [14]. Previous studies showed that natural immunity is involved in the pathogenesis of pregnancy loss. The subpopulation of blood leukocytes and their state in the decidua were studied in women with habitual abortions. During uncomplicated pregnancy and repeated spontaneous abortions, leukocytes with normal karyotype are activated to a greater degree than leukocytes with trisomy 16 in their karyotype (fatal disorder). In women with repeated spontaneous abortions of unknown cause the count of natural killer $CD56^+$ cells in the decidua is lower than during normal pregnancy [62].

Early spontaneous abortions are accompanied by the inflammatory reaction in maternal *d. parietalis* and *d. basalis* with high count of maternal macrophages, large granular lymphocytes, and T cells [92]. Various immunological parameters of the peripheral blood, including the number of $CD3^+$, $CD4^+$, $CD8^+$, $CD3^+$/$HLA\text{-}DR^+$, and $CD16^+/CD56^+$ cells, proliferative response of lymphocytes to mitogens and alloantigens, and chemiluminescence of neutrophils, were studied in nonpregnant women with a history of habitual abortions and in healthy nonpregnant women with a history of normal pregnancies. In nonpregnant women with a history of habitual abortions changes in the populational composition of peripheral blood T lymphocytes manifested in a lower count of $CD8^+$ cells and higher ratio of $CD4^+/CD8^+$ cells compared to healthy nonpregnant women with normal pregnancies. However, functional activity of lymphocytes and neutrophils remained unchanged [49]. It was hypothesized that nonpregnant women with repeated habitual abortions of unknown etiology and healthy pregnant women have various immunological statuses. These differences are related to humoral immunity and manifested in the absence of blocking antibodies in the plasma and presence of antinuclear and anticardiolipin antibodies in women with habitual abortions of unknown etiology [50].

Massive chronic intervillousitis is a rare placental disease leading to fetal growth retardation and unfavorable pregnancy outcome. These changes were believed to be associated with spontaneous abortions or repeated pregnancy loss. One patient had 10 spontaneous abortions and repeated massive chronic intervillousitis [34]. Histological characteristics of the placenta included massive infiltration of the maternal intervillous space with cells of chronic inflammation and fibrin. The cellular infiltrate was presented by large granular lymphocytes, immunoreactive $CD68^+$ cells, individual $CD45RO^+$ compatible with monocytes and macrophages, and T lymphocytes (rarely). Two pregnant women had high content of maternal serum α-fetoprotein. The present study supports the hypothesis that this placental disorder is associated with immune disturbances and can serve as a histologically verified cause of habitual abortions.

Antiphospholipid syndrome

Little is known about the role of phagocytes in pregnancy disorders associated with antiphospholipid syndrome (APS). Pregnancy loss in women with APS results from repeated thromboses and thrombocytopenia. The mechanism of thrombosis remains unclear. Cell glycoprotein, a tissue factor expressed by monocytes, is the main initiator of coagulation *in vivo*. Expression of monocyte tissue factor plays a role in the pathogenesis of thrombosis in patients with primary APS [30]. Pregnancy loss in women with APS is related to induction of monocyte tissue factor due to the activation of β_2-glycoprotein-I-specific T lymphocytes. Induction of monocyte tissue factor was not observed in patients with antiphospholipid antibodies, but without APS [93]. It was reported that patients with APS have circulating $CD4^+$ (Th1) T cells that undergo proliferation and secrete interferon-γ after *in vitro* stimulation with β_2-glycoprotein-I. Therefore, induction of monocyte tissue factor with β_2-glycoprotein-I requires the presence of T lymphocytes $CD4^+$ and MHC class II molecules. Antiphospholipid antibodies can stimulate the release of platelet-activating factor in patients with low acetylhydrolase activity, which probably contributes to preterm labor [8].

Fetal growth retardation

Little is known about the role of phagocytes in the pathogenesis of pregnancy complications leading to fetal growth retardation (FGR). Particular attention is given to studies of its consequences in the postnatal period. FGR and gestosis are associated with similar developmental disorders of the placenta [70]. FGR can result from inadequate blood supply to the fetus due to disturbances in implantation and growth of the placenta. Macrophages participate in these processes [66, 72,84]. Growth factor expressed in vascular endothelial cells plays a role in angiogenesis in the maternal and fetal placenta. It should be emphasized that macrophages are the primary source of this factor [77]. Previous studies showed that during normal pregnancy the intensity of division of fetal blood mononuclear cells decreases with gestational age. However, in fetuses with chromosomal aberrations the rate of cell division and count of erythroblasts markedly increased. These parameters decreased in fetuses receiving erythrocytes. In fetuses with FGR the number of erythroblasts increased, while the count of monocytes remained unchanged [87]. During normotensive pregnancy and FGR, apoptosis in neutrophils was delayed (compared to cells from women with normal pregnancy) [31].

Late gestosis

Late gestosis is the main cause of perinatal morbidity and mortality. Its clinical manifestations include edema, proteinuria, nephropathy, arterial hypertension, preeclampsia, and eclampsia [5]. Gestoses of various types and severities are immune-related disorders. The severity of gestosis in various stages of pregnancy should be evaluated to make the decision on preterm delivery

stimulation and to predict the outcome of pregnancy [12]. The increase in the concentration of proinflammatory cytokines and degree of hemodynamic disturbances are proportional to the severity of gestosis [3]. Gestosis is accompanied by changes in immunological parameters, including those related to functional activity of phagocytes. Production of IL-1α, IL-1β, IL-8, and TNF-α is markedly intensified during late gestosis [3]. Phagocytes act as the source and target for these cytokines. Studies of functional activity of the immune system during pregnancy complicated by late gestosis revealed a decrease in the total count of T cells, intensification of spontaneous proliferation, increase in phagocytic activity of venous blood monocytes, and stimulation of spontaneous and LPS-induced production of TNF-α [13]. The development of gestosis is accompanied by changes in the count of cells reactive to placental proteins. Most these cells were presented by neutrophils [10]. Various immunological tests, including studies of circulating immune complexes, nitro blue tetrazolium test, and measurements of the cytological index for neutrophil activity, can be used to estimate the severity of gestosis [12].

Gestosis proceeds via two pathogenetic stages. Stage I includes processes that reduce the size of spiral arteries, which results in shallow trophoblast invasion into these vessels during the development of placentas [20,21,64,67,90]. Consequences of placental ischemia appear in stage II. This period is characterized by dysfunction of the maternal endothelium [64, 90]. FGR and gestosis result from impaired relationships between maternal and fetal cells in the placenta. Their pathogenesis is considered taking into account the concept "fetus as allotransplant" [20,64] and role of the natural immune system [20,65]. Factors that suppress the development and adherence of the placenta can abolish maternal tolerance to genetically foreign fetus, which at least results in immune-related abortions [64]. The regulation of trophoblast invasion depends on internal processes (*e.g.*, production of proteolytic enzymes and expression of MHC class I antigens) and the state of maternal cells in the decidua [20]. Leukocytes are an important component of the decidua in human uterus. During the first trimester leukocytes are presented by granular lymphocytes, macrophages, and T lymphocytes. They activate natural killer cells, secrete cytokines, present antigens, and are responsible for immunosuppression [20,91]. Suppressed invasion of the trophoblast into uteroplacental spiral arteries is associated with the presence of excessive amounts of macrophages inside and around these vessels [66]. As differentiated from normal pregnancy, during gestosis a negative correlation was found between local infiltration with macrophages and trophoblast invasion. The presence of excess macrophages in the placenta of women with gestosis can attenuate invasion of the extravillous trophoblast into segments of spiral arteries. This is realized via apoptosis that results from TNF-α secretion and tryptophan deficiency [67].

Migration of the intravascular trophoblast and maternal macrophages is impaired during gestosis [21]. Myeloid cells expressing HLA-DR antigens were assayed in the peripheral blood of the mother and fetus and the placenta from women with normal pregnancy and gestosis. Studies of peripheral blood monocytes from women with normal pregnancy and gestosis revealed no considerable changes in the size of $CD14^+$/$CD16^+$ monocytes. The count and localization of macrophages in the placental villi were similar. In women with normal pregnancy the basal layer of normal decidua contained numerous $CD14^+$ and $HLA\text{-}DR^+$ tissue macrophages carrying mannose receptors. However, in women with gestosis these phagocytizing cells were not found in the decidua [21]. The mechanisms underlying activation and recruitment of macrophages into the placenta remain unclear. It cannot be excluded that these mechanisms play a central role in the pathogenesis of gestosis [66].

It was believed previously that immune mechanisms are not involved in stage II of gestosis and activation of neutrophils contributes to the development of endothelial damages [64]. Numerous studies indicate that components of innate immunity play a role in the pathogenesis of gestosis. The syndrome of gestosis is similar to the systemic inflammatory response. Recent studies showed that gestosis is accompanied by pronounced activation of circulating monocytes and granulocytes [65]. Local inflammatory reactions in the uteroplacental area can contribute to the pathogenesis of gestosis [56]. In patients with gestosis the absolute count of neutrophils was much higher than in women with normal pregnancy. It should be emphasized that in women with severe gestosis the increase in cell count was more pronounced than in patients with moderate gestosis [47]. The intensity of apoptosis in peripheral blood neutrophils decreases in women with normal pregnancy, FGR with normal blood pressure and, to a greater extent, in patients with gestosis (compared to nonpregnant women). Suppression of apoptosis in neutrophils probably contributes to high neutrophil count in normal pregnancy. In women with gestosis activated neutrophils can circulate for a long time after labor [31].

Plasma lactoferrin content and stimulated expression of CD11b, CD18, and L-selectin (CD62L) did not differ in women with uncomplicated pregnancy and gestosis. Moreover, baseline production of superoxide, expression of CD11b, CD18, and L-selectin, and secretion of lactoferrin were similar in these women

[27]. These data suggest that activation and priming of circulating neutrophils do not proceed in pregnant women with gestosis *in vivo*. Priming of neutrophils suggests exposure to factors (*e.g.*, cytokines) that do not activate cells, but potentiate their further stimulation. Other authors reported that in neutrophils from women with FGR and gestosis expression of CD11b is higher, while expression of CD62L is lower than in cells from healthy pregnant women [70]. Gestosis is associated with intensification of CD11b expression on granulocytes and monocytes and inhibition of CD62L expression on granulocytes. It should be emphasized that basal secretion of intracellular ROS increases in monocytes, but not in granulocytes. However, the respiratory burst is enhanced in cells of both types [36]. In women with gestosis basal expression of CD11b markedly increases before parturition compared to women with normal pregnancy [15]. The increase in basal expression of CD11b in women with gestosis probably reflects *in vivo* neutrophil activation. In women with gestosis a positive correlation was found between CD11b expression on neutrophils and uric acid level in the plasma. Therefore, the severity of gestosis correlates with the degree of neutrophil activation [15]. Intensive generation of ROS indicates that neutrophils play the major role in oxidative stress during gestosis [27]. Severe gestosis complicated by HELLP syndrome is characterized by suppressed spontaneous production of ROS, which probably results from exhaustion of the cellular response by plasma factors in these patients [106].

Neutrophil activation during gestosis manifested in a systemic increase in myeloperoxidase activity [57]. In patients with gestosis enzyme activity was much higher than in women with normal pregnancy. However, these women did not differ in the content of lactoferrin. Migration of phagocytes is intensified in pregnant women. During gestosis the intensity of this process was lower than in women with normal pregnancy [17].

Published data show that gestosis and FGR are related to similar placental disorders [70]. In women with gestosis and FGR the concentration of plasma L-selectin markedly surpassed the control. No differences were found in the contents of CD11b, CD62L, and plasma L-selectin in women with gestosis and FGR.

Repeated attempts were made to determine the cause of neutrophil hyperactivation during gestosis. The activation of neutrophils estimated by expression of CD11b and CD18 after incubation with the autologous plasma before and after parturition was induced by a stable circulating factor [16]. It is hypothesized that activating factors are secreted and released from the placenta [98]. Placental factors can activate neutrophils due to generation of superoxide and modulation of expression of adhesive molecules [98]. During gestosis plasma elastase activity is higher than in women with normal pregnancy. This reflects a greater activation of neutrophils in patients with gestosis. A correlation was found between elastase activity, endothelin-1 content, and systolic and mean blood pressure. These data indicate that activation of neutrophils during gestosis is associated with the influence of endothelin-1 [39].

Gestosis is accompanied not only by pronounced activation of neutrophils, but also by stimulation of other granulocytes, monocytes, and macrophages [72]. It should be emphasized that high activity is typical of maternal and fetal monocytes [84]. Spontaneous term labor and preterm delivery during gestosis and HELLP syndrome (hemolysis, high activity of liver enzymes, and low platelet content) are accompanied by intensive production of IL-6 by fetal monocytes, which reflects strong activation of these cells [84]. The activation of fetal monocytes as effectors of natural immunity is probably involved in the mechanisms of spontaneous and term labor. Gestosis can be associated with immune dysfunction in the mother and fetus [84]. Intact plasma IL-2 expressed by antigen-presenting cells, including mononuclear phagocytes, is more frequently detected in patients with gestosis [32]. Plasma IgG and IgA levels in women with gestosis are lower than in the control. These patients are characterized by prestimulation of macrophages *in vivo* (expression of transferrin receptors) [18]. During gestosis thromboxane A_2 content in mononuclear cells is much higher than in women with normal pregnancy. However, prostacyclin concentration tends to decrease in patients with gestosis [23]. Chemiluminescence of monocytes decreases or remains unchanged during normal pregnancy, but markedly increases in women with gestosis [17]. There is no evidence for the involvement of granulocytes in the pathogenesis of gestosis [42]. The density and volume of platelets undergo considerable changes in women with gestosis. These data suggest that severe gestosis is characterized by an increase in the average volume of platelets.

Increased phagocyte activity can be associated with complement activation. In women with severe gestosis the content of C5a, elastase activity in neutrophils, and neopterin concentration during labor are higher than in women with normal pregnancy [38]. Severe gestosis is characterized by systemic complement activation. The only exception is fragment C4, whose amount in patients with gestosis is lower than that in women with uncomplicated pregnancy [57]. Other authors reported that the content of fragments C3 and C4 remains unchanged in women with gestosis [17]. The amount of complement C3d markedly increases during severe gestosis [51].

Oxidative stress that accompanies gestosis can be associated with intensive generation of ROS, impaired regulation of this process [27], and decrease in the activity of antioxidant enzyme [95]. The respiratory burst is enhanced in women with gestosis, which is probably related to a decrease in the concentration of circulating gravidin that acts as the endogenous inhibitor of phospholipase A_2 [28]. Intensification of TNF-α and lipid peroxide production in the placenta due to inhibition of placental antioxidants suggests the loss of fetal protective systems. The intensity of lipid peroxidation (LPO) in the peripheral blood increases. Moreover, the concentrations of monocytic IL-6, IL-8, and TNF-α increase. Stimulated monocytes generate free radicals that cause oxidative damages. Plasma and intracellular antioxidants protect maternal cells. Gestosis is accompanied by an imbalance between oxidative and antioxidant agents. Changes in membrane oxidation affect membrane stability. Genetic differences in secretion of TNF-α and NO also modify the course of this disease [95]. In women with gestosis LPO in the plasma, plasma leukocytes, and urine is intensified before and after parturition, which indicates that they are predisposed to oxidative stress that contributes to weak inflammatory reactions [16]. Plasma IL-6 level is high in women with severe gestosis, which probably determines exhaustion of plasma antioxidants and hemolysis [81]. High expression of cyclooxygenase-1 in the placenta is probably responsible for pathophysiological changes in gestosis [100]. Gestosis is characterized by the presence of neutrophil-activating agents, which intensifies superoxide generation and probably contributes to the development of pathophysiological changes [88].

Hyperactivation of granulocytes and monocytes produces damages to endothelial cells, which is mediated by the inflammatory response. These changes are terminal manifestations of gestosis. The process is maintained by various infections [72]. The mechanisms of changes in endothelial cells during gestosis remain unclear. Probably, in women with gestosis the placenta produces modulators that enter the maternal blood system and affect functional activity of the endothelium [97]. In women with gestosis adhesion of neutrophils to cultured endothelial cells of the umbilical vein was more intensive than during normal pregnancy [96,97]. This is probably associated with the effect of platelet-activating factor [97]. Moreover, in cultured endothelial cells of the umbilical vein from patients with gestosis expression of P-selectin was higher, while expression of ICAM-1 was lower than in cells from women with normal pregnancy. Expression of VCAM-1 and E-selectin did not differ in women with normal pregnancy and gestosis [96]. Intensive adhesion of neutrophils to the endothelium induced by placental factors from women with gestosis is probably related to increased expression of surface adhesive CD11 molecules [98]. Other authors reported that the plasma from women with gestosis does not contain factors modulating adhesion of neutrophils to endothelial cells via the direct influence on the endothelium. It was demonstrated that the complement system inhibits adhesion [24].

The role of NO in the pathogenesis of gestosis is a controversial issue. Placental ischemia causes dysfunction of the endothelium in maternal vessels, which is followed by intensive formation of endothelin and thromboxane, increase in the sensitivity of vessels to angiotensin II, and suppression of NO and prostacyclin production [37]. However, plasma nitrate concentration increases in women with gestosis, which can reflect intensive production of NO from an unknown source or inhibition of urine nitrate excretion [80]. The concentrations of nitrates, nitrites, and endothelin-1 increase during gestosis. No correlation was found between the contents of these substances. These data indicate the loss of mechanisms that compensate overproduction of NO [94]. Cultured endothelial cells of the umbilical vein from women with gestosis were characterized by changes in membrane permeability for cations and activities of endothelial NO synthase and guanylate cyclase [85].

Blood levels of inflammatory markers and characteristics of the liver and kidneys suggested that in women with normal pregnancy the inflammatory response during parturition is more pronounced than in women with gestosis. The activation of neutrophils increases in women with normal pregnancy, but decreases in patients with gestosis [63].

Fetal infections and gestosis produce pronounced and opposite effects on the phenotype of immune blood cells. Fetal infections were accompanied by accumulation of immature $CD11a^{-}CD20^{+}$, $CD40^{+}$ $CD19^{-}$, and $CD14^{+}$HLA-DR cells. However, the count of naive $CD4^{+}CD8^{+}$ and $CD5^{+}CD19^{+}$ cells and ratio of $CD11a^{+}$, $CD14^{+}$, and $CD14^{+}$HLA-DR^{+} cells decreased in women with gestosis. These data indicate that the ratio between mononuclear cells undergoes considerable variations in the third trimester. Moreover, the effect of gestational age is less pronounced than the influence of other neonatal and maternal factors [44]. Expression of CD15s, CD49d/CD29, and CD31 on neutrophils, as well as expression of CD15s, CD11c, and CD54 on monocytes, decreased in newborns from mothers with gestosis. Activation of neutrophils and monocytes was accompanied by an increase in the content of IL-8 and decrease in the concentrations of soluble E- and L-selectins in the plasma. IL-8 concentration was high only in newborns of mothers with gestosis and high blood pressure. Activation of fetal

neutrophils and monocytes during gestosis is followed by intensive secretion of cytokines, which probably contributes to high mortality associated with this disorder [55].

Intrauterine infections

The stage of placentation can be accompanied by the development of weak inflammatory reactions in the uteroplacental area (without leukocytic infiltration and exudative component) [7]. Macrophages and natural killer cells act as the main effectors in this process. In the first trimester the inflammatory response involves maternal cells. Developing embryonic tissues are characterized by alterative inflammation. Various intrauterine infections, including measles, toxoplasmosis, and cytomegalovirus, damage germ layers and developing organs and produce congenital defects. Immune organs develop at 20 week's gestation, and further productive processes involve mesenchymal cells and exudation from local capillaries. However, the inflammatory reaction is reduced even in the third trimester of pregnancy. Therefore, intrauterine infections are extremely hazardous.

Intrauterine infections are the main cause of fetal infections. These disorders increase the risk of perinatal morbidity, mortality, purulent, and septic complications in the maternal organism. Most studies are devoted to the diagnostics of intrauterine infections. Prospective studies of a correlation between histopathological changes in the placenta, risk factors, and early bacterial infections attract much attention [43]. Invasion of PMNL into the amniochorionic plate, amniotic membranes, and umbilical cord, premature rupture of membranes, low weight of infants, gestational age 34 weeks, unpleasant odor, and hyperthermia during parturition are typical of early bacterial infections in newborns. During microbial invasion of the amniotic sac neutrophils are accumulated in the amniotic fluid [68]. Fluorescence assay specific for the Y chromosome showed that PMNL in the amniotic fluid have X and Y chromosomes (male cells). This confirmes fetal response to intraamniotic infections [74]. In fetuses of mothers with chorioamnionitis detected in the placenta during preterm labor, neutrophils in the bronchi are of the fetal origin [75]. IL-8 concentration in neutrophils increases in response to microbial invasion of the amniotic sac. The content of this cytokine is high during preterm and term labor [68]. Intraamniotic infections are accompanied by intensive production of cytokines in the maternal peripheral blood (IL-8 and TNF-α), umbilical blood (IL-1α, IL-1β, and TNF-α), and amniotic fluid (IL-1β, IL-8, and TNF-α) [2]. High content of TNF-β and interferon-γ in supernatants of *in vitro* cultured blood leukocytes and increased concentration of IL-6 in the mucus and cells of the cervical canal in the second and third trimesters of pregnancy are associated with chorioamnionitis, activation of disseminated intravascular coagulation, intrauterine fetal hypoxia, and preterm labor [11]. Other authors reported that high vaginal concentration of IL-8 (not IL-6), abnormal Gram staining of vaginal flora, absence of H_2O_2-producing *Lactobacillus*, and presence of anaerobic vaginal bacteria can be used as criteria for infections of the amniotic fluid in women with preterm labor [40]. However, measurements of urine IL-8 level cannot serve as the screening test for asymptomatic bacteriuria in pregnancy [78].

Inflammation that accompanies infections can be responsible for preterm labor during prelabor rupture of the amnion. These women have histologically verified chorioamnionitis [76]. Leukocytic infiltration of the chorion is the initial stage of chorioamnionitis, which is considered as polymicrobial infection. Studies of leukocytic enzymes in the amniotic fluid (*e.g.*, esterases) and measurements of IL-6 content are informative for the diagnostics of chorioamnionitis [9]. As differentiated from healthy women, the total count and the ratio of viable vaginal neutrophils and IL-8 content markedly increase in patients with chorioamnionitis [101]. The concept developed by these authors suggests that women, in which viability of vaginal PMNL is equal to or more than 11%, are at high risk of histologically verified chorioamnionitis. These data indicate that PMNL continuously migrate into the vagina. Infection of the amniotic fluid is associated with bacterial vaginosis or intermediate vaginal flora (Gram staining), absence of *Lactobacillus*, and presence of vaginal *Bacteroides ureolyticus* and *Fusobacterium* [40]. Microbial invasion into the amniotic sac and clinical chorioamnionitis were closely related to inflammation of the umbilical cord (funiculitis) accompanied by neutrophilic infiltration of the vascular wall or Wharton's jelly [105]. Funiculitis is associated with infection of the amniotic fluid, congenital sepsis of newborns, and syndrome of the fetal inflammatory response. Infants with umbilical inflammation were small for gestational age and had high plasma IL-6 content in the median umbilical artery, which is considered as a sensitive and highly specific test for funiculitis [105]. However, this hypothesis is open to question. Spontaneous preterm labor is associated with intensive production of IL-6 by myelomonocytic cells [82]. Fetal monocytes serve as the source of IL-6 in term labor. The activation of umbilical blood T cells was not found during spontaneous term labor and uncontrolled premature labor. The increased release of IL-6 from fetal monocytes plays a role in the mechanisms underlying normal term labor, but not premature labor. These data suggest that term and preterm

labors are realized via various mechanisms [82]. Published data show that premature labor is related to phenotypic and metabolic changes in maternal granulocytes and monocytes [35].

During infections and inflammatory processes premature labor is induced by macrophages that serve as the main cell component of the uterus, placenta, and extraplacental membranes [41] and source of various proinflammatory cytokines [91]. Secretion of platelet-activating factor by decidual macrophages plays a role in the pathogenesis of premature labor and preterm rupture of membranes produced by endotoxins and further activation of cytokines (IL-1α, IL-1β, and TNF-α) [58]. Immature pregnancy complicated by endometrial infection is characterized by changes in the subpopulational composition of immunocompetent cells in decidual tissues (increase in macrophage count) and intensive secretion of TNF-α [6]. Previous studies showed that macrophages act as the source of TNF-α, whose release is associated with parturition; placental endothelial cells secrete IL-6 and IL-1β [83]. Intracellular signal pathways involved in the induction of TNF-α by streptococci B in blood monocytes from the umbilical vein were extensively studied. These pathogens induce secretion of TNF-α in host cells by activating signal pathways. These pathways initiate mitogen-activated 38-kDa kinases and transcriptional factors NF-κB and AP-1, which activates genes and prevents apoptosis [89]. The onset of spontaneous delivery during premature and term labors associated with infection can be due to TNF-α-induced stimulation of production of prostaglandins $F_{2\alpha}$ [59] and D_2 [60] by macrophages of the decidual membrane. Clinical studies of procalcitonin (sensitive and specific marker of systemic infections) showed that this substance was more informative parameter for the diagnostics of septic abortion than other inflammatory markers. Peripheral blood monocytes act as a potential source of procalcitonin [69].

Prostaglandins play a particular role in the induction of labor. Fetal membranes and amniotic fluid transduce the signal that initiates uterine contractions and labor. PMNL and decidual macrophages promote premature labor and activate secretion of prostaglandins in fetal membranes. The increase in prostaglandin content contributes to the onset of labor. Activation of monocytes and macrophages by bacterial products stimulates cytokine formation, which initiates prostaglandin synthesis [9]. Some microorganisms, including those causing bacterial vaginosis, produce considerable amounts of phospholipase A_2. Intensive synthesis and release of prostaglandins also contribute to premature labor associated with bacterial vaginosis [1]. Intrauterine infections enhance the release of platelet-activating factor from phagocytes, endotheliocytes, and other cells. Secretion of platelet-activating factor in infected pregnant women is followed by a considerable increase in the concentrations of prostaglandin E_2, lipoxygenase metabolites of arachidonic acid, and leukotrienes B_4 and C_4 in the amniotic fluid, which probably contributes to premature labor [8].

Published data show that neutrophils play a role in premature labor [52,53,103]. During premature labor fetal membranes accumulate considerable amounts of activated PMNL (as confirmed by morphological, enzymatic, and histochemical assays). As differentiated from peripheral blood leukocytes, leukocytes of fetal membranes are characterized by intensive phagocytosis and presence of peroxidase and alkaline phosphatase activities on plasma membranes of phagosomes [53]. Activated vaginal PMNL were present during preterm labor [52,103]. These women had considerable amounts of viable leukocytes [103]. Morphological and cytochemical assays demonstrated that vaginal PMNL in women with premature labor are stimulated *in situ*. Phagosomes, phagocytolysis of bacteria, and adhesion of primary granules to phagosome membranes were found on vaginal leukocytes, but not on peripheral blood leukocytes. Peroxidase activity was detected on the surface of cells, phagosome membranes, and primary granules. NADPH oxidase activity was revealed on the surface of leukocytes [52]. The degree of PMNL infiltration into the region of umbilical cord separation was high after fetal death in labor. During cesarean section the degree of PMNL infiltration was lower than after vaginal delivery [61]. Functional activity of umbilical blood neutrophils in infants of mothers without inflammatory changes in the afterbirth estimated by the content of lysosomal cationic proteins was higher than in infants of mothers with intraamniotic infections. It should be emphasized that no differences were found in functional activity of peripheral blood neutrophils in pregnant women [2]. The use of recombinant granulocytic colony-stimulating factor before preterm labor produced to adverse effect on the mother and fetus, but stimulated production of neutrophils and improved the prognosis for infants [22].

One of the causes of fetal morbidity and mortality is meconium, which produces necrosis of umbilical vessels at various gestational periods, abortions, and delivery of stillborn child [79]. Bilirubin was found in the Wharton's jelly and macrophages between myocytes of umbilical vessels. These macrophages contain also IL-1β. Cytokines and other factors associated with meconium can cause fetal death. Survived fetuses often present with intraventricular hemorrhage, periventricular leukomalacia, and other diseases [79]. In the absence of clinically verified infections, cloudy meconium-stained amniotic fluid displayed chemotactic activity for PMNL. In this fluid the contents of

TNF-α, IL-1β, and IL-8 were higher than in the transparent amniotic fluid. A positive correlation was found between IL-8 concentration in the amniotic fluid and its chemotactic activity. Antibodies against IL-8 added into the amniotic fluid abolished chemotactic activity of cloudy amniotic fluid. These data show that meconium-stained amniotic fluid meconium serves as a chemoattractant for PMNL. IL-8 probably plays a role in these changes [102].

Published data indicate that phagocytes constituting an important cell component of natural immunity play a role in the course of pregnancy and determine its development, maintenance, and favorable outcome. It should be emphasized that these cells are involved in the pathogenesis of various pregnancy complications. An understanding of the pathophysiological mechanisms that occur in the mother—placenta—fetus system would allow us to improve diagnostic procedures, perform pathogenetically substantiated therapy, and decrease the incidence of obstetrical complications.

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