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Dysregulation of the Fas/FasL system in mononuclear cells recovered from peritoneal fluid of women with endometriosis

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ABSTRACT

In endometriosis, regurgitating endometrial cells fail to undergo apoptosis and implant themselves outside the uterus, particularly in the peritoneum. We studied Fas and FasL behaviour by evaluating the percentages of mFas and mFasL-bearing mononuclear cells from peritoneal fluid, the level of Fas and FasL gene expression at both mRNA and protein levels in the same cells, and the sFas and sFasL values in peritoneal fluid of 80 endometriotic women, at four stages of disease severity. We found no variation in percentage of mFas-bearing mononuclear cells; high and unchanging levels of Fas mRNA and protein, and high and invariable sFas values. Overproduction of sFas antagonises mFas function and plays a role as a decoy in the peritoneal fluid. The mFasL-bearing mononuclear cells and protein levels decreased from the minimal to the severe stage of disease. In contrast to FasL protein, FasL mRNA was overexpressed throughout the course of the disease. sFasL values were high and increased as the disease worsened. Our results showed a non-linear ratio between FasL mRNA and FasL protein levels. Abnormally elevated FasL mRNA may be due to dysregulation in several mechanisms controlling mRNA turnover. The high level of sFasL would be expected to down-regulate FasL activity and compete with the membrane form for mFas binding. As a consequence, mFas-bearing mononuclear cells may be unable to kill and in turn, may themselves become targets for killing by FasL-expressing endometriotic cells.

1. Introduction

Endometriosis is a disorder characterised by the ectopic presence and growth of functional endometrial tissue, glands and stroma, outside the uterus (Baldi et al., 2008; Bulun, 2009). Endometriotic foci can be found anywhere in the pelvis, including the peritoneal surface of endopelvic structures and the ovaries. Endometriosis is classified depending on the number, size and superficial and/or deep location of endometrial implants, plaques, endometriomas and/or adhesions, as follows: stage I (minimal, 1–5 points), stage II (mild, 6–15 points), stage III (moderate, 16–40 points) and stage IV (severe >40 points) following the revised American Society for Reproductive Medicine classification for Endometriosis (American Society for Reproductive Medicine, 1996).

Of the several theories that have been proposed to explain the aetiology of endometriosis, the implantation theory (Sampson, 1927) has been widely accepted.

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During retrograde menstruation, eutopic endometrial cells reflux throughout the tubes to the peritoneal cavity, adhere to the peritoneal wall, proliferate and form endometriotic lesions, thereby triggering and advancing the disease (Baldi et al., 2008; Bulun, 2009; Khan et al., 2008). These changes in the peritoneal microenvironment involve peritoneal macrophages and attract peripheral mononuclear cells, recruited from the blood into the peritoneal cavity (Arici et al., 1997; Braun et al., 2002; Cao et al., 2004; Pizzo et al., 2002; Tariverdian et al., 2009). These peritoneal fluid mononuclear cells (PFMCs), as well as endometriotic cells, secrete different patterns of cytokines (Kalu et al., 2007; Pizzo et al., 2002), which may polarise towards either a Th1 or Th2 profile and regulate cellular processes, including apoptosis (Giudice and Kao, 2004).

During endometriosis a breakdown occurs in endometrial and peritoneal homeostasis caused by cytokine-induced cell proliferation and dysregulation of apoptosis (Agic et al., 2009; Harada et al., 2007; Pizzo et al., 2002; Ulukus and Arici, 2005). Execution of the programmed cell death is a process that can be triggered by many factors such as radiation, chemotherapeutic drugs and apoptotic signalling and it occurs via two main pathways. Both pathways stimulate an intracellular cascade of events that leads to cell death. The intrinsic pathway is initiated from mitochondria, whereas the extrinsic pathway is activated by death receptors (DRs) that engage their respective ligands on the surface membrane of target cells. Fas (DR2/CD95/Apo-1) is a type I cell membrane protein (mFas) with an extracellular domain that binds FasL and a cytoplasmic domain that transduces the death signal (Peter et al., 2007; Strasser et al., 2009). FasL (CD95L/CD178/Apo-1L) is a type II cell membrane protein (mFasL) which is inducibly expressed in lymphocytes and constitutively expressed in cells present in immune-privileged organs (Lettau et al., 2009; Suda et al., 1993). In cytotoxic T and NK cells, FasL is also stored in so-called secretory lysosomes (Lettau et al., 2008). Soluble forms may be produced either by proteolytic cleavage of membrane-bound molecules (sFas and sFasL) or by alternative splicing (sFas) (Cascino et al., 1996; Voss et al., 2008). These soluble forms act as antagonists to the membrane-bound FasL (Lettau et al., 2009; O'Reilly et al., 2009; Weinlich et al., 2010) in several cellular systems, including in immune and endometriotic cells.

In a previous study, we showed a prevalence of Th1 profile cytokines in the peritoneal fluid (PF) of women with endometriosis at minimal and mild stages whereas a Th2 profile cytokines prevailed in severe stages (Pizzo et al., 2002). Since Roberts et al. (2003) demonstrated that the polarisation of T cells towards Th1 is driven by a Fas/FasL interaction, the aim of this work was to verify whether the Fas/FasL system undergoes modifications related to different stages of endometriosis. Therefore, we evaluated the percentages of mFas and mFasL-bearing PFMCs, the levels of the Fas and FasL gene expression at both mRNA production and protein synthesis in the same cells and also the values of sFas and sFasL in the PF of women with endometriosis at minimal, mild, moderate and severe stages.

2. Materials and methods

2.1. Study population

Ninety-six women of reproductive age attending the infertility outpatients clinic of the University of Messina were eligible for this study. Patients (aged 20–45 years) were diagnosed with endometriosis ($n = 80$) following laparoscopy. Diagnosis was later confirmed by histopathological examination. In accordance with the revised American Society for Reproductive Medicine classification for Endometriosis (American Society for Reproductive Medicine, 1996) the patients were divided into four groups: minimal ($n = 26$), mild ($n = 28$), moderate ($n = 14$) and severe ($n = 12$) stages of endometriosis. The controls ($n = 16$) consisted of women (aged 21–43 years) affected by non-immunological infertility, who were undergoing explorative laparoscopy. All diagnostic laparoscopic procedures were performed during the proliferative phase of the menstrual cycle. All women involved in this study had no other pelvic disorders, chronic circulatory, autoimmune or neoplastic disease and had not been taking any anti-inflammatory or hormonal or immunomodulatory medications in the preceding six months. Investigation protocols were approved by the local Ethics Committee and prior to participating in this study all the subjects involved signed an informed consent form allowing us to use their biological fluids and cells. None of the Authors has any proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company.

2.2. Sample collection

2.2.1. PF sample preparation

Peritoneal fluid samples were collected under sterile conditions at the time of the laparoscopy prior to any operative manipulation in order to minimise blood contamination. PF samples were centrifuged at $200 \times g$ for 5 min, the supernatants removed, aliquoted and stored at $-80\,^{\circ}C$ until use.

2.2.2. Cell preparation and identification

The cell pellet was suspended in PBS, layered onto Histopaque-1077 (Sigma, St. Louis, MO, USA) and centrifuged at $150 \times g$ for 30 min, as previously described by Yoshino et al. (2003). The cells were collected at the interface, washed twice in PBS, suspended, counted and identified as PFMCs by light microscopy observation (purity >97%) and indirect immunofluorescence as described below.

Anti-CD3, anti-B19, anti-CD56 and anti-CD64 mAbs (Biosource, Camarillo, CA, USA) characterised the cells as T lymphocytes (controls: 10–20%; patients: 35–40%), B lymphocytes (controls: 0.8–1%; patients: 0.5–1%), NK lymphocytes (controls: 2–3%; patients: 5–7%) and macrophages (controls: 75–80%; patients: 45–60%) respectively, in accordance with Oosterlynck et al. (1992). An aliquot of these cells was immediately used to evaluate mFas and mFasL expression by indirect immunofluorescence. The remaining cells were stored at $-80\,^{\circ}C$ until assay