The excessive presence (percentage or number) of endometrial immune cells in patients with chronic endometritis cannot be associated with reduced endometrial receptivity or recurrent pregnancy failure

In their study, Li et al. (1) analyzed immune cells in peripheral blood and endometrial tissue samples from patients with recurrent reproductive failure (recurrent miscarriage [RM]/implantation failure [RIF]). They observed an increased percentage of CD68⁺ macrophage, CD8⁻ T cells, and Foxp3⁺ Treg cells in the endometrium of patients with chronic endometritis (CE) compared with non-CE.

We were interested to read that they observed no significant differences between CE and non-CE patients with RM and RIF. They, the authors, Li et al. (1), showed that neither endometritis nor maternal-fetal tolerance failure (RM/RIF), both tissue-restricted processes, have a systemic impact; tests based on the % or number of peripheral blood immune cells, Th1 cytokine or NK cytotoxicity assays did not reflect endometrial imbalance in patients with endometritis or RM/RIF.

Li et al. (1) studied endometrial cells using immunohistochemistry staining (IHC) with a single molecular marker for immune cells and they observed differences in some immune cell percentages between patients with CE and those without CE. They diagnosed CE based on the presence of CD138 cells, a nonspecific test that very often does not correlate accurately with hysteroscopy and microbial culture findings. They concluded that the excessive presence of endometrial immune cells in patients with CE could be associated with reduced endometrial receptivity and recurrent pregnancy failure, also that CE antibiotic therapy modulates the immune status of the endometrium, which potentially improves pregnancy outcomes.

They analyzed endometrial samples collected during the midluteal phase on luteinizing hormone days 7–9. It would have been interesting to have information regarding the aneuploidy rate of embryos transferred or lost in miscarriages (patients aged ≤45 years), oocyte origin (own or donated), embryo quality, embryo transfer day, and number of embryos per transfer. The study did not analyze the pregnancy, miscarriage, or live birth rates after CE antibiotic therapy or compare those rates before and after treatment. All of these factors represent important limitations, which make it difficult to support their conclusions. Observational data provide interesting hypotheses, but they need to be supported by experimental data and clinically relevant interventions.

Regarding immune cell distribution, function, and behavior in a local infection or pregnancy failure [RIF/RM] context, some important issues must be pointed out. Most immune cells in the endometrium are tissue-resident cells and their number, type, and activation state are highly dependent on the hormonal environment. Maternal uterine immune cells actively respond to fetal antigens, promoting maternal-fetal immune tolerance, as observed in a normal pregnancy. Uterine natural killer (uNK) cells regulate trophoblast invasion and enhance vascular remodeling by extravillous trophoblast (3) and Treg cells (Foxp3⁺ Treg), promoting maternal-fetal immune tolerance. Human trophoblasts express many immune inhibitory molecules predominantly targeting T cells (CD4⁺ or CD8⁻), such as Fas ligand or indoleamine 2,3-dioxygenase (IDO), which are potent inducers of T-cell apoptosis as a protective mechanism of maternal-fetal tolerance. The interaction between trophoblast HLA-G dimers and LILRB1 receptors expressed by macrophage and dendritic cells also promotes maternal-fetal tolerance.

Apart from these changes induced by the presence of trophoblast molecules, macrophage and dendritic cells (DC) protect against infections (3) by increasing their number and activity. Their increased number, observed during local infections or menses, is attributed to their phagocytic properties and active role in clearing infected, apoptotic cells and cell debris. Macrophages express TLR4 (Toll-like receptor 4), a coreceptor of CD14 that recognizes lipopolysaccharides, major cell-surface components of Gram-negative bacteria found in CE samples, and this interaction precipitates macrophage-mediated bacterial phagocytosis and cell death.

Therefore the increased percentage of CD68⁺ macrophages, as observed in this study in CE endometrial samples compared with those without CE, is the consequence of the presence of bacterial lipopolysaccharides as part of a normal immune response against pathogens. The consequence of macrophage activation is Treg recruitment and immunoregulation to reestablish endometrial tissue homeostasis.

In the late secretory phase (luteinizing hormone days 7–9), changes in the distribution of the local immune cells in a healthy endometrium have been observed. The uNK cell percentage increases to 70%–80% of total leukocytes, percentage of macrophages to 30%, and T cells decrease to <10% in response to estradiol and progesterone regulation.

For IHC, the study used CD68 as a macrophage marker and, separately, CD163 for the M2 macrophage subset. CD68 is also expressed by other cells including NK cells, DC, basophils, endometrial cells, and fibroblasts. Therefore IHC does not provide accurate cell identification. There are two macrophage subsets: M1, which secretes proinflammatory factors, and M2, involved in angiogenesis, anti-inflammatory processes, and tissue repair coordination, and both express CD68 (4).

CD163 is expressed by M2 macrophages, the anti-inflammatory subset, and this study found no differences between CE and non-CE samples. Li et al. (1) did find significant differences with increased macrophage and Treg cell percentages between CE and non-CE samples. They reported a macrophage distribution of <10% of total leukocytes in CE and non-CE samples, which, compared with the macrophage distribution in the late secretory phase of a normal endometrium,
can be considered normal. The differences observed between CE and non-CE samples would be a normal response to the presence of pathogens to restore endometrial homeostasis, and cannot be associated with reproductive failure in patients with RIF or RM. The antibiotic treatment also supports pathogen clearance and its consequence is endometrial immune homeostasis. It cannot be considered an immunomodulator that improves pregnancy outcomes, as the study concluded.

The maternal immune system actively responds to fetal antigens and a dysregulation in this cross-talk could partly explain pregnancy complications such as RM. Apart from uNK cells, many immune cells are present at the maternal-fetal interface and play a role in promoting embryo implantation and immune tolerance. Monocyte-derived IDO may enhance fetal tolerance through either the generation of CD4⁺ Treg cells or the selective apoptosis of effector T cells (including CD8⁺ T cells). Some studies have reported a diminished Treg population in women suffering from RM. Another interesting study (5) has shown that in uncomplicated pregnancies with an HLA-C mismatched child, the percentage of activated Treg cells increases in decidual tissue compared with uncomplicated HLA-C matched pregnancies. This shows how the presence of Treg cells may suppress T-cell activation in such a way that no detrimental allogeneic response is initiated.

So, the Treg increased in endometrial samples from CE compared to non-CE patients is an immune protective response to prevent tissue damage secondary to the presence of pathogens and the immune response to them (increased % of CD68⁺ macrophage, CD83⁺ mature DC and CD8⁺ T cell population). Previous studies (5) have shown that an increased Treg population at the maternal-fetal interface is a protective and adaptive process inducing tolerance. It cannot be associated with RIF or RM.

The study by Li et al. (1) concluded that “a dysregulation of the uterine immune status due to CE may affect materno-fetal tolerance, alter the endometrial receptivity, and lead to RRF.” The study did not perform a transcriptomic analysis of the endometrium to detect steroid hormone signaling and up-regulation of pathways involving lymphocyte activation, antigen presentation, or proinflammatory cytokines. It did not use any assays based on embryo-maternal cell binding and interactions that affect maternal-fetal immune tolerance (i.e., KIR-HLA-C, LILRB1-HLA-G, Fas ligand, and IDO-T cells). Although the hypothesis is interesting, the study results cannot be used to support its conclusions. The study observed differences in endometrial immune cells between patients with and without CE as an immune response to pathogens, and cannot be associated with endometrial receptivity or maternal-fetal tolerance failure.

More studies based on a careful selection of patients, euploid embryo transfers, and transcriptomic and genetic analysis of the molecules and cells involved in maternal-fetal interactions are needed to identify whether the presence of CE could affect endometrial receptivity or maternal-fetal tolerance. At present, the question remains unanswered.

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