Inflammatory cytokine profile of co-cultivated primary cells from the endometrium of women with and without endometriosis

ADRIANA LUCKOW INVITTI, EDUARDO SCHOR, RAFAEL MARTINS PARREIRA, ALEXANDER KOPELMAN, GIL KAMERGORODSKY, GIOVANA APARECIDA GONÇALVES and MANOEL JOÃO BATISTA CASTELLO GIRÃO

Department of Gynecology, Paulista School of Medicine, Federal University of São Paulo, São Paulo, SP 04024-002, Brazil

Received November 7, 2017; Accepted March 6, 2018

DOI: 10.3892/mmr.2018.9137

Abstract. Endometriosis is a chronic gynecological disorder defined as the presence of endometrial tissue within extra-uterine sites. The primary symptoms are infertility and chronic pain. The inflammatory environment and aberrant immune responses in women with endometriosis may be directly associated with the initiation and progression of endometriotic lesions. In the present study, the secretion of inflammatory cytokines was evaluated in cultures of primary endometrial cells (ECs) isolated from the endometrium of women with and without endometriosis. The presence of endometriotic cells leads to alterations in the secretory profile of healthy ECs. The expression of the inflammatory cytokines interleukin (IL)-6 and IL-8 was significantly increased in endometriotic and co-cultured cells compared with healthy ECs. IL-6 expression was strongly correlated with IL-8 expression in endometriotic cells. IL-1β expression was increased on day 10 of co-culture to 48.30 pg/ml and may be associated with the long-term co-culture, rather than IL-6 and IL-8 expression. IL-6 expression was strongly correlated with cell number, whereas IL-8 expression was moderately correlated with cell number. Additionally, it was observed that co-cultured cells exhibited a different population of cells, with expression of the mesenchymal stem cell marker cell surface glycoprotein MUC18, indicating a putative role of endometrial mesenchymal stem cells in the secretion of cytokines and disease development. These results indicate a predominant role of primary endometriotic cells in the secretion of cytokines, which contributes to the disrupted peritoneal and endometrial environment observed in the women with endometriosis.

Introduction

Endometriosis is a chronic gynecological disorder defined as the presence of endometrial tissue within extra-uterine sites (1,2). It affects 10-15% of women of reproductive age and results in a markedly reduced quality of life (3-5). The primary symptoms are infertility and chronic pain (6,7). It is a hormone-dependent and chronic inflammatory disease (1,2,8), indicating that the endometrium and the peritoneal environment are directly associated with its pathogenesis.

The progression of endometriosis is dependent on genetic, endocrine, immunological and environmental factors (9,10). The endometrium, serum and peritoneal fluid of women with endometriosis have abnormal levels of inflammatory cytokines, angiogenic, growth and adhesion factors, and cancer-like molecules (6,11,12). These soluble factors are thought to have predominant involvement in disease initiation and progression. Interleukin (IL)-1β has a proliferative effect on endometriotic cells that does not occur in healthy endometrial cells (13). IL-1β stimulates the production of IL-6 and IL-8 in endometriotic cell cultures, which further induce proliferation (14,15) and reduce the apoptotic rate (16-19). Additionally, IL-1β increases the shedding of intercellular adhesion molecule (ICAM)-1 from peritoneal mesothelial cells, indicating a role in the neovascularization mediated by IL-6 and vascular endothelial growth factor (VEGF) (20). Furthermore, higher levels of IL-1β may indicate the conversion of inflammation from an acute to a chronic form (21).

Tumor necrosis factor (TNF)-α, IL-6, IL-8, IL-10, VEGF and C-C motif chemokine 2 (MCP-1) expression is increased in the peritoneal fluid of women with endometriosis (11,18,21-24). TNF-α is associated with pluripotency mediation and inflammatory cytokine production, particularly IL-8, in endometriotic tissues (25). IL-8 increases the adhesion of endometrial stroma to extracellular matrix proteins, in addition to increasing metalloproteinase expression and proliferation (26-28). IL-8 may be a key cytokine in the progression of endometriotic lesions, by stimulating growth and indirectly protecting implants against apoptosis (18). IL-6 is secreted by endometrial cells and may have an important role in the pathology of endometriosis with interferon-γ. IL-6 increases macrophage expression of ICAM-1 in patients with endometriosis (29,30). Increased peritoneal concentrations...
of IL-6 and IL-8 are associated with different stages of the disease (23,31). In addition, the resistance to progesterone observed in endometriosis (32-34) is linked to the aberrant expression of cytokines (35,36).

The aberrant peritoneal environment, in addition to discrepancies in apoptosis and proliferation, result in abnormal immune cell clearance in women with endometriosis, providing a longer survival of the endometrial cells regurgitated by uterine tubes and the establishment of endometrial implants (5,24,31,37). Recently, the evaluation of peritoneal cytokines in patients with endometriosis indicated that the inflammatory environment observed in these patients is triggered by the establishment and growth of endometrial implants (38). Therefore, the present study investigated inflammatory cytokine secretion in the culture media of healthy and endometriotic primary cells. It was hypothesized that co-culture with endometriotic cells may lead to inflammatory cytokine secretion and phenotypic modifications in healthy endometrial cells associated with endometriosis implant establishment.

Materials and methods

**Human tissues and cell culture.** The protocol of the present study was approved by the Federal University of São Paulo ethical committee (registration no. 1044/11, São Paulo, Brazil). Written informed consent was obtained from all patients. Endometrial cells (ECs) from healthy individuals and patients with endometriosis were obtained from the Endometrium and Endometriosis Cell Bank of the Pelvic Pain and Endometriosis Unit of the Federal University of São Paulo. The selected samples were collected from August 2013 to April 2014. Human endometrium samples were collected from fertile cycling women aged 18-45 undergoing laparoscopic surgery for endometriosis stage IV (endometriosis group; n=3) and tubal ligation (control group; n=3). Patients had not taken exogenous hormones, given birth or breastfed for 3 months prior to surgery; patients with co-morbidities including teratoma, endometrial polyps or any other proliferative disease were excluded. The collected endometrial tissue was separated into two for either histological analysis (39,40) (data not shown) or cell culture.

The tissue for cell culture was immediately placed in Dulbecco's modified Eagle's medium with nutrient mixture F12 (DMEM/F12; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 400 U/ml penicillin and 400 µg/ml streptomycin (Gibco; Thermo Fisher Scientific, Inc.), stored at 4°C and processed within 2-24 h. The endometrial tissue was dissociated with 255 units of collagenase type IA (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and 3 units of dispase (Gibco; Thermo Fisher Scientific, Inc.) in DMEM/F12 and was subsequently incubated for 40 min in a 37°C water bath under constant agitation. The cell suspension obtained was centrifuged at 500 x g for 5 min at room temperature and the pellet was resuspended in 5 ml EC medium supplemented with 10% FBS at 37°C until approximately 70% confluence was reached. At this stage, cells were subcultured. The cells were stored in liquid nitrogen.

**Healthy and endometriotic cell co-culture.** Co-cultures were performed in 12-well culture plates. Healthy ECs were pre-seeded with 2 µg/ml Calcein-AM (Thermo Fisher Scientific, Inc., Waltham, MA, USA) for 30 min at 37°C with 5% CO₂. Cell number and viability were assessed with a Countess™ Automated Cell Counter (Invitrogen; Thermo Fisher Scientific Inc.); 10 µl cell suspension was thoroughly mixed with 10 µl 0.4% Trypan Blue (Invitrogen; Thermo Fisher Scientific Inc.), then, 10 µl was added to the chamber slide, the cells were settled for 30 sec at room temperature prior to counting; according to the manufacturer's protocols. Healthy and endometriotic cells were mixed at a 1:1 ratio and seeded onto 12-well plates at a density of 5,000 cells/cm² and cultured in EC medium supplemented with 10% FBS for 10 days. The adhesion of green (healthy) and unstained (endometriotic) cells was reported by light microscopy (magnification, x50, Axio Observer, Zeiss GmbH, Jena, Germany) 24 h after the initial cell seeding, the stained and non-stained cells were visually counted in four different fields for each triplicate. The culture medium was changed every 2 days and in the day prior to each control point (1st, 3rd, 7th and 10th days of culture). Cells were harvested during the 1st, 3rd, 7th and 10th days of culture, suspended in FBS with 10% dimethyl sulfoxide and stored in liquid nitrogen. The culture medium was collected and stored at -80°C on days 1, 3, 7 and 10 of culture. As a
control, healthy and endometriotic cell groups were cultured separately as described above.

**Culture medium cytokines detection.** IL-1β, IL-6, IL-8, IL-10, IL-12p70 and TNF-α expression levels were detected in the supernatant culture medium samples of single cell or co-cultured cells at days 1, 3, 7 and 10 by flow cytometry using the phycoerythrin (PE)-conjugated beads from the Human Inflammatory Cytokine cytometric bead array kit (BD Biosciences, Franklin Lakes, NJ, USA). Events acquisition was performed in a CANTO II 6-colour flow cytometer (BD Biosciences) and data were analyzed with FlowJo software (version 10.0.7; FlowJo LLC, Ashland, OR, USA).

**Flow cytometry of membrane markers.** Healthy and endometriotic cells prior to the co-culture seeding and co-cultured cells harvested in the 1st, 3rd, 7th and 10th days of culture were thawed on ice, centrifuged at 500 x g for 5 min 4°C, resuspended in pH 7.4 1X PBS (Gibco; Thermo Fisher Scientific, Inc.) with 1% FBS (Gibco; Thermo Fisher Scientific, Inc.) for blocking and incubated with directly conjugated antibodies for the surface markers aminopeptidase N [CD13-allophycocyanin (APC); cat no. 555394; BD Biosciences] at 1:5 dilution ratio and cell surface glycoprotein MUC18 [CD146-PE; cat no. 561013; BD Biosciences] at 1:20 dilution ratio for 1 h at 4°C. Cells were washed with PBS 1X pH 7.4 and centrifuged at 500 x g for 5 min 4°C, resuspended in PBS 1X pH 7.4 and subsequently analyzed with a BC FACSCanto II flow cytometer (BD Biosciences). The experiment controls were performed using immunoglobulin G1 isotype controls conjugated with PE (cat no. 550617) and APC (cat no. 550854) both at 1:5 working dilution ratio (BD Biosciences). Data were analyzed in FlowJo software (version 10.0.7; FlowJo LLC).

**Statistical analysis.** The data were analyzed with PASW Statistics 18.0.0 (IBM Corp., Armonk, NY, USA). All cell cultures were performed in experimental triplicates. Data are presented as the mean ± standard error (SE). Cytokine data were analyzed with repeated measures analysis followed by Dunnett’s T3 pairwise comparisons post-hoc test between the groups that presented unequal variances. Cell marker data were analyzed with repeated measures analysis followed by a Bonferroni corrected pairwise comparisons test within-subjects factors. The curves obtained were tested for sphericity using Mauchly’s test and within-subject effects were corrected using the Greenhouse-Geisser test when sphericity assumption was violated. Simple and multiple linear regression were performed to identify the correlation between the variables obtained, where 0< r <0.25 was null; 0.25 < r <0.50 was weakly correlated; 0.50 < r <0.75 was moderately correlated; and 0.75 < r <1 was strongly correlated (41). P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Competitive relationship between healthy and endometriotic cells in co-culture systems.** The adhesion of endometriotic (unstained) and healthy (Calcein-AM stained) cells in the first day of the co-culture is presented in Fig. 1. The amount of stained and not stained cells was visually semiquantitative evaluated in four microscopy fields in each triplicate. No observable difference was determined in the amount of endometriotic or healthy cells attached, indicating an equal number of each type of cell at the beginning of co-cultivation.

**Inflammatory cytokine profile of healthy, endometriotic and co-cultured cells.** The levels of inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12p70 and TNF-α) secreted into the culture medium were determined over time in endometriotic and healthy cells cultured alone, in addition to co-cultured cells. Insignificant levels of IL-10, IL12p70 and TNF-α were detected in the culture medium of all tested combinations of cells (data not shown). However, the IL-6 concentration increased over time in all groups (Fig. 2A). The variation observed for the endometriotic cells was similar to that observed in the co-cultured ECs (P=1.000, across all time points). The healthy ECs had a significantly different profile of IL-6 secretion, compared with co-cultured ECs (P=0.017, across all time points). As presented in Fig. 2, IL-6 secretion
was higher in the endometriosis and co-culture groups. A strong correlation was detected between the number of cells and the concentration of IL-6 in the medium of healthy and endometriotic cells (Table I).

Pro-angiogenic IL-8 was differentially secreted in healthy, endometriotic and co-cultured ECs (Fig. 2B). The most significant variation occurred from day 7, when the concentration increased in all groups. The differences between the curves of IL-8 concentration were statistically significant (P=0.010). The secreted IL-8 level in the healthy EC culture was significantly lower compared with the endometriosis (P=0.026, across all time points) and co-cultured (P=0.006, across all time points) groups. Similar to the trend observed for IL-6, the secretion of IL-8 was similar in the endometriotic and co-cultured cells (Table I). The number of cells and IL-8 concentration were moderately correlated in healthy and endometriotic cells (Table I). Additionally, there was a moderate correlation between IL-6 and IL-8 secretion in the healthy and co-cultured cells. A strong correlation between the secretion of these cytokines was observed in the endometriotic cells (Table I).

The co-cultured cells had 48.30 pg/ml (SE, 9.68) IL-1β at day 10 of culture (data not shown); IL-1β levels at days 1, 3 and 7 were undetectable. The secretion of IL-1β appears to be associated with the effects of long-term co-culturing of the cells, although not directly to the secretion of IL-6 or IL-8 (Table I). Population profile of co-cultured ECs. Flow cytometry analysis of the cells cultured alone and co-cultured cells using the size (forward scatter) and complexity (side scatter) parameters demonstrated the presence of two main populations in the
samples. One and two. Population one represented <5% of the total events collected in the healthy (Fig. 3A) and endometriosis (Fig. 3B) samples alone (2.95%; SE, 0.44). The percentage of this population increased by 15-fold in the first day of co-culture (Day 1, 45.93%; SE, 3.58; P=0.000179 vs. cells cultured alone; Fig. 3C). No population differences were observed in the ECs individually cultured over time (data not shown).

Population one diminished at day 3 (32.35%; SE, 2.16; P=0.028412 vs. day 1 of co-culture; Fig. 3D) and day 7 (11.89%; SE, 2.33; P=0.000076 vs. day 3 of co-culture; Fig. 3E) of co-culture and appeared to stabilize between days 7 and 10 (Fig. 3F) of culture (10.40%; SE, 1.79; P=0.302732, day 7 vs. day 10 of co-culture).

Population two was more prevalent in the EC samples cultured alone (87.36%; SE, 1.52; Fig. 3A and B). This percentage reduced by one-half on day 1 of co-culture (43.78%; SE, 3.03; P=0.000264 vs. cells cultured alone; Fig. 3C). Despite this population size increasing over time (day 3, 59.50%; SE, 2.41; P=0.000449 vs. cells cultured alone; day 7, 78.36%; SE, 2.19; P=0.015462 vs. cells cultured alone; Fig. 3D and E, respectively), the original population size detected in ECs cultured alone was not completely reestablished at day 10 of co-culture (80.73%; SE, 2.06; P=0.000622 vs. cells cultured alone; Fig. 3F).

Phenotypic profile of co-cultured ECs over time. According to the variations detected within the populations of ECs cultured alone or co-cultured, the stromal endometrial cell marker CD13 (42,43) and the endometrial mesenchymal stem cell (eMSC) marker CD146 (44-46) were selected to further characterize these populations. CD13 expression was not significantly different within co-cultured or individually cultured populations (Fig. 4).

The expression of eMSC marker CD146 varied significantly over time (Fig. 5). Population one had negligible expression of CD146, with the mean expression at all time points in the co-cultured group of 1.21% (SE, 0.25). In population two, an increase in CD146 expression was observed between days 1 and 7 of co-culture (1st vs. 3rd days, P=0.037; 3rd vs. 7th days, P=0.017), with a slight decrease in expression between days 7 and 10 of co-culture (P=0.071). The expression of CD146 in population 2 at 1st day of co-culture was more similar to endometriotic cells (P=0.102010) compared with healthy ECs (P=0.051579) (data not shown). These results demonstrated that population two was predominantly composed of eMSCs, and that there was an increase in these cells when healthy and endometriotic cells were co-cultured.

Discussion

The first and most acceptable theory for endometriosis pathogenesis was proposed almost a century ago (47), yet the pathophysiology of this disease remains unclear. As a hormone-dependent and chronic inflammatory disease (1,2,8), cytokines and soluble factors appear to serve an important role in its pathophysiology. The inflammatory content of the peritoneal fluid and serum, in addition to the eutopic and ectopic endometrium has been extensively studied (6,11,12,18,21,23,38). However, there are a number of questions which remain unanswered.

The increased levels of inflammatory cytokines in the peritoneal fluid, serum and endometrium of women with endometriosis has been reported in numerous studies (18,23,28,31,38,48). The principal identified factors include TNF-α, IL-6, IL-8, IL-10, VEGF and MCP-1 (11,18,21-24,49-51). In the present study, increased levels of IL-6 and IL-8 were observed in endometriotic cells cultured alone and in co-culture with healthy ECs, compared with individually cultured healthy ECs. The levels of IL-1β were additionally increased in the co-culture at day 10.

Sikora et al (18) proposed that IL-8 is important for progression of endometriosis. IL-8 increases the adhesion of endometrial stroma to extracellular matrix proteins, in addition to metalloproteinase expression and cell proliferation (26-28). The surgical excision of endometrial lesions leads to a decrease in the peritoneal levels of IL-8 (38). In the present study, increased secretion of IL-8 by endometriotic and co-cultured cells was detected. A previous study indicated that peritoneal immune cells, particularly macrophages, are the main source of IL-8 (18). The results of the present study indicated that ECs additionally contribute to IL-8 secretion. Furthermore, IL-8 expression was moderately correlated with cell number and IL-6 expression, suggesting that IL-8 secretion may be independent of cell proliferation and IL-6 levels.

IL-6 is secreted by the endometrium and implants, and is involved in diverse aspects of reproductive physiology, including ovarian steroid production, folliculogenesis and early embryonic implantation (28,49,52,53). IL-6 is associated with the increased expression of ICAM-1 by macrophages in patients with endometriosis (29,30). The present study demonstrated that the secretion of IL-6 by endometriotic cells was higher compared with healthy or co-cultured ECs. The increased levels of IL-6 were strongly correlated with cell numbers, indicating that the increased proliferative rate may be due to IL-6 signaling. The increased cell number also increases the IL-6 secretion. It has been well established that endometriotic stromal cells have a higher proliferative rate compared with healthy endometrial stromal cells (54,55). This increase in proliferation rate may be mediated by IL-6.

The levels of IL-6 may be associated with IL-1β secretion (20). The results of the present study revealed the presence of IL-1β at day 10 of co-culture only, with no significant alterations in the expression of IL-1β in individually cultured healthy or endometriotic cells. Furthermore, there was no statistically significant correlation between IL-6 and IL-1β expression. The secretion of IL-1β by the primary co-cultured ECs may be associated with long-term co-culture, which may indicate a switch from an acute to chronic inflammatory response in the endometrial cells (21). The proliferative effects of IL-1β have been reported in endometriotic cells (13). Furthermore, an increase in IL-1β expression occurs in the co-culture of endometrial primary cells and MSCs (56).

The endometrium contains MSCs that are involved in the cyclical regeneration of this tissue (57-59). The role of stem cells in the pathogenesis of endometriosis has been reported (37,60,61). The most widely accepted theory for endometriosis pathogenesis was proposed by Sampson (47) and indicates that the implants establish from cells undergoing...
retrograde menstruation; this theory alone is unable to explain all clinical presentations of the disease. The combined theories of Sampson (47) and the involvement of stem cells provide a better explanation for endometriosis physiopathology. The dysregulation of eMSCs has been proposed as a key mechanism of endometriosis, in concordance with the retrograde menstruation theory (62).

The mesenchymal stem cell marker CD146 (46) was used in the present study in order to identify the presence of MSCs in the pool of primary cells, and to report the variations in the MSC population during co-culture. An increase in the number of eMSCs detected by CD146 expression was observed in the co-culture system. These alterations in CD146 expression over time, together with the alterations in cytokine secretion, may...
indicate the involvement of MSCs in cytokine secretion. A pool of primary cells at a low passage number were selected, as this contained various cell populations and was more similar to the composition of the eutopic endometrium. In previous work (56), the co-culture of umbilical cord blood MSCs with primary endometrial cells resulted in increased IL-1β expression. Furthermore, co-culture eliminated the effect of p27 gene therapy on endometriotic stromal cells. These observations, together with the identification and characterization of endometrial MSCs (37,45,58,60,63), have provided an insight into the involvement of MSCs in endometriosis pathogenesis. In the present study, the co-cultivation of a pool of healthy and

![Figure 5](image)

Table: CD146 expression in each population.

| Co-culture | P1 p-Value | P2 p-Value |
|------------|------------|------------|
| 1st x 3rd Day | 1.000 | 0.037 |
| 3rd x 7th Day | 0.038 | 0.017 |
| 7th x 10th Day | 0.394 | 0.071 |

ECs, endometrial cells; CD146, cell surface glycoprotein MUC18; P1, population one; P2, population two; H, healthy; EIV, endometriosis ECs; D1, day 1 of co-culture; D3, day 3 of co-culture; D7, day 7 of co-culture; D10, day 10 of co-culture.
diseased primary cells, containing endometrial MSCs, led to the secretion of cytokines that may be directly involved in the pathogenesis of endometriosis. This supports the theory that eMSCs have an important role in the secretion of cytokines and endometriosis.

MSCs express CD13 (44,46), which is additionally expressed by stromal endometrial cells (42,43). CD13 is an N-aminopeptidase involved in the inactivation of IL-8 (64). Conflictingly, increased levels of CD13 expression due to cell-to-cell contact may be associated with IL-8 inactivation resistance (65) and the inhibition of apoptosis mediated by IL-8 (66). In the present study, CD13 expression was not significantly different between the co-cultured and individually cultured cells, indicating no physiological implications. Therefore, CD13 expression in the present study may be indicative of fibroblastoid cells.

Furthermore, the present study observed that a mixed cell population derived from the endometrium exhibited a secretory profile similar to that of endometriotic cells, even in the presence of healthy ECs. The results obtained demonstrated that the communication between endometriotic and healthy cells results in a secretory profile similar to what is reported in the peritoneal fluid of women with endometriosis (24,28,31,52). The increased secretion of IL-6 and IL-8 is additionally associated with the progesterone resistance observed in endometriosis (32,34). The results of the present study support this finding, as the endometriotic cells secreted higher levels of IL-6 and IL-8 compared with the healthy cells. Additionally, the findings of the present study were consistent with the retrograde menstruation theory and clarified certain aspects of the disrupted peritoneal environment of women with endometriosis. For example, despite the presence of healthy endometrial cells in retrograde menstruation, the presence of endometriotic cells may be decisive in the secretory profile of the cell pool (18,21,37). The endometrial cells regurgitated into peritoneal cavity may be responsible for the induction of the aberrant cytokine profile observed in the peritoneal fluid and eutopic endometrium of women with endometriosis (18,19,21,37,38). The variations observed in the CD146+ population support the putative role of eMSCs in the pathogenesis of endometriosis.

The secretion alterations observed in the individually cultured ECs and the co-culture indicate a critical role for ectopic endometrial cells in the initiation of peritoneal environment disruption, which is in accordance with a recently published report (38). Thus, the cytokine profile observed may not only be generated by molecules secreted from immune cells. The main limitation of the present study is that, as an in vitro study, the effects of EC-secreted cytokines on immune cells present in the peritoneum of women with endometriosis was not demonstrated. Studies which isolate each population of cells observed in the present study may clarify the cell type responsible for the secretion of the cytokines or reveal the association between healthy and disease cell contact.

In accordance to previous research (18,19,21,23,37,38), the findings of the present study suggest that the soluble factors secreted by endometriotic cells may have an important role in the disruption of the cell cycle and in the establishment of the peritoneal environment of women with endometriosis. The endometriotic cell pool may be responsible for the establishment of an inflammatory peritoneal environment favorable to the initiation and progression of endometrial cell adhesion and clustering.

Acknowledgements

We thank Colsan Blood Bank (São Paulo, Brazil) for all the technical support.

Funding

The present study was supported by the São Paulo Research Foundation (grant no. 2011/14683-7) and the Brazilian National Council of Technological and Scientific Development (grant no. 480303/2013-4).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ALI, GAG, MJBCG and ES provided major contributions to the conception and design of the present study. ALI, RMP, AK and GK conducted the collection of samples, experimentation and acquisition of data. ALI and ES were involved in analysis and interpretation of data. All authors were involved in manuscript drafting and critical discussion, as well as in the final approval of the version to be published.

Ethics approval and consent to participate

The protocol of the present study was approved by the Federal University of São Paulo ethical committee (São Paulo, Brazil). Written informed consent was obtained from all patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Ulukus M, Cakmak H and Arici A: The Role of Endometrium in Endometriosis. J Soc Gynecol Investig 13: 467-476, 2006.
2. Falconer H, D’Hooghe T and Fried G: Endometriosis and genetic polymorphisms. Obstet Gynecol Surv 62: 616-628, 2007.
3. Eskenazi B and Warner ML: Epidemiology of endometriosis. Obstet Gynecol Clin North Am 24: 235-258, 1997.
4. Vinatier D, Cosson M and Dufour P: Is endometriosis an endometrial disease? Eur J Obstet Gynecol Reprod Biol 91: 113-125, 2000.
5. Bulun SE: Endometriosis. N Engl J Med 360: 268-279, 2009.
6. Burney RO and Giudice LC: Pathogenesis and pathophysiology of endometriosis. Fertil Steril 98: 511-519, 2012.
7. Giudice LC and Kao LC: Endometriosis. Lancet 364: 1789-1799, 2004.
8. Berkley KJ, Rapkin AJ and Papka RE: The Pains of Endometriosis. Science 308: 1587-1589, 2005.
A potential autocrine growth factor. J Clin Endocrinol Metab 83: 1201-1205, 1998.

Arici A, Seli E, Zeyneloglu HB, Senturk LM, Oral E and Olive DL: Adhesion of endometrial stromal cells to fibronectin. Fertil Steril 88: 730‑735, 2003.

Expression. J Clin Endocrinol Metab 97: 1380‑1386.e1, 2012.

Herington JL, Bruner-Tran KL, Lucas JA and Osteen KG: Immune interactions in endometriosis. Expert Rev Clin Immunol 7: 611‑626, 2011.

Young VJ, Brown JK, Saunders PT and Horne AW: The role of the peritoneum in the pathogenesis of endometriosis. Hum Reprod Update 19: 558‑569, 2013.

Barcz E, Milewski Ł, Dzniycz P, Kamiński P, Płoski R and Malejczyk J: Peritoneal cytokines and adhesion formation in endometriosis: An inverse association with vascular endothelial growth factor concentration. Fertil Steril 97: 1380-1386.e1, 2012.

Young SL and Lessey BA: Progestrogen function in human endometrium: Clinical perspectives. Semin Reprod Med 28: 5‑16, 2010.

Barragan F, Irwin JC, Balayan S, Erikson DW, Chen JC, Houshdaran S, Piltonen TT, Spitzer TL, George A, Rabban JT, et al: Human endometrial fibroblasts derived from mesenchymal progenitors inherit progesterone resistance and acquire an inflammatory phenotype in the endometrial niche in endometriosis. Biol Reprod 94: 118, 2016.

Lessey BA and Young SL: Homeostasis imbalance in the endometrium of women with implantation defects: The role of estrogen and progesterone. Semin Reprod Med 32: 365‑375, 2014.

Wing LY, Chuang PC, Wu MH, Chen HM and Tsai SJ: Expression and mitogenic effect of fibroblast growth factor‑9 in human endometriot implant is regulated by aberrant production of estrogen. J Clin Endocrinol Metab 88: 5547‑5554, 2003.

Wu MH, Lu CW and Tsai SJ: Prostaglandin E2: The master of endometriosis? Exp Biol Med (Maywood) 235: 668‑677, 2010.

Gargett CE, Schwab KE, Brosens JJ, Puttemans P, Benagiano G and Brosens I: Potential role of endometrial stem/progenitor cells in the pathogenesis of early‑onset endometriosis. Mol Hum Reprod 20: 591‑598, 2014.

Monsanto SP, Edwards AK, Zhou J, Nagarkatti P, Nagarkatti M, Young SL, Lessey BA and Tayade C: Surgical removal of endometriotic lesions alters local and systemic proinflammatory cytokines in endometriosis patients. Fertil Steril 105: 968-977.e5, 2016.

Moen MH and Halvorsen TB: Histologic confirmation of endometriosis in different peritoneal locations. Acta Obstet Gynecol Scand 71: 337‑342, 1992.

Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril 67: 817‑821, 1997.

Vegh F, Žitelská B, Žitelský B, Rábová A and Šimáčková M: Peritoneal cytokines and adhesion formation in endometriosis. J Reprod Immunol 77, 2017.

Gazvani R and Templeton A: Peritoneal fluid cytokines related to endometriosis in patients with peritoneal endometriosis and controls. J Reprod Immunol 84: 193‑198, 2010.

Vetvicka V, Laganà AS, Salmeri FM, Triolo O, Palmaro VI, Vitale SG, Soto V and Králiková M: Regulation of apoptotic pathways during endometriosis: From the molecular basis to the clinic. Arch Gynecol Obstet 283: 1291‑1296, 2011.

Sikora J, Smycz‑Kubańska M, Mielczarek‑Palacz A and Kondera‑Anasz Z: Abnormal peritoneal regulation of chemokine activation‑The role of IL‑8 in pathogenesis of endometriosis. Am J Reprod Immunol 57: 558-569, 2007.

Jørgensen H, Hill  AS, Beste  MT, Herington JL, Bruner‑Tran KL, Lucas JA and Osteen KG: Immune interactions in endometriosis. Expert Rev Clin Immunol 7: 611‑626, 2011.

Young VJ, Brown JK, Saunders PT and Horne AW: The role of the peritoneum in the pathogenesis of endometriosis. Hum Reprod Update 19: 558‑569, 2013.

Barcz E, Milewski Ł, Dzniycz P, Kamiński P, Płoski R and Malejczyk J: Peritoneal cytokines and adhesion formation in endometriosis: An inverse association with vascular endothelial growth factor concentration. Fertil Steril 97: 1380-1386.e1, 2012.

Young SL and Lessey BA: Progestrogen function in human endometrium: Clinical perspectives. Semin Reprod Med 28: 5‑16, 2010.

Barragan F, Irwin JC, Balayan S, Erikson DW, Chen JC, Houshdaran S, Piltonen TT, Spitzer TL, George A, Rabban JT, et al: Human endometrial fibroblasts derived from mesenchymal progenitors inherit progesterone resistance and acquire an inflammatory phenotype in the endometrial niche in endometriosis. Biol Reprod 94: 118, 2016.

Lessey BA and Young SL: Homeostasis imbalance in the endometrium of women with implantation defects: The role of estrogen and progesterone. Semin Reprod Med 32: 365‑375, 2014.

Wing LY, Chuang PC, Wu MH, Chen HM and Tsai SJ: Expression and mitogenic effect of fibroblast growth factor‑9 in human endometriot implant is regulated by aberrant production of estrogen. J Clin Endocrinol Metab 88: 5547-5554, 2003.

Wu MH, Lu CW and Tsai SJ: Prostaglandin E2: The master of endometriosis? Exp Biol Med (Maywood) 235: 668‑677, 2010.

Gargett CE, Schwab KE, Brosens JJ, Puttemans P, Benagiano G and Brosens I: Potential role of endometrial stem/progenitor cells in the pathogenesis of early‑onset endometriosis. Mol Hum Reprod 20: 591‑598, 2014.

Monsanto SP, Edwards AK, Zhou J, Nagarkatti P, Nagarkatti M, Young SL, Lessey BA and Tayade C: Surgical removal of endometriotic lesions alters local and systemic proinflammatory cytokines in endometriosis patients. Fertil Steril 105: 968-977.e5, 2016.

Moen MH and Halvorsen TB: Histologic confirmation of endometriosis in different peritoneal locations. Acta Obstet Gynecol Scand 71: 337‑342, 1992.

Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril 67: 817‑821, 1997.

Vieira S: Introdução à Bioestatística. 4th edition. Elsevier Ltd, São Paulo, 2008.

Kato Yoshi moto M, Kato K, Adachi S, Yamayoshi A, Arima T, Asanoma K, Kyo S, Nakahata T and Wake NK: Characte risation of stromal cell population in human normal endometrium. Hum Reprod 22: 1212‑1223, 2007.

Seli E, Senturk LM, Bahityar OM, Kayisli UA and Arici A: Expression of aminopeptidase N in human endometrium and regulation of its activity by estrogen. Fertil Steril 75: 1172‑1176, 2001.

Schwab KE and Gargett CE: Co‑expression of two perivascular cell markers isolates mesenchymal stem‑like cells from human endometrium. Hum Reprod 22: 2903‑2911, 2007.

Schwab KE, Hutchinson P and Gargett CE: Identification of surface markers for prospective isolation of human endometrial stromal colony‑forming cells. Hum Reprod 23: 934‑943, 2008.

Gargett CE, Schwab KE, Zillwood RM, Nguyen HP and Wu D: Isolation and culture of epithelial progenitors and mesenchymal stem cells from human endometrium. Biol Reprod 80: 1136‑1145, 2009.

Sampson JA: Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol 140: 242‑469, 1972.

Milewski Ł, Dzniycz P, Barcz E, Radomski D, Roszkowski P, Czajkowska‑Kawa O, Kamiński P and Malejczyk J: Increased levels of human neutrophil peptides 1, 2, and 3 in peritoneal fluid of patients with endometriosis: Association with neutrophils, T cells and IL‑8. J Reprod Immunol 91: 64‑70, 2011.

Jacobs AL, Sehgal PB, Julian J and Carson DD: Secretion and hormonal regulation of interleukin‑6 production by mouse uterine stromal and polarized epithelial cells cultured in vitro. Endocrinology 131: 1037‑1346, 1992.
50. Szylo K, Tchorzewski H, Banasik M, Glowacka E, Lewkowicz P and Kamer-Bartosinska A: The involvement of T lymphocytes in the pathogenesis of endometriotic tissues overgrowth in women with endometriosis. Mediators Inflamm 12: 131-138, 2003.

51. Kang YJ, Jeung IC, Park A, Park YJ, Jung H, Kim TD, Lee HG, Choi I and Yoon SR: An increased level of IL-6 suppresses NK cell activity in peritoneal fluid of patients with endometriosis via regulation of SHP-2 expression. Hum Reprod 29: 2176-2189, 2014.

52. Harada T, Yoshioka H, Yoshida S, Iwabe T, Onohara Y, Tanikawa M and Terakawa N: Increased interleukin-6 levels in peritoneal fluid of infertile patients with active endometriosis. Am J Obstet Gynecol 176: 593-597, 1997.

53. Bedaiwy MA, Falcone T, Sharma RK, Goldberg JM, Attaran M, Nelson DR and Agarwal A: Prediction of endometriosis with serum and peritoneal fluid markers: A prospective controlled trial. Hum Reprod 17: 426-431, 2004.

54. D'Amora P, Maciel TT, Tambellini R, Mori MA, Pesquero JB, Sato H, Girão MJ, Guerreiro da Silva ID and Schor E: Disrupted cell cycle control in cultured endometrial cells from patients with endometriosis harboring the progesterone receptor polymorphism PROGINS. Am J Pathol 175: 215-224, 2009.

55. Li MQ, Luo XZ, Meng YH, Mei J, Zhu XY, Jin LP and Li DJ: CXCL8 enhances proliferation and growth and reduces apoptosis in endometrial stromal cells in an autocrine manner via a CXCR1-triggered PTEN/AKT signal pathway. Hum Reprod 27: 3107-3116, 2012.

56. Gonçalves GA, Invitti AL, Parreira RM, Kopelman A, Schor E and Girão MJ: p27kip1 overexpression regulates IL-1β in the microenvironment of stem cells and eutopic endometriosis co-cultures. Cytokine 89: 229-234, 2017.

57. Prianishnikov VA: On the concept of stem cell and a model of functional-morphological structure of the endometrium. Contraception 18: 213-223, 1978.

58. Gargett CE, Chan RW and Schwab KE: Endometrial stem cells. Curr Opin Obstet Gynecol 19: 377-383, 2007.

59. Sasson IE and Taylor HS: Stem cells and the pathogenesis of endometriosis. Ann N Y Acad Sci 1127: 106-115, 2008.

60. Gargett BE and Chan RW: Endometrial stem/progenitor cells and proliferative disorders of the endometrium. Minerva Ginecol 58: 511-526, 2006.

61. Gargett CE and Gurung S: Endometrial mesenchymal stem/stromal cells, their fibroblast progeny in endometriosis, and more. Biol Reprod 94: 129, 2016.

62. Hufnagel D, Li F, Cosar E, Krikun G and Taylor H: The role of stem cells in the etiology and pathophysiology of endometriosis. Semin Reprod Med 33: 333-340, 2015.

63. Gargett CE and Masuda H: Adult stem cells in the endometrium. Mol Hum Reprod 16: 818-834, 2010.

64. Kanayama N, Kajiwara Y, Goto J, el Maradny E, Maehara K, Andou K and Terao T: Inactivation of interleukin-8 by aminopeptidase N (CD13). J Leukoc Biol 57: 129-134, 1995.

65. Kehlen A, Egbert I, Thiele K, Fischer K, Riemann D and Langner J: Increased expression of interleukin-8 and aminopeptidase N by cell-cell contact: Interleukin-8 is resistant to degradation by aminopeptidase N/CD13. Eur Cytokine Netw 12: 316-324, 2001.

66. Mishima Y, Matsumoto-Mishima Y, Terui Y, Katsuyama M, Yamada M, Mori M, Ishizaka Y, Ikeda K, Watanabe J, Mizunuma N, et al: Leukemic cell-surface CD13/aminopeptidase N and resistance to apoptosis mediated by endothelial cells. J Natl Cancer Inst 94: 1020-1028, 2002.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.