Current Trends in Immunology
Vol. 19, 2018

Original Article

The type of peripheral blood and peritoneal fluid B-lymphocyte differentiation and autoantibody production in women with endometriosis and infertility

Yulia Antsiferova*, Anna Malyshkina, Natalia Sotnikova and Leila Abdullaeva

Ivanovo's Research Institute of Maternity and Childhood named after V. N. Gorodkov, Health Ministry of Russian Federation, 153045, Ivanovo, Pobedy St., 20, Russia.

ABSTRACT

It has been proposed that endometriosis development is associated with autoimmune disorders. The aim of this study is to elucidate the type of B-cell differentiation and autoantibody production at the systemic and local levels in women with different stages of endometriosis and infertility. 35 women with stages I-II of endometriosis, 25 women with stages III-IV of endometriosis and 25 gynecologically healthy women (control group) were recruited into the study. B-lymphocyte phenotype was assessed using three-color flow cytometry and concentration of anti-ovary and anti-Zona Pellicida (ZP) autoantibodies was measured using enzyme-linked immunosorbent assay (ELISA). It was found that all women with endometriosis were characterized by low level of switched memory CD19+IgD-CD27+ B-cells, high level of unswitched naïve CD19+IgD+CD27- B-lymphocytes and elevated number of plasma cells in peripheral blood and peritoneal fluid in comparison to the control group. In the peritoneal fluid of all women with endometriosis the amount of unswitched memory CD19+IgD+CD27+ B-cells was increased. Changes at the local level were significantly more pronounced in the group of women with severe endometriosis than in the group with mild endometriosis. In women with stages I-II of endometriosis we noted the elevation of serum content of anti-ZP and anti-ovary antibodies, whereas women with stages III-IV of endometriosis were characterized by higher level of anti-ovary antibodies both at the systemic and local levels. But in all cases the concentrations of autoantibodies were lower compared to that in persons with true autoimmune disorders. It could be concluded that endometriosis development is associated with increased autoreactivity but not with true autoimmunity.

KEYWORDS: endometriosis, naïve B-lymphocytes, memory B-cells, autoantibodies, autoimmunity, autoreactivity, blood, peritoneal fluid.

INTRODUCTION

Endometriosis is a common benign gynecological disease, which is usually defined as the presence and growth of endometrial glands and stroma outside of the uterus, predominantly in the peritoneal cavity [1]. According to the literature data endometriosis affects 5-10% of all women in reproductive age. The more frequent clinical symptoms of endometriosis are associated with local inflammation, pelvic pain and infertility [2]. It was shown that infertility is diagnosed in 40-60% of women with endometriosis and there are not yet effective medical treatments for infertility associated with endometriosis [3]. Despite the decades of study of endometriosis the pathogenesis of this disease is still unknown. Now it is widely accepted that the immune mechanisms play an important role in infertility and endometriosis development [2].

*Corresponding author: niimid.immune@mail.ru

At the end of the last century it was noted that different autoantibodies, usually observed in autoimmune diseases, such as antiendometrial, antiphospholipid, anticardiolipin, antinuclear autoantibodies, etc. circulated in the peripheral blood and peritoneal fluid of women with endometriosis [4]. According to these data the theory about the autoimmune nature of endometriosis was proposed by Gleicher and coworkers in the 1980s and is widely accepted even now [5]. But this autoimmune theory is based only on previous results about the presence of autoantibodies in endometriosis patients. In current literature we found only sparse data about the functioning of B-lymphocytes, the main source of autoantibodies, in women with endometriosis. Both increase in the amounts of B-cells in the peripheral blood and peritoneal fluid of patients with endometriosis [6] and diminishing in the levels of peripheral B-cells and subsets of CD20 cells, coexpressing either HLA-DR or high level of CD44 molecules, have been demonstrated in women with endometriosis [7]. In women with endometriosis the elevation of the peritoneal level of transcriptional factor B-lymphocyte-induced maturation protein-1 (Blimp-1), which regulates differentiation of plasma cells, has also been demonstrated [8]. High production of BLyS (B-lymphocytes stimulator) has been noted in endometriosis lesions [9] and the heterozygozity of this gene correlated with the severity of endometriosis [10]. But another research group was not able to confirm these data [11]. Thus, there is no consensus about the functional activity of B-lymphocytes and their role in autoantibody production in endometriosis [12]. Literature data concerning differentiation of B-cells from naïve to memory cells in women with endometriosis, are currently practically absent.

It is well known that immunological memory in the process of humoral response development is maintained by pools of antibody-producing plasma cells (PCs) and memory B-cells, which can respond to antigens more rapidly and effectively than naive B cells of the same specificity [13]. Earlier memory B-cells differed from naïve B-cells by their absence of the ATP-binding cassette (ABCB1) transporter, but now surface CD27 and IgD markers are widely used for separation of B-cells into memory and naïve pools. According to these markers CD19+ B-cells are classified into: classical unswitched naïve B-cells (IgD+CD27-), unswitched memory B-cells (IgD+CD27+), switched memory B-cells (IgD-CD27+) and double-negative memory B-cells (IgD-CD27-) [14]. Functionally these populations have not been completely studied yet. CD19+IgD+CD27- and CD19+IgD-CD27+ cells are considered as classical naïve and memory B-cells, which are differentiated consistently during interaction between B-cells and antigens with subsequent mutations of IgD genes and membrane switching of IgD molecules. The nature of unswitched IgD+CD27+ memory B-cells remains to be clarified. It was suggested that these cells are circulating marginal zone B-cells, formed independently of the germinal centers [14]. These cells are termed “innate-like B-cells” or “natural effector B-cells”. Likely, the non-specific but rapid production of low affinity but high-avidity IgM is a main function of this unswitched memory B-cells subset [14]. The CD19+IgD-CD27- cells are also memory cells, because this pool shares similar morphology with CD27+ cells and have no ABCB1 transporter [15]. IgD-CD27- memory B-cells are present at birth; their level account for < 5% of peripheral blood B-cells in healthy subjects and the increase in patients with active SLE [14]. It must be specifically noted that the study of B-cell differentiation with simultaneous estimation of autoantibody production in women with endometriosis has not been performed earlier.

Thus, the aim of our work is to establish the type of B-cell differentiation and autoantibody production at the systemic and local levels in women with different stages of endometriosis and infertility to improve our understanding of the autoimmune nature of endometriosis in infertile women.

MATERIALS AND METHODS

Patients

60 infertile women with laparoscopically proven endometriosis, including 35 patients with stages I-II of disease (mild endometriosis) and 25 women with stages III-IV of endometriosis (severe stages of disease), who underwent examination for sterility, were recruited into the study after obtaining their informed consent. The stages of endometriosis were graded according to the revised American Fertility Society criteria. 25 fertile healthy women,

admitted for surgical sterilization, formed the control group. All patients ranged in age from 25 to 40 years and had not been taking any hormone therapy for at least 6 months before examination.

Heparinized peripheral blood taken from the cubital vein before surgery, and peritoneal fluid taken during laparoscopy were used as the materials for investigation. Informed consent for participation in the study was received from every woman. This investigation was approved by the local ethical committee of FSBE "Ivanovo's Research Institute of Maternity and Childhood" of Health Ministry of Russia.

Isolation of mononuclear cells from blood and peritoneal fluid

Enriched factions of mononuclear cells (MNC) were isolated from peripheral blood and peritoneal fluid by standard method of density gradient centrifugation using Ficoll (d = 1,078). Cells from interphase Ficoll (d = 1,078) – culture media RPMI 1640 were collected, washed twice using phosphate buffer solution and later used for flow cytometry at a concentration of 1 x 10^6 cells/ml.

Flow cytometry analysis

We assessed the relative amount of CD27+ (memory cells), CD27- (naive cells), CD20-CD38+ (plasma cells), and CD27+IgD-, CD27+IgD+, CD27-IgD+ CD27-IgD- lymphocytes in population of CD19+ cells. For membrane staining we used monoclonal antibodies (mAB): anti-CD19, conjugated with PerCP-Cy5.5 (peridin chlorophyll protein, conjugated with cyanin) (eBioscience, USA); anti-CD27 and CD38, conjugated with phycoerythrin (PE) (Beckman Coulter, USA); anti-CD20, conjugated with allophycocyanin (APC) (Beckman Coulter, USA) and anti-IgD, conjugated with Fluorescein ispthyocyanate (FITC) (Beckman Coulter, USA). We performed three-color flow cytometry analysis using cytometer FACSCanto II (Becton Dickinson, USA). Lymphocyte gate (region 1) was built using dot plot data from forward and side scatters. Then in lymphocyte gate we built the CD19+ gate (region 2) using staining with anti-CD19 mAB. All results were estimated in the region 2. Data analysis was performed using FACSDiva software (Becton Dickinson, USA).

Enzyme-linked immunosorbent assay (ELISA)

Anti-Zona Pellucida and anti-ovary autoantibody concentrations in serum and peritoneal fluid were assessed by using commercial ELISA kits according to the manufacturer's instructions (BIOSERV Diagnostics, Germany). Reactions were measured on the reader (Multiscan EX Labsystems, Finland) according to the manufacturer's protocol.

Statistics

Statistical analysis was carried out according to generally accepted methods of variation statistics after checking the variables for normal distribution. When distribution was normal, we used the parametric Student's t-test and results in these cases are presented as mean ± SD. When distribution did not correspond to the law of normal distribution, we used the nonparametric Mann-Whitney test and data are shown as median, 25% and 75% percentiles. A p value < 0.05 was considered to denote a significant difference. Statistical analysis was performed using Statistica 6.0 software.

RESULTS

We found that all women with endometriosis regardless of disease stage, were characterized by high levels of peripheral and peritoneal CD19+CD20-CD38+ plasma cells and naïve CD19+CD27- B-lymphocytes, but low level of memory CD19+CD27+ B-cells ($p < 0.05$ in all cases, Table 1). It must be noted that in the peritoneal cavity the changes in the number of naïve and memory B-cells were significantly more pronounced in women with severe endometriosis in comparison to that in women with mild endometriosis (Table 1).

We also studied the amount of switched and unswitched B-cells in the blood and peritoneal fluid of women with endometriosis and found the elevation of classical unswitched naïve B-cells and diminishment of switched memory B-cells in all women with endometriosis compared to the control group ($p < 0.05$ in all cases, Table 2). We found a decrease in the pool of unswitched memory B-cells with the phenotype CD19+CD27+ IgD+ only in the peritoneal cavity of the women with endometriosis ($p < 0.05$ in all cases, Table 2).

Table 1. The content of plasma cells, naïve and memory B-cells in the peripheral blood and peritoneal fluid of women with different stages of endometriosis.

Parameter	Control group (n = 25)	Women with stages I-II of endometriosis (n = 35)	Women with stages III-IV of endometriosis (n = 25)
Peripheral blood			
CD19+CD20-CD38+	2.03 ± 0.38%	5.58 ± 1.06% p1 = 0.003	4.62 ± 1.03% p1 = 0.026
CD19+CD27-	66.98 ± 1.34%	73.55 ± 1.12% p1 = 0.000	75.05 ± 1.27% p1 = 0.000
CD19+CD27+	33.09 ± 1.32%	26.45 ± 1.11% p1 = 0.000	24.90 ± 1.27% p1 = 0.000
Peritoneal fluid			
CD19+CD20-CD38+	3.03 ± 0.53%	6.67 ± 0.74% p1 = 0.000	7.48 ± 1.15% p1 = 0.001
CD19+CD27-	58.82 ± 2.46% p3 = 0.008	70.92 ± 1.46% p1 = 0.000	75.14 ± 1.17% p1 = 0.000, p2 = 0.028
CD19+CD27+	41.25 ± 2.45% p3 = 0.01	28.96 ± 1.41% p1 = 0.000	25.10 ± 1.20% p1 = 0.000, p2 = 0.042

Data are shown as mean ± SD. p1 – is given in comparison to the control group, p2 – is given in comparison to the group with stages I-II of endometriosis, p3 – is given in comparison to the same parameters in peripheral blood.

Table 2. The content of switched and unswitched naïve and memory B-cells in the peripheral blood and peritoneal fluid of women with different stages of endometriosis.

Parameter	Control group (n = 25)	Women with stages I-II of endometriosis (n = 35)	Women with stages III-IV of endometriosis (n = 25)
Peripheral blood			
CD19+CD27-IgD+	42.21 ± 1.58%	50.11 ± 2.13% p1 = 0.006	50.01 ± 2.30% p1 = 0.009
CD19+CD27+IgD+	10.45 ± 0.67%	9.05 ± 0.95%	9.26 ± 0.91%
CD19+CD27+IgD-	22.81 ± 1.23%	19.02 ± 1.15% p1 = 0.032	18.09 ± 1.68% p1 = 0.032
CD19+CD27-IgD-	24.59 ± 1.48%	22.72 ± 1.35%	22.78 ± 1.73%
Peritoneal fluid			
CD19+CD27-IgD+	28.6 ± 4.24% p3 = 0.009	44.51 ± 2.67% p1 = 0.005	43.17 ± 3.69% p1 = 0.016
CD19+CD27+IgD+	22.98 ± 2.52% p3 = 0.000	14.81 ± 1.69% p1 = 0.014, p3 = 0.007	13.28 ± 1.74% p1 = 0.005
CD19+CD27+IgD-	18.95 ± 2.64%	12.34 ± 1.71% p1 = 0.049, p3 = 0.003	11.76 ± 1.85% p1 = 0.037, p3 = 0.019
CD19+CD27-IgD-	29.68 ± 3.22%	28.33 ± 2.8%	30.60 ± 3.89%

Data are shown as mean ± SD; p1 – is given in comparison to the control group, p3 – is given in comparison to the same parameters in peripheral blood.

Local immune reactions, which develop in the peritoneal cavity during the process of direct contact of immune cells with ectopic endometrial cells, seemingly play an important role in endometriosis pathogenesis [1]. To decipher the mechanisms of local humoral immune response formation in endometriosis, we compared the type of B-cell differentiation in the blood and peritoneal fluid of healthy women (control group) and women with endometriosis. It was established that in healthy women peritoneal B-lymphocytes were characterized by higher content of memory B-cells and lower numbers of naïve unswitched B-cells compared to B-lymphocytes in the blood (Table 1). It must also be noted that elevation of memory B-cells took place predominantly in the population of peritoneal unswitched CD19+IgD+ CD27+ B-cells and the level of these cells increased more than two-fold in comparison to that in the peripheral blood (Table 2). In patients with endometriosis local humoral immune response was different from the systemic immune response. All changes to the amount of cells were noted only in subsets of switched and unswitched memory B-cells. Women with mild endometriosis were characterized by an increase in unswitched memory B-cells and both groups of women with endometriosis, unlike the control group, were characterized by the diminishment of classical switched memory B-cells in the peritoneal fluid compared to that in the peripheral blood (Table 2).

At the next step of our work we tried to define the connection between the changed functional activity of B-cells and production of autoantibodies against tissues and organs of the reproductive system. We also studied two organ-specific autoantibodies, against zona pellucida (anti-ZP) and anti-ovary antibodies, which can compromise the initial steps of embryogenesis and implantation. It was found that in women with endometriosis stages I-II all changes were more pronounced at the systemic level ($p < 0.05$ in all cases, Table 3). On the contrary, in women with severe endometriosis the content of serum anti-ZP autoantibodies was lower than that in women with stages I-II of endometriosis, and anti-ovary autoantibodies both in serum and peritoneal fluid were elevated ($p < 0.05$ in all cases, Table 3). But concentrations of all studied autoantibodies did not exceed 10 U/ml in any of the groups. According to the instructions of the ELISA kit manufacturer, values of indicators characteristic of persons with autoimmune diseases should be 10 U/ml or higher for both antibodies. Hence, all changes in the

Table 3. The content of anti-Zona Pellucida and anti-ovary autoantibodies in the serum and peritoneal fluid of women with different stages of endometriosis.

Parameter	Control group (n = 25)	Women with stages I-II of endometriosis (n = 35)	Women with stages III-IV of endometriosis (n = 25)
Peripheral blood			
anti-ZP autoantibodies	5.295 U/ml [4.378-8.161]	7.627 U/ml [4.484-10.21] p1 = 0.041	4.7355 U/ml [3.54-7.015] p2 = 0.017
anti-ovary autoantibodies	3.698 U/ml [1.673-4.658]	5.376 U/ml [4.668-6.01] p1 = 0.000	4.554 U/ml [3.99-5.815] p1 = 0.009, p2 = 0.033
Peritoneal fluid			
anti-ZP autoantibodies	3.11 U/ml [2.34-4.25]	3.7 U/ml [1.74-4.96]	3.19 U/ml [1.62-5.57]
anti-ovary autoantibodies	1.35 U/ml [0.0-3.56]	1.637 U/ml [0.76-2.72]	2.66 U/ml [1.62-3.44] p2 = 0.016

Data are shown as median, 25% and 75% percentiles. p1 – is given in comparison to the control group, p2 – is given in comparison to the group with stages I-II of endometriosis.

groups of women with endometriosis statistically distinguish these patients from healthy women, but these changes do not allow us to conclude on the appearance of true autoimmune manifestations in endometriosis.

DISCUSSION

Thus, differentiation of peripheral and peritoneal B-lymphocytes in women with endometriosis significantly differs from that in healthy women. In all women with endometriosis and infertility the amount of plasma cells and CD19+IgD+ CD27- naïve B-cells was increased and the content of CD19+CD27+ memory B-cells was decreased, both at systemic and local levels, compared to that of control values, and the additional diminishment of unswitched CD19+IgD+CD27+ memory B-cells was found only in the peritoneal fluid of women with endometriosis. Changes at the local level were more pronounced in women with severe forms of endometriosis. In the peritoneal fluid of healthy women the level of unswitched memory B-cells was significantly higher and the level of switched CD19+IgD+CD27- naïve B-cells was lower than that in peripheral blood. A slight elevation of unswitched memory B-cells was found in the peritoneal cavity compared to the peripheral blood only in patients with mild endometriosis. In both groups of women with endometriosis the content of classical switched memory B-cells in peritoneal fluid was lower than that in the peripheral blood, which distinguishes these women from control group.

According to our results, we can conclude that the type of B-lymphocyte differentiation and local humoral immune response development in healthy women and women with endometriosis are different. In the peritoneal fluid of healthy women the accumulation of cells at the final stages of differentiation, such as memory B-cells, with simultaneous decrease of naïve B-cells shows a higher effector potential of peritoneal B-lymphocytes which are able to quickly and effectively respond to antigenic stimulus. Also, special attention in this respect should be paid to the unswitched peritoneal B-cells. These cells are the circulating marginal zone B-cells which are formed independently of the T-cells [16]. This pool has been termed “innate-like” B-cells. It was shown that IgD+CD27+ B-cells would represent an immediate line of immune defense with none of the functional characteristics of memory cells [16]. It has been proposed that natural antibodies and IgM antibodies, which are essential for immediate protection against infection are secreted by these unswitched memory B-cells [16]. At the same time, it has been shown that unswitched memory B-cells contain a large amount of IL-10-producing regulatory B-lymphocytes or B-10+ cells possessing suppressor activity [14]. Hence, in physiological conditions unswitched memory B-cells both in blood and especially in the peritoneal fluid are involved in the rapid production of natural antibodies and IgM antibodies. Simultaneously, this pool likely promotes suppression of the humoral immune response due to high activity of regulatory B-10 cells to prevent unwanted hyperactivation of B-cells.

Earlier it was demonstrated that the unswitched memory B-cells play an important role in the development of autoimmune diseases. In patients with systemic lupus erythematosus (SLE) the level of peripheral CD19+IgD+CD27+ lymphocytes was significantly decreased, and reduction of this subset correlated with higher levels of serum anti-nuclear autoantibody production [17]. We established the diminishment of the level of peritoneal CD19+IgD+CD27+ B-cells in all women with endometriosis in comparison to the control group. It can be supposed that the decrease in the activity of these cells in the peritoneal fluid of women with endometriosis may lead to a predisposition for local inflammatory processes with expressed autoimmune component.

Thus, our results showing the high level of unswitched naïve B-cells and low level of memory B-cells including unswitched memory B-cells, indicate a shift in B-lymphocyte differentiation in endometriosis. Earlier it was shown that similar changes accompany many autoimmune diseases, such as systemic sclerosis, rheumatoid arthritis and SLE [17]. And in the population of classical unswitched CD19+IgD+CD27- naïve B-lymphocytes, autoreactive B-cells with B-cell receptor (BCR), capable of reacting with autoantigens, are quite common [18]. It has been demonstrated that the number of these cells is significantly increased in rheumatoid arthritis and correlates with the activity of the pathological processes [19]. Thus, our results are generally in good accordance with the

previous hypothesis about the autoimmune nature of endometriosis.

On the other hand, impairment of B-cell differentiation might be connected with infertility development in patients with endometriosis. Sung N. *et al*. (2016) has shown that in infertile women the level of naïve B-cells increased and the amount of memory B-cells decreased compared to those in fertile women [20]. In our work we studied the production of anti-ZP and anti-ovary autoantibodies and found that in women with mild endometriosis the serum concentration of both autoantibodies was maximal and was higher compared to the control group and the group of women with severe endometriosis. The distinctive characteristic of the group with stages III-IV of endometriosis was the high systemic and local level of anti-ovary autoantibodies. In this group we often diagnosed ovarian cysts, and anti-ovary antibodies seemingly participated in ovarian cyst development. Thus, the systemic production of autoantibodies against autoantigens of the reproductive system was not correlated with the severity of endometriosis and was maximal in patients with mild endometriosis. Likely, increased production of these autoantibodies is associated predominantly with infertility but not with endometriotic lesion formation. This suggestion is in good accordance with literature data. It has been shown that concentration of anti-ZP and anti-ovary autoantibodies rises in women with infertility, especially those with primary infertility [21]. But, it must be specifically noted that all values of the studied autoantibodies in all groups were below diagnostic level. Among our patients we recorded antibody concentration values of more than 10 U/ml only in two cases (data not shown). Hence, we believe that endometriosis cannot be considered as a true autoimmune disease but only as an autoreactive condition.

Although autoantibodies are an important serologic feature of autoimmune diseases, their presence is not exclusive to these conditions. Autoantibodies of different specificities were also found in cancer patients, in cases of massive tissue damage, and even in healthy subjects [22]. Endometriosis is associated with accumulation of activated immune cells and proinflammatory cytokines and growth factors in peritoneal fluid, creating a local inflammatory environment [1]. Disruption of tolerance and autoantibody production often take place during inflammation, with accumulation of cell debris in the extracellular space. It is known that in endometriosis the massive transport of endometrial fragments and cell debris to the peritoneal cavity takes place. Hence, at the local level the amount of autoantigens increases, activating both the humoral and innate immune response. Antibodies can directly activate phagocytes and participate in antigen opsonization and in immune phagocytosis induction [12]. Increased inflammation can induce the development of immunologic autoreactivity, driving the selection and development of weakly auto-reactive antibodies through affinity maturation [2]. And increased activation of phagocytes, in particular, peritoneal macrophages, noted by numerous researchers in endometriosis patients [1], may be the trigger for the enhanced production of autoantibodies. Since inflammation, especially local, is the main pathophysiological mechanism associated with endometriosis development, the enhanced production of autoantibodies can be considered as one of the factors promoting phagocyte activation and endometriotic lesion formation and growth. On the other hand, circulating autoantibodies, capable of reacting with antigens of reproductive system organs, certainly participate in development of infertility in women with endometriosis.

CONCLUSIONS

1. In women with endometriosis, regardless of disease degree the systemic and local levels of plasma cells, and naïve B-cells increase, memory B-cells and peritoneal unswitched memory cells decrease in comparison to that in healthy women. These changes are maximally expressed in the peritoneal fluid in women with severe endometriosis.
2. Comparison of the local and systemic humoral immune response shows that in healthy women the local level of the memory B-cells and unswitched memory cells exceed that at the systemic level and the number of naïve B-cells in the peritoneal fluid was significantly lower than that in the blood. We did not observe the same changes in endometriosis patients.

3. In women with mild endometriosis we found elevation of the serum concentrations of anti-ZP and anti-ovary autoantibodies in comparison to that in control group. In women with severe endometriosis we noted the elevation of anti-ovary autoantibodies both at systemic and local levels. In all groups the autoantibody values were lower than those in subjects with true autoimmune disorders.
4. We think that changes in B-cell differentiation with slight accumulation of autoantibodies capable of reacting with organs of the reproductive system, indicate the presence of autoimmune reactions but it does not speak of the true autoimmune process. This situation might be responsible for both chronic local inflammation and fertility impairment in women with endometriosis.

CONFLICT OF INTEREST STATEMENT

The authors do not have any conflict of interest to report for this work.

REFERENCES

1. Burney, O. and Giudice, L. 2012, Fertil. Steril., 98, 511.
2. Barrier, B. F. 2010, Clinical Obstetrics and Gynecology, 53, 397.
3. Gupta, S., Goldberg, J. M., Aziz, N., Goldberg, E., Krajcir, N. and Agarwal, A. 2008, Fertil. Steril., 90, 24.
4. Ulcová-Gallová, Z., Bouse, V., Svábek, L., Turek, J. and Rokyta, Z. 2002, Am. J. Reprod. Immunol., 47, 269.
5. Gleicher, N., el-Roeiy, A., Confino, E. and Friberg, J. 1987, Obstet. Gynecol., 70, 115.
6. Odukoya, O. A., Bansal, A., Wilson, P., Lim, K., Weetman, A. P. and Cooke, I. 1996, Hum. Reprod., 9, 2018.
7. Gagné, D., Rivard, M., Pagé, M., Shazand, K., Hugo, P. and Gosselin, D. 2003, Fertil. Steril., 80, 43.
8. Yeol, S. G., Won, Y. S., Kim, Y. I., Lee, J. W., Choi, Y. J. and Park, D. C. 2015, Clin. Exp. Obstet. Gynecol., 42, 156.
9. Hever, A., Roth, R. B., Hevezi, P., Marin, M. E., Acosta, J. A., Acost, H., Rojas, J., Herrera, R. Grigoriadis, D., White, E., Conlon, P. J., Maki, R. A. and Zlotnik, A. 2007, Proc. Natl. Acad. Sci. USA, 104, 12451.
10. de Graaff, A. A., Dunselman, G. A., Delvoux, B., van Kaam, K. J., Smits, L. J. and Romano, A. 2010, Fertil. Steril., 94, 1108.
11. Christofolini, D. M., Cavalheiro, C. M., Teles, J. S., Lerner, T. G., Brandes, A., Bianco, B. and Barbosa, C. P. 2011, Scand. J. Immunol., 74, 628.
12. Riccio, L. G. C., Baracat, E. C., Chapron, C., Batteux, F. and Abrão, M. S. 2017, J. Reprod. Immunol., 123, 29.
13. Blanchard-Rohner, G., Pulickal, A. S., Jol-van der Zijde, C. M., Snape, M. D. and Pollard, A. J. 2009, Blood, 114, 4998.
14. Torigoe, M., Iwata, S., Nakayamada, S., Sakata, K., Zhang, M., Hajime, M., Miyazaki, Y., Narisawa, M., Ishii, K., Shibata, H. and Tanaka, Y. 2017, J. Immunol., 199, 425.
15. Wu, Y. C., Kipling, D. and Dunn-Walters, D. K. 2012, Front. Immunol., 2, 81.
16. Weller, S., Braun, M. C., Tan, B. K., Rosenwald, A., Cordier, C., Conley, M. E., Plebani, A., Kumararatne, D. S., Bonnet, D., Tournilhac, O., Tchernia, G., Steiniger, B., Staudt, L. M., Casanova, J. L., Reynaud, C. A. and Weill, J. C. 2004, Blood, 104, 3647.
17. Malkiel, S., Jeganathan, V., Wolfson, S., Manjarrez, O. N., Marasco, E., Aranow, C., Mackay, M., Gregersen, P. K. and Diamond, B. 2016, Arthritis Rheumatol., 68, 2210.
18. Malkiel, S. D., Balogh, P., Bognár, A., Kellermayer, Z., Engelmann, P., Németh, P., Farkas, N., Minier, T., Lóránd, V., Czirják, L. and Berki, T. 2016, Clin. Exp. Rheumatol., 100, 30.
19. Duty, J. A., Szodoray, P., Zheng N. Y., Koelsch, K. A., Zhang, Q., Swiatkowski, M., Mathias, M., Garman, L., Helms, C., Nakken, B., Smith, K., Farris, A. D. and Wilson, P. C. 2009, J. Exp. Med., 206, 139.
20. Sung, N., Byeon, H. J., Garcia, M. D. S., Skariah, A., Wu, L., Dambaeva, S., Beaman, K., Gilman-Sachs, A. and Kwak-Kim, J. 2016, J. Reprod. Immunol., 118, 70.
21. Huo, Y., Xu, Y., Wang, J., Wang, F., Liu, Y., Zhang, Y. and Zhang, B. 2015, International Journal of Clinical and Experimental Medicine, 8, 14048.
22. Lleo, A., Invernizzi, P., Gao, B., Podda, M. and Gershwin, M. E. 2010, Autoimmun. Rev., 9, A259.