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Review

The biological role of Treg cells in ectopic endometrium homeostasis

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Summary. Although retrograde menstruation is observed in up to 90% of women, endometriosis actually develops in only15% of women. There is considerable evidence in the literature that ectopic endometrial cells are able to evade immune surveillance and that the immune response in the microenvironment of ectopic lesions is limited. Endometriosis develops when a deficiency in the local immune response has been generated, and progression of the disease is related to the intensity of this process.

Over the last couple of decades it has been well known that T regulatory lymphocytes (Tregs) play a crucial role in controlling a variety of physiological and pathological immune responses. In this review we have focused on the physiological alteration of Treg cell infiltration into the endometrium during the reproductive processes of women. We discuss how a disturbance in Treg cell expansion is involved in generating such pathological processes as miscarriage and ectopic pregnancy development. We hypothesize about the role Treg cells might play in the survival of endometriosis foci in ectopic localization and in the evasion of such lesions from host immune surveillance.

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Introduction

Endometriosis is a chronic disease that has been the subject of many studies over the past one hundred years. Women suffering from endometriosis typically experience abdominal pain and infertility. Although there have been intensive studies on endometriosis, the pathogenesis of this disease remains obscure (Borrelli et al., 2013). Endometriosis was first described by Carl von Rokitansky in the second half of the nineteenth century. In 1927, John A. Sampson presented the first etiological theory of the disease based on the idea that endometriosis is linked with retrograde menstruation (Sampson, 1927). Menstrual blood has been found in the peritoneal fluid of 90% of patients with patent fallopian tubes (Halme et al., 1984). In the animal model of endometriosis the development of the disease from utero-pelvic fistulas or by the obstruction of antegrade menstruation demonstrates a high prevalence of pelvic endometriosis when there is retrograde menstruation (Nap et al., 2004; Braundmeier et al., 2012).

Endometriosis develops when a deficiency in the local immune response has been generated, and the progression of the disease is related to the intensity of this process (Wu et al., 2005, Zhang et al., 2006). In the

1990s, Dmowski et al. suggested that the mechanism determining immune escape by endometriosis lesions is linked with the ethiopathogenesis of the disease (Braun and Dmowski, 1998; Dmowski et al., 2001; Braun et al., 2002). Lebovic et al. have demonstrated that although retrograde menstruation is observed in up to 90% of women, endometriosis actually develops in only 15% of women (Lebovic et al., 2001). There is considerable evidence in the literature that ectopic endometrial cells are able to evade immune surveillance and that the immune response in the microenvironment of the ectopic lesions is limited (Wu et al., 2005). Some authors have proposed that local immune deficiencies are secondary to the development of the endometriotic foci. For example Vernet-Tomas et al. have demonstrated that ectopic endometrial cells overexpress HLA-A and are resistant to NK lysis (Vernet-Tomas Mdel et al., 2006). Other authors have suggested that an immune deficiency is primary to the development of endometriotic lesions. Maeda et al. have demonstrated that the expression of NKG2A on NK increases during the development of endometriosis (Maeda et al., 2004). With regard to the two primary or secondary involvements of immune system deficiencies in the development of endometriosis it is well known that endometriotic tissue in ectopic localization creates a microenvironment capable of suppressing the immune response, thus allowing the evasion of endometrial cells.

The microenvironment of endometriosis is related to a disturbance in the immune response and once this pro-inflammatory environment has been created, it persists (Braundmeier et al., 2012). Furthermore, patients with endometriosis are typified by a greater predisposition to autoimmune disorders (Nielsen et al., 2011).

Endometriosis is a chronic disease and many factors give rise to its pathology. We know that the disease develops over many years, but its precise etiopathology remains unclear. Moreover, it is difficult to distinguish the factors causing the disease from the factors facilitating and supporting the development of the endometriotic lesion. The symptoms of endometriosis tend to be confusing for patients and their chronic nature contributes to a delay in medical treatment; on average it takes 8-11 years between the initiation of endometriosis and the onset of symptoms and diagnosis (Arruda et al., 2003). At the beginning of this chronic disease, as the endometriosis nest starts to self-organize, the disturbance of the general homeostasis is slight. As endometriotic lesions grow, however, the local disease starts to generalize, and there is a significant disturbance of the overall homeostasis even to the point of generating the conditions favorable for carcinogenesis. Indeed, women suffering from endometriosis have a significantly greater predisposition for developing ovarian cancer (SIR from 1.43 to 8.95) (Heidemann et al., 2014), while patients with scar endometrioma following cesarean section have a greater predisposition for developing clear-cell carcinoma endometriotic lesions (Li et al., 2012; Mert et al., 2012; Shalin et al., 2012). Although the development of a cancer nest requires multifactorial support and carcinogenesis is itself a multistep process, in both endometriosis and cancer there is an evasion of immune surveillance and the progression of the disease is supported by an immune system whose functioning has been disturbed. For the last couple of decades it has been well known that T regulatory lymphocytes (Tregs) play a crucial role in controlling a variety of physiological and pathological immune responses (Sakaguchi et al., 2009). In this review we have focused on the physiological alteration of Treg cells in the reproductive processes of women and their potential role in endometriosis foci survival in ectopic localization, as well as the evasion of such lesions from host immune surveillance.

T regulatory lymphocytes

Immune system function is to protect the human body against dangers to its integrity. For proper functioning, the immune system must be continuously stimulated. Moreover, this continuous stimulation requires the existence of suppressive mechanisms (Sakaguchi, 2004; Sakaguchi et al., 2010) that allow the immune response to be controlled. A disturbed immune response is potentially harmful for the integrity of the body, and therefore the immunological mechanisms linked with the maintenance of self-tolerance are crucial (Wing and Sakaguchi, 2010).

In the 1970s, Gershon predicted the role of T suppressor cells in the regulation of the immune system (Gershon et al., 1972), but T regulatory cells were only discovered by Sakaguchi in the 1990s (Sakaguchi et al., 1995, 1996). The main subset of CD4+T cells involved in the suppression of the immune response include regulatory T-cell type I (TrI) (Groux et al., 1997), T-helper-3 cells (Th3) (Weiner, 2001; Saito et al., 2007, 2008), and T regulatory cells (FOXP3+) (Treg cells) (Sakaguchi et al., 1995; Guerin et al. 2009; Sakaguchi, 2011). It has been suggested that TrI and Th3 represent different states of CD4+CD25-T cells and that only Treg cells consist of a unique T-cell lineage (Weiner, 2001; Guerin et al., 2009). Recent studies have revealed that the main subset of T cells involved in immune system regulation in the female reproductive tract is that of Treg cells (Guerin et al., 2009).

Additionally, two main subpopulations of Treg cells have been identified in the human body: the first is made up of innate thymus-derived Treg cells while the second consists of Treg cells generated in the periphery (inducible/adoptive Treg cells (Bluestone and Abbas, 2003; Sakaguchi et al., 2008, 2013). Both sub-populations of Treg cells are typified by the intracellular expression of the forked head/winged-helix family, known as Forkhead box P3 (FOXP3 (Yagi et al., 2004; Sakaguchi, 2005; Wing and Sakaguchi, 2010; Sakaguchi et al., 2013). In the first group, expression of transcription factor FOXP3 occurs in the thymus, and in the second group, the expression of this transcription factor is induced on the periphery.

The alteration of Treg expansion can be exploited in the different types of therapies both to reduce immune suppression in cancer therapy and to generate immune tolerance during transplantation.

Antigen-typified phenotype of Treg cells

The suppressive activities of these cells are linked with the expression of the following markers: transcription factor forkhead BOX P3 (FOSXP3 (Ohkura and Sakaguchi, 2010b; Ohkura et al., 2013), cytotoxic T lymphocyte-associated antigen 4 (CTLA4/CD152) (Yamaguchi et al., 2013), human leukocyte antigen-DR (HLA-DR), the IL-7 receptor (CD 127) (Liu et al., 2006), the glucocorticoid-induced tumor necrosis factor receptor related protein (GITR) (Shimizu et al., 2002), transforming growth factor (TGF-beta), Nrp 1 (neuropilin-1collaborating with TGF-beta receptors) (Glinka and Prud'homme, 2008; Hansen et al., 2012), lymphocyte activation gene 3 (LAG-3, CD223) (Okamura et al., 2009; Sierro et al., 2011),GARP (transmembrane protein present on stimulated human regulatory T lymphocytes responsible for the cleavage of the pro-TGF-beta1 precursor) (Gauthy et al., 2013), ectonucleoside triphosphate diphosphohydrolase (CD39), ecto-5-nucleotide enzyme (CD73 (Sakaguchi et al., 2009; Smyth et al., 2013), the forkhead box O (Foxo) family of transcription factors (FOXO 1) (Ohkura and Sakaguchi, 2010a) and other markers, CD4, CD25 (IL-2R alfa-chain), CD122, CD132, OX40 (CD134, which is a member of the TNF-receptor family that is transiently expressed after TCR triggering). Treg cells express chemokine receptors CCR4 and CCR8 and are attracted to the place where immune cytotoxic activities correlative to chemokine action (CCL1, CCL17 and CCL22) appear (Iellem et al., 2001; Mjosberg et al., 2010).

Suppressive function of Treg cells

Sakaguchi et al. have revealed that the suppressive activities of Treg are related to both cell-contact dependent and cell-contact independent (that is humoral factor-mediated) mechanisms of suppression (Sakaguchi et al., 2009). As far as the mechanisms of cell to cell contact are concerned, Treg may inhibit effector T cells by the following molecular mechanisms: a) granzyme and perforin-dependent (Gondek et al., 2005), b) alterations of the intracellular cyclic adenosine monophosphate (cAMP), which is linked with an increased level of cAMP early repressor (ICER). ICER induces transcription of the nuclear factor of activated T cell c1/alfa (NFATc1/alfa) which finally suppresses NFAT-driven transcription, including that of IL-2. Additionally, cAMP via ICER alternates CTLA-4 expression, which in turn, via CD80/86, modulates interactions with APC cells (Bopp et al., 2007; Bodor et al., 2012); c) CD39 and CD73 generation, that may be secreted within membrane vesicles, such as exosomes by Treg cells (Sakaguchi et al., 2009; Smyth et al., 2013); d) all iterating on the CD80, CD86 on APCs expression; e) inducing indoleamine 2,3-dioxygenase (IDO); this enzyme is responsible for proper local tryptophan catabolism (Oderup et al., 2006; Sakaguchi et al., 2009; Xu et al., 2013); f) Lymphocyte activation gene 3, the CD4-like molecule present on the Treg cell surface that modulates Treg-APC interaction (Sierro et al., 2011); CTLA-4 expression on Treg cells is responsible for the down-regulation of CD80/86 on antigen-presenting cells (Wing et al., 2008). Finally, activation of naive T cells via CD28 is disturbed. CTLA-4 expression is necessary for the functioning of Treg suppression. Wing et al. have presented the results they obtained from blocking these receptors in mice. Without these receptors, Treg cells are unable to control immune homeostasis. Treg cells most likely use multiple mechanisms to suppress the immune response. The exact mechanism of suppression depends on the conditions related to the microenvironment and to the immune response context. However, when the CTLA-4 dependent manner of suppression is excluded, the other mechanisms of compensating for the CTLA-4 function prove insufficient. The blockade or deficiency of CTLA-4 in mice abrogates Treg suppression *in vitro*, causing the mice to die of systemic lymphoproliferation (Wing et al., 2008). Onishi et al. have suggested that antigen-activated Treg cell suppression is related to the presence of the leukocyte function-associated antigen-1 (LFA-1) (CD11a/CD18) which allows Treg and dendritic cells (DCs) to aggregate. The authors have revealed a "two-step model" as a possible mechanism: a) the LFA-1-dependent initial formation of Treg aggregates on DCs and (b) LFA-1- and CTLA- 4-dependent active down-modulation of CD80/86 expression on DCs. Aggregating Treg cells and DCs exerts down-regulation of CD80/86 on DCs. This down-modulation of CD80/86 (B7-1/B7-2) expression on DCs might be realized not only by LFA-1 manner but by CTAL-4 dependent way. Moreover, blocking LFA-1 does not influence CTAL-4 function. The influence of Treg cells on the down-regulation of CD80/86 is greater because the concentration of such factors in the microenvironment such as granulocyte macrophage colony-stimulating factor (GM-CSF), TNF alfa, and INF gamma increase (Onishi et al., 2008). Treg cell function realized in a contact-dependent manner is focused on the DCs that are the target for Treg cells, as these cells are crucial for the initiation of the immune reaction because they activate naive T cells.

The surface markers GITR and CTLA-4 on Treg cells play a role in competing with the co-stimulatory molecule CD28 by binding B7-1 (CD80) and B7-2 (CD86). This reaction may result in the up-regulation of dendritic cells and a higher level of IDO (indoleamine 2,3-dioxygenase) expression by these cells (Scherjon et al., 2011). Treg cells are typified by a higher level of expression of both LFA-1and CTLA-4 than naıve T cells (Tn). An *in vitro* study has revealed that a deficiency or blockade of CTLA-4 stops the suppressive function of Treg cells and might promote intensive autoimmuno-

logical diseases such as colitis in experimental models (Takahashi et al., 2000).

Recently, Ohkura et al. have described the new receptor for the lipid mediator sphingosine 1-phosphate. The interaction between the ligand and these receptors is essential for Treg trafficking. Moreover, these molecular processes participate in the generation, maintenance, and suppressive properties of Treg (Ohkura and Sakaguchi, 2009). TNF receptor 2 (TNFR2) is present on Treg cells, as demonstrated by the high level of suppressive activities, and TNF2-positive cells were found in the ascites of ovarian cancer patients (Govindaraj et al., 2013). Moreover, these cells were marked by a high level of FOXP3 intracellular expression as well as by more abundant CTLA-4 expression within the Treg cell membrane. The TNFR2-positive Treg cells were also observed in various diseases related to immune suppression, including malaria (Minigo et al., 2009). Indeed, Minigo et al have shown that the TNFR2-positive Treg cell population significantly increased in patients with hyper parasitemia and that these Treg cells were typified by an increased expression of FOXP3 and suppressive activities, which are determinants for the development of severe and fatal malaria (Minigo et al., 2009). In mice, TNFR2 was expressed on 30-40% of the Treg cells of the peripheral blood. These cells are able to activate the memory phenotype and maximal suppressive activity (Chen et al., 2008). TNFR2-positive Treg cells localized in the ascites of ovarian cancer patients expressed higher levels of regulatory factors such as CD39, CD73 GARP (glycoprotein, a repetition predominant protein). It has also been demonstrated that TNFR2-positive Treg cells influence TH1 effector cytokine production such as INF gamma and IL-2 in ascitic fluid. In this way, Treg cells reduce the antitumor immune response. Govidaraj et al. have demonstrated that TNFR2+ Treg cells migrate at a significantly higher rate than TNFR2-negative Treg cells, and that Treg cells expressing TNFR2 are the most active. The level of suppression induced by cells constitutes a continuous process taking place within the context of the immune response. For example, ovarian cancer cells present neuropilin-1 (Nrp-1), a molecule on their surfaces able to activate the latent TGF-beta bound with GARP. In this way, TNFR2+Treg cells infiltrating the ovarian cancer microenvironment may enhance the suppressive profile of the tumor microenvironment (Glinka and Prud'homme, 2008; Glinka et al., 2011; Govindaraj et al., 2013). It is likely that certain features of Treg cells, namely their ability to change the level of suppression relative to the immune response context, may be responsible for the ability to modulate the immune response locally within the tumor microenvironment. Zheng et al. have proposed that Treg cells create "a barrier" around the tumor. In order to give the effector T cells (which are important for anticancer immunotherapy) access to the tumor, it is necessary to destroy the Treg cell barrier in the tumor microenvironment. Such destruction must be linked with a functionally effective decrease in the number of Treg cells (Zheng et al., 2013). This suppressive micro-environment dominated by Treg cells may eventually promote a local disturbance of the immune response within the ectopic endometrial environment.

Treg cells are responsible for protecting the integrity of the human body against an uncontrolled immune response (Guerin et al., 2009). Sakaguchi has suggested that the phenotype of the FOXP3(+) Treg cell is unstable and may differ according to the inflammatory conditions of the microenvironment in which the immunological processes take place (Sakaguchi et al., 2013). Endometriosis is characterized by such an inflammatory microenvironment. The immune tolerance to Treg cell expansion is plastic and varies according to the particular type of immune response. Recently, Ono et al. have described the transcription factor AML1 (acute myeloid leukemia Runx1) as cooperating with Foxp3 to control physiological and pathological T-cell-mediated immune responses. They have presented some evidence that the interaction between these factors suppresses both IL-2 and IFN-gamma production, which in turn increases the suppressive activity of Treg cells (Ono et al., 2007).

Not only are cell to cell contact mechanisms involved in the suppressive function of Treg cells, but Treg cells may modulate the profile of the microenvironment through cell contact-independent (that is, humoral factor-mediated) mechanisms of suppression. Treg cells suppress cytokine production (especially IL-2) by CD4+ and CD8+ lymphocyte (Sakaguchi et al., 2009). The main cytokines involved are TGF beta and IL-10 (Kryczek et al., 2009). It has been demonstrated that Treg cells CD4+CD25-LAG3+ are able to secrete IL-10, which is responsible for the negative control of T-cell proliferation (Okamura et al., 2009). However, since the suppressive function of Treg cells is linked with the context of immune response IL-10 and with TGF-beta - the main cytokines presenting suppressive activates - it was at first suspected that they were not sufficient to mediate Treg inhibitory function. Blocking either IL-10 or TGF-beta does not abrogate suppression of T cells in an *in vitro* model (Thornton and Shevach, 1998; Sakaguchi et al., 2009). Recently, it has been demonstrated that Foxp3+ natural Treg cells predominantly produce immunosuppressive IL-35, a member of the IL-12 family (Collison et al., 2007). These interleukins have been detected in both non-activated and activated Treg cells and demonstrate suppressive activity against effector T cells (such as Th1, Th2, and Th17) (Chaturvedi et al., 2011; Zheng et al., 2013). IL-35 may recruit Treg cells to the tumor microenvironment (Chaturvedi et al., 2011), and IL-35 action in cooperation with IL-9 causes the suppressive function of Treg cells to develop (Gorczynski et al., 2014).

Transforming growth factor-beta (TGF-beta) induces Foxp3 expression in antigen-stimulated naive T cells (Sakaguchi et al., 2010; Ohkura et al., 2011), and Foxo1

is necessary for its induction (Kerdiles et al., 2010). Using the genetically modified clones of Treg cells, it has been shown that Treg cells (but not other T lymphocytes) produce the active form of TGF-beta1 after T cell receptor (TCR) stimulation (Stockis et al., 2009), and bind them to the cell membrane. Both CD4+ and CD8+T cells secrete soluble latent TGF-beta1 after TCR stimulation (Stockis et al., 2009; Gauthy et al., 2013).

The evolution of the immune system creates the mechanism that protects against the attack of healthy self-tissues. An adaptive immune system is capable of an antigen-specific defense response, but does not attack healthy self-tissue (Luo and Li, 2013). The final suppressive activities of Treg cells are achieved in multistep processes. On the one hand, this is a result of the evolution of the immune system and its exceptional adaptive ability; on the other hand, however, the nature of these multistep processes makes them extremely difficult to control. Treg cells are therefore a markedly independent part of the human immune system. The difficulties in controlling these suppressive processes mean difficulty in manipulating them; this constitutes a distinct limitation in the development of immunotherapy. Recently, Baur et al. have described the possibility of inducing the tolerogenic phenotype of dendritic cells by using Denileukindifitox (ONTAK) to deplete Treg cells. In cancer patients, generating tolerogenic DCs helps Treg cells to survive (Baur et al., 2013), and this significantly limits the efficacy of immune therapy. Furthermore, peripherally generated Treg cells may promote immune tolerance to environmental antigens (Luo and Li, 2013; Sakaguchi et al., 2013). The expression of the FOXP3 genes are controlled by the promoter known as conserved non-coding sequences (CNS1-3). Recently, it has been suggested that through evolution, placental mammals acquire the promotor CSN1 to control the processes on the maternal fetal interface (Josefowicz et al., 2012a,b). Treg cell differentiation in the periphery is related to TGF-beta presence in the tumor microenvironment because in peripheral CD4+Tcell TGF-beta binds Smad3 at CSN1 (a Smad3 is essential for histone acetylation in the enhancer region and induction of Foxp3) (Tone et al., 2008). The state of Treg cells is controlled not only by genetic and epigenetic imprinting, but also by transcriptional programs responding to extracellular signals (Luo and Li, 2013). Treg cell homeostasis is controlled by the differentiation factor FOXP3 (an X-chromosome encoded member of the forkhead TF family) (Littman and Rudensky, 2010). Other crucial factors are also involved in the regulation of the Treg cell suppressive phenotype, such as Aktkinase and the forkhead box O (Foxo) family of transcription factors (Foxo1) (Ouyang et al., 2012). Foxo1 has the ability to control gene expression for the chemokine receptor CCR7, thereby modulating the migration of Treg cells (Ouyang et al., 2012). Foxo1 ablated Treg cells induce fatal inflammatory disease. Foxo1 also promotes the expression of the gene for CTAL-4 (Ouyang et al., 2012). Kerdless has constructed Foxo1-deficient mice and observed that the expression of genes related to Treg cells, particularly Ctla-4gen, were substantially reduced, (Kerdiles et al., 2009, 2010).

The role of Fox-transcription factors (foxo 1,2,3) is to create complexes with other transcription factors acting as transcriptional activators or repressors. Foxo transcription factors cooperate with b-catenin, STAT3, Runx3, Smad3, or Smad4, thus controlling the differentiation program for regulatory T cells (Sakaguchi et al., 2008; Ohkura and Sakaguchi, 2009, 2010a,b; Ohkura et al., 2011, 2013).

FOXP3 was originally reported to be the gene that mutations may supply in generating the following autoimmune disorders in humans: enteropathy, X-linked syndrome (IPEX), type 1 diabetes, thyroiditis, inflammatory bowel disease, atopic dermatitis, and food allergy (Bennett et al., 2001). Yagi et al. (2004) have demonstrated that ex vivo retroviral gene transfer of FOXP3 can convert human naive CD4+ T cells into a regulatory T cell phenotype. The gene for transcription factor Foxp3 is encoded on chromosome X. The authors have further suggested that FOXP3 may be one of the main genes involved in the regulation of the generation of the suppressive phenotype (Yagi et al., 2004), and have proposed that FOXP3 may be used as a marker for Treg cells. Additionally, ex vivo expanded Treg cells derived from naive T cells through FOXP3 transduction would probably be useful for cell-oriented therapies for autoimmune disorders (Yagi et al., 2004).

According to the "transcriptional program" hypothesis, the final suppressive phenotype of Treg cells is also regulated by other transcription factors. This final Foxp3 activation is related to the realization of genetically defined programs of specification. Additionally, such genetic expression is controlled by epigenetic alterations and by transcriptional as well as post-transcriptional regulation (Josefowicz et al., 2012a; Samstein et al., 2012). The following transcription factors are involved in such a process: Eos, interferon regulatory factor (IRF4), special AT-rich sequence-binding protein-1 (Satb1), lymphoid enhancer-binding factor-1 (Lef-1), and GATA1. These factors cooperate with FOXP3 in establishing the final suppressive phenotype of Treg cells (Luo and Li, 2013).

Based on their biochemical and mass-spectrometric studies, Rudra et al. have stated that Foxp3 forms are transcriptional complexes. This analysis has revealed that Foxp3 forms multi-protein complexes of 400–800 kDa, and additionally, numerous associated complexes were identified, the majority of which were linked with transcription. FOXP3 complex cooperates with several hundred partners (including Nuclear Factor of Activated T cells (NFAT), and Runx1-Cbfbeta complex) (Rudra et al., 2012). Onkura at al. have suggested that NFAT and Runx transcription factors are crucial for the development of the suppressive regulatory function of Treg cells through direct binding to the Foxp3 gene

(Kitoh et al., 2009, Akdis and Akdis, 2009).

Treg cells and chemokines in the endometrium and the endometriosis microenvironment

Chemokines are small chemotactic cytokines (size 7-12 kDa) that coordinate the immune response and are directly involved in leukocyte maturation, migration, and trafficking within the membrane or tissue (Hannan et al., 2006; Borrelli et al., 2013). Various cells are able to produce chemokines, including leukocytes, fibroblasts, endothelial, hematopoietic, and endometrial cells. The presence of chemokines and their ligands has been documented in the microenvironment of the endometrium (Hannan et al., 2006). Hannan et al have suggested that an inflammatory microenvironment alters the production of chemokines. The final repertoire of chemokines may be related to the activities of such cytokines as TNF-alfa and IL-1 (Bergqvist et al., 2000; Borrelli et al., 2013)

The endometrium, as a place in which feto-maternal cooperation takes place, is a specific chemokine-cytokine-rich microenvironment, and there are many similarities in the trafficking of trophoblast cells within the decidua and leukocyte migration. During the implantation window the mRNA for the cytokines CX3CL1 and CCL7, as well as their receptors (CX3CR1, CCR1, CCR2, CCR3, and CCR5) were found in the endometrium (Hannan et al., 2006). Furthermore, the human endometrial epithelium can produce CCR1 ligands CCL7, CCL14, CCL16, and CCL4 (Jones et al., 2004). The course of many reproductive events in the endometrium linked with the different levels of the immune system activities are related to alterations in the concentration levels of various types of cytokines in the microenvironment of the endometrium. Hamilton et al. have demonstrated that the following mRNA and protein levels increase during labor: CCL2, CCL4, CCL5, CCL8, CXCL8, and CXCL10 (Hamilton et al., 2013). The expression of several chemokine ligands in the endometrium differs between healthy and arresting tissue, for example, in cases of healthy pregnancy compared to miscarriage (Wessels et al., 2011). Reproductive processes mainly require that the suppressive profile of the endometrial microenvironment be generated. Of the many types of leukocytes infiltrating the endometrium at the time of these events, Treg cells seem to be one of the most important. However, the proper course of reproductive processes also requires the incidental increase of immune cytotoxic responses. This situation is observed especially during such events as labor and abortion. As has been demonstrated in many studies, a strong change in the infiltration of Treg cells into the deciduas is observed during such events and is associated with changes in the repertoire of endometrial chemokines. Since Treg cells suppress T cells by using the mechanism required of cell to cell contact, the number of Treg cells in the endometrial tissue is crucial. Therefore, having a way to control the intensity of the Treg cell infiltration may be fundamental to the final activities of immune T cells. As mentioned above, at the spontaneous beginning of labor, Treg cell infiltration starts to decrease, and this is linked with the increased activity of the T and NK cells present in deciduas (Galazka et al., 2009, 2010). Moreover, at the beginning of labor, the chemokine repertoire in deciduas starts to change.

Human Treg cells are expressed on the cell surface of chemokine receptors CCR4 and CCR8. *In vitro* studies have demonstrated that the migration of Treg cells takes place in response to macrophage-derived chemokine (MDC)/CCL22, thymus and activation-regulated chemokine (TARC)/CCL17, I-309/CCL1, and to the MIP-I (ligands of CCR4 and CCR8). Such chemokines as CCL1, CCL17, and CCL22 that are produced in the precise site where the cytotoxic activities appear may influence the final result of the immune response by modulating the recruitment of Treg cells expressing CCR4 and CCR8 (Iellem et al., 2001). Treg cells accumulate in the ovarian cancer microenvironment in response to the concentration of chemokine CCL22 which binds its receptor CCR4. Zou et al. have demonstrated that Treg cells from bone marrow may be recruited to the periphery by a reducing CXCL12 expression in the bone marrow (in response to G-CSF). Clinically, this observation may be important because blocking the CXCL12/CXCR4 signal may increase the migration of Treg cells from the bone marrow to the periphery (Zou et al., 2004). Furthermore, Sugiyama et al. have shown that the administration of anti-CCR4 mAb strongly reduces the Treg cell population (Sugiyama et al., 2013). Since the chemokines TARC/CCL17 and MDC/CCL22 (specific ligands for CCR4) are produced by cancer cells to enhance the migration of Treg cells to the tumor microenvironment, the monoclonal antibody anti-CCR4 mAb is used in cancer therapy (Ishida and Ueda, 2006). By directly blocking the chemokine and its receptor in order to alter Treg cell migration, it is possible to indirectly influence the chemokine repertoire expression, as happens during the menstrual cycle phase. The menstrual cycle is controlled by many factors, of which factors steroid hormones are crucial.

It has been observed in mice that estrogen (17-beta-estradiol-E2) enhances Foxp3 expression in CD25+ T cells both *in vitro* and *in vivo* (Polanczyk et al., 2004). In an *in vitro* study in mice, Mo et al. have demonstrated that the female gender is associated with increased CD4(+) T cell CCR1-CCR5 gene expression. The alteration of CCR expression results in achemotactic response to MIP-1beta (CCL4). The author suggested that estrogens may be responsible for the increased incidence of autoimmune diseases in females (Mo et al., 2005). Estrogen has been called a Janus molecule because, in addition to being a physiologic mediator, it participates in the pathogenesis of such disease processes as atherosclerosis and autoimmune disorders (Shim et al., 2004).

Borrelli et al. have suggested that chemokine alterations are involved in the pathogenesis of endometriosis and infertility. This chemokine repertoire, including CCL2, CCL5, CCL11, CXCL8, CXCL12, and CXCR4, differs between the ectopic and eutopic endometrial microenvironments (Borrelli et al., 2013). In 2008, Braudmeier observed that Treg cells present in ectopic endometrial lesions in a baboon model of endometriosis were typified by an increased level of expression of CXCL12 (Braundmeier et al., 2012). Ruiz et al. have shown that the deregulation of the CXCR4/CXCL12 axis homeostasis may play a critical role in the pathogenesis of endometriosis. Specifically, they demonstrated that the gene expression of CXCR4 and CXCL12 may be modulated by steroid hormones. This axis is linked with the promotion and invasion of endometrial cells in ectopic localization. In the rat model of endometriosis the authors found a higher level of CXCR4 expression. It may be that high levels of estrogens in ectopic lesions disrupt the CXCR4-CXCL12 signal (Ruiz et al., 2010). Yang et al. have shown that the RANTES/CCR1 axis may be used as a biomarker of the severity of the symptoms of the deep infiltrating endometriosis correlating with the intensity of the disturbance of the inflammatory response (Yang et al., 2013). Bellelis et al. have suggested that Treg function in the disturbance of the inflammatory response in endometriosis may itself be related to the alteration in the expression of CCL17, CXCL12, and CX3CL1 (Bellelis et al., 2013).

Estrogen helps to create a special kind of cooperation between the hormonal and immunological systems in the female reproductive tract. Since the development of endometriosis is typified by a condition of high endogenous estrogen levels, most likely the Treg immune homeostasis in ectopic lesions is disturbed both directly and indirectly by estrogens. Estrogens directly modulate Treg cell function, but indirectly they deregulate the chemokine repertoire, in turn influencing Treg cell trafficking and activation.

Treg cell trafficking in endometrial cell dissemination

The thymus and peripheral lymph nodes are not the only places where Treg cells host. Recently, it was discovered that the bone marrow is a significant reservoir of Treg cells (Zou et al., 2004). The activity of chemokines facilitates the recruitment of Treg cells from the bone marrow to the periphery (Zou et al., 2004). Treg cells from the thymus and bone marrow can be found in deciduas, but the main population of these cells in the female reproductive tract is linked with their expansion in to the uterine-draining lymph nodes as a response to paternal and fetal antigens appears in the vagina, uterine cavity, and the fallopian tubes (Sakaguchi et al., 2003; Nomura and Sakaguchi, 2007). Additionally, Berbic et al. have found numerous endometrial CD10+ stromal cells in the uterine-draining lymph nodes during menstruation (Berbic and Fraser, 2011). This observation is crucial for understanding the role of Treg cells in generating the maternal immune tolerance phenomenon. This phenomenon has a local function, and the molecular mechanism of its background must be limited to the female reproductive organs. The immune system is anatomically linked not only with the reproductive organs, but also with the lymph nodes that are located in the neighborhood of the reproductive organs, such as the pelvic lymph nodes. However, the lymph nodes are located relatively far from the uterus as in the para-aortic space. It has also been demonstrated that DCS traffics to the para-aortic lymph nodes just after activation through paternal antigens (Berbic et al., 2009, 2010, 2013; Berbic and Fraser, 2011, 2013). Probably this dispersion of the places in which immune cells mature allows the pathology to spread, as the molecular mechanisms of maternal immune tolerance are part of the etiopathology. Since endometrial cells may be dispersed in the pelvis and lower abdomen, it is likely that the immune cell response modulation in reproductive processes may not be limited to the vagina and uterine cavity. Endometriosis seems to be a strictly limited pathological process. Therefore, at the beginning, ectopic endometrial tissue modulates the immune response locally, as it is realized in the endometrium in the uterine cavity during the development of maternal immune tolerance. The phenomenon of maternal immune tolerance might be independent of the general immune response control since it is linked with the development of the semi-allograft fetus within the uterus. This independence of the molecular control on the maternal fetal interface is conditioned by the ability to regulate the immune system response by endometrial cells. This process can be realized in two ways. First, endometrial cells may directly suppress the activity of immune cells, for example, by the expression of proteins that are able to induce the apoptosis of activated immune cells, such as RCAS1 and HLA-G (Wicherek et al., 2008, 2012; Basta et al., 2009, 2011; Knafel et al., 2009; Mach et al., 2010). However, the endometrial cells might, through expression of chemokines and interleukins, influence the trafficking of immune cells to the endometrial microenvironment; both effector immune cells, such as cytotoxic T lymphocytes and NK cells, and regulatory cells, including Treg cells. The endometrium seems to be responsible for generating the proper microenvironment for the development of immune tolerance phenomena. The endometrial tissue works as a buffer. On the one hand, it might exert on the suppressive local microenvironment, but on the other hand, the endometrial tissue also helps the increase of the cytotoxic immune response necessary for proper implantation and the beginning of labor (Wicherek, 2008). However, the endometrium fulfills its role only within the archimetera, and these processes are strictly local in character. When the endometrium starts to disperse from the archmietra (internal part of uterine smooth muscles and mucosa membrane), endometriotic

lesions initially appear as lesions independent of the general control of the immune system.

Recently, Yamaguchi et al. have reviewed and discussed two modes of suppression realized by Treg cells. When the immune response is controlled within the physiological conditions, inhibition of the activation of naïve T cells is a main function of natural Treg cells; this might be linked with the deprivation of activation signals, including CD28 signal and IL-2 from antigen-reactive T cells. In an inflammatory microenvironment, however, this mechanism seems to be insufficient for immune response control. For example, Yamaguchi et al. have described the suppression of Treg cells during microbial infection when a highly inflammatory microenvironment is created and activation of such mechanisms as granzyme/perforin formation or IL-10 secretion are needed for adequate suppression (Yamaguchi et al., 2011). Endometriosis is typified by such an inflammatory microenvironment. The chronic inflammatory process associated with the endometriosis lesions has been described by many authors along with the high local concentration of estrogens (Colette et al., 2013). Since two factors, namely, the inflammatory microenvironment and a high level of estrogen concentration - both of which are crucial for the development of endometriosis - might also be related to the activation of Treg cells, we must increase our understanding of the immune tolerance phenomena and the participation of Treg cells in these.

Probably, a precise understanding of the immune response regulation will help us to recognize the development of endometriosis as a disease state and not just a physiological phenomenon of retrograde menstruation. Furthermore, this information might help us to treat patients suffering from the disease. Immunotherapy has advanced markedly in recent years. Perhaps in the near future selected forms of immunotherapy for cancer patients will be useful for treating women with endometriosis.

Treg and reproductive processes

Treg cells are crucial for generating the suppressive profile of the microenvironment in patients with endometriosis because these cells constitute a major component of the physiological regulation of maternal immune tolerance against fetal antigens.

Treg lymphocytes hosting in decidua are typified by a more distinctive suppressive phenotype (Wing and Sakaguchi, 2014). Treg cells are responsible for controlling a number of processes during the development of pregnancy (Guerin et al., 2009; Wang et al., 2011): a) Treg cells increase in decidua, in the lymph nodes draining the uterus, and in the peripheral blood during pregnancy development, and this increase is directly linked with the generation of maternal immune tolerance against fetal antigens (Guerin et al., 2009); b) disturbance in Treg cell function is linked with the pathogenesis of preeclampsia and miscarriages (Guerin et al., 2009); c) Treg cell expansion is associated with the suppression of the autoimmune response and allograft rejection; d) tDC or tolerogenic DC cell activation is related to the presence of spermatozoa within the reproductive tract and induces Treg cell expansion, allowing for maternal immune tolerance against paternal antigens (Guerin et al., 2009; Robertson et al., 2009, 2013); e) Treg cell alteration at the spontaneous beginning of labor allows for the immune response activation necessary for the proper course of spontaneous labor (Galazka et al., 2009).

All the different types of regulatory CD4+ T lymphocytes are present in decidua, including Tr1, Th3, Treg, and Th17 (Mjosberg et al., 2010). Pregnancy development is associated with a significant expansion of Treg cells, which make up the predominant population of regulatory cells. For the proper development of pregnancy not only the increased suppression of the immune response, but also moderate Th1 activity, is required. Mijsoberg et al. have demonstrated that in early pregnancy the populations of both CCR6-Th1 and aggressive CCR6+TH1 cells as well as Th17 cells decrease. The lack of Th1 action at the beginning of the course of pregnancy and excessive cytotoxic activity lead to pregnancy failure. Only a balance between an increase of Th1 action and a parallel expansion of Treg cells can ensure the proper development of pregnancy (Mjosberg et al., 2010).

Three different kinds of phenotypes of FOXP3-positive T lymphocytes have been found in decidua during pregnancy: CD4+CD25++FOXP3+, CD4+ CD25+FOXP3+, and CD4+CD25-FOXP3+ lymphocytes T. All of these subpopulations of Treg cells accumulate in decidua. During pregnancy development, the CD4+CD25-FOXP3+ subpopulation increases 10 times in comparison to the corresponding subpopulation in the peripheral blood (Dimova et al., 2011). Among the various types of cytokines, only the mRNA for TGF beta has been found in all three types of Treg lymphocytes in both decidua and in the peripheral blood of pregnant women, and it has been found in the peripheral blood of non-pregnant controls as well (Dimova et al., 2011). Additionally, it has been demonstrated that CD25-FOXP3+ produces the greatest amount of TGF-beta in comparison to other types of Treg cells. TGF-beta seems to be a major factor in maintaining the suppressive profile of the endometrium and thereby enabling pregnancy development. The increasing concentration levels of such cytokines creates the optimal conditions for the maturation of Treg cells, and TGF beta produced by Treg cells is partly responsible for generating the specific suppressive profile of the endometrium. This suppressive profile determines the proper course of reproductive processes, and the suppressive profile is disturbed in women experiencing failure of early pregnancy development.

Regardless of the cause of miscarriage - whether due to the genetic aberration of the fetus or abnormal immune response - a reduction in Treg cell expansion

can be found. The authors have examined Treg cell populations in the decidua at the time of the miscarriages of fetuses that exhibited both abnormal and normal chromosomal content. The number of Ki67-, Foxp3+CD4+ T cells decreases during miscarriage and is at its lowest in miscarriages where the embryo exhibits a normal chromosomal content. Since this cell population increases in decidua in the first trimester during normal pregnancy, the alteration of Ki67-Treg cells would seem to be linked with immune tolerance during pregnancy development (Inada et al., 2013). Patients with recurrent miscarriage (RM) demonstrated a disturbance of the interaction between macrophages and Treg cells. Interaction between these two kinds of cells is generally realized by cell to cell contact (CD80/86 via CTLA-4), and cytokines such as TGF-beta and IL-10 are crucial for these processes. Wang et al. have observed that macrophages from the decidua of patients with RM are characterized by a lower level of secretion of IL-10. The deregulation of macrophages is thus correlated with the reduced regulatory capacity of Treg cells (Wang et al., 2011). In patients with unexplained recurrent spontaneous abortion (URSA), a reduced level of Treg cell expansion within both the peripheral blood and decidua has been observed in comparison with normal pregnant controls (Yang et al., 2008). Additionally, during miscarriage, a decreased level of the rate of CTLA4+/CD28+ in CD4 (+)CD25(bright) T has been seen. Such observations would seem to support suggestions that Treg cells participate in pregnancy development mainly via the upregulation of CTLA-4 (Jin et al., 2009). The magnitude of the population of Treg cells within CD4+ lymphocytes may be used as a clinical marker to predict the outcome of the first trimester of pregnancy in the group of women who have lost pregnancies due to immunological issues (Winger and Reed, 2011).

The disturbance of Treg cell expansion during miscarriage causes continuous processes to deregulate. Many reports have shown that Treg cells constitute the principal component of maternal immune tolerance during pregnancy. However, this immune phenomenon is a progressive process that begins prior to implantation and continues even after labor. It is understood that the first symptoms that maternal tolerance is being created occur at the time of intercourse. Antigens derived from seminal fluid activate the Treg cell population that is related to the initiation of T-cell tolerance (Robertson et al., 2009). Just prior to ovulation, increasing estrogen levels in the proliferative cycle phase lead to an increased expression of chemokines CCL3, CCL4, and CCL5 (chemokine C-C motif ligand) in decidua, which affect the systemic expansion of the Treg cell population. During coitus, seminal fluid, which contains TGF-beta and PGE2, leads to dendritic cell activation. The maturation of DCs begins and tolerogenic DCs appear in the uterine tissue. After antigen presentation in the vagina (paternal antigens), dendritic cells traffic to the lymph nodes where the maturation of Treg cells from naïve CD4+CD25-T lymphocytes is completed (Scherjon et al., 2011). As a result of such processes in the lymph nodes draining the uterus, an expansion of the subsets of antigen-reactive Treg cells is initiated. Implantation of the ovum within the endometrium stimulates the further recruitment of Treg cells to decidua, where it is linked with increased CCL4 expression in the decidua. Cytokines such as G-CSF, GM-CSF, IL-4, IL-10, and IDO are responsible for the differentiation of DC into its tolerogenic phenotype. However, Th1 cytokines, such as IL-2 and IL-15, are essential for antigen presentation by tDCS and further Treg cell activation and proliferation. The maternal immune tolerance phenomenon is a progressively changing and dynamic process. Further expansion of Treg cells correlates with increased concentration levels of TGF-beta and PGE2. Treg cells are able to secrete the following factors: IDO, IL-4, and IL-10 (Robertson et al., 2009; Scherjon et al., 2011), which help to generate the conditions favorable to Treg cell expansion. Decidual cells express CCL4; as CCL4 attracts Treg by way of CCR5, it is essential for trafficking Treg cells from the regional lymph nodes to the decidua. Additionally, Treg cells express chemokine receptors CCR4 and CCR8. Moreover, the expression of CCL17 has been observed in the endometrium, and this chemokine, which produces the ligand for CCL22, is needed for Treg cell interaction with macrophages. This particular chemokine also participates in the infiltration of Treg cells to the decidua (Scherjon et al., 2011). Using a mouse model, it has been demonstrated that the expression of chemokines changes in accordance with the menstrual cycle phase. The increase in the levels of estrogens is linked with a higher level of CCL4 expression in the endometrium. Furthermore, Jin et al. have observed a greater correlation between CCL4 and FOXP3 expression in the endometrium (Jin et al., 2009).

The beginning of immune tolerance based on Treg cell accumulation in decidua depends on the hormonal milieu (estrogen and progesterone levels) as well as on the presence of paternal antigens (Scherjon et al., 2011). There are two crucial factors for the start of these processes. The biological evidence of the proper level of immune tolerance generation is the invasion of trophoblasts within the endometrium (changes in blood vessels). When invasion into deep placenta increta or, conversely, insufficient invasion is observed, symptoms of preeclampsia may occur (Scherjon et al., 2011). Thus, the microenvironment of the endometrium creates the special conditions that support a multistep process of controlling immune tolerance intensity levels. Such a microenvironment is generated by endometrial cells and the maternal immune system, and later correlates with the development of pregnancy by fetal cells. The endometrium and decidua support these processes in accordance with the necessary regulation. On the one hand, the endometrium secretes factors that reinforce immune response (for example, implantation of the ovum requires TH1 immune response increases), and on

the other hand, the endometrium secretes factors that create the suppressive profile (which enables Treg cell infiltration into the endometrium). Thus the endometrium works as a buffer between maternal and fetal immune cells.

The phenomenon of the selective suppression of cytotoxic immune cells consist of a number of processes that increase progressively. Different molecular mechanisms are involved in these processes which can be presented sequentially as occurring in at least four phases: a) invisibility to immune cells; b) aggressive killing of immune cells; c) changes in the microenvironment of immune cells inhibiting the maturation of immune cytotoxic cells; d) the use of suppressive mechanisms belonging to the maternal immune system (Dutsch-Wicherek et al., 2013). These selective suppression mechanisms progressively increase, causing the phenomenon of selective suppression to advance from a local process at implantation to a general mechanism during labor. Finally, trophoblast cells recruit Treg cells and other regulatory immune cells, such as B7H4 positive macrophages, to the decidua (Wicherek et al., 2009).

The endometrium microenvironment normally functions only within the uterus. When the endometrium or decidua is located outside the uterus, the multistep process of immune control involving Treg cells and other cells is disrupted. During ectopic pregnancy implantation, a disturbance in the generation of the local suppressive profile leads to an increase in the maternal immune system response, and as a result of this increased response, Fallopian tube rupture occurs (Wicherek et al., 2006). In our recent studies we have shown that implantation of the ovum within the Fallopian tube is associated with an alteration in the amount of Treg cell infiltration into the Fallopian tube mucosa when compared to the amount of Treg cell infiltration into the endometrium at the time of intrauterine implantation (Basta et al., 2010). In cases of ectopic pregnancy, different kinds of infiltrations of macrophages to the endometrium have been observed. Similarly, in cases of intrauterine pregnancy in RM patients, an aberrant interaction between macrophages and Treg cells has been found (Wang et al., 2011). It is likely that a disturbed interaction between macrophages and Treg cells can also be seen in patients with endometriosis. The ectopic endometrium micro-environment function is disrupted, and this may lead to improper control on Treg cell expansion (trafficking by chemokine expression, maturation by high local estrogen concentration levels, and cooperation with other immune cells by high cytokine concentration levels, for example, TGF-beta).

Treg in endometriosis

Berbic et al. have demonstrated the presence of Treg cells within both the ectopic and eutopic endometrium of women suffering from peritoneal endometriosis. Endometriotic foci (including endometrial cell antigens) lack proper antigen presentation by DCs (Berbic and Fraser, 2011). Schulke et al. have observed a significant drop in the number of mature DCs CD83+ cells in the eutopic endometrium of women with endometriosis during both the secretory and proliferative cycle phases (Schulke et al., 2009). The change involves the functional as well as the basal part of the endometrium over the course of the various menstrual cycle phases. The density of CD1a+ cells is greater in eutopic endometrium in women during the proliferative cycle phase. Similarly, a greater number of CD68+ positive cells have been observed in the eutopic endometrium of women compared to that found in normal endometrium as confirmed by immunohistochemistry analysis (Berbic et al., 2009). In a consecutive study, Berbic et al. have analyzed the Treg cell population in the endometrium by assessing Foxp3-antigen immunoreactivity over the course of the different menstrual cycle phases. They have shown that the level of FOXP3 expression within the eutopic endometrium derived from women with peritoneal endometriosis differs from that observed in the tissue of healthy women (Berbic et al., 2010). Moreover, they have identified an increasing mean density of FOXP3+ cells in eutopic endometrium during the secretory cycle phase (and this increase has been observed during both the early and mid-secretory cycle phases). During the other phases, the number of infiltrating Treg cells is lower in the eutopic endometrium of women with endometriosis when compared to that found in the endometrium of healthy women, except during the early proliferative phase when the number of Treg cells is actually higher. In ectopic endometrium the presence of Foxp3+ positive cells has been observed in only 30% of the tissue samples, and differences in the number of Treg cells correlating with the different menstrual cycle phases have not been observed in peritoneal endometriotic lesions (Berbic et al., 2010; Berbic and Fraser, 2011).

Utilizing the induced non-human primate (*Papioanubis*) model of endometriosis, Braundmeier et al. have investigated the Treg cell population. The presence of ectopic foci significantly reduced the Treg cell population during the secretory, proliferative, and menstrual cycle phases. Natural (CD4+/CD25+ /FOXP3+) and adoptive Treg (CD4+/CD25+/FOXP3- or CD4+/CD25-/FOXp3+) cells in both eutopic and ectopic endometrium as well as in the peripheral blood have been assessed for FOXP3 transcript levels. The level was significantly higher in ectopic endometrial lesions. Similarly, an increased number of FOXP3+ cells have been observed in ectopic lesions while in eutopic endometrium the number remained unchanged. In endometriosis foci Treg cells have been localized in the mesenchymal/endometrial border, particularly in invasive lesions (Abbott et al., 2003; Littman et al., 2005). Removal of these endometriotic lesions which were active immuno-regulatory foci promotes the rebound from a pro-inflammatory to a normal

immunological environment (Braundmeier et al., 2012). This process of normalization of immune system activity has also been observed in an animal model. In this model, the effect of the surgical removal of endometriotic lesions on the Treg cell population confirmed the suitability of surgery for treatment of women with endometriosis. It also demonstrated the direct influence of surgery as a therapeutic method on the immune system. Such a relation has also been demonstrated in our studies concerning the effects of surgical treatment on women with advanced ovarian cancer (Wicherek et al, 2011).

In our previous study we assessed the subpopulation of CD4+CD25+FOXP3+ positive cells in tissue samples from ovarian endometriotic lesions and compared them to endometrial tissue samples taken from women during the secretory cycle phases. We observed a significant increase in Treg cell population in ectopic endometrium compared to eutopic endometrium (Basta et al., 2010).

Immunologic disturbance during the development of endometriosis is associated with two processes: first of all, decreased DC numbers in ectopic lesions are related to ineffective antigen presentation; secondly, increasing Treg accumulation during the secretory cycle phases is associated with Treg-mediated suppression (Berbic and Fraser, 2011). These processes are essential for the proper functioning of the immunoregulative endometrium. After functional awakening, tDCs presenting paternal antigens in the uterine cavity balance the suppressive activity of the increasing Treg cell population. The beginning of pregnancy and proper ovum implantation involve not just the suppression of theTh1 response, but more importantly, a balance between the activity and suppression of the immune response. Just prior to the implantation there is an increase in Th1 and Th17 response that has been observed (Robertson et al., 2009, 2013).

Sakaguchi has suggested that Treg phenotypes can be altered in response to inflammatory conditions (Sakaguchi et al., 2013). Since the microenvironment of ectopic endometrium constitutes a special kind of inflammatory environment that can modified by the activation of many molecular mechanisms linked with immunoregulation, Treg cell function may be disturbed. Additionally, the endometriosis foci microenvironment influences genetic changes in endometrial ectopic cells. Many studies have revealed genetic and epigenetic changes in ectopic endometrial cells in comparison to eutopic endometrial cells. Recently, Onkura has suggested that Treg cell activation may ultimately be linked with epigenetic changes in these immune cells. Moreover, the process of Treg cell specification is crucial for protecting against the autoimmune response. This process may result from its own potential plasticity and would seem to be linked with epigenetic changes (Ohkura et al., 2013). Most likely, the microenvironment of ectopic endometrial tissue influences not only the function of the genome of ectopic endometrial cells, but also the function of Treg cells.

The participation of the endometrium in the immune tolerance triangle (interactions between maternal immune cells, decidual cells and trophoblast cells) seems to be disturbed in women with endometriosis. Similarly, in the absence of the endometrium such as in the decidua of the ectopic pregnancy, the mucosa of the Fallopian tube is able to regulate immune system cells, but not to the same extent as the endometrium with in the uterine cavity. Development of improper immune system cell regulation in ectopic endometrium is a result of a disturbance (apart from numerous functional differences) in the discrete balance which characterizes the regulation of the immune system cell response to fetal antigens within the uterine cavity. This disturbance, together with hyperestrogenization which can disturb the multistep mechanism of Treg cell activation, promotes the inflammatory microenvironment of endometriosis and results in the excessive expansion of Treg cells, which disables the removal of the ectopic foci of the endometrium.

Endometriosis is a condition characterized by a disturbance in the regulation mechanism. This mechanism operates according to a discrete balance or tolerance between the activation and suppression of the immune response. In order to control Treg cell expansion and the proper course of pregnancy within the uterine cavity, numerous molecular changes must begin prior to ovulation and occur in the proper sequence. These changes lead to the development of the immune tolerance phenomenon that enables proper embryo development, and Treg cell expansion is one of the most important mechanisms controlling this process.

Perspectives

The etiopathogenesis of autoimmune disorders is linked with the disturbance of Treg cell function (Asano et al., 1996; Sakaguchi, 2000; Miyara et al., 2011). When CD25+ T cells, which constitute 5-10% of peripheral CD4+ T, were eliminated in mouse models, the development of various autoimmune diseases was observed (Takahashi et al., 1998). While endometriosis is not an autoimmune disorder, strictly speaking, in both autoimmune diseases and endometriosis there is a disturbance of the immune response, and the severity of this disturbance is directly related to the advancement of the disease. In endometriotic lesions, the improper immune response is related to the special nature of the endometriosis microenvironment, which is itself directly linked with immune regulatory ability of endometrial cells. Such features of the endometrium are related to the ability to accumulate Treg cells. Similarities between autoimmune disorders and endometriosis are apparent in the relationship between the intensity of self-tolerance and an increased population of Treg cells. The degree of self-tolerance is also related to an increase in the Treg cell population during the development of an allergy (Wing and Sakaguchi, 2006). We might then understand that the survival of the transplanted ectopic endometrial

cell is linked to Treg cell activities. Similarly, the accumulation of Treg cells may be linked to the ability of cancer cells to evade the immune response (Yamaguchi and Sakaguchi, 2006). It is likely that such an observation will lead to new therapeutic possibilities for the treatment of endometriosis. Allergy therapy uses Treg cell modification with the potential prospect of a safe and long-term alleviation of allergic diseases (Wing and Sakaguchi, 2006; Miyara et al., 2009; Sakaguchi et al., 2013). The depletion of Treg cells in mice or abrogation of their function is related to the development of various autoimmune diseases such as gastritis and thyroiditis (Sakaguchi et al., 1995). Nagaham et al. have presented the results of their observations on the expansion of both donor-specific Treg cells and effector T cells in response to Mls allo-antigen in an experimental skin transplant model. Graft-tolerance might be achieved by a combination of allo-antigen exposure and the manipulation of T cell function (for example, usage of by donor-specific transfusion (DST) and anti-CD4 mAb) (Nagahama et al., 2009). Blocking Treg cells by the use of anti-CD154 [CD40 ligand (CD40L)] mAb leads to an enriched population of CD25+CD4+ Treg cells that help to suppress allograft rejection (Jarvinen et al., 2003). The IL-2 signal inhibitor Rapamycin may enhance the population of CD25+CD4+ Tregs both *in vitro* and *in vivo* (Battaglia et al., 2005; Nagahama et al., 2009). Moreover, the literature provides a great deal of evidence that it is possible to modulate the Treg cell population (Ohkura et al., 2011). Foxp3-transduced T cells are able to inhibit the rejection of an allogenic transplant; furthermore, T-cell Foxp3 gene transfer may have therapeutic value in clinical transplantation (Chai et al., 2005). However, control of the Treg population is instrumental in effectively controlling immune responses. Such cytokines as interleukin-2 and transforming growth factor-beta can either enhance or dampen the suppressive activity of Treg cells. Increased suppressive activity of Treg cells is one of the possible directions of immune therapy. Conversely, manipulation to decrease the suppressive activity of these cells is also possible. Moreover, the ligand of GITR (GITRL) contributes to T-cell responses. Consequently, the combined use of a GITRL tumor vaccine with methods aimed at enhancing the activation of host antigen-presenting cells in secondary lymphoid tissues may be a promising strategy for tumor immunotherapy (Piao et al., 2009). Yamaguchi has suggested that the attenuation of T(R) cell-mediated suppression in ongoing anti-tumor immune responses (by, for example, altering signaling through CTLA-4 or GITR expressed by natural T(R) cells), can enhance those responses and thereby eradicate advanced forms of cancer (Yamaguchi and Sakaguchi, 2006).

Once we understand precisely how Treg cells participate in the generating of the suppressive microenvironment of ectopic endometrial cells, it will be possible to effect the depletion of the Treg cell population in endometriosis therapy. Controlling the population of Treg cells in the endometriosis microenvironment can stimulate the local cytotoxic immune response needed to eradicate ectopic endometrial cells. Such modulation may be realized by the alteration of chemokine receptor expression or by other methods used in anticancer therapy. The limitation of such planned therapy lies in its local nature, as the general modulation of the Treg cell population would seem to be dangerous for homeostasis.

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