ORIGINAL ARTICLE: REPRODUCTIVE SCIENCE

Mycoplasma genitalium can modulate the local immune response in patients with endometriosis

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Objective: To detect Mollicutes in women with endometriosis and healthy peritoneal tissues and evaluate the participation of these bacteria in the immune response during endometriosis.
Design: Cross-sectional study.
Setting: University hospitals.
Patient (s): Women with endometriosis (n = 73) and without endometriosis (n = 31).
Intervention(s): Endocervical swabs, peritoneal fluid, and biopsied lesions of endometriosis of women with endometriosis (study group) and healthy peritoneal tissues (control group) were collected during surgery. Clinical characteristics were registered before surgery.
Main Outcome Measure(s): We determined the infectious agents with the use of quantitative polymerase chain reaction (PCR). The cytokine secretion profile was determined with the use of Luminex. The expression of immune response related genes was determined with the use of a PCR array kit.
Result(s): All target microorganisms were detected at least once in the swab samples analyzed. It was possible to observe higher diversity of microorganisms in the samples of swab and peritoneal fluid in the study group compared with the control. *Ureaplasma parvum* was associated with the severity of the symptom dyspareunia. *Mycoplasma genitalium* was associated with higher production of interferon-γ and interleukin-1β. Genes of inflammatory response activation and antigen presentation were up-regulated in biopsied tissue of women with endometriosis. In women with endometriosis, peritoneal fluid cells showed a down-regulation of genes associated with the inflammatory response. This down-regulation profile was higher in presence of *M. genitalium*.
Conclusion(s): *Mycoplasma genitalium* may play a key role in the immune tolerance process and, especially, the aggravation of this profile. More studies are needed to understand this immune tolerance profile of bacterial infections. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Mollicutes, endometriosis, theory of contamination

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Endometriosis is an estrogen-dependent gynecologic disorder characterized by the implantation of endometrial tissue outside of the uterus (1, 2). The symptoms are variable which contributes to delayed diagnosis. Although some patients are asymptomatic, most women present dysmenorrhea, dyspareunia, intestinal and urinary alterations, pelvic pain, chronic fatigue, and infertility (3–6).

The pathogenesis and pathophysiology of endometriosis remain unclear (7). Among the most plausible explanations, the theory proposed by Sampson in 1927 (8) is based on the flowback of endometrial tissue. This happens through the fallopian tubes flowing into the peritoneal cavity with potential implantation and growth of endometrial tissue (7). Retrograde menstruation could explain the majority of events associated with the disease. Even so, only 10% of women with normal tubal patency develop the disease (8). Two other plausible hypotheses suggest that: abnormalities of the eutopic endometrium confer intrinsic resistance to elimination by the immune system; and the disease could be a consequence of the inability of macrophages and natural killer cells to eliminate endometrial implants (1).

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It is still difficult to identify a single mechanism that consistently explains the pathogenesis of endometriosis (9).

The exposure of the lower genital tract to microbes allows them to enter the upper genital tract (10). In this context, some studies investigated the participation of microorganisms in endometriosis (9,11–14). Recently, researchers demonstrated menstrual blood contamination by *Escherichia coli* in women with endometriosis and in control women without endometriosis. Furthermore, one study reported increased levels of bacterial endotoxin (lipopolysaccharide [LPS]) in menstrual fluid and peritoneal fluid of women with endometriosis compared with control women and an LPS/Toll-like receptor (TLR) 4 cascade in the growth of endometriosis (12). The same research group demonstrated that intra-uterine microbial colonization considered to be a subclinical infection was significantly higher in women with endometriosis than in the control. Therefore, the "theory of contamination" or "infectious theory" was proposed to explain these findings. During retrograde menstruation, it was suggested that endometrial tissue and microorganisms are carried to the ovary and peritoneal cavity (9).

Mollicutes are the smallest self-replicating free-living microorganisms (15). Mollicutes are found in genital disorders, but they are also found in healthy individuals. However, five decades ago, some Mollicutes were considered to be infectious agents of the human urogenital tract (16). This inconsistent history has made it challenging to clarify the role of these bacteria (17). *Mycoplasma hominis* is associated with nongonococcal urethritis (NGU), bacterial vaginosis, and post-birth fever (18). In turn, *M. genitalium* was identified as a possible etiologic agent of NGU and nonchlamydial urethritis (19). This species has been detected in cervical samples from patients with salpingitis and acute endometritis (20). Furthermore, *M. genitalium* has been strongly associated with cervicitis (21). In women, an increase of vaginal discharge may be reported as well as dysuria, but the infection may be asymptomatic (22). The Mollicutes mentioned above also present a complex relationship in the host immune response (23). Based on the above considerations, information about the participation of Mollicutes in the pathogenesis of endometriosis is lacking. There is a strong indication that microorganisms could be carried to the upper reproductive tract by means of retrograde menstruation. Therefore, the present study aimed to evaluate the influence of microorganisms with gynecologic importance in the peritoneal cavity and their association with the local immunologic response with endometriosis.

MATERIALS AND METHODS

Study Population

Subjects included in this study were women of reproductive age. Endocervical swabs were collected before laparoscopy, and peritoneal fluid and biopsy tissue samples were collected during laparoscopy, from 104 women with endometriosis (n = 73) and without endometriosis (n = 31). Patients were evaluated from August 2014 to August 2015. Women 15–51 years of age were recruited. Endometriosis was clinically suspected based on clinical symptoms of chronic pelvic pain, severe or incapacitating dysmenorrhea, deep dyspareunia, cyclic urinary abnormalities (pain and/or bleeding) or cyclic bowel abnormalities (pain and/or bleeding), and infertility. All patients were submitted to videolaparoscopy. According to the surgical findings, patients were divided into two groups: with and without endometriosis. Endometriosis was diagnosed when lesions were observed during surgery and subsequently confirmed by histology. The control group consisted of women without any evidence of endometriosis and who had been operated on for other reasons (Supplemental Table 1; Supplemental Tables 1–4 are available online at www.fertstert.org). The exclusion criteria for both groups were presence of any autoimmune or neoplastic disease and antibiotic therapy 1 month before collecting samples. The study complied with the Declaration of Helsinki standards and was approved by the Research Ethics Committees of the University of São Paulo, São Paulo, Brazil, and of the Hospital Sírio-Libanês, where the study was conducted (1170/CEPSH and AVAP/GBC260, respectively).

The patients in the endometriosis group were staged according to the 1996 classification established by the American Society for Reproductive Medicine (ASRM). Stages I and II were grouped as "initial stages" and stages III and IV as "advanced stages." Thirty-seven patients were classified as being in the initial stages of the disease and 35 in advanced stages. The severity of the disease in the affected organ was used to determine the most important site of the disease. Three categories were established: peritoneal disease, ovarian disease, and deep endometriosis. Among the deep lesions, retrocervical lesions (deep lesions >5 mm in depth), bowel lesions, and lesions of the urinary tract (in both cases affecting muscle layers) were considered. Sites affected by the disease were defined according to the following criteria. For the disease to be considered peritoneal, foci had to be exclusively peritoneal without presence of the deep disease (retrocervical, posterior vaginal wall and rectosigmoid regions, ureters, and bladder) or ovarian disease. Ovarian endometriosis was defined as the absence of deep disease regardless of peritoneal foci. And in the definition of deep endometriosis, no restrictions were made regarding the presence of peritoneal or ovarian disease. The phase of the menstrual cycle of the patient at the time of videolaparoscopy was evaluated (4).

Collection of Clinical and Demographic Data

Before surgery, each woman included in the study self-completed validated questionnaires. Demographic data collected included age, ethnicity, level of education, marital status, weight and height, and familial history of endometriosis. Clinical data collected included previous use of hormones before surgery (which type and time since last use), previous use of an intrauterine device, average length of menstrual bleeding and average length of menstrual cycle, age at menarche, stage of endometriosis (ASRM, 1996), menstrual phase of the cycle, and severity of pain during menses, during intercourse, or at other times. Patients indicated on a visual analog scale (VAS) the intensity of five types of pain