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Autoimmune Response in Women with Endometriosis

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PROBLEM: The aim of this study was to investigate the humoral immune response to the female reproductive tissues associated with endometriosis (grades I–III) ($n = 52$), compared with a group of healthy fertile women ($n = 6$).

METHOD OF STUDY: An ELISA with cultured endometrial cell lines in monolayer was used to determine the presence of anti-endometrial antibodies (AEA). For anti-zona pellucida antibodies (AZPA) assessment a conventional ELISA was employed. The presence of antibodies to human sperm (ASA) was performed by the tray agglutination test (TAT).

RESULTS: Endometriosis grade III was associated with AEA in serum in the 45.4% of patients. The presence of AEA in serum is correlated to endometriosis severity. The 8.7% of women with endometriosis showed ASA, and the 10.9% of them were positive for AZPA. Antibodies specific for endometrial cells do not show reaction to any gamete antigen (sperm or oocyte), suggesting that they are not cross reactive.

CONCLUSIONS: Severity of endometriosis is correlated with high titers of AEA.

Key words:
Autoantibodies, endometriosis, sterility

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INTRODUCTION

Endometriosis is one of the most common gynecological disorders in women of reproductive age, and the prevalence of the disease appears to be higher among women with sterility.[1] It is a disease characterized by the ectopic uterine implantation of endometrial cells that are biologically and morphologically similar to eutopic endometrium. Several studies have suggested a link between endometriosis and sterility[2,3] although the mechanism by which endometriosis causes sterility is still unclear. Altered immunity and autoantibodies have been related to sterility.[4]

The association between endometriosis and autoantibodies was first proposed by Weed and Arquenburg[4] who hypothesized that ectopic endometrial implants may be perceived by the host as foreign and trigger an autoimmune response. Increased amounts of endometrial proteins in extrauterine locations may provoke humoral or cell mediated autoimmune response.[5] The presence of anti-endometrial antibodies (AEA) would not to be related with the growth of the endometriotic implants, but would be rather a consequence of the disease. These antibodies have been detected in patients with endometriosis using different methodologies[6] such as immunodiffusion,[7] passive hemagglutination,[8] indirect immunofluorescence,[9] western blot[10] and enzyme-linked immunosorbent assay.[11,12]

Anti-sperm antibodies (ASA) are present in a small percentage of sexually-active women which could present sterility problems. The presence of ASA in biological fluids manifest their effects through decreased sperm motility,[13] reduced fertilization rates,[14] or decreased rates of cell division in the early pre-embryo.[15] Specific antibodies directed against zona pellucida glycoproteins are able to inhibit sperm interaction and penetration into the ovum *in vitro*.[16] The role of IgG and IgM antibody isotypes are reported to have a negative effect on fertilization.[17] Sperm and zona pellucida antibodies are related to fertilization failure.

In this study, sera and peritoneal fluids obtained from fertile women and sterile patients with several grades of endometriosis were screened for the presence of antibodies specific to endometrium or to human gametes.

MATERIAL AND METHODS

Patients and Clinical Diagnosis

Serum and peritoneal fluid were obtained from 52 women, with ages between 26 and 40, from the Department of Obstetrics and Gynecology at the Charles University of Pilzen, with several degrees of endometriosis: grade I ($n = 27$), grade II ($n = 14$) and grade III ($n = 11$). The extent of disease was scored according to the revised American Fertility Society Classification System.[18] Six fertile women without any gynecological pathology and clear ovulation were used as a control for this study. Clinical laparoscopic examination was performed at time of ovulation to exclude anovulation as a cause of sterility. Other pathologies associated with endometriosis were described in order to compare them with the immunological analysis. During examination and shortly before laparoscopy the patients were tested for vaginal and cervical infection. This group of infertile women had no gynecological infection at the time of their examination.

Anti-endometrial Antibodies Determination

ELISA technique was developed on human endometrial adenocarcinoma monolayer cell culture from two commercial cell lines (ATCC) as described previously in Palacios et al.[11] Briefly, endometrial cells were cultured until monolayer phase in microtiter sterile plates. After cell fixation in 1.25% glutaraldehyde in PBS–Tween20 0.05%, sera or peritoneal fluid samples were incubated for 2 hr at 37°C. We washed twice to remove excess of antibody and incubated for 2 hr at 37°C with an appropriate secondary antibody conjugated to peroxidase enzyme (The Binding Site, Labclinics, Barcelona, Spain). We added chromogenic substrate (DMAB/MBTH, Sigma), and the reaction was stopped with 2 M sulfuric acid and read at 620 nm in a multiscan plate reader (Anthos-Labtech). Samples with absorbances higher than the mean value (A_{620}), obtained in an ELISA developed with sera from fertile healthy women, plus one SD were considered positive as was described previously.[11] Samples positive for the two endometrial cell lines tested were considered positive for the presence of anti-endometrial antibodies. Samples positive for one cell line alone was considered slightly positive.

Anti-sperm Antibodies Determination

The tray agglutination test (TAT) was performed for anti-sperm antibodies determination. Briefly, sera were centrifuged at 5000 rpm for 30 min. Five microliters of supernatant and 1 μL of treated motile donor sperm isolated by the 'swim-up technique', in average counts of 40×10^6 spermatozoa/mL, were posed into microchambers covered with paraffin oil. Backer solution used for dilution was: $CaCl_2$ 50% (0.44 g), KCl (0.2 g), $MgSO_4$ $0.7{\cdot}H_2O$ (0.07 g), glucose (0.5 g), phenol red (8.3 mL) and distilled water (500 mL). After incubation for 2 hr at 37°C, the agglutination was evaluated and observed under the inverted Zeiss Jena microscope at $\times 200$ magnification. Agglutination lower than a 1:16 dilution was considered slightly positive and a dilution 1:16 was considered a positive result.[17]

Anti-zona Pellucida Antibodies Determination

Conventional procedure of ELISA for detection of anti-zona antibodies of IgG isotype was performed (BioGen, Germany). The type of ZP antigens coating the plates was not informed by the kit. Positive and negative controls with known antibody concentration were provided.

TABLE I. Humoral IgG Anti-endometrium Response in Several Grades of Endometriosis ($n = 52$)[a]

				Serum		Peritoneal fluid	
		n	%	AEA+	%	AEA+	%
Endometriosis	Grade I	27/52	51.9	2/27	7.4[ns]	3/27	11.1[ns]
	Grade II	14/52	26.9	2/14	14.3[ns]	0/14	0[ns]
	Grade III	11/52	21.1	5/11	45.4**	1/11	11.1[ns]

[a] *Serum from six healthy women was used as a control.*
[ns] $P > 0.05$; ** $P < 0.01$.

Statistical Analysis

All statistical analyses were performed by SPSS software (SPSS Inc, Chicago, IL). $P < 0.01$ was considered statistically significant. Z was calculated to observe different significance between groups of percentages.

RESULTS

The presence of autoantibodies to the endometrium and to gametes (anti-sperm and anti-zona pellucida antibodies) was evaluated in serum and peritoneal fluid from women with several grades of endometriosis: grade I ($n = 27$), grade II ($n = 14$) and grade III ($n = 11$). Six healthy fertile women without any gynecological disorder were used as control.

Table I shows the results obtained for the presence of AEA in several groups. All antibodies were IgG. Autoantibodies of IgA isotype were not detected. A good correlation between severity of endometriosis and presence of AEA in sera was observed. A low percentage of patients with endometriosis grade I show antibodies in serum that recognize endometrial antigens (7.4%). On the other hand, a higher percentage of patients with endometriosis grade III presented AEA in their serum (45.5%). This difference was statistically significant. Results obtained from the analysis of peritoneal fluid did not show any correlation between the grade of endometriosis and AEA.

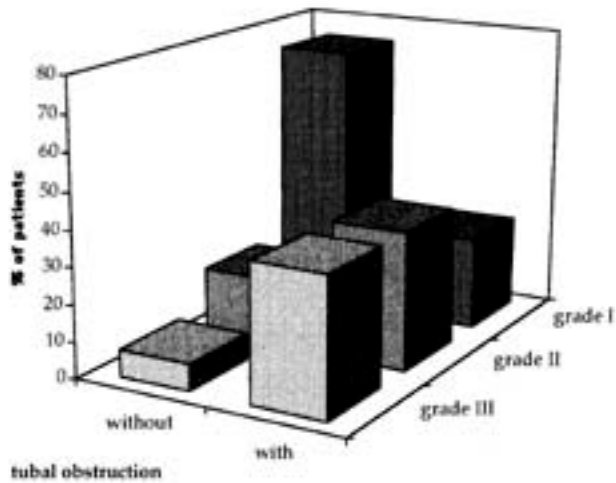

Fig. 1. Percentage of patients with several grades of endometriosis, related to tubal obstruction.

Some women with endometriosis have other pathologies associated, such as ovarian dysfunction or tubal obstruction. The possible influence of ovulatory dysfunction on endometriosis severity was evaluated in 45 women. Table II shows the relationship between ovulatory dysfunction associated with endometriosis, and AEA. It seems that ovulatory dysfunction is neither related to endometriosis development nor to the presence of autoantibodies to endometrium.

We also investigated the existence of tubal obstruction related to the severity of endometriosis, and to AEA in the sera of 43 women. Fig. 1 shows tubal

TABLE II. Humoral IgG Anti-endometrium Response and Ovulatory Dysfunction in Women with Endometriosis ($n = 45$)

			Serum		Peritoneal fluid	
	n	%	AEA+	%	AEA+	%
With ovulatory dysfunction	9/45	20	3/9	33.3[ns]	1/9	11.1[ns]
Without ovulatory dysfunction	36/45	80	8/36	22.2[ns]	4/36	11.1[ns]

[ns] $P > 0.05$.

TABLE III. Humoral IgG Anti-endometrium Response and Tubal Obstruction in Women with Endometriosis ($n = 43$)

			Serum		Peritoneal fluid	
	n	%	AEA+	%	AEA+	%
With tubal obstruction	16/43	37.2	5/16	31.2[ns]	2/16	12.5[ns]
Without tubal obstruction	27/43	62.8	3/27	11.1[ns]	2/27	7.4[ns]

[ns] $P > 0.05$.

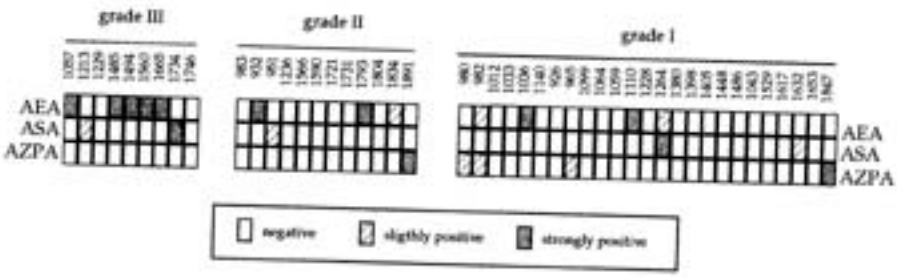

Fig. 2. Severity of endometriosis is classified in three groups. Lines correspond to the antigen analyzed (AEA, or ASA or ZP). Columns (numbers) show the patient code. The fill/empty pattern show the titer or concentration of antibodies in the serum of the patients (positive, or slightly positive, or negative for every antigen tested) ($n = 46$).

obstruction versus severity of endometriosis. No tubal obstruction is found in women with mild endometriosis (74%), but grade III of endometriosis seems to be related to tubal obstruction. A high percentage of women with a moderate endometriosis (grade III) have an associated tubal obstruction associated (37.5%). Table III (all grades) shows the relationship between tubal obstruction and endometriosis in the presence of AEA. The percentage of AEA positive women with endometriosis and tubal obstruction was found to be not statistically significant.

Clinical laparoscopy helped us to determine the sites of ectopic implantation of endometrial cells in 44 cases. Endometriosis was classified according to the ectopic implantation. Implantation of ectopic endometrium in Fallopian tubes is highly correlated with the presence of tubal obstruction (70%) and negativity for AEA (0%). On the other hand, peritoneum, Douglas cavity, and ovary implantation sites are associated with lower percentages of tubal obstruction (20–30%) as compared to the Fallopian tubes ectopic implantation, although in these localizations AEA were detected in serum.

In order to determine if the AEA induced in endometriosis could recognize gamete antigens, the presence of anti-sperm antibodies (ASA) by the TAT-technique and anti-zona pellucida antibodies (AZPA) by ELISA was evaluated. We found that 8.7% of samples were ASA positive and 10.9% were AZPA positive. Fig. 2 shows the results of every sample for all the antibodies screened. In only two cases the sera were not strictly specific for the endometrium: patient number 982 was AEA/AZPA positive and patient number 1264 was AEA/ASA positive. The two patients have endometriosis grade I and the reactivity of their sera is low. Anti-endometrial antibodies seem to be unrelated to anti-gamete antibodies.

DISCUSSION

In the present study, the presence of AEA in the serum of patients with endometriosis is shown. An ELISA using human endometrial adenocarcinoma cell lines was described previously. This technique was also performed in an ELISA using endometrial cells from biopsy and comparable results were obtained.[11]

A significant increase in the percentage of women with AEA, in sera, according to the severity of endometriosis was observed. The response in terms of AEA when endometriosis is more advanced seems to indicate that AEA are a consequence of ectopic endometrial implants growth rather than an efficient humoral response developed in order to eliminate the first cells implanted ectopically in the peritoneal cavity.

Fernández-Shaw[19] described that ectopic endometrial proteins are immunogenic. Endometriosis implants would result in an increase of some antigens that could induce an autoimmune response. Other authors describe that endometriosis could promote an oxidative stress that modify structure and immunogenicity of some endometrial antigens.[20] This could explain the higher titers of AEA that are found in severe endometriosis (grade III) as compared with mild endometriosis (grade I).

The results for peritoneal fluid were variable, probably because peritoneal fluid antibodies are more diluted than in the sera. Alternatively, they could be bound to endometrial tissue, or could form immunocomplexes rather than being in the soluble form.[21] AEA of IgA isotype was not detected by ELISA. Other authors[22] describe the presence of different immunoglobulin isotypes in serum and peritoneal fluid with specificity for different antigens by western blot.

The development of endometriosis is related to a chronic inflammatory response. It is possible that this inappropriate microenvironment of endometrial stressed cells may help humoral response development. These conditions of chronic inflammatory response involve changes in the expression of peritoneal fluid cytokines, such as IFN_γ,[23] TNF_α, IL-1,[24] and an elevated concentration of nitric oxide[25] that could promote tissue damage and induce antibody secretion.

On the other hand, our results seem to indicate that the location of ectopic endometrial implants might be crucial in eliciting AEA associated with endometriosis. Two types of tubal obstruction associated with endometriosis are shown: a mechanical tubal obstruction, with implants located in Fallopian tubes, that cause tubal obstruction in many women, without AEA development, and a non-mechanical tubal obstruction, associated with implants in the peritoneum, ovary or Douglas cavity that are poorly associated with tubal obstruction, but that are accompanied with the presence of AEA. We believe that the location of implants might determine or not the induction of autoimmune response.

Some authors[26,27] have described that anti-gamete antibodies are involved in reproductive failure. Our results indicate that AEA and anti-gamete antibodies (ASA, AZPA) are induced to separated antigens and support the idea that AEA are a consequence of development of endometriosis rather than its cause.[18] AEA, ASA and AZPA indicate that several causes for reproductive failure associated with autoantibodies in women with endometriosis may be found. The establishment of a chronical inflammatory response associated with the development of the endometriosis might promote changes in adherence of endometrial tissue that inhibits embryo implantation,[28] while the production of anti-gamete antibodies might inhibit sperm–oocyte interaction and sperm penetration.[29,30]

In conclusion, detection of AEA, of the IgG isotype, in sera of women with endometriosis may be an indicator of the severity of endometriosis. The presence of AEA is a consequence of the extrauterine implantation and growth of endometrial cells. Samples positive for AEA do not result positive for ASA or AZPA.

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