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The placental membrane microbiome and preterm birth

Corina Grigoriu[1,2], Vlad Dima[3], Nicolae Bacalbasa[1], Tiberiu Augustin Georgescu[4], Bianca Mihai[3], Ioana Teodora Vladareanu[5], Consuela-Madalina Gheorghe[6], Roxana Bohiltea[1,3]

[1] Department of Obstetrics and Gynecology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2] Department of Obstetrics and Gynecology, University Emergency Hospital, Bucharest, Romania
[3] Filantropia Clinical Hospital, Bucharest, Romania
[4] Department of Pathology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[5] Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania
[6] Department of Marketing and Medical Technology, "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania

ABSTRACT

According to the World Health Organization, it is estimated that 15 million children are born prematurely each year – more than 1 in 10 children worldwide and this number is rising.
The impact of infections is recognized starting from the first trimester (endometritis, miscarriage, poor embryo quality implantation), then, in the second trimester, by probably other mechanisms, the infection is responsible for the abortion, raising the problem of transmission to the fetus and amniotic fluid. In the third trimester, the infection is shown to cause spontaneous rupture of the membranes, with or without the onset of premature birth. All these clinical situations raise major difficulties in choosing the therapeutic conduct, the implications being the more severe the younger the pregnancy. In these cases, observation is delicate, with major maternal and fetal consequences.
The mechanisms underlying the onset of uterine contractions during pregnancy are not fully understood. At present, it is accepted that proinflammatory cytokines are key mediators in the inflammatory response that occurs during term or premature labor. However, the triggers of inflammation during premature labor have not been fully identified.

Keywords: placental microbiome, premature birth, trophoblast-microbiome interaction, chorioamnionitis

INTRODUCTION

According to the World Health Organization, it is estimated that 15 million children are born prematurely each year – more than 1 in 10 children worldwide and this number is rising.

The impact of infections is recognized starting from the first trimester (endometritis, miscarriage, poor embryo quality implantation), then, in the second trimester, by probably other mechanisms, the infection is responsible for the abortion, raising the problem of transmission to the fetus and amniotic fluid. In the third trimester, the infection is shown to cause spontaneous rupture of the membranes, with or without the onset of premature birth. All these clinical situations raise major difficulties in choosing the therapeutic conduct, the implications being the more severe the younger the pregnancy. In these cases, observation is delicate, with major maternal and fetal consequences.

Numerous studies that have been done in recent years have shown that the fetus does not live *in utero* in a sterile environment, as expected (1). The placenta was considered a sterile organ in the absence of an infection, but this theory is refuted by the evidence of the existence of a community of microorganisms at this level - the so-called placental microbiome.

Corresponding authors:
Consuela-Madalina Gheorghe
E-mail: consuela.gheorghe@umfcd.ro
Roxana Bohiltea
E-mail: r.bohiltea@yahoo.com

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The adaptive immune system was formed in response to the interaction of the human body with the microbiome, managing to create a functional mechanism that preserves the symbiotic relationship between the body and the commensal flora. Autoimmune diseases, inflammation, infection, are a disruption of the connection between the microbiome and the immune system, leading to exacerbation of the immune response to self, microbiome, or pathogens. However, the interaction between the host and the microbiome differs depending on the tissue and the type of commensal flora, being subject to continuous regulation between the two entities.

Bacterial infections are a threat to pregnancy, both for the mother and the fetus. There are three ways for bacteria to enter the decidua, placenta, and fetal membranes: ascending from the lower genital tract, hematogenous, or translocating into the uterus from the peritoneal cavity (2).

In more than 40% of cases, infections are responsible for premature birth. In 80% of cases of premature birth under 30 weeks, the bacterial infection was incriminated. Current research has also shown that premature birth is a polymicrobial disease (involving more than one microorganism).

The mechanisms by which an infectious agent can cause premature birth are incompletely elucidated, involve the nonspecific immune response that occurs in the infectious agent and triggers an exaggerated inflammatory response and/ or apoptosis at the maternal-fetus interface.

Laboratory methods used in the past to highlight bacteria in the placenta have used cultures on special media (dependent cultures). Currently, independent molecular-based techniques are used: polymerase chain reaction (PCR) and 16S ribosomal RNA molecules sequencing. 16S ribosomal RNA analysis revealed low-molecular-weight endogenous bacterial communities in the placenta. The term placental microbiota is mainly composed of Lactobacillus sp., Propionibacterium spp. and Enterobacteriaceae. There was a decrease in the number of Lactobacillus sp. in the placental tissue prelevated from women who gave birth prematurely (3).

The way microorganisms reach the placental tissue are: ascending from the vagina, bacterial translocation from the intestinal or hematogenous level. Intercellular junctions in the intestinal and oral mucosa allow a small number of circulating bacteria to enter, thus inoculating the placental tissue (3). Current researches have shown that the oral microbiota is the primary source of bacterial origin.

The placenta has a distinct bacterial signature from other microbiota in the body, the greatest phylogenetic resemblance being to the oral flora of the non-pregnant woman, suggesting that the fetus does not grow in a sterile environment. The theory is that the placental microbial population occurs hematogenously or by translocation from the oral and intestinal bacterial niches than by ascending translocation from the vaginal flora (4). Bacteria common to microbiomes in the body belong to 4 classes: Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria. Of these, the most common species are: Prevotella, Streptococcus and Veillonella, the latter being a species that it is not found in the vaginal environment. In the placental microbiota, the most frequently present bacteria are Lactobacillus, Streptococcus, Veillonella, Haemophilus, Acinetobacter, Pseudomonas. At the taxonomic level, the similarity between microbiomes is decreasing as the taxonomic level decreases, suggesting the uniqueness of the placental bacterial flora, even if at the phylum level the similarity with the oral and intestinal commensal flora is about 90% and at the taxonomic unit level of 30% with oral flora and 6% with intestinal flora. In addition, studies show that there is no difference between the microbiome of placentas delivered intraoperatively or those delivered naturally, suggesting the minimal importance of the vaginal passage in colonization. Also, the placental microbiota is different in the case of pregnant women with gestational diabetes, possibly due to the alteration of the placental structure, but also of the intestinal commensal flora (4).

TROPHOBLAST-MICROBIOME INTERACTION

The complex immune relationship between fetus and the mother during pregnancy was considered similar to that of a transplanted allograft and recipient, thus assuming that the maternal immune system is suppressed (5). However, current studies show that during pregnancy, the mother's body is gradually exposed to paternal/ fetal antigens, thus appearing the phenomenon of tolerance, not rejection. Pregnancy does not induce suppression of the maternal immune system.

One chapter, less addressed over the years, is the one that highlights the active role of the placenta in modulating the maternal immune response.

The presence of the fetus and placenta is responsible for mediating the maternal immune response, essential in supporting and protecting the pregnancy. The cellular and molecular basis of this immune interaction between the placenta and the maternal uterus shows that implantation predominantly involves a new alloantigen recognition system, based on NK cells rather than T cells.

Between the fetus and the maternal organism there is a hybridized area made up of the trophoblast and the decidua. The embryo does not come into direct contact with the mother's body, but with

the tissue of the lining, represented by the endometrium, which becomes decidua by contact with the embryo, and as it grows, even at term, is contained in two layers: trophoblast (embryonic tissue) and decidua (maternal layer). The cells through which communication takes place are cytotrophoblasts on the one hand and macrophages together with large granulocyte lymphocytes (LGLs) on the maternal side.

At the level of the intervillous space, small maternal-fetal hemorrhages take place, through which fetal cells pass in the maternal circulation, especially red blood cells, fetal leukocytes (carriers of the immunological message). Villous fragments, made up of syncytiotrophoblast and cytotrophoblast, reach the maternal circulation.

The interaction between the placenta and the decidua determines a special environment that prevents the release of proinflammatory cytokines, inhibits the recruitment of T cells with cytolysis function, modulates the immune system to facilitate fetal development, controls the bacterial population and protects the fetus from various infectious agents.

From many points of view, we can consider that the placenta acts through a mechanism similar to a tumor process and thus induces the release of immunosuppressive regulatory factors that act locally, at the decidua and systemic level (5,6).

There are more and more studies that have shown that not only the bacterium alone is responsible or able to induce an inflammatory response that triggers birth. The trophoblast can recognize lipopolysaccharides (LPS) or specific bacterial peptidoglycans. In the placenta, there is a population of Toll-like receptors (TLRs), capable of recognizing the infectious agent and triggering an adequate immune response through the TLR4 trophoblast. Like other cells involved in the innate immune response, once the TLR4 interaction of the trophoblast with LPS occurs, local production of cytokines and chemokines begins. Basically, the trophoblast can recognize and initiate the immune response (6).

The microbiota plays an important role in regulating the host's immune response, prevents the onset of an inflammatory response to saprophytic flora or its degradation products, and intervenes in maintaining homeostasis. Any imbalance of the host's immune response to the microbiota causes an inflammatory reaction. The complex process by which the nonspecific or adaptive immune response is modulated against the commensal flora (self) and against pathogens is a broad research topic (7).

At the implantation site, there are populations of cells that specialize in integrating local signals, such as cytokines, chemokines, and microbial factors, that determine an immune response, which ensures tissue integrity and functionality. The expression of the 10 TLRs and other co-receptors and CD11 accessory proteins demonstrates the ability of the trophoblast to recognize bacteria, but also its ability to induce an adequate response in maintaining homeostasis. The trophoblast expresses TLR4 and thus recognizes LPS, but the LPS-TLRL interaction does not induce a classical NF kB inflammatory response with production of inflammation mediators, but promotes the production of regulatory cytokines (IL 10, IFN-γ 1) (8).

The trophoblast-commensal flora (microbiome) relationship is important in maintaining homeostasis at the maternal-fetal interface and altering this relationship can trigger a proinflammatory response that can induce premature labor (9).

CHORIOAMNIONITIS

Chorioamnionitis is the consequence of the inflammatory response of the maternal organism, at the level of the chorion and amniotic sheets, to an aggression that can be infectious or non-infectious (known as "sterile" intra-amniotic inflammation). Considering the infectious mechanism, imbalance of the placental microbiota by overpopulation of local bacteria or invasion of a pathogenic exogenous agent can cause acute chorioamnionitis as a maternal inflammatory response and funisitis or chorionic villi following the fetal inflammatory response (10). Acute chorioamnionitis has a decreased prevalence with the increase of gestational age, from 3-5% in term placentas to 94% in placentas delivered at 21-24 weeks. This trend highlights the need to underline the factors responsible for the occurrence of acute chorioamnionitis more frequently in the second trimester and with a much more resonant clinical picture (11). Signs of chorioamnionitis in the absence of rupture of membranes show inflammatory changes and intra-amniotic bacterial overpopulation without the existence of direct contact with the vaginal environment. These data indicate the possibility of initiating the inflammatory process following an imbalance between the placental microbiota and the maternal and fetal immune system. Funisitis and villitis are the consequences of the fetal inflammatory response, a condition characterized by elevated fetal plasma IL-6 and associated increased fetal mortality and morbidity. Associated with chorioamnionitis, it increases inflammation, recruitment of neutrophils, secretion of chemokines (CXCR-2), pro-inflammatory cytokines (TNFα, IL-1β, IL-6, IL-8) and MMP (metalloproteinases), which will finally lead to the rupture of the amniotic membranes (11). The final effectors of choriodecidual inflammation are the

prostaglandins that induce uterine contractions and subsequent cervical changes (12). A lesser known and discussed pathophysiological mechanism is deciduitis. In embryogenesis, we know that the embryo, once fully integrated into the decidua, begins to develop and the decidua is anatomically and histologically divided into the basal, capsular, and parietal. At the end of the first trimester, the amnion, the chorion, and the decidua fuse, creating a "sealing" of the choriodecidual space. Thus, the parietal decidua, which has a special protective role of the embryo, can be found at the level of the internal os, interposed between the chorionic membrane and the endocervix. The first signs of inflammation in case of contact with bacteria in the endocervix determine the inflammatory response of the decidua, deciduitis, having as final effects in the cascade of inflammation, prostaglandins, which generate uterine contractions. Chorioamnionitis is an inflammation that occurs inside the amniotic sac, with a much more resonant clinical response, but it is a histological form with a different mechanism and consequences than deciduitis. Chorioamnionitis represents the presence of inflammation/ infection in direct contact with the fetus, but deciduitis represents inflammation in the maternal environment, with the generation of uterine contractions, which have as final goal the complete elimination of the conception product (13). One of the hypotheses developed is that the decidua has a well-established potential to protect the embryo against any microorganism. Bonding of amniotic, chorionic and decidual structures would be a physiological mechanism for closing any gateway of microorganisms to the amniotic cavity. According to this theory, chorioamnionitis is the consequence of a hematogenous dissemination or bacterial translocation, while deciduitis occurs as a result of the ascension of microorganisms from the vagina and endocervix (14).

CORRELATION BETWEEN INTRAUTERINE INFECTION AND PRETERM BIRTH

Additional evidence that intrauterine infection is associated with the onset of preterm birth is derived from histopathological studies of the placenta. Placental inflammation is a host-response mechanism to a variety of stimuli such as infection and immune system disorders. Traditionally, acute inflammation of the chorioamniotic membranes has been considered an indicator of amniotic fluid infection (15). This opinion was based on indirect evidence. Previous studies have shown an association between acute inflammatory lesions of the placenta and the recovery of microorganisms from the subchorionic fibrin plaque and the chorioamniotic space. Bacteria were recovered from the subchorionic fibrin plaque from 72% of placentas with histological chorioamnionitis (16).

It was found that there was a close correlation between positive cultures of amniotic fluid for microorganisms and histological chorioamnionitis. The studies were based on the evidence of placental inflammatory changes in patients who had premature births. Thus, the evidence indicates a direct association between premature birth and the appearance of acute chorioamnionitis (16).

There is a relationship between the postpartum evolution of the newborn, infection, and premature birth. The prevalence of neonatal sepsis is 4.3/ 1000 live births in premature infants compared to 0.8/ 1000 live births in full-term infants. The lower the birth weight, the higher the prevalence of sepsis (164/1,000 for 1,000-1,500 grams, 91/1,000 for 1,501-2,000 grams and 23/1,000 for 2,001-2,500 grams). These data show that premature infants are more susceptible to infection (17).

The observation that at least half of sepsis cases are diagnosed within 48 hours after birth, together with the increased incidence of intra-amniotic microbial invasion in women with premature labor and premature rupture of membranes, calls for a reassessment of this classic concept, suggesting that the increased number of cases of sepsis in the premature newborn is partly due to the increased frequency of intrauterine infection in women with premature labor. The initiation of premature labor is determined by the effect that the infection has on the host organism (18).

Two paradigms are debated in literature regarding the uterine environment and its sterility. The first theory developed since the nineteenth century supports the absence of microorganisms in the intrauterine and intra-amniotic environments and the sterility of meconium (Theodor Escherich, 1885). In contrast, new detection methods that determine bacterial DNA propagate the idea of the existence of a specific placental microbiota with the role of antepartum population of the fetal intestine. The fine line between the two paradigms is the sensitivity of the tools to highlight viable bacteria in the uterus. The theory of "sterile womb" is based on studies conducted on bacterial culture methods and support the need for the existence of viable and cultivable bacteria to define the formation of a microbiota. On the other hand, the detection methods of 16S rRNA or bacterial DNA highlight only the traces of a lysed bacterium following the interaction with the immune system and not the bacterium itself. However, the weakness of this theory in many studies is the insufficiency and simplicity of detection methods represented only by the culture media and the cultivation of amniotic fluid in cases of premature birth or premature rupture of membranes, in which

there is indeed an increased microbial load, which makes it easily detectable (15).

The two theories require extensive molecular studies, because understanding the possibility of the presence of an intrauterine microbiota influences the understanding of the mechanism of intestinal colonization of the newborn and the consequences of disrupting this fine adjustment between bacteria and the immune system. Also, the question how to colonize the intestine of the newborn extracted by cesarean section procedure in pregnancies with non-infectious complications, with mothers who underwent postpartum ablation, remains unanswered.

Infection triggers labor through various cellular mechanisms. The main pathways involved in this process are contraction-associated proteins (CAPs), prostaglandin E2 (PGE2), Rho-associated protein kinase, and matrix metalloproteinases (MMPs). Arachidonic acid (AA), toxins, 15-hydroxyprostaglandin dehydrogenase (15-PGDH), infection-induced amplifier nuclear factor of κ-light chain of activated B cells (NF-κB) mediates the onset of PGE2-induced myometrial contraction. CAPs, PGE2, Rho/ ROCK, NF-κB and infection-induced MMPs act directly on the myometrial fiber initiating labor (11).

CONCLUSIONS

The mechanisms underlying the onset of uterine contractions during pregnancy are not fully understood. At present, it is accepted that proinflammatory cytokines are key mediators in the inflammatory response that occurs during term or premature labor. However, the triggers of inflammation during premature labor have not been fully identified. Major advances have been made in understanding the molecular and triggering mechanisms of uterine contractions. The infection is closely correlated with the onset of labor and the mechanisms underlying these phenomena have become apparent. Also, understanding these cascades of events is important in understanding the mechanisms that affect the fetus and the newborn.

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