Immunotherapy in Endometriosis

Role of immune response

Monocytes/macrophages

- One of the main functions of macrophage is phagocytosis mediated by scavenger receptors for cell recognition and regulated by various cytokines and growth factors.
- The defect in phagocytic ability of macrophages in endometriosis has been associated with downregulation of scavenger receptors expression and reduced function of the CD36, a class B scavenger receptor.
- Women affected of endometriosis have a significantly higher concentrations of PF macrophages.

- Akoum et al. demonstrated that peritoneal macrophages of these women had an increased capacity to secrete MCP-1 (monocyte chemotactic protein-1), which plays a role in the recruitment of monocytes to sites of injury and inflammation.
- Macrophages are not only involved in the early immune escape of endometriotic cells, but also are favored later establishment and growth with the cytokines produced.
- Aberrant increased inflammatory cytokines produced by the endometriotic lesion and peritoneal immune cells, especially macrophages, could critically contribute to the pathogenesis of endometriosis. They can interact with each other to convey a cascade of functions.

Cytokines

- Cytokines are proteins produced and secreted by a variety of cells including stromal cells, fibroblasts, and endothelial cells. In the immune system they are produced by leukocytes and exert their function on other leukocytes or tissues that express the cytokine receptor.
- Pro-inflammatory cytokine activated macrophages have an essential role in the onset and progression of edometriosis. Both the endometrial and infiltrating immune cells produce inflammatory cytokines, such as TNF-α, IL-1β, IL-4, IL-6, IL-8, IL-17 that further stimulate a cascade of inflammatory response.

- TNF-a mainly acts as a precursor to initiate inflammatory response at the acute phase by activating a cascade of other cytokines, such as IL-1, IL-6 and VEGF. In addition it can promote the adherence of ectopic endometrial cells to the peritoneum and plays a crucial role on increasing the invasiveness of endometrial fragments by upregulating MMP and reducing the effect of tissue inhibitors of MMP.
- IL-1 has been found to onset cascade of other cytokine production in endometriotic stromal cells, such as vascular endothelial growth factor (VEGF) to promote angiogenesis.
- IL-6 stimulate stromal cell proliferation, sICAM-1 and is able to impair NK cell activity, B cells proliferation and autoantibody production.

- IL-8 concentration in the peritoneal fluid and serum seems to be higher in women with endometriosis than women without endometriosis.
- In addition to macrophages, eutopic and ectopic endometrial cells are another important source of IL-8, and also IL-17A released by endometriotic lesions significantly increase angiogenic cytokines (IL-8,VEGF,) and proinflammatory cytokines (IL-6 and IL-1β) in the peritoneal cavity favoring the establishment, proliferation and migration of endometriotic cells.

T-cells

- T-helper 1(Th1) T-helper 2 (Th2) imbalance has been associated with endometriosis wherein the pro-inflammatory Th1 profile dominates over the Th2 anti-inflammatory response.

- One of the key regulators of immune processes in endometriosis are regulatory T cells (Tregs) derived from CD4 lineage.
- Tregs suppresse the response of effector T cell proliferation and play an indispensable role in the maintenance of self-tolerance and immune homeostasis and are involved in various human diseases, such as autoimmune diseases, allergies, and cancer.
- Tregs induce immune tolerance by production of IL-10, TGF-β, and anti-inflammatory cytokines that inhibit T helper cell activation.
- In line with a role for a modified immunity in the pathogenesis of endometriosis, Tregs are present in endometriotic lesions.

Dendritic cells

- Dendritic cells (DCs) are bone marrow-derived hemotopoietic cells which act as a “bridge” between innate and adaptive immunity. They are critically involved in the initiation and modulation of the adaptive immune response by recognizing and capturing antigens to naïve T cells as well as immune tolerance in inflammatory and neoplastic diseases.
- Schulke et al. demonstrated significant increase of immature DCs (iDCs) in peritoneal endometriotic lesions and in the surrounding peritoneum when compared with paired eutopic endometrium and peritoneum distant from the lesion.

Natural killer cells (NK)

- In endometriosis, the cytotoxic activity of peripheral and peritoneal NK cells was obviously decreased with the severity of the disease.
- The decrease in NK-mediated cytotoxicity in the peritoneal fluid might promote retrograded endometrial cells to escape immune surveillance and promote its lesion establishment on the peritoneal cavity.

Myeloid derived suppressor cells

- Based on the well-known immune suppressive role of MDCSs in tumors, it is believed that MDSCs might contribute to the escape of ectopic endometrial cells from immune surveillance through

  1) inducing differentiation of Tregs

  2) dysfunction of NK cells cytotoxic activity

  3) promoting polarization of M2 macrophages which was characterized with decreased phagocytosis but increased tissue remodeling and angiogenesis

- thereby contributing to the development of endometriosis.

Adhesion/invasion, angiogenesis and proliferation/growth

- Cell adhesion molecules, including integrins, cadherins and ICAM-1, are expressed in endometrial and mesothelial cell surface to promote endometrial-mesothelial adhesion.
- Following adhesion, endometrial cells invade the peritoneal tissue by proteolytic digestion of extracellular matrix (ECM), such as serine proteases and matrix metalloproteinases (MMPs) and have increased adhesive capacity to various components of the ECM including collagen type IV, laminin, vitronectin and fibronectin.

- Furthermore growth and development of endometrial cells will heavily depend on neovascularization that is mediated by cytokines produced by immune cells, peritoneal cavity and retrograde sheds.
- In particular the expression of VEGF, which is dependent on estradiol, hypoxia and peritoneal inflammatory cytokines, is known to be an important precursor toward promoting the pathological angiogenesis for the endometrial lesions to grow and develop.

B-cells and autoantibodies

- Endometriosis shares many charateristics with autoimmune diseases, since women affected exhibit altered immune surveillance with abnormal functions of T and B cells, heightened humoral immune response (high serum levels of IgG, IgA, IgM autoantibodies, and anti-endometrial antibodies) and inflammatory tissue damage.

- The increased B-cell function was firstly documented in the 1980's. Around the same year, Weed and coworkers reported that C3 and IgG deposited in the endometrium of women with endometriosis is associated with a reduction in the serum total complement level, which suggested an intra-endometrial antigen-antibody reaction.
- Further studies demonstrated higher incidence of autoantibodies in sera, cervical and vaginal secretions from women with endometriosis.
- However, the correlation between autoantibodies concentration and the severity of endometriosis remains controversial.
- As anti-endometrial antibodies are the most well-studied autoantibodies, it has been characterized as a potential diagnostic or follow-up marker on the assessment of treatment and recurrence toward endometriosis.
- Such profound anti-endometrial antibodies include transferrin and alpha 2 Heremans Schmidt (α2-HS) glycoprotein.

- The levels of endometrial transferrin in the peritoneal fluid of patients with endometriosis are significantly elevated, whereas the serum levels are lower when compared with controls.
- With the concentration of transferrin and α2-HS glycoprotein being found higher in peritoneal fluid from women with endometriosis, it has been showed that transferrin attenuates FSH-induced differentiation of granulosa cells and might consequently lead to the varying degrees of ovarian dysfunction.
- α2-HS glycoprotein, a negative acute phase protein, suppresses the maturation conversion of mouse embryo zona pellucida protein ZP3 to ZP3f and causes polyspermia.

- Furthermore the addition of antibodies specifically to transferrin and a 2-HS glycoprotein in in vitro can inhibit motility and survival of sperm.
- This can lead to a disturbance on endometrial receptivity, oocyte quality, sperm motility, fertilization failure and embryo toxicity.

- On the other hand, the reason causing the elevated purge of autoantibodies in women with endometriosis is still uncertain.
- Recently identification of shared molecular signature indicate the susceptibility of endometriosis to MS, with shared genes up-regulating both endometriosis and MS such Neuronal growth regulator 1 (NEGR1), leptin receptor (LEPR), Cholinergic receptor muscarinic 3(CH3M3), Inositol 1,4,5-triphosphate receptor type 1 (ITPR1), the last able to disregulate oocyte meiosis and calcium signaling pathway.
- Different shared genes are able to down regulate both endometriosis and MS, such as Solute carrier family 8 member A1 (SLC8A1), Erb-b2 receptor tyrosine kinase 3 (ERBB3), Cadherin 1 (CDH1), Integrin subunit beta8 (ITGB8) Protein tyrosine phosphatase, non receptor type 11 (PTPN11), and Protein phosphatase 2 regulatory subunit B epsilon (PPP2R5E). The last one is able to disregulate oocyte meiosis.

- A comparative evaluation of clinical and humoral immunologic abnormalities between SLE and endometriosis has observed that the diseases are associated.
- Endometriosis was also associated with a lesser magnitude with RA risk.
- Also Graves disease is linked with endometriosis.
- This association was explained for the high incidence of positive antinuclear antibodies (ANA) in both the disease and for differential expression of the estrogen receptor beta gene (ESR2) confirmed in patients with endometriosis and associated with susceptibility to Graves disease.

- Another possible explanation that can lead to self-tolerance breakdown women with endometriosis is the overexpression of B lymphocyte activating factor (BAFF).

- Other studies showed that significantly elevated IgG antilaminin-1 antibodies are strongly associated with endometriosis in infertile patients.
- Laminin is a major multifunctional basement membrane glycoprotein with at least 15 known isoforms.
- It plays a critical role during embryo implantation by synthesizing network-forming complement during early embryo development.
- Also, it has been found to elevate the trophoblast adhesion to the maternal extracellular matrix and then decidua in endometrium, which further promote proliferation and differentiation of trophoblast when interacts with integrin receptors.

- In a study conducted by Inagaki and coworkers, they found significant association between IgG anti-laminin-1 antibodies and infertile patients with endometriosis for stage II or more.
- The same group also demonstrated that anti-laminin-1 antibodies are significantly higher in women with recurrent miscarriage when compared with healthy controls, which causes a disruption in early reproductive stages by interfering with embryogenesis and placental development.

- Moreover anti-laminin-1 in follicular fluid was noted to be related with oocyte maturation, it affected oocyte quality resulting in reduced fertility.
- It was demonstrated that these antibodies may directly interfere with the function of laminin-1 to disrupt early reproductive stages and be involved in the development of endometriosis.
- In light of these findings, anti-laminin-1 antibodies might be clinically important in development of autoimmune-mediated reproductive failure and the antibody assessment may provide a novel non-invasive diagnosis of endometriosis.

Immunomodulatory therapies

Steroids

- In a murine embryo assay, the addition of dexamethasone to murine embryo culture with endometriotic peritoneal fluid improved the rates of blastocyst development.
- Few studies evaluated the benefit of steroids for endometriosis-associated infertility.

- Twenty-one patients with endometriosis received steroids before IVF, at 10mg/day from the 3rd day of the cycle until the day of oocyte retrieval and the dosage was increased to 60 mg/day from the evening of oocyte retrieval and for 4 days.
- The rates of clinical pregnancies after IVF were compared to 44 controls with IVF without steroids.
- The clinical pregnancies rates were at 42.6% in the steroid treated group vs. 22.8% in the absence of steroid therapy ($p<0.05$), without differences in the miscarriage rates.
- In women with positives autoantibodies the clinical pregnancy rates were higher in steroid treated group as compared to non-treated group (40.9% vs 14.8%; $p<0.05$).

- In 84 infertile women with endometriosis, steroids were added during the entire IVF cycle or 5 days before embryo transfer and various autoantibodies were tested before IVF cycle.
- In 35 autoantibodies-positives patients, steroid use during IVF cycle was associated with clinical pregnancy in 8/10 cases versus 0/25 in those without steroid treatment (80% vs 0% $p < 0.05$).
- In 35 autoantibody-negative patients, pregnancy was obtained in 7/15 cases with steroid treatment during IVF versus 9/20 in the group without steroid treatment (46.7% vs 45%, p=NS) [49].

Antimalarials: Hydroxychloroquine

- Antimalarials particularly hydroxychloroquine (HCQ) exerts pleiotropic effects other than antiinfectious.
- Antimalarials have many anti-inflammatory, immune-regulatory and anti-aggregant properties.

- They inhibit phospholipase activity, stabilize lysosomal membranes, block the production of several pro-inflammatory cytokines, impair complement-dependent antigen-antibody reactions and attenuate antigen processing, and inhibit cell-mediated cytotoxicity.
- Besides, HCQ reduce the proliferative response of T-lymphocytes and NK cell activity.
- In addition, inhibition of autophagy prevents immune activation of different cell types, which inhibits cytokine production and modulates CD154 expression on the surface of T cells.

- HCQ also inhibits macrophage TNF mRNA transcription and endotoxin-induced secretion of TNF-α, IL-1, and IL-6.
- Furthermore, HCQ include anti-inflammatory properties through the blocking effect on the arachidonic acid cascade (phospholipases A2 and C), which contribute to the down-regulation of proinflammatory prostaglandins.

- Accordingly, the therapeutic effects of HCQ were assessed in an established mouse model of endometriosis.
- HCQ significantly altered the endometriotic cells survival, by interfering with the inflammatory response, altering the organization of ectopic growth and modulating the expression of autophagic markers.
- Nevertheless, clinical data about the potential benefit of hydroxychloroquine are lacking.

Intralipids

- Increased NK cells rates and cytotoxicity have been demonstrated in endometriosis-associated infertility, and in vitro and clinical studies raise the potential interest to use intravenous intralipid infusion to modulate the NK cells cytotoxicity in infertility.
- The data about the use of intralipids in endometriosis-associated infertility report 3 women with increased uterine NK (uNK) which received intralipid infusion on the day of the embryo transfer in frozen embryo transfer cycles, 2nd infusion after the positive pregnancy test and following infusions every 2 weeks until 12th week of pregnancy which resulted in live birth in 2 cases.
- However, uNK cells are necessary to achieve a normal implantation and further correct placentation. Like this, only cases with high uNK cells in endometrial fluid could be suitable for a treatments directed to reduce them. Thus, the role played for intralipids appears to be anecdotic.

TNF-α antagonists

- In baboon model of endometriosis, the neutralization of TNF-α activity with recombinant TNFRSF1A (the soluble form of TNF receptor type 1) significantly decreased the endometriotic lesions without causing the hypoestrogenic effects.

- The increased apoptosis and embryotoxicity of TNF-α was abrogated by the addition of adalimumab, a human monoclonal anti-TNF-α antibody.
- In a randomized placebo-controlled blinded study using rat endometriosis model, female rats were randomized to receive either etanercept 0.4 mg/kg sc once weekly during 4 weeks or placebo.
- The volume and extension of endometrial implants were significantly reduced in female rats under etanercept in comparison to untreated controls, but the effect on the clinical pregnancy was not analyzed in this study.

- Etanercept, a TNF-α blocker, a potential immunomodulatory agents was used in a baboon model, and led to a statistically significant decrease in red lesion surface area in the treatment group with a trend toward a decrease in the absolute number of red lesions.

- So far no well-designed studies evaluated the clinical value of TNF-α antagonists in women with endometriosis-associated infertility.
- In a woman with severe endometriosis under etanercept for rheumatoid arthritis, no spontaneous pregnancy occurred, and she became pregnant several months later after IVF preceded by laparoscopic surgery.

- Nineteen women with endometrioma received etanercept 50 mg on the second day of the menstrual cycle preceding IVF cycle.
- The clinical pregnancy rate was higher in patients who received etanercept as compared to non-treated women, with odd ratio 4.17 [95% CI 1.23-14.14).

- Data about the safety of TNF-α antagonists during the pregnancy are extensively reported, in particular in women with Crohn's disease and rheumatoid arthritis.
- The main concern is the increased risk of infectious adverse events.

- In the only trial of infliximab in 21 women with endometriosis, an acute tonsillitis and a mild infusion reaction were noted.
- In this randomized trial analysis the efficacy of infliximab versus placebo in patients with endometriosis and chronic pelvic pain prior to surgery is done. The decrease of pain severity was noted in 30% of infliximab treated women and was similar to those under placebo.
- The volume of endometriotic nodules, the lesions extension evaluated during the surgery and the extent of endometriosis were not significantly changed after infliximab use in comparison to placebo. The impact on the infertility was not analyzed in this trial.

Other TNF-a inhibitor: Pentoxifylline

- Pentoxifylline is a phosphodiesterase inhibitor commonly used in the treatment of peripheral vascular disease.
- A wide range of immunomodulatory properties has been described, including of down-regulating TNF-alpha synthesis.
- Furthermore, animal models showed a reduction in fetal resorption, thereby diminishing TNF-alpha and increasing IL-10 and IL-4 placentally produced levels.

- In nude mouse model with implanted human endometrial tissue from endometriosic women, the use of oral pentoxifylline reduced the number and the volume of endometriosis-like Lesions.
- Recent Cochrane review included four trials involving 334 participants and pentoxifylline had no significant effect on reduction in pain. There was no evidence of an increase in clinical pregnancy events in the pentoxifylline group compared with placebo.

- Another immunomodulator (loxoribine) caused a reduction in NK cells and endometriotic lesions in a rat model and a similar reduction of endometriotic lesions was observed with lipoxin, rapamycin, and pentoxifylline.

- So, even if these novel therapeutic agents appear promising on the treatment of endometriosis, further studies from multi-center clinical trials are still needed prior to be recommended as a routine regime for women with endometriosis.

- Mariani et al. and other groups have demonstrated that rodents supplemented with 1,25(OH)2D3 showed a regression of endometriotic lesion by significantly reducing VEGF and MMP-9, and increasing MMP-2 inhibitors.
- Importantly, dairy products rich in calcium and VD have been linked to lower the risk of developing endometriosis in humans.

- Also a wide variety of antiangiogenic agents has been evaluated in vitro as potential treatments for enometriosis.
- These include growth factor inhibitors, endogenous angiogenesis inhibitors, fumagillin analogues, statins, cyclooxygenase-2 inhibitors, phytochemical compounds, immunomodulators, dopamine agonists, peroxisome proliferator-activated receptor agonists, progestins, danazol, and gonadotropin-releasing hormone (GnRH) agonists.
- However, clinical evidence for the efficacy and safety of most of them is still lacking.