Circulating cytokines during the blastocyst peri-implantation period

Human reproduction is an inefficient process, primarily because of frequent aneuploidy, but also because the maternal-fetal interface requires an immune homeostasis with precise conditions to enable pregnancy. Classical immune studies of peripheral blood had suggested that maternal immunosuppression is required to tolerate the presence of an allogeneic fetus. Although this theory was inspired by solid organ transplantation studies, it is now understood that the immunology of transplantation and that of pregnancy are quite different.

Successful maternal adaptation to the semiallogeneic fetus occurs in the uterus (specifically, the endometrium) at the site of placentation. Most immune cells in the endometrium are tissue-resident cells; their number, type, and activation state are highly dependent on the local hormonal environment. Consequently, progesterone and estradiol may regulate the expression of specific chemokines from immune cells in the endometrium. At the same time, fetal antigens may induce changes in the maternal uterine and circulating immune cells. This is possible because the syncytiotrophoblast is in contact with immune cells circulating in the maternal blood in the intervillous space, and extravillous trophoblast cells invade the uterine decidual lining at implantation and during placentation to transform arteries and establish the blood supply to the placenta, an essential process in implantation (1). Further, the search for potential markers of embryo viability and implantation, along with the development of advanced gene expression quantification methods and other high-throughput "-omics" approaches, established that human embryos produce and secrete a wide variety of cytokines, growth factors, hormones, microRNAs, and metabolites before implantation that may trigger a maternal response at the embryo-maternal interface (2). Dysregulation of the complex maternal-fetal crosstalk may underlie many cases of pregnancy failure.

The present study (3) suggests that cytokines in peripheral blood must change from a proinflammatory to an anti-inflammatory profile to enable human blastocyst implantation. Human embryos express proinflammatory cytokines, such as interleukin 1 (IL-1), IL-6, IL-8, IL-12, interferon γ (IFN-γ), and tumor necrosis factor α (TNF-α), as well as anti-inflammatory cytokines such as IL-10, transforming growth factor β, and IL-1 antagonists. In vitro models provided valuable information about the dialogue between mother and embryo at the endometrial level and established that the presence of embryos in coculture induces significant changes in gene expression and the secretome of endometrial cells (2). However, in vitro approaches provide limited information regarding the in vivo maternal interface during the early stages of implantation, where all factors interact simultaneously. It, therefore, remains unknown whether local cytokine changes at the endometrium can be detected at the circulation level. An overactive maternal proinflammatory, T helper lymphocyte 1-type (Th1) response from the mother, as well as defective Th2 cytokine production by maternal immune cells and genetic predisposition to Th1-type response, may explain some instances of infertility. However, more studies are needed to further investigate this mechanism.

Interestingly, in vitro production of mitogen-activated peripheral mononuclear cells from pregnant women showed that during the first trimester of pregnancy, these cells have less capacity to produce Th1-type cytokines such as IFN-γ or IL-2 and a higher capacity to secrete Th2-type cytokines (IL-4 and IL-10). Miscarriage was associated with elevated production of type 1 cytokines. Other studies comparing pregnant and nonpregnant women also demonstrated that the levels of cytokines such as IFN-γ were significantly lower in pregnant than in nonpregnant women and the level of IL-10 was higher in pregnant than in nonpregnant women (4).

This straightforward, prospective observational study does not focus on recurrent pregnancy loss but rather highlights the immune profile of women around the time of implantation, comparing successful implantation with the absence of implantation (3). The study compares circulating inflammatory cytokines in women undergoing selective single-blastocyst transfer who did or did not become pregnant. The investigators hypothesized that successful implantation would be characterized by cytokine profile changes at the peripheral level corresponding to key events of early implantation. A successful transition from the proinflammatory Th1 type to the anti-inflammatory Th2 type would indicate a successful pregnancy up to at least 20 weeks' gestation.

The study (3) used a small number of participants to show that women who did not conceive had a pronounced proinflammatory cytokine profile that did not convert to an anti-inflammatory profile, a phenomenon observed in women with similar demographic characteristics who became pregnant. When women who did not conceive and pregnant women were compared, only the level of IL-17, a proinflammatory cytokine, was higher in women who did not conceive as early as 3 days after embryo transfer, whereas the level of the anti-inflammatory cytokine transforming growth factor β was significantly lower in women who did not conceive 6 days after embryo transfer. Similarly, the proinflammatory to anti-inflammatory ratios of IFN-γ to IL-10 and TNF-α to IL-10 were higher in women who did not conceive than in pregnant women. Interestingly, basal cytokine levels at the moment of embryo transfer were similar in the two groups.

The mechanisms driving the switch in cytokine profile in peripheral blood as early as 6 days after blastocyst transfer are unknown. Significant immune changes occur in the transition from nonpregnant to pregnant endometrium (1), but the variations observed in peripheral blood may or may not reflect the change in the endometrial immune status of the mother. Whether these early changes are because of cell count and phenotypic variations of a subset of maternal peripheral blood leukocytes and what factors are involved in these changes need to be investigated.

In the late secretory phase (LH7-9), there are significant changes in the distribution of local immune cells in a normal endometrium. The percentage of uterine natural killer cells

increases to 70% to 80% of total leukocytes and the percentage of macrophages increases to 30%, whereas the percentage of T cells decreases to less than 10% in response to estradiol and progesterone regulation. The cytokine profiles of CD4 T cells dictate their classification as Th17, Treg, Th1, or Th2 cells, among others. Treg cells are potent suppressors of inflammatory immune responses. Th17 cells are a subset of proinflammatory T CD4 cells. The signal that promotes Th17 differentiation inhibits differentiation of Treg cells: Th17 and Treg cells are lymphocyte populations with opposite actions.

Zhao et al. (3) compared pregnant women at the early stages of conception with nonpregnant women. Unfortunately, the average age of the nonpregnant women was higher than that of the pregnant women, and circulating cytokine levels were not categorized according to whether a euploid or an aneuploid embryo was transferred; because maternal-fetal crosstalk may be influenced (including immune cell changes) by aneuploidy, this is an important factor to consider. Moreover, the investigators did not exclude patients with endometriosis, adenomyosis, chronic endometritis, other infectious conditions, or metabolic disorders that could affect the cytokine profile in both the endometrium and the peripheral blood. Indeed, an inflammatory state was described in the endometrium of patients with endometriosis, adenomyosis, chronic endometritis, and metabolic disorders such as insulin resistance (5).

As suggested by the investigators (3), measurements of peripheral blood cytokines in early pregnancy may have clinical implications and may guide the understanding of spontaneous early losses and recurrent implantation failure after in vitro fertilization. However, more studies with careful patient selection, transfers of euploid embryos, and transcriptomic and genetic analysis of the molecules and cells involved in maternal-fetal interactions are needed to conclusively determine whether peripheral blood cytokine profiles in the early stages of embryo implantation may be useful in informing clinical practice.

Maria J. de los Santos, Ph.D.[a,b]
Diana Alecsandru, M.D., Ph.D.[c]
[a] IVF Laboratory of IVIRMA Valencia, Spain; [b] Fundación IVI, Valencia, Spain, and Department of Reproductive Endocrinology and Infertility, IVI RMA Madrid, Spain; and [c] Department of Immunology, IVI RMA Madrid, Spain

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