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ENDOMETRIOSIS-INDUCED CHANGES IN REGULATORY T CELLS – INSIGHTS TOWARDS DEVELOPING PERMANENT CONTRACEPTION

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Abstract

Endometriosis is a gynecological disorder which is associated with alterations in the immune system that contributes to its pathology as well as its associated infertility. This brief report summarizes our findings related to the changes in T regulatory cells (Tregs) which may affect the uterine environment and impact the fertility of women and non-human primates with endometriosis. Targeted therapies that could reduce Tregs within the reproductive tract may have a potential as long-lasting or permanent contraception.

Keywords

Endometriosis; inflammation; immune cells; uterine environment

Endometriosis is a common gynecological disease affecting up to 10 to 15 percent of women of reproductive age [1] and is characterized by the development of hormonally responsive endometrial glands and stroma outside of the uterine cavity [2]. Endometriosis is a major cause of pelvic pain and profoundly impacts fertility, and it has been reported that 50% of women with endometriosis are infertile compared to 5–10% of women without disease [3]. In addition, previous studies have shown that aberrant gene expression in the eutopic endometrium of women and baboons with endometriosis may contribute to disease-based implantation failure and infertility [4, 5]. Infertility has been associated with aberrant expression of immune modulators, including leukemia inhibitory factor (LIF), soluble gp130, and IL-11 [6–8]. The peritoneal cavity of patients with endometriosis has been well

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characterized as a proinflammatory environment [9]. Additionally, the immune cell profile of the peritoneal cavity, eutopic endometrium and lymph nodes of human patients and baboons with endometriosis has an enhanced proinflammatory phenotype indicated by the Th1/Th2 cell ratio, macrophage activation and also natural killer cell activity [10–12]. Peritoneal fluid from patients with endometriosis has elevated levels of inflammatory cytokines which is believed to result from improper clearance of ectopic fragments. Many studies have shown aberrant levels of cytokines in the peritoneal cavity of women with endometriosis compared to those of control women, including IL-1, IL-6, IL-10, tumor necrosis factor- α (TNF α), and transforming growth factor- β (TGF β) [13–17]. The chemotactic activity of peritoneal fluid of patients with endometriosis is higher than that of women with no disease and it is well documented that there is a greater number of macrophages in the peritoneal cavity of women with endometriosis than in that of women with no disease [18, 19]. The inflammatory peritoneal environment created by the presence of endometriotic lesions induces a uterine immunological environment that is not conducive to the establishment of pregnancy. Progesterone has well described anti-inflammatory properties and endometriosis is associated with attenuated progesterone action at the level of the endometrium [20,21]. Progesterone resistance may also simulate a constant menstrual phase phenotype within the endometrium and in endometriotic lesions, leading to a chronic inflammatory state [22]. Thus, the relief from endometriosis associated pain following high-dose progesterone treatment, or a viable pregnancy, may be due to a decrease in inflammation.

Immune factors are likely to contribute to early implantation failure when the specialized mechanisms that contribute to the maternal tolerance of the fetus are compromised [23]. Tolerance is the holy grail in the field of immunology and the immune system within the female reproductive tract has evolved to protect against pathogens without compromising fetal viability. Regulatory T cells (Tregs) have been implicated in regulating the immune tolerance required for host-graft-transplantation and mediating an immunosuppressive environment in humans [24]. This immune tolerance is the same as that required at the maternal fetal interface during the window of implantation. CD4⁺ CD25⁺ Tregs maintain tolerance through secretion of immunosuppressive cytokines that promote differentiation of naïve CD4⁺ T cells into Tregs, while also inhibiting Th1 mediated inflammation [25]. It is interesting to note that surface bound molecules such as CTLA-4, which is upregulated in the baboon endometrium during the window of receptivity [26] appears to contribute to this suppressive function [27]. Data strongly suggests that CD4⁺CD25⁺ Tregs are essential for normal pregnancy [28–30]. Development of Tregs in the thymus and periphery is dependent on expression of the transcription factor, forkhead box P3 (FoxP3), [31–33] and Foxp3 mRNA expression is decreased two-fold in women with primary infertility compared to fertile patients. Likewise, adoptive transfer of Tregs into abortion-prone CBA/J mice alleviates fetal rejection in the mice [34, 35]. Thus, dysregulated Treg activity may contribute to pregnancy loss and/or infertility.

Data suggests that subfertility and ectopic lesion growth in patients with endometriosis may be attributed to dysregulation of Tregs [36–38]. Tregs may provide a protective environment for ectopic lesions similar to the immunologically protective microenvironment that promotes tumor metastasis [34,39]. The importance of Tregs for embryo implantation and

tumor growth prompted us to investigate Treg expression associated with the pathogenesis of endometriosis using a baboon model of endometriosis. The baboon (*Papio anubis*) is an excellent model to the study the pathophysiology of gynecological conditions such as endometriosis. Baboons develop spontaneous endometriosis with ectopic lesions that are similar to those observed in women. An added advantage to the baboon model is that the disease can also be induced by injection of menstrual effluent into the pelvic cavity, which permits the study of disease progression from the initial onset of the disease [3, 40, 41].

In our studies, the induction of endometriosis resulted in a dramatic and rapid decrease in both nTregs and adaptive Tregs in the peripheral circulation and endometrium. This reduction was most evident in nTregs and Th3 adaptive Tregs by the reduction of Foxp3-positive cells in the peripheral circulation and secretory endometrium but also by the reduction of Foxp3 transcript in the secretory endometrium. In contrast, higher levels of Foxp3 transcript and an increased abundance of Foxp3-positive cells were detected in ectopic lesions [37]. The growth and maintenance of ectopic lesions can be modeled after the establishment and growth of tumors in the abdominal cavity where the primary method to acquire immune tolerance is through recruitment and active differentiation of Tregs in the abdominal tumor microenvironment [39]. Our data suggests that in contrast to the eutopic endometrium, Tregs in the ectopic tissue may enhance immune tolerance and contribute to survival of the lesions. In summary, the aberrant expression of Tregs in both the eutopic and ectopic endometrium likely contributes to the classification of endometriosis as a reproductive inflammatory immune disorder which ultimately has a negative effect on fertility and the high degree of pain associated with the disease.

The immune imbalance and resulting infertility that is induced by the growth of ectopic lesions indicates that a novel contraceptive therapy could be the reduction of peripheral activated Tregs. This concept has been utilized in several studies for the reduction of immune suppression by tumor infiltrating lymphocytes for anti-tumor immunity. Reduction of immune tolerance can be broadly achieved by a few mechanisms 1) reduction of immune tolerance promoting cytokines such as IL-10, TGF- β and colony stimulating factor (CSF-1), 2) blocking the activation of immune tolerant cells (ie. Tregs, uterine natural killer cells) important for pregnancy, or 3) enhancement of inflammatory lymphocyte populations (ie. Th17 cells). The challenge for any immune modulatory therapy is to find the lowest effective dose for contraception but to not promote harmful systemic immune activation. Inhibition of tolerant cytokines has been achieved in cancer models through the utilization of an anti-TGF- β monoclonal antibody [42]. Inhibition of TGF- β resulted in an increase of cytotoxic T cells but did not deplete systemic Treg populations important for overall immune balance. Cyclophosphamide (CTX) has been shown to be very effective at depletion of Tregs and modulation of the Th2/Th1 cytokine ratio [43]. However CTX does have a high level of gonadotoxicity and even low dose treatment results in premature ovarian failure [44] so this would not be an appropriate option for women. In, addition the use of anti-CD4 and anti-CCR4 antibodies has been shown to inhibit activated Treg recruitment in different cancer models [45, 46]. Finally Paclitaxel is an approved chemotherapy agent that when used at low doses can modulate Treg inhibition of immune activation through interference of the toll-like receptor 4 (TLR4) pathway that is important for Treg inhibition of dendritic cells [47]. The therapies mentioned above should be

evaluated with great caution for application in regards to contraception due to their cytotoxic side effects. Ultimately the best concept for an effective immune therapy should be one that can be administered locally within the uterus (such as an IUD), at a non-cytotoxic dose, that does not alter systemic immunity and that does not result in permanent sterilization. However information from cancer models does provide evidence that an immune therapy may exist that can also be used for contraceptive purposes.

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