Inflammosome in the human endometrium: further step in the evaluation of the "maternal side"

Silvia D'Ippolito, M.D.,[a] Chiara Tersigni, M.D.,[a] Riccardo Marana, M.D., Ph.D.,[a,b] Fiorella Di Nicuolo, Ph.D.,[a] Raffaele Gaglione, M.D.,[a] Esther Diana Rossi, M.D., Ph.D., M.I.A.C.,[c] Roberta Castellani, L.T.,[a] Giovanni Scambia, M.D., Ph.D.,[a] and Nicoletta Di Simone, M.D., Ph.D.[a]

[a] Department of Obstetrics and Gynecology, [b] International Scientific Institute Paolo VI, ISI, and [c] Division of Anatomic Pathology and Histology, Università Cattolica Del Sacro Cuore, A. Gemelli Universitary Hospital, Rome, Italy

Objective: To investigate the expression of inflammosome components (NALP-3, associated speck-like protein containing a CARD [ASC]) and their activation (caspase-1, interleukin [IL]-1β, and IL-18 secretion) in the human endometrium from fertile and women with history of recurrent pregnancy loss (RPL).
Design: Experimental study.
Setting: University hospital.
Patient(s): Ten fertile women (control group [CTR]) and 30 women with RPL.
Intervention(s): None.
Main Outcome Measure(s): Endometrial samples were collected by hysteroscopy during the putative window of implantation and evaluated for chronic endometrial inflammation by hystopathological analysis. Inflammosome expression was analysed by immunohystochemical staining (27 RPL and 10 CTR women). The expression of NALP-3 and ASC protein was quantified by Western blot (30 RPL and 10 CTR women). Caspase-1 activation and IL-1β and IL-18 secretion was quantified by ELISA (30 RPL and 10 CTR women).
Result(s): We observed a significantly increased expression of inflammasome NALP-3 and ASC protein, an increased activation of caspase-1, and increased levels of IL-1β and IL-18 in RPL endometrium compared with CTR.
Conclusion(s): Abnormal activation of endometrial innate immunity by means of inflammosome, stimulated by pathogen- or damage-associated molecular patterns, may represent an additional mechanism, currently not investigated, negatively interfering with endometrial receptivity. More studies are required [1] to identify the primary trigger of endometrial inflammosome activation and its clinical impact in the occurrence of RPL; and [2] to validate the inflammosome components as a novel family of endometrial biomarkers and promising therapeutic targets in RPL. (Fertil Steril® 2016;105:111–8. ©2016 by American Society for Reproductive Medicine.)
Key Words: Endometrium, inflammosome, NALP-3, interleukins, miscarriage

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Spontaneous pregnancy loss is the most common complication of pregnancy, occurring in 15% of clinically recognized pregnancies (1). Of all women 1%–5% suffer from recurrent pregnancy loss (RPL) (2). Although RPL has been associated to various hematologic, anatomic, hormonal, immune, and genetic defects, in 30%–40% of the cases, screening tests included in the RPL workup may have negative results (3, 4). Thus, the identification of factors involved in RPL and a clearer understanding of its causes are urgently needed.

The establishment and maintenance of pregnancy occurs through the interaction of maternal endometrial and trophoblast tissues, therefore the pathogenic mechanisms underlying pregnancy loss must directly or indirectly affect this interaction (5). There is evidence that the dynamic endometrial balance between proinflammatory and anti-inflammatory mediators required for normal pregnancy is altered in recurrent miscarriage (6–10). Increased levels of proinflammatory molecules like tumor necrosis factor

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Reprint requests: Nicoletta Di Simone, M.D., Ph.D., Department of Obstetrics and Gynecology, Università Cattolica Del Sacro Cuore, A. Gemelli Universitary Hospital, Largo Agostino Gemelli 8, 00168 Rome, Italy (E-mail: nicolettadisimone@rm.unicatt.it).

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(TNF)-α, interferon (IFN)-γ, interleukin (IL)-6, IL-10, and inflammatory leukocytes are reported in women with RPL compared with women with normal pregnancy (11, 12). An abnormal cytokine endometrial environment might be a nonspecific consequence of endometrial innate immunity activation by exogenous or endogenous stimuli, thus leading to reduced endometrial receptivity and RPL.

Several causes, including microorganisms, trauma, ischemia, metabolic disorders, can trigger tissue inflammation. Despite the multiplicity of agents, the final common event is the cell damage (13) with the consequent release of damage-associated molecular pattern molecules in circulation. These molecules, which are absent in the extracellular environment under healthy conditions, are released when a foreign microorganism enters and multiplies within our body (infection) or as consequence of tissue injury (sterile inflammation). In the initial phases of inflammation, the coordinated action of damage-associated molecular patterns and/or of pathogen-associated molecular patterns are, in turn, able to induce activation of inflammosomes. Inflammasomes are intracellular, multiprotein assemblies, belonging to innate immunity system, involved in the amplification of inflammatory processes to eliminate possible noxious agents and/or minimize their detrimental effects (14). At present four main types of inflammosomes have been characterized: the NALP-3 family represents the most studied component. Upon activation of NALP-3, apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1 are assembled to the inflammosome. Once recruited, this multiprotein complex enables the caspase-1-mediated proteolytic processing of the proinflammatory cytokine IL-1β, IL-18, and IL-33, generating their respective mature secretory forms. These events are necessary for the induction of further systemic responses and spreading of inflammation (15).

At present there is no information available on the expression and the role of inflammosomes in the human endometrium, as well as on their relevance in patients with RPL. Our study aimed at investigating the expression of this structure in the endometrial tissues obtained from women with history of RPL in the absence of any positive result at the RPL workup. We demonstrated the expression of NALP-3 and ASC protein in the human endometrium and we observed significantly increased levels of NALP-3 and ASC in the human endometrium from women with RPL compared with the control group. In addition, an increased caspase-1 activation and secretion of IL-1β and IL-18 has been documented in endometrial tissue from women with RPL. These data would suggest that NALP-3 inflammosome and ASC might represent a novel family of marker proteins involved in the establishment of a proinflammatory endometrial environment and/or therapeutic targets in RPL. Their use would be of great promise to [1] better understand the process of normal and abnormal implantation and [2] provide clues to the causes and therapy of a subgroup of early pregnancy losses and/or unexplained infertility.

MATERIALS AND METHODS

Patients

The study population included 10 women with previous uncomplicated term pregnancies (control group) and 30 women with RPL in the absence of any positive result at the screening tests used for RPL workup (anatomic, hormonal/metabolic, hematologic, genetic, autoimmune, thrombophilic tests). The inclusion criteria for both groups were as follows: white, age $\leq$39 years, healthy, regular ovulatory cycles (28–32 days), normal endocrine profile, normal serum levels of FSH ($<$10 MIU/mL), LH ($<$10 mIU/mL), and antimüllerian hormone (AMH $>$2 ng/mL) on day 3 of the menstrual cycle, absence of abnormal ovarian and endometrial ultrasonographic features, no use of any contraceptive drugs or intrauterine device (IUD) in the past 6 months, or vaginal infections. Women with RPL have had three or more sequential early ($\leq$10 weeks of gestation) spontaneous pregnancy losses clinically documented by ultrasonography and/or histopathology examination, after excluding known causes of RPL (uterine abnormalities, hormonal and metabolic disorders, severe male factor infertility, known clinical autoimmune diseases, thrombophilic conditions). All women did not received any medications (including anti-inflammatory, antibiotics, insulin-sensitizing drugs) in the past 3 months before inclusion in the study.

All women gave their informed consent to use, anonymously, their data for research purposes, and the protocol was approved by the ethics committee of A. Gemelli Universitary Hospital, Università Cattolica del Sacro Cuore in Rome, Italy. All the women were advised to avoid pregnancy in the month in which hysteroscopy was carried out.

Endometrial Samples

Women underwent diagnostic mini-hysteroscopy during the putative window of implantation (days 19–24). The timing of the biopsy was dated according to the last menstrual period, monitoring the follicle size using a transvaginal ultrasound and was confirmed by histologic assessment (16). Serum P and β-hCG levels were determined in the day of biopsy. Endometrial biopsies were performed using a 3-mm Novak curette (Cooper Surgical Inc.) for cultural, histologic, and functional examination. Extreme care was taken during endometrial sampling to avoid any contact between the curette and the vaginal walls. Endometrial samples were investigated for the following infectious agents: *Chlamydia trachomatis*, *Mycoplasma* spp., *Ureaplasma urealyticum*, *Neisseria gonorrhea*, and yeast. A specific antibiotic therapy was prescribed in case of infection and patients were excluded from the study (about 18%). In consideration of the literature reports that hysteroscopic evaluation of the endometrial inflammatory disease could have a higher sensitivity than the endometrial cultures (17), patients with evidence of chronic endometritis (CE) at hysteroscopy (about 20%) were not included in the study.

The collected samples were washed immediately in normal saline and divided into two parts: one was placed in 10% buffered formalin for paraffin embedding (18) and the other was stored at -80°C. Endometrial histologic dating was performed according to standard criteria (16) by a single investigator who was blinded to clinical outcomes. Histologic diagnosis of CE was based on criteria previously described (19, 20). We analyzed in detail the following features: