



Increased IL-25 levels in the peritoneal fluid of patients with endometriosis

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ABSTRACT

Previous studies have indicated a higher prevalence of allergic disease among women with endometriosis. It is already well established that type 1 allergies develop in a Th2 cytokine environment. Recent studies have shown, however, that IL-25 induces a Th2 development of naive T lymphocytes and is central in the Th2 response.

The aim of this case-control study was to investigate the presence of IL-25 in the peritoneal fluid of women suffering from endometriosis.

PF was obtained both from women undergoing laparoscopic surgery due to endometriosis (25 cases) and from women wanting sterilisation (19 controls). IL-25 levels were then investigated by ELISA.

Women with endometriosis showed significantly higher levels of IL-25 in their PF ($p = 0.019$) compared to controls. IL-25 levels did not correlate with the stage of endometriosis.

Both Th2-cells and mast cells express IL-25, which could favor the development of allergies by perpetuating a hypersensitivity reaction. Further, IL-25 may also hold a role as a diagnostic tool.

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1. Introduction

Endometriosis is defined by the presence of endometrium-like tissue outside the uterine cavity (Burney and Giudice 2012). Affecting up to 10% of women in their reproductive years, it has extensive impact on the affected women's work and social life (Simoens et al., 2012) and is a major cause of hospitalization in developed countries.

It is widely accepted that the immune system plays a crucial role in the pathogenesis of endometriosis. Considerable research have reported on changes in the peritoneal microenvironment, where defect or altered functions of peritoneal immune cells allows endometriotic lesions to develop and aggravate (Lagana et al., 2013). Further, up- and down-regulation of genes involved in decidualization and implantation, seems to be a contributing factor to the inflammatory environment seen in the chronic pelvic inflammation of endometriosis (Kobayashi et al., 2014).

(Hsu et al., 1997; Antsiferova et al., 2005) describe increased expression levels of IL-4 in peripheral blood cells, in cells in the ectopic endometrium, and in the peritoneal fluid (PF), as well as elevated serum levels, in women with endometriosis. (Gazvani et al., 2001) however found no difference in IL-4 levels. Furthermore, several other pro-inflammatory cytokines like IL-17, IL-6, IL-10, TNF α and growth factors have been found in the PF of women with endometriosis, supporting the theory that an inflammatory response plays a crucial part in the pathogenesis of endometriosis (Harada et al., 2001).

Recently, we performed a review of the literature concerning the association between endometriosis and allergic disease, but due to poor quality of studies and lack of definitions of allergic disease, no firm conclusions could be made (Bungum et al., 2014). A trend toward a correlation between the two diseases, however, seems to exist.

Type 1 allergies develop in a Th2 cytokine environment under the influence of IL-4, -6 and -10. The cytokine IL-25 (also called IL-17E) has drawn considerable attention in the search for cytokines central to the development and maintenance of the Th2 response, since it induces a Th2 development of naive T lymphocytes (Fort et al., 2001; Ikeda et al., 2003). IL-25 is expressed by Th2-cells and

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mast cells. IL-25 could be a part of the cytokine milieu, which favors the development of allergies by perpetuating a hypersensitivity reaction.

This study was undertaken to evaluate the level of IL-25 in the peritoneal fluid of women with and without endometriosis, hence exploring a possible cause and effect between IL-25 and the inflammatory response seen in endometriosis.

2. Materials and methods

This study is based on the STROBE guidelines for observational studies.

PF was obtained both from women wanting sterilisation (controls) and from women undergoing laparoscopic surgery to diagnose and/or to remove endometriotic lesions (cases).

The local Regional Ethics Committee approved the protocol and all women had oral and written information prior to signing a form of consent. PF was aspirated from 25 cases with histologically confirmed endometriosis and American Fertility Score stage 1 ($n=6$), 2 ($n=9$), 3 ($n=9$) or 4 ($n=1$), and 19 controls with no history of endometriosis. No women in the control group revealed signs of endometriosis during their procedure. The study population is further described in (Vestergaard et al., 2010, 2011a,b).

The PF was obtained as soon as the laparoscopic instruments were in place and before any surgical manipulation. 5 mL of isotonic saline solution was released in the pelvic cavity, and aspirated by a syringe. If no fluid could be aspirated after the first infusion, another 5 mL of saline was installed.

Information on age, menstrual phase, contraceptive use as well as levels of follitropine, LH, estrogen and progesterone were obtained. Data on last menstrual period were obtained, evaluated alongside hormone levels, and used to determine menstrual cycle phase.

Operative details regarding the location and degree of endometriosis were recorded. The extent and severity of endometriosis was graded according to the revised American Fertility Society (rAFS).

The concentration of IL-25 was measured by enzyme-linked immunoabsorbant assay (ELISA). The IL-25 ELISA kit used in our analyses was commercially available from PeproTech (900-K234 Lot# 1208234, PeproTech, Rocky Hill, USA). It was used according to the manufacturers protocol with two exceptions. Firstly, the reagent diluent contained 0.5% bovine serum albumin instead of the recommended 0.1% which increased recovery. Secondly, we extended the standard-ladder and hence lowered the detection limit from 32 pg/mL to 8 pg/mL. These changes were made after meticulous validation prior to use in accordance with the publication from Kragstrup et al. (2013).

2.1. Statistics

The distribution of data was evaluated from scatter plots and histograms, supported by the skewness and kurtosis tests for normality ($p=0.00001$). As expected, data were not normally distributed. Non-parametric test were used for all statistical analyses except for differences in age, menstrual phase and use of contraceptives, where Student's *t*-test, Fisher's exact test and Pearson's chi-squared test were used, respectively. All statistics were done in STATA version 12.0 (StataCorp, College Station, Texas, USA). Graphs were made in GraphPad Prism version 6 (GraphPad software, San Diego, USA).

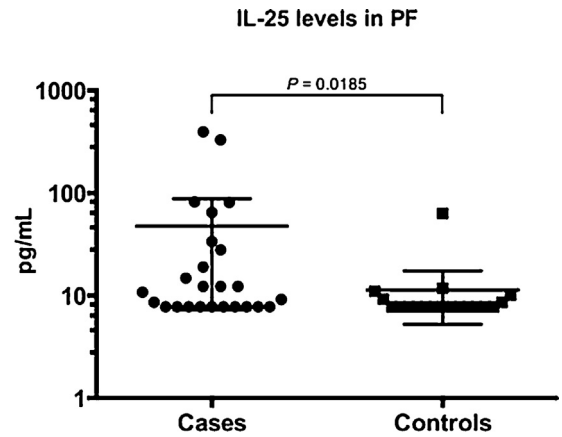


Fig. 1. The concentrations (pg/mL) of IL-25 in PF of patients with endometriosis and healthy controls. Values are presented in a scatter plot with mean and 95% CI. Please note the logarithmic y-axis.

3. Results

Samples of peritoneal fluid from 25 women with endometriosis and 19 controls were suitable for assessments of IL-25. Fig. 1 illustrates the concentrations of IL-25 in cases and controls.

There were significantly higher concentrations of IL-25 in women with endometriosis compared to controls, ($p=0.019$). No significant differences between cases (women with endometriosis) and controls were found regarding menstrual phase ($p=0.293$), levels of estrogen ($p=0.5601$), progesterone ($p=0.1805$), Luteinizing Hormone (LH) ($p=0.6417$) or use of hormonal contraception ($p=0.4247$). There was however, a significant difference regarding age ($p=0.0126$) and follitropin ($p=0.0020$) (Table 1). The presence of IL-25 in the peritoneal fluid showed no significant correlation to age ($p=0.9358$), levels of follitropin ($p=0.1058$), lutropin ($p=0.7973$), estrogen ($p=0.7344$) or progesterone ($p=0.0676$) (Table 2). Finally, there were no significant differences in the presence of IL-25 and menstrual phase ($p=0.2338$) or rAFS stages ($p=0.0908$) (Table 2).

4. Discussion

This study showed women with endometriosis had significantly higher levels of IL-25 in their peritoneal fluid compared to women without endometriosis ($p=0.019$). The result could not be correlated to differences in demographics, phase of menstrual cycle or severity of endometriosis. The latter can be due to the fact that the inflammatory response seems to be low at more severe stages of endometriosis (rAFS) (Salmeri et al., 2015).

Limitations of our study include a low number of participants and a lack of tissue for additional analyses and histology to evaluate the possible origin of IL-25. The greatest limitation however is the fact that we do not know the exact dilution factor of our samples as the volume of the saline solution injected differed and we had no knowledge of the amount of fluid in the peritoneal cavity prior to injection of saline—hence absolute values are of no value in this study. Despite this, the *p*-value reflects a significant gap in the levels of IL-25 between women with endometriosis and healthy controls.

We recently reported that there seemed to be an association between endometriosis and type 1 allergy. This present study suggests that the common factor could be IL-25, a member of the IL-17 family—crucial in a Th2 response and now also found to be present in the peritoneal fluid of women with endometriosis. This finding supports the theory of a pathophysiological significance of Th2 cytokines in the development of endometriosis. This is in line with our previous hypothesis that ectopic endometrial cells

Table 1
Comparison of the demographics/clinical characteristics between cases and controls.

	Demographics		Clinical characteristics							
	N	Age (year)	M ^a	P ^a	L ^a	Estrogen	Follitropin	Progesterone	LH	Contraceptives ^a
Controls	19	36.5 ± 6.5	7 (37)	3 (16)	9 (47)	236.7 ± 181.9	6.2 ± 2.9	4.2 ± 6.2	3.2 ± 2.0	9 (47)
Cases	25	31.5 ± 6.3 [*]	3 (12)	10 (40)	12 (48)	208.9 ± 160.2	3.9 ± 3.4 [*]	5.0 ± 7.9	3.7 ± 2.7	17 (68)

Data are mean ± SD unless otherwise noted.
M (menstrual phase), P (proliferative phase), L (luteal phase), LH (luteinizing hormone), Contraceptives (hormonal contraceptive users).
^{*} P < 0.05 compared with controls.
^a N (%).

Table 2
Correlation between IL-25 and age, hormone levels, menstrual phases and stage of endometriosis.

Secondary statistical analysis							
	Kruskal–Wallis test [#]		Spearman's rank correlation [#]				
	M/P/L	rAFS score	Age	Estrogen	Follitropin	Progesterone	LH
IL-25	X ² = 2.91, p = 0.23	X ² = 6.47, p = 0.09	r = −0.01, p = 0.94	r = −0.06, p = 0.73	r = −0.26, p = 0.11	r = 0.31, p = 0.07	r = −0.04, p = 0.80

M (menstrual phase), P (proliferative phase), L (luteal phase), rAFS (revised American fertility Society), LH (luteinizing hormone), X² (chi-squared), r (Spearman's rho), [#](adjusted for ties).

might also participate in the production of these cytokines, which could lead to allergic inflammation. The pathophysiology could involve macrophages producing IL-1 β and TNF α, found in peritoneal fluid from women with endometriosis (Halme 1989), which stimulates production of RANTES (Regulated on Activation, Normal T cell Expressed and Secreted) and MCP-1 (Monocyte Chemotactic Protein-1) (Akoum et al., 1995; Hornung et al., 2001). RANTES and MCP-1 may be responsible for recruiting macrophages, producing Histamine Releasing Factor (HRF), into endometriotic implants. In turn HRF might induce production and release of IL-4 and maybe IL-25 from mast cells, which in turn stimulates IgE production, exacerbating a Th2 response.

It is presently not known if IL-25, like IL-4, also stimulates proliferation of endometriotic stromal cells, but studies indicate that other members of the IL-17 family could induce the development of endometriosis by the upregulation of cytokines (Hirata et al., 2011).

Other subsets of effector T cells, like the regulatory T cells, may be responsible for a pathway of immunosuppression leading to a dysfunctionel peritoneal homeostasis, which in turn allows endometriotic cells to implantate and proliferate.

The implantation and survival of endometriotic lesions could be generated through several mechanisms; environmental toxins such as 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), but also as a result of dysfunctional Fas/FasL mediated apoptosis (Sturlese et al., 2011).

Changes in the microenvironment of the pelvic cavity also seem to be responsible for altered innervation and central modulation of pain resulting in chronic pelvic pain (Triolo et al., 2013; Neziri et al., 2014).

In this present study we hypothesize that, if IL-25 is specific for endometriotic tissue present in the pelvic cavity, this might lead to a precise and easy diagnostic test for endometriosis. For the affected women, this could improve the time frame from onset of symptoms to definite diagnosis, resulting in earlier initiation of medical therapy and hence better quality of life.

5. Conclusion

In conclusion this study supports a potential connection between endometriosis and allergy and indicates that IL-25 might be a specific marker for endometriosis. Further studies are needed to clarify if there is a common pathophysiology between allergy and endometriosis, involving proinflammatory cytokines such as IL-25.

Furthermore, prospective studies are urgently needed to confirm if IL-25 could be a diagnostic marker for endometriosis.

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