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Local and systemic levels of cytokines and danger signals in endometriosis-affected women

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ABSTRACT

Endometriosis is a prevalent gynaecological disorder with a still unclear pathogenesis. So far inflammatory mechanisms are associated with disease progression and critical reviews have discussed the so-called 'danger theory' related to endometriosis. Hence, we performed immunoassays to evaluate whether local inflammation is linked to the severity of the disease. In addition, we investigated the role of recently described cytokines IL-33, IL-32α and the 'alarmin' high mobility group box 1 (HMGB1). We confirmed a dysfunctional immune response in the local environment of women suffering from endometriosis. However, we found no direct evidence for a significant up-regulation of danger signals in endometriosis, irrespective of the severity of the disease.

1. Introduction

Endometriosis is a chronic disorder defined by the presence of endometrial tissue outside the uterus (Giudice and Kao, 2004). Inflammation and fibrosis frequently cause pain and infertility in affected women (Hickey et al., 2014) and patients often face diagnostic delays due to non-specific symptoms. Moreover, only invasive methods for diagnosis are currently available (Giudice and Kao, 2004). Therefore, it is essential to better understand the pathomechanism of the disease, in order to improve management.

In the context of inflammation, toll-like receptors play a crucial role and they are considered to recognize endogenous molecules called danger-associated molecular patterns (DAMPs) or 'alarmins' (Kobayashi et al., 2014). DAMPs reflect tissue damage to the host. They are released upon cell death in the course of strong inflammation, while they have different intracellular physiological roles. HMGB1 and IL-33 are prototypic DAMP molecules with different characteristics in inflammation and tissue repair. HMGB1 is involved in the recruitment of immune cells as well as the migration and proliferation of stem cells. Further, IL-33 triggers the secretion of pro-inflammatory and Th2 cytokines, but also acts in epithelial cell proliferation (Venereau et al., 2015). Sterile inflammation leads to the release of IL-33 or HMGB1, which triggers immune responses similar to the process seen during infection (Klune et al., 2008; Venereau et al., 2015). Although articles have been arguing about sterile inflammation theories in endometriosis, data providing information about expression as well as release of danger signals and underlying mechanism is scarce (Kajihara et al., 2011; Kobayashi et al., 2014).

We checked for IL-33 and HMGB1 in peritoneal lavage fluid and plasma of endometriosis- affected women. Additionally, we measured a panel of cytokines to underline the inflammatory cytokine pattern of the selected patients.

2. Material and methods

2.1. Subjects

Eighty women who underwent laparoscopy at the certified Endometriosis Center of the Department of Obstetrics and Gynecology, Medical University of Vienna (Vienna, Austria), participated in this study between December 2010 and April 2012. All subjects gave written informed consent and the study protocol was approved by the ethics committee of the Medical University of Vienna. Indications for all laparoscopies were dysmenorrhea, fertility work-up, fibroids and

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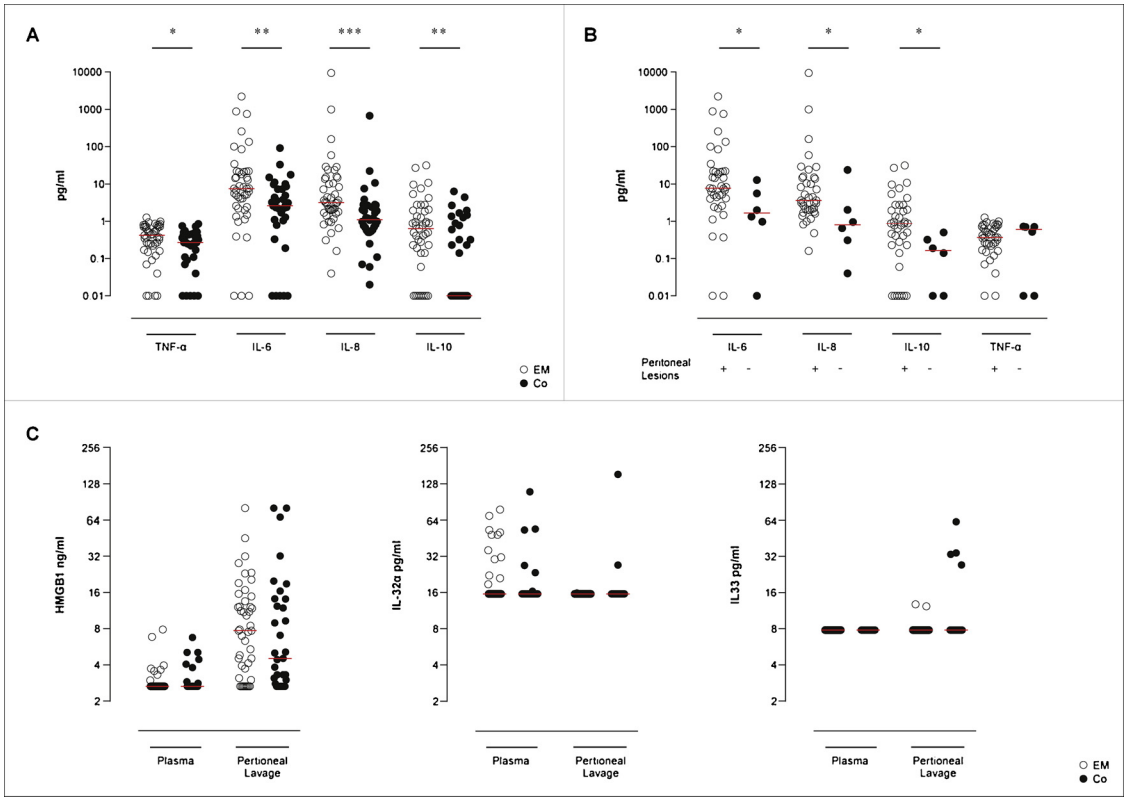

Fig. 1. A. Endometriosis patients display enhanced local inflammation profiles. Cytokine levels in peritoneal lavage fluid were measured by Luminex technology. Peritoneal IL-6 ($p = 0.005$), IL-8 ($p < 0.001$), IL-10 ($p = 0.006$) and TNF-α ($p = 0.045$) levels are significantly elevated in endometriosis patients (n = 45) as compared to controls (n = 35). **B. The presence of peritoneal lesions in endometriosis favors the local elevation of inflammatory cytokines.** In peritoneal fluid IL-6 ($p = 0.034$), IL-8 ($p = 0.022$) and IL-10 ($p = 0.031$) values are significantly higher in endometriosis patients with peritoneal lesions (n = 39) compared to patients without peritoneal lesions (n = 6). +: endometriosis patients with peritoneal lesions; -: endometriosis patients without peritoneal lesions. **C. HMGB1, IL-32α and IL-33 show similar expression between women suffering from endometriosis and control individuals.** The respective cytokines and HMGB1 were measured via ELISA. (Endometriosis group: n = 45 for lavage and n = 44 for plasma samples, control group: n = 35). IL-32α was detected in 18 (22.8%) of all plasma samples, six (17.1%) in the control group and twelve (27.3%) in the endometriosis-affected group. We could detect HMGB1 in the plasma of eight (18.2%) endometriosis patients and eight controls (22.9%). In 63 (78.8%) of all peritoneal samples HMGB1 was detectable, 36 (80.0%) in the peritoneal lavage of endometriosis group and 27 (77.1%) in the control group. We failed to detect IL-33 in any of the plasma samples. However, we could detect it in six (7.5%) peritoneal lavage samples.
Mann Whitney *U* Test, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.
EM: endometriosis-affected women; Co: control individuals.

ovarian cysts. Based on the laparoscopic outcome, patients were assigned either to the endometriosis-affected group or the endometriosis-free control group (Dunselman et al., 2014). Patients were staged according to the revised American Fertility Society (rAFS) and the existence of lesions was specified. Controls have been well defined, showing representative control samples with no signs of endometriosis. A standardized questionnaire was used to obtain all clinically relevant information and a visual analogue scale (VAS) to examine subjective pain intensities.

2.2. Sample collection

Plasma samples were collected from all participants at the time of inclusion before the surgical procedure. Peritoneal lavage fluid was obtained at the beginning of the laparoscopy, using a standardized protocol by collecting the free fluid in the pouch of Douglas, followed by rinsing the genital organs with 10 cc of saline solution. This fluid was collected again and mixed with the first portion. After a subsequent centrifugation step, supernatants were collected and preserved at −80 °C.

2.3. Sandwich enzyme-linked immunosorbent- and Luminex assays

IL-6, IL-8, IL-10, IL-1β, TNF-α and INF-γ levels were quantified via a Bead-Based Multiplex assay (Merck Millipore, Billerica, MA, USA). Levels of IL-32α (Biolegend, San Diego, CA, USA), IL-33 (polyclonal antibody, eBioscience, San Diego, CA, USA) and HMGB1 (IBL International, Hamburg, Germany) were measured with sandwich enzyme-linked immunosorbent assays according to the manufacturers' instructions. The ranges of determination were 7.8–500 pg/ml for IL-33/IL-32α measurements and 2.5–80 ng/ml for HMGB1 levels.

2.4. Statistics

Data was analyzed using GraphPad Prism version 4.03 (GraphPad Software, Inc., La Jolla, CA, USA). Different groups were compared using nonparametric Mann-Whitney *U* test or Fisher's exact test. Correlations were analyzed with non-parametric Spearman's rank correlation test. P-values of ≤0.05 were considered significant.

3. Results & discussion

In this study we enrolled 45 women with histologically proven endometriosis (mean age 34.2 ± 7.8 years) and 35 women without