T helper (Th)1, Th2, and Th17 interleukin pathways in infertile patients with minimal/mild endometriosis

In the present study, interleukin (IL)-10, IL-12, IL-17, and IL-23 levels were measured in serum and peritoneal fluid of women with minimal or mild endometriosis and compared with levels in controls without endometriosis. Higher IL-23 levels were encountered in the peritoneal fluid of women with endometriosis, suggesting a possible role of this cytokine in these women's infertility. (Fertil Steril® 2011;95:2477–80. ©2011 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, Th1, Th2, Th17, interleukin, infertility

Endometriosis is characterized by the presence of endometrial tissue outside the uterine cavity (1, 2) and affects approximately 10% of the female population of reproductive age (2–4).

The association between endometriosis and infertility has long been known (5, 6). Numerous mechanisms may provoke impaired fertility in patients with endometriosis, such as ovulatory (7–10), hormonal (11–13), endometrial (14, 15), and immunologic disturbances (16, 17).

The discovery of another type of T cell revised the conventional T helper (Th)1/Th2 hypothesis (18). T helper 17 cells produce interleukin (IL)-17A, IL-17F, IL-21, and IL-22 and mediate host defensive mechanisms against infections (19). Current evidence suggests that IL-23 is responsible for the differentiation and expansion of Th17/ThIL-17 cells (20). Several studies revealed the association between IL-23, Th17 cells and many autoimmune diseases (19, 21).

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It is currently accepted that an increase in IL-1, -4, -5, -6, -8, and -10, tumor necrosis factor-α, and vascular endothelial growth factor occurs in endometriosis (22). Only one study found higher levels of IL-17 in the peritoneal fluid (PF) of women with minimal/mild endometriosis, especially when infertility coexisted (23). Our group studied IL-18 and found no correlation with endometriosis (24). However, there are no data regarding IL-23 and endometriosis to date, and moreover, to date there has been no study of a subgroup of minimal/mild endometriosis with respect to infertility and Th1/Th2/Th17 pathway profiles.

In view of the emerging significance of Th17 cells in immunology, we investigated, for the first time, the role of IL-10, -12, -17, and -23 in infertile patients with minimal/mild endometriosis.

A cross-sectional study was performed on 80 patients aged ≥ 18 years who were enrolled from March 2007 to December 2008 at Hospital de Clínicas de Porto Alegre when seeking care for the investigation of infertility or for tubal ligation.

Patients submitted for laparoscopy indicated by infertility were enrolled. Infertility was defined as inability to achieve pregnancy after 1 year of regular unprotected sexual intercourse (25). Forty patients who presented peritoneal implants and met criteria for minimal or mild endometriosis, according to the classification proposed by the American Society for Reproductive Medicine (25), were included in the study. The control group consisted of 40 fertile patients who underwent laparoscopy for tubal ligation and were endometriosis free.

The same surgical staff performed all laparoscopies. Exclusion criteria comprised presence of autoimmune disease, absence of peritoneal liquid during laparoscopy, coexistence of other causes of infertility, and having used hormonal medication in the 3 months before surgery. Other causes of infertility were excluded by hysterosalpingography, spermiogram, and measurements of serum FSH, PRL, and TSH levels on the third day of the menstrual cycle. Laparoscopy was done during the first phase of the menstrual cycle in all patients.

During laparoscopy, samples of PF (3–6 mL) were aspirated after the introduction of the second trocar, and blood samples were collected. All samples were centrifuged and stored at −80°C until analyzed.

All patients who participated in this study signed an informed consent form. The study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (institutional review board equivalent).

Serum and peritoneal cytokines were measured by ELISA using Human Ready-SET-Go! commercial kits (eBioscience, San Diego, CA), including IL-10, IL-12(p70), IL-17A, and IL-23(p19/p40). The sensitivity was 2 pg/mL, 4 pg/mL, 4 pg/mL, and 15 pg/mL, respectively.

For statistical analysis, data with normal distribution are reported as means ± SD with the 95% confidence interval. Student's *t*-test was used to compare means. Samples were considered statistically different at $P \leq .05$.

The clinical and demographic characteristics of the two groups did not differ. Mean age in the study group and controls was 32.48 ± 4.99 years and 33.63 ± 6.51 years, respectively. Mean body mass index was 23.75 ± 4.37 kg/m^2 in cases and 25.31 ± 3.95 kg/m^2 in controls. Mean FSH level in the study group and controls was 5.72 ± 2.44 mIU/mL and 6.24 ± 1.96 mIU/mL ($P>.05$), respectively. Although the indication for laparoscopy was infertility, 16 patients (40%) with endometriosis had a complaint of pelvic pain. In the study group (40 patients), 26 had minimal endometriosis, and 14 had mild disease. When regarding endometriosis stage (minimal vs. mild), all cytokines did not significantly differ ($P>.05$).

Mean IL-23 levels measured in the PF were higher in patients with endometriosis when compared with the control group ($P=.006$). No statistically significant differences were found in serum concentrations of IL-23 between cases and controls. Statistically significant differences were not found in levels of IL-10, -12, and -17 in blood or PF between the endometriosis and control groups as demonstrated in Table 1.

Moreover, we performed a receiver operating characteristic analysis using PF IL-23 as a screening test. The area under the curve was 0.703 (95% confidence interval 581–825); the best discriminatory cutoff was 10.755 pg/mL, and sensitivity and specificity were 0.744 and 0.647, respectively.

Interleukin-23 levels in PF were found to be significantly higher in patients with endometriosis when compared with controls. In serum, this difference was not detected. We demonstrate for the first time not only the presence of IL-23 in PF but also higher levels of this cytokine among patients with endometriosis.

Previous studies have found increased levels of IL-10 in the PF of patients with endometriosis (22). However, there are still conflicting data. Pogdaec et al. (26) found significantly higher levels of interferon-γ and IL-10 in the PF of patients with endometriosis when compared with controls. In this study, all patients were symptomatic, and controls were women without detectable endometriosis during laparoscopy.

The literature about IL-12 and endometriosis is also conflicting. Fairbanks et al. (27) and Galinelli et al. (28) found higher levels of IL-12 in the PF of patients with endometriosis when compared with healthy controls. However, Bedaiwy et al. (29) found similar levels in serum and PF of women with and without endometriosis. Moreover, Gazvani et al. (30) found no correlation between presence of endometriosis, endometriosis grade, or menstrual cycle phase and IL-12 but could not detect the cytokine in 67% of the patients with endometriosis.

The existing literature on IL-17 and endometriosis is sparse. Zhang et al. (23) showed no difference in IL-17 levels between patients with endometriosis and controls. However, the concentrations in PF were correlated with severity, being significantly higher in patients with minimal/mild endometriosis than in those with moderate/severe endometriosis and those without endometriosis. Interleukin-17 levels also correlate with severity of autoimmune diseases (31–33). Thus, similar IL-17 levels between groups in the present study may be due to selection of early-stage endometriosis.

Interleukin-23 has been implicated in the differentiation of naïve T cells into Th17 cells, a novel route for immune response.

TABLE 1

Comparison of IL levels in serum and PF between endometriosis (n = 40) and control groups (n = 40).

Cytokine level (pg/mL)	Endometriosis group	Control group	*P* value[a]
IL-10 serum	13.05 ± 29.55	10.43 ± 7.56	.604
Detectable IL	37/40 (92)	39/40 (97)	
IL-10 PF	70.51 ± 88.50	50.41 ± 42.59	.209
Detectable IL	40/40 (100)	40/40 (100)	
IL-12 serum	7.95 ± 3.14	14.39 ± 11.20	.203
Detectable IL	6/40 (15)	7/40 (17)	
IL-12 PF	3.39 ± 7.31	2.24 ± 2.17	.344
Detectable IL	14/40 (35)	21/40 (52)	
IL-17 serum	4.83 ± 8.60	2.35 ± 2.40	.325
Detectable IL	13/40 (32)	13/40 (32)	
IL-17 PF	30.03 ± 64.27	3.66 ± 2.26	.445
Detectable IL	6/40 (15)	4/40 (10)	
IL-23 serum	6.49 ± 4.71	10.12 ± 9.87	.209
Detectable IL	14/40 (35)	22/40 (55)	
IL-23 PF	13.89 ± 5.48	9.96 ± 6.31	.006
Detectable IL	39/40 (97)	35/40 (87)	

Note: Values are mean ± SD or number (percentage).
[a] Student's *t*-test.

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Several publications have associated IL-23 with many autoimmune and immune-mediated diseases, such as inflammatory bowel disease (19) and rheumatoid arthritis (34). There are also publications linking the IL-23/Th17 axis with psoriasis, multiple sclerosis, lupus, asthma, vasculitis, and many other diseases (21, 34).

An association between endometriosis and autoimmune diseases is probable: many authors have described a higher prevalence of diseases such as systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis, hypothyroidism, and others (35–39) in patients with endometriosis. Until now, IL-23 has not been studied in endometriosis or even detected in the PF, which makes our results unique. For the first time we demonstrate higher IL-23 levels in the PF of infertile patients with endometriosis. Furthermore, our receiver operating characteristic analysis also demonstrated the effect of this cytokine on mild/minimal endometriosis compared with endometriosis-free controls.

The composition of our groups makes our study original, because we included only infertile patients with minimal/mild endometriosis and compared them with fertile asymptomatic patients without endometriosis. For this reason, we were not able to make associations between cytokines and endometriosis grade. Because our patients had early-stage disease, the levels of the cytokines may actually be lower, and therefore, we were not able to detect them. Because the indication for laparoscopy in our study was infertility and not pain, this may also influence our results. Different sample sizes among studies may hamper comparison, not showing statistical difference.

Most authors used ELISA assays because of their availability and cost when compared with other refined techniques. However, ELISA has limitations, such as lower sensitivity, requiring the consumption of a relatively large amount of sample, and a complex protocol. Other methods, like flow cytometry, Luminex, or real-time polymerase chain reaction for messenger RNA, may provide more accurate results and reduce the conflicting data previously reported.

The limitation of our study is the fact that several patients did not reach the cutoff for IL detection. We could infer from this result that, in fact, these ILs have a low relevance in mild/minimal endometriosis. Moreover, our conclusion is valid only for infertile patients with minimal/mild endometriosis, in contrast with the majority of studies, which included patients with endometriosis with pain and an obviously greater inflammatory component.

The discovery of Th17 provides us another path to follow and introduces a new research trend. Evidence linking IL-23, Th17 cells and autoimmune and immune-mediated diseases could provide insights into the role of this pathway in the pathogenesis of endometriosis.

In conclusion, we found similar levels of IL-10, -12, and -17 in serum and PF of infertile women with endometriosis compared with fertile healthy controls. Interleukin-23 levels were similar in serum but significantly higher in PF of infertile women with minimal/mild endometriosis, and this may be implicated in the subfertility of these patients.

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