Trophoblast-microbiome interaction: a new paradigm on immune regulation

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The immunologic paradigm of pregnancy led to the conceptualization of pregnancy as an organ transplant that requires, for its success, suppression of the maternal immune system. Growing scientific evidence suggests that in many ways the placenta functions as a tumor rather than a transplant and the immune regulation of the maternal-fetal interface is the result of the coordinated interaction between all its cellular components, including bacteria. Examining the role of microbiota in reproduction is in its infancy, but there is growing literature that supports its relevance. We discuss a potential normal function of bacteria in the establishment of immune tolerance and compelling evidence that a viral infection might be the underlying cause of perturbation of homeostasis. There is compelling evidence that many infectious diseases of human beings are caused by >1 microorganism and are defined as polymicrobial infections. We propose that pregnancy complications, such as preterm birth, are the result of polymicrobial infections. We examine the potential cellular and molecular mechanisms by which a viral infection of the placenta might disrupt the normal interaction between the cellular component of the implantation site and bacteria. As we better understand the normal homeostasis among the maternal immune system, placenta, and commensal, we will be able to elucidate pathogenic conditions and design better approaches to treat pregnancy complications associated with infection.

Key words: bacteria, inflammation, preterm birth, trophoblast, virus

The immunologic paradigm of pregnancy was described for the first time by a transplant immunologist, Sir Peter Medawar,[1,2] who observed the antigenic mixture of the fetus containing paternal antigens that are not rejected by the maternal immune system. This unique observation led to the conceptualization of pregnancy as a "semi-allograft" and it was proposed that pregnancy represents a natural model of transplantation that could help explain the immunologic bases for transplant rejection and acceptance.[2] Consequently, the study of the immunology of pregnancy followed the immunological models associated with organ transplant. Over the years several mechanisms have been proposed to explain the immune privileged state of the fetus, all of them with the basic understanding that the maternal immune system is antagonistic to the fetus/placental unit.[3] Since the success of an organ transplant is obtained by inducing immune suppression of the host, it was postulated that pregnancy may have a natural mechanism to induce systemic suppression of the maternal immune system. This concept has been studied by numerous investigators and over many years has become the conventional wisdom.[4] Indeed, a wide array of factors in human serum have been found to possess profound in vitro immunosuppressive activities.[5] However, if we carefully analyze this hypothesis it is difficult to imagine how, from an evolutionary point of view, pregnancy involves a stage of profound immune suppression. Early human beings were not able to wash their hands or clean their food and, with the absence of antiseptics, were continually exposed to bacteria, parasites, and other microorganisms. If pregnant women were systemically immunologically suppressed, they would not have survived and the human species would have become extinct. Even today, in many parts of the world, pregnant women are continually exposed to harsh, unsanitary conditions and a suppressed immune system would make it impossible for the mother and fetus to survive. Furthermore, in countries such as Africa where HIV is pandemic, HIV-positive women do not develop AIDS during pregnancy.[6] In fact, there are recent studies clearly demonstrating that the maternal antiviral immunity is not affected by pregnancy.[7,8] Together, these observations argue against the existence of such nonspecific immune suppression.

Medawar's original observation was based on the assumption that the placenta was akin to a "piece of skin" with paternal antigens, which, under normal immunological conditions, should be rejected.[1,2] However, the placenta is more than just a transplanted organ. Our knowledge of placental biology has significantly increased over the last 50 years. We now know that the placenta is a complex organ, which has evolved from the original "egg cover." Pregnancy and implantation, contrary to graft implants, has been taking place for >1 million years. Therefore, from an evolutionary point of view it is difficult to conceive that the placenta and the maternal immune system still maintain

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an antagonistic status. Thus, while there should be an active mechanism preventing the potential recognition of paternal antigens by the maternal immune system, the trophoblast and the maternal immune system have evolved and established a cooperative status, helping each other against common enemies: infectious microorganisms.[9-11]

Therefore, it is important to evaluate the immunologic aspects associated with pregnancy to further understand the potential biological reasons associated with the risk of pregnancy complications potentially triggered by infection. One wonders why the model of transplantation may not represent the correct immunological situation of pregnancy: during organ transplantation there is a major influx of foreign antigens as a result of the introduction of a fully foreign organ. Under this circumstance the host immune system acutely reacts to the foreign antigens and mounts an immunologic response to reject the source of foreign antigens. During pregnancy the process is different. Pregnancy is a slow and gradual process where paternal/fetal antigens are released in a gradual and increasing manner as the blastocyst grows into an embryo and then into a fetus. The exposure of small amounts of foreign antigens during this process may actually induce *tolerance* rather than rejection.[12] Consequently, pregnancy, contrary to transplantation, does not require systemic immune suppression.

A second aspect that has been poorly evaluated for many years is the active role of the placenta in the modulation of the maternal immune system. Pregnant women represent an immunologically unique population because their immune system is influenced by signals originating from the placenta.[13,14] The presence of the fetus and placenta alters maternal immunity and physiology to sustain and protect the pregnancy. We and others have shown that the placenta may function as an immune-modulatory organ that regulates the immune responses of cells present both at the implantation site as well as systemically.[10,15-17] However, this modulation is not suppressive, but protective. The placenta together with the decidua are responsible for establishing a unique microenvironment at the implantation site that: (1) prevents a proinflammatory cytokine storm; (2) inhibits the recruitment of T cells with cytolytic function; (3) educates the local immune system to facilitate the fetal development; (4) controls bacterial growth; and (5) protects the fetus from viral infections.

In many ways, the placenta functions as a tumor rather than a transplant. Tumors secrete an array of factors that establish a local immunosuppressive microenvironment in which dysfunction and even death of tumor-specific T cells can occur. This immune-regulatory effect occurs at 2 sites: (1) locally at the tumor-host interface where cancer cells condition the tumor stroma, and (2) systemically where cells and/or factors mediate suppression of antitumoral T-cells in the blood and lymphoid organs. The placenta has also 2 regulatory sites that are: (1) locally, at the decidua where immune cells are differentiated toward a pregnancy supporting function, and (2) systemically, affecting the maternal immune system and preventing the expansion of T-cell clones that recognize paternal antigens.

The placenta and bacteria, friends or foes?

Bacterial infections are thought to pose a significant threat to a pregnancy and to the well-being of the fetus, by gaining access to gestational tissues, such as the decidua, the placenta, and the fetal membranes, through 1 of 3 major routes: by ascending into the uterus from the lower tract; by descending into the uterus from the peritoneal cavity; or via the maternal circulation.[18-23] There are strong clinical links between bacterial infection and preterm birth.[23-27] Indeed, infections have been reported as responsible for up to 40% of preterm birth cases.[28,29] Furthermore, 80% of preterm deliveries occurring at <30 weeks of gestation have evidence of infection.[26,27] While many of the pathways involved are still largely undefined, growing literature suggests that the way in which a microorganism can induce a pregnancy complication, such as preterm birth, involves innate immune responses toward the pathogen, leading to excessive inflammation and/or apoptosis at the maternal-fetal interface.[30-32] Indeed, experimental in vivo models have demonstrated that delivery of infectious components (bacteria and bacterial products) to a variety of animals triggers preterm delivery.[33-36] Clinical studies have correlated placental infection/inflammation with prematurity[37-39] and this is supported by experimental studies.[14,34,40,41] In spite of the strong literature linking bacterial infection and pregnancy complications, targeting bacterial infections have failed to prevent pregnancy complications.[42,43] Furthermore, growing evidence suggests bacteria are a normal component of the pregnant and nonpregnant uterus.[44-48] These observations suggest that in most cases bacteria alone may not be sufficient to induce an inflammatory event leading to parturition and that the immune response to commensal bacterial product at the maternal-fetal unit is tightly controlled by regulatory mechanisms.[17,48] When and why bacteria become detrimental for pregnancy has not been defined.

Trophoblast cells as a component of innate immune system

The placenta is in direct contact with maternal component, thus it is imperative that a high level of immune protection is present at the maternal-fetal interface to protect the fetus against any infectious agent that reaches the placenta.[49] It is well known that classic immune cells, such as macrophages and natural killer cells, are present at the interface to facilitate innate immune responses. In addition to these immune cells, previous studies demonstrated that trophoblasts, the major constituents of the placenta, are also able to sense and respond to pathogen-associated molecular patterns such as lipopolysaccharide (LPS) or peptidoglycan.[41] We have confirmed the expression by the placenta of Toll-like receptors (TLRs), membranous receptors known to recognize microbial products and have demonstrated that via TLR4 trophoblasts elicit an immune response.[50-53] Similar to other innate immune cells, ligation of TLR4 in