Apoptosis in human endometrium and endometriosis

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Apoptosis plays a critical role in maintaining tissue homeostasis and represents a normal function to eliminate excess or dysfunctional cells. Accumulated evidence suggests that apoptosis helps to maintain cellular homeostasis during the menstrual cycle by eliminating senescent cells from the functional layer of the uterine endometrium during the late secretory and menstrual phase of the cycle. The BCL-2 family and Fas/FasL system have been extensively studied in human endometrium and endometriotic tissues. Eutopic endometrium from women with endometriosis reportedly has some fundamental differences compared with normal endometrium of women without endometriosis. The differences could contribute to the survival of regurgitating endometrial cells into the peritoneal cavity and the development of endometriosis. One mechanism that recently gained a lot of interest is the finding that apoptosis appeared in eutopic and ectopic endometrium of patients with endometriosis. This study is a current review of the literature focused on the physiological role of apoptosis in normal endometrium and the alterations in regulation of apoptosis in eutopic and ectopic endometrium from women with endometriosis. Similarities in characteristics of endometriosis at a molecular level with gynaecological tumours are also discussed. Finally, the role of apoptosis in the treatment of endometriosis is reviewed to link the basic research findings into clinical applications.

Key words: apoptosis/bcl-2/endometriosis/Fas/FasL system

Introduction

Historically, menstruation has been associated with ischaemic necrosis of the functional layer of the endometrium caused by the contraction of the spiral arteries, with the process being dependent on sex hormone concentration (Markee, 1940; Bartelmez, 1957). Endometriosis, defined by endometrium-like glandular tissue and stroma outside the uterus, is a common disease affecting 5–15% of women in the general population and 40% of women seeking infertility evaluation (Eskenazi and Warner, 1997). Although the high incidence of endometriosis and the fact that three-quarters of a century has passed since the initial description of the disease by Sampson (1927), our current understanding of the aetiology and pathophysiology of endometriosis remains obscure. Several theories have been proposed, including development by metaplasia, development from Müllerian remnants, and after implantation and growth of endometrium following retrograde menstrual reflux.

Nearly all women of reproductive age exhibit some degree of reflux of endometrial debris (Halme et al., 1984a). Menstrual effluents retrogradely shed into the peritoneal cavity were observed to contain viable endometrial cells (Keettel and Stein, 1951; Nisolle et al., 1990; Kruitwagen et al., 1991; Arumugam and Lim, 1997; Vercellini et al., 1997). These mechanisms are necessary but insufficient to explain why only some patients develop the disease. A couple of views have been presented (Vinatier et al., 2001). The first theory is based on disorders of the endometrium in which it resists normal peritoneal means of cleaning. The second theory suggests that the disease is secondary to abnormalities of the cellular and humoral immunity that induce excessive receptivity of the peritoneal mesothelium, hyperactivated macrophages, and abnormalities of NK cells. It seems that the peritoneal environment may alter a genetically predisposed endometrium, which then becomes favourable for invasion. It is also possible that an excess of refluxing endometrium or altered endometrium has the potential to form a proinflammatory or hormonal environment favourable to establish the disease (Vinatier et al., 2001).

The fact that the eutopic endometrium of women with endometriosis shares changes with ectopic tissue and that these changes are not found in the eutopic endometrium of disease-free women has advanced the view that the primary defect in endometriosis is to be found in the eutopic endometrium (Sharpe-Timms, 2001). Cells and tissue elements, derived from such an altered eutopic endometrium and shed into the peritoneal cavity, have been proposed to have a higher potential for implantation and growth on peritoneal surfaces and development.
Table I. Morphological and biochemical features of apoptosis

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<th>Morphological features</th>
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<tr>
<td>Membrane blebbing</td>
<td>Energy (ATP)-dependent process</td>
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<td>Aggregation of chromatin at the nuclear membrane</td>
<td>Non-random mono- and oligonucleosomal length fragmentation of DNA</td>
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<td>Shrinking of cytoplasm and condensation of nucleus</td>
<td>Release of cytochrome c, apoptosis-inducing factor (AIF) and other factors</td>
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<td>Fragmentation of cell into smaller bodies</td>
<td>Into cytoplasm by mitochondria</td>
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<td>Formation of apoptotic bodies</td>
<td>Activation of caspase cascade</td>
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<td>Pore formation in mitochondrial membrane, involving proteins of the bcl-2 family</td>
<td>Alterations in membrane biochemistry (i.e. translocation of phosphatidylserine from the cytoplasmic to the extracellular side of the membrane)</td>
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into endometriosis. (Noble et al., 1997; Jolicoeur et al., 1998; Leyendecker et al., 1998). On the other hand, many differences observed between eutopic endometrium and ectopic tissue of a patient with endometriosis can be explained as the direct consequence of the different environment of peritoneal fluid (Koninckx et al., 1998; Kupker et al., 1998; Harada et al., 1999; Harada et al., 2001). One of the endometrial alterations appearing in eutopic and ectopic endometrium from women with endometriosis refers to the regulation of apoptosis. Electron microscopic studies have revealed the presence of apoptotic bodies in human endometrial epithelial cells during the late secretory phase (Hopwood and Levison, 1976; Otsuki et al., 1994).

Molecular basis of apoptosis pathways

Apoptosis is a distinctive form of programmed cell death that is defined by characteristic morphological and biochemical events that result in the efficient elimination of cells from tissue without eliciting an inflammatory response (Kerr et al., 1972). Apoptosis is critical for tissue modelling during embryogenesis and cellular homeostasis, and it is a defense mechanism against pathogens.

The programmed cell death cascade can be divided into at least three phases: signal activation–induction of apoptosis, regulation and execution, and cellular structural alterations (Table I).

Signal activation

Apoptosis can be induced by a broad range of stimuli such as deprivation of growth factors, glucocorticoids, DNA damage, exposure to ionizing radiation, chemotherapeutic drugs, and/or stress.

Regulation and execution

At least three different models have been described for the function of apoptotic molecules (reviewed by O'Reilly and Strasser, 1999). According to the first model, cell death signals lead to the activation of both caspases (cysteine proteases) and pre-apoptotic members of the bcl-2 family. Protein–protein inter-

actions can cleave and inactivate certain vital cellular proteins leading to apoptosis. The second model is based on the ability of a pre-apoptotic member of the bcl-2 family to form ion channels in cytoplasmic membranes of mitochondria, nuclear envelope and endoplasmic reticulum (Schendel et al., 1997). Disruption in the mitochondrial membrane results in the release from mitochondria apoptosis-inducing factors that activate caspases and subsequently kill cells by apoptosis (Chinnaiyan et al., 1997). The third model is based on the function of members of the tumour necrosis factor (TNF) receptor family and their corresponding ligands. A few members of the receptor’s family (e.g. Fas) contain a cytoplasmic region called the ‘death domain’ (Tartaglia et al., 1993). Upon receptor activation, the death domain undergoes homotypic interaction with a death domain in the adaptor protein FasL, resulting in the initiation of apoptosis (O’Reilly and Strasser, 1999).

Structural alterations

Apoptotic cells are characterized by many morphological and biochemical alterations (Table I). Morphologically, apoptotic cells present with condensed chromatin, multiple membrane-bound organelles (apoptotic bodies) and a shrunken appearance. Biochemically, apoptosis is characterized by monomeric or multimeric 180 base pair (bp) nucleosomal fragments resulting from the cleavage of double-stranded nuclear DNA via activation of a calcium–magnesium-dependent endonuclease (Kerr et al., 1972). However, the absence of low mol. wt DNA ladders does not confirm that apoptosis is not occurring. Cleavage of DNA into higher mol. wt structures (rosettes, 300 kb) and loops (50 kb) may be detected with histochemical techniques or by pulsed-field gel electrophoresis and may indicate apoptosis (Garcia-Velasco and Arici, 2003). Another biochemical characteristic of apoptosis is the translocation of phosphatidylserine to the outer surface of the plasma membrane. This translocation constitutes one of the principal targets of phagocyte recognition (Savill, 1998).

Apoptosis in the normal endometrium (Table II)

The endometrial cycle in regularly menstruating women consists of three distinct phases (proliferative, secretory and menstrual). Hopwood and Levison (1976) reported the presence of apoptosis in human endometrium following advancement of the concept of apoptosis by Kerr et al. (1972). Accumulated evidence suggests that apoptosis helps to maintain cellular homeostasis during the menstrual cycle by eliminating senescent cells from the functional layer of the uterine endometrium during the late secretory and menstrual phase of the cycle (Hopwood and Levison, 1976; Kokawa et al., 1996; Shikone et al., 1996). This is followed by the proliferative phase of the cycle.

Apoptosis was detected in the glandular epithelium of late secretory and menstruating endometrium, while very little apoptosis was detected during the proliferative phase or at the beginning of the secretory phase (Kokawa et al., 1996; Tao et al., 1997; Vaskivuo et al., 2000). The proliferation of endometrial cells in the proliferative phase has been related generally to the action of estrogens, while progesterone is thought to direct the cells into the differentiation pathway, resulting in growth arrest. Considering the cyclical nature of apoptosis in normal endometrium, it seems likely that estrogen and progesterone can regulate
irreversibly committed to apoptosis (Diebold, chromosome 18, is important in determining whether a cell will be.
The Bcl-2 (B cell lymphoma/leukaemia-2) gene, located on.
Bcl-2 in normal endometrium

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ND = not determined.
The expression levels summarize the data derived from immunohistochemical staining in published literature. BCL-2: Watanabe et al. (1997), Jones et al. (1998), Konno et al. (2000), Otsuki (2001); Bax: Meresman et al. (2000), Vaskivuo et al. (2001); BCL-X: Tao et al. (1997); BAK: Tao et al. (1998); Fas/FasL: Watanabe et al. (1997), Yamashita (1999), Selam et al. (2001, 2002).

the signals that result in apoptosis in this tissue. Vaskivuo et al. (2000) showed that the pattern of apoptosis negatively correlated to serum estradiol concentrations in the proliferative phase.

Bel-2 in normal endometrium

The Bcl-2 (B cell lymphoma/leukaemia-2) gene, located on chromosome 18, is important in determining whether a cell will be irreversibly committed to apoptosis (Diebold et al., 1996). The BCL-2 protein is probably the best characterized of apoptosis-related molecules, and data now unequivocally support a role for the BCL-2 protein as a cell death repressor (Reed, 1997).

To date, the precise mechanism of apoptosis in the human endometrium is not yet fully clarified, although BCL-2 has been considered to inhibit apoptosis in the human endometrium during the proliferative phase (Otsuki et al., 1994). BCL-2 cyclically expressed in endometrial glandular and stromal cells peaks during the late proliferative phase and decreases during the late secretory and menstruating phases (Watanabe et al., 1997; Konno et al., 2000; Otsuki, 2001). In contrast, myometrial smooth muscle cells showed consistent Bcl-2 immunoreactivity throughout the menstrual cycle (Otsuki, 2001). In Bcl-2-deficient mice, many apoptotic cells and apoptotic bodies were often observed in the glands and the myometrium (Daikoku et al., 1998). Therefore, BCL-2 may be an essential gene product for the survival of both endometrial glandular cells and myometrial smooth muscle cells.

Rogers et al. (2000) demonstrated that the cyclical pattern of the BCL-2 expression had no longer occurred after administration of levonogestrel, suggesting that a constant administration of steroid hormones can affect the expression of BCL-2. Using immunohistochemical staining, the authors observed that BCL-2, Fas and caspase-3 showed different expression levels in the functional layer versus basal layer of normal endometrium (Rogers et al., 2000). The anti-apoptotic protein BCL-2 presented with higher expression in the basal layer, whereas death receptor Fas and caspase-3 were higher in the functional layer of the endometrium. These results fit well with the functional biology of endometrium. Since the basal layer remains relatively constant throughout the menstrual cycle, apoptosis is less common in this layer. In contrast, the functional layer that undergoes cyclical growth, differentiation and shedding, appears with an increased level of apoptosis.

Some transcriptional molecules can regulate the expression of Bcl-2 in human endometrium. p53 immunoreactivity was not observed in either glandular cells or stromal cells throughout the cycle (Otsuki, 2001). In contrast, the staining pattern of c-jun and Sp-3 in glandular cells was similar to that of Bcl-2, in terms of pattern, intensity and cellular distribution throughout the menstrual cycle, suggesting that c-jun and Sp-3 may have a role in regulating BCL-2 expression (Otsuki, 2001).

Although expression of the protein product of bcl-2 gene in the human endometrium has been described (Gompel et al., 1994; Otsuki et al., 1994; Koh et al., 1995), bcl-2 is only one member of this multi-gene family, consisting of numerous proteins homologous to BCL-2 (Boise et al., 1993; Oltvai et al., 1993; Hanada et al., 1995). Other members of the bcl-2 gene family likely play important roles in controlling apoptosis by mechanisms that are independent of or complementary to the action of BCL-2. Members of the BCL-2 family interact through homodimeric and heterodimeric associations (Oltvai et al., 1993; Hanada et al., 1995), such that the susceptibility of any given cells to a potential apoptotic stimulus may be determined by the ratio of pro-apoptotic and anti-apoptotic BCL-2 family members present in the cell at that time (Oltvai et al., 1993).

BAX is a BCL-2 family member that promotes cell death susceptibility, possibly by countering the effect of BCL-2 on cellular survival through heterodimer interaction (Oltvai et al., 1993). Another member of this family of genes, bcl-x, provides an interesting example of a single gene that, via alternative splicing mechanisms, encodes both a positive and a negative regulator of apoptosis (Boise et al., 1993). The long form of BCL-X (BCL-X\textsubscript{long}) contains an open reading frame of 233 amino acids with two domains homologous to BCL-2, whereas BCL-X\textsubscript{short} is a 170 amino acid truncated form of BCL-X\textsubscript{long} in which the region of highest homology to BCL-2 has been deleted (Boise et al., 1993). These two forms of BCL-X have opposing functions in that BCL-X\textsubscript{long} renders cells resistant to apoptotic cell death upon deprivation of growth factors, whereas BCL-X\textsubscript{short} counters the resistance to apoptotic cell death provided by BCL-2 (Boise et al., 1993; Oltvai et al., 1993).

Tao et al. (1997) reported that levels of BAX protein were modest in proliferative endometrium and increased dramatically in the secretory phase when apoptosis was most prevalent. Immunoreactive BCL-X protein was observed mostly in glandular epithelial cells of the human endometrium. Compared with proliferative endometrium, secretory endometrium showed stronger BCL-X staining, especially in the functional layer. These data collectively suggest that BCL-2 and BCL-X\textsubscript{long} are important anti-
apoptotic factors in the human endometrium, whereas BAX induced in secretory phase endometrial cell turnover may be associated with menses.

BAX (BCL-2 homologous antagonist/killer) is another pro-apoptotic member of the BCL-2 family, and is believed to accelerate apoptosis in mammalian cells, at least in part, by interacting with BCL-2 and BCL-X long (Chittenden et al., 1995). Immunohistochemical analysis revealed that staining for BAX protein was localized almost exclusively to the glandular epithelial cells, especially in the functional layer of the secretory endometrium. Immunoreactive BAX was absent from most of the cells of the proliferative endometrium (Tao et al., 1998). These data imply the existence of a dynamic interplay among many members of the BCL-2 family in triggering apoptosis in this system.

Several scientists have suggested that ovarian steroids may control endometrial apoptosis by up- and down-regulation of Bcl-2 and BAX expression (Rotello et al., 1992; Koh et al., 1995; Tabibzadeh, 1995). The cyclic pattern of BCL-2 expression in endometrial glandular cells was related to changes in estrogen receptor and progesterone receptor patterns throughout the cycle (Tabibzadeh, 1995). The cyclic pattern of BCL-2 expression in endometrial glandular cells was related to changes in estrogen receptor and progesterone receptor patterns throughout the cycle (Otsuki et al., 1994). In this context, Critchley et al. (1999) reported an increase in BCL-2 protein expression in glandular and surface epithelium of antiprogestin-treated endometrium. These data suggest that this gene’s expression may be stimulated by estrogen and down-regulated by progesterone.

**Fas/Fasl system in normal endometrium**

Fas (CD95) is a 45 kDa type I membrane protein that belongs to the TNF/nerve growth factor receptor family (Nagata and Golstein, 1995). The Fas ligand (FasL) is a 37 kDa protein that belongs to the TNF superfamily. The Fas–FasL interaction is essential in inducing apoptosis (Suda et al., 1995). Fas-bearing cells undergo apoptotic cell death when they interact with FasL (Nagata and Golstein, 1995). The Fas/FasL system plays a crucial role in normal tissue homeostasis and pathological conditions, including the escape of tumour cells from immune surveillance (Lincz, 1998). FasL expression by cells from such tissues as the eye, testis and placenta may be involved in maintaining the immune privilege of these tissues by inducing apoptosis of Fas-positive immune effector cells (Bellgrau et al., 1995; Griffith et al., 1995; Mor et al., 1998).

Fas and FasL are expressed in human endometrium throughout the menstrual cycle (Garcia-Velasco et al., 1999; Yamashita et al., 1999; Song et al., 2002). During the late proliferative phase, these proteins are primarily retained within the cell’s Golgi apparatus and cytoplasmic vesicles and are unable to interact and induce apoptosis (Otsuki, 2001; Song et al., 2002). In contrast, during the secretory phase, these proteins are extruded as part of the cellular membranes, where Fas can bind FasL and turn on apoptotic signals (Otsuki, 2001; Song et al., 2002). Fas immunostaining on human endometrial glandular cells was determined to be stronger during the secretory phase than during the proliferative phase (Watanabe et al., 1997; Yamashita et al., 1999). Peng et al. (1998) demonstrated that FasL exhibits peak expression during the secretory phase. FasL reportedly has a cycle-dependent expression in both glandular and stromal cells. Relatively higher expression of FasL during the secretory phase compared with the proliferative phase goes along with the increased rate of apoptotic cell death.

Song et al. (2002) demonstrated that withdrawal of estrogen and/or progesterone from endometrial cells in culture induced apoptosis, causing a significant decrease in cell viability. This coincided with increased Fas and Fasl expression. These data indicate that Fas-mediated apoptosis is important for endometrial cycling. Selam et al. (2001) also studied the regulation of FasL expression by estrogen and progesterone in cultured endometrial stromal and glandular cells. They showed that estradiol and progesterone up-regulated FasL protein expression in cultured endometrial glandular and stromal cells. However, in an *in vitro* culture system, it is difficult to mimic the production of endogenous estrogen and progesterone levels and to assess their effect on Fasl expression. Therefore, the exact mechanism by which endogenous steroid hormones regulate Fasl expression cannot be adequately elucidated.

There are two forms of FasL: membrane-bound and soluble. The membrane-bound FasL is converted to the active soluble FasL by the action of some matrix metalloproteinases (MMP) (Tanaka et al., 1996). In the proliferative phase, endometrial glandular cells scarcely undergo apoptosis. This may be due to the localization of FasL in the glandular cells in a membrane-bound inactivated form (Otsuki, 2001) and/or its localization into the cell’s Golgi apparatus. There are increased activities of MMP, such as MMP-1, -3 and -9, before and during the menstruating phase (Salamonsen and Woolley, 1996). The membrane-bound FasL localized on the apical membrane of glandular cells is cleaved by the action of MMP during the secretory phase, resulting in an increase in the amount of soluble Fasl in the glandular lumen and therefore can bind with Fas on the cell surface of glandular cells, inducing their death (Otsuki, 2001).

The possible inhibition of Fas-induced apoptosis by Bcl-2 during the proliferative phase suggests that Bcl-2 can control the Fas/FasL apoptotic pathway. Bcl-2 inhibits Fas-mediated apoptosis via inactivation of an interleukin-converting enzyme (ICE)-like protease that is located downstream of Fas/Fasl in the apoptotic pathway (Enari et al., 1995; Shimizu et al., 1996).

**Apoptosis in endometriosis**

**Spontaneous apoptosis of eutopic and ectopic endometrium in endometriosis**

Eutopic endometrium from women with endometriosis has some fundamental differences compared with normal endometrium of women without endometriosis. These include a variety of abnormalities in structure, proliferation, immune components, adhesion molecules, proteolytic enzymes and their inhibitors, steroid and cytokine production and responsiveness, gene expression and protein production (Sharpe-Timms, 2001). These differences could contribute to the survival of the regurgitating endometrial cells into the peritoneal cavity and the development of endometriosis. One of the mechanisms that recently gained a lot of interest is the finding that apoptosis appears in eutopic and ectopic endometriosis of patients with endometriosis.

Gebel et al. (1998) reported that the percentage of apoptosis in sloughed endometrial cells was greatly reduced among women with endometriosis, implying that the number of surviving cells that enter the peritoneal cavity is greater in women who develop endometriosis. Dmowski et al. (2001) demonstrated that the
Apoptosis in endometriosis

The sources of the elevated levels of soluble FasL, in the peritoneal cavity were endometriotic lesions and peritoneal fluid leukocytes. Several authors have shown that endometrial glandular and stromal cells express FasL, at both the mRNA and protein levels. This membrane-bound FasL can be shed by matrilysin, producing an active, soluble form of the ligand (Powell et al., 1997). Peritoneal fluid leukocytes are another plausible source for high levels of soluble FasL in women with endometriosis, because human-activated peripheral blood mononuclear cells were shown to express FasL messenger RNA (Suda et al., 1995).

Macrophase-derived growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF), are increased in the peritoneal fluid of women with endometriosis (Oosterlynck et al., 1994). Garcia-Velasco et al. (1999) showed that macrophase-conditioned media containing PDGF and TGF-β induced FasL expression by endometrial stromal cells, suggesting that peritoneal macrophages in endometriosis might stimulate a Fas-mediated apoptosis of immune cells. Expression of FasL by the endometriotic cells may protect them from attack by T-cells. Consequently, ectopic endometrial cells escaping from immune surveillance in the peritoneal cavity of women with endometriosis may contribute to the maintenance of the disease.

It is therefore possible that many endometriotic cells not only become resistant to Fas-mediated apoptosis, but also acquire the ability to utilize this pathway to their advantage by launching a ‘Fas counter-attack’ against the host’s immune system. The increased expression of FasL by endometriotic cells coincides with their inherent resistance to Fas-mediated apoptosis which protects them from a ‘suicidal’ death.

MMP have been implicated in the conversion of TNF-α and FasL to active soluble forms, suggesting that these molecules can activate or release factors involved in the apoptotic process (Gearing et al., 1994; Kayagaki et al., 1995). MMP-2, MMP-9 and Mti-MMP mRNA expression levels are significantly higher in endometriotic lesions compared with normal eutopic endometrium (Ueda et al., 2002), implying that endometriotic tissue has a greater capacity for invasion.

Up-regulation of FasL expression by endometriotic cells could be induced after the adhesion of these cells to the extracellular matrix proteins laminin, fibronectin and collagen IV (Selam et al., 2002a). FasL expressed on endometriotic cells may induce apoptosis of the local immune cells including activated T lymphocytes, thereby reducing attacks by host immune surveillance and promoting the survival of endometrial stromal cells during the initial attachment of endometrial implants. Early lesions

apoptotic index in glandular epithelium was significantly lower in women with endometriosis than in controls. This difference was caused primarily by a significant decrease in apoptosis during the late secretory/menstrual and early proliferative phases in women with endometriosis. The cyclic variability of apoptosis was lost in these women.

One can speculate that if the decrease in apoptosis facilitates ectopic survival and implantation of the endometrial cells then there may be an inverse correlation between the level of apoptosis and the severity of the disease. To test this hypothesis, Dmowski et al. (2001) analysed the apoptotic index according to the stage of endometriosis and found that there was a trend toward decreased apoptosis with increasing stage of the disease, but the difference lacked statistical significance.

**Bcl-2 in eutopic and ectopic endometrium in endometriosis**

Watanabe et al. (1997) examined Bcl-2 expression in eutopic and ectopic endometrium in patients with endometriosis, demonstrating that the expression of Bcl-2 in endometrial glandular cells has a cyclic pattern in eutopic endometrium in patients with endometriosis, but that cyclic changes were not apparent in peritoneal and ovarian endometriotic tissues. In a study by Jones et al. (1998), the authors did not detect apoptosis in stromal cells from peritoneal endometriotic tissues. In accordance with these findings, Bcl-2 is expressed to a greater extent in stromal cells from ectopic tissues (Jones et al., 1998). This overexpression may be directly correlated to the increase in the number of estrogen receptors expressed by ectopic stroma (Fujishita et al., 1997).

An increased expression of Bcl-2 protein was found in proliferative eutopic endometrium from women with endometriosis when compared with normal endometrium from healthy women (Mereden et al., 2000). In the same study, Bax expression was absent in proliferative endometrium, whereas there was an increase in its expression in secretory endometrium from women with endometriosis and healthy women. The altered expression of BCL-2 in eutopic endometrium of women with endometriosis resulted in a decrease number of apoptotic cells and consequently to their abnormal survival in the ectopic locations (Meremen et al., 2000).

**Fas/FasL system in eutopic and ectopic endometrium in endometriosis**

Few studies have been published on the expression of Fas in endometriotic tissues. Moreover, to our knowledge, there are no studies showing quantitative comparison of Fas expression between endometriotic tissues and endometrium from disease-free women. Harada et al. (1996) found that Fas is expressed randomly in both eutopic and ectopic endometrical tissues. The authors suggested that the expression of Fas antigen may be less involved in apoptosis of eutopic and ectopic endometrial tissues as an apoptosis-regulator. In accordance with this finding, Watanabe et al. (1997) also observed Fas expression in glandular cells of both ectopic and eutopic endometrium. In contrast with the cyclic expression pattern of Bcl-2, Fas expression was constant in both tissues throughout the menstrual cycle. Differences in the expression of Fas were found between ovarian, cervical and endometrial carcinoma tissues compared with normal tissues. Tumour cells had significantly decreased levels of Fas (Das et al., 2000). In addition, there was a higher Fas expression in ovarian endometriotic cells compared with benign ovarian tumours but the difference did not reach significance (Fauvet et al., 2003).

In contrast with Fas, there are many studies indicating that higher expression of FasL by endometriotic tissues contributes to their survival and the development of endometriosis. A recent study by Garcia-Velasco et al. (2002) suggests that levels of soluble/active FasL are higher in serum and peritoneal fluid in women with moderate to severe endometriosis than in women with early-stage disease or in disease-free women. Higher levels of soluble FasL in the peritoneal fluid of women with endometriosis may contribute to increased apoptosis of Fas-bearing immune cells in the peritoneal cavity, leading to their decreased scavenger activity (Garcia-Velasco et al., 2002). This may result in prolonged survival of endometrial cells in the peritoneal cavity.

Up-regulation of FasL expression by endometriotic cells could be induced after the adhesion of these cells to the extracellular matrix proteins laminin, fibronectin and collagen IV (Selam et al., 2002a). FasL expressed on endometriotic cells may induce apoptosis of the local immune cells including activated T lymphocytes, thereby reducing attacks by host immune surveillance and promoting the survival of endometrial stromal cells during the initial attachment of endometrial implants. Early lesions
of endometriosis reportedly invade the extracellular matrix of the peritoneum (Spuijkbroek et al., 1992). FasL expression that occurs when endometrial stromal cells attach to the extracellular matrix may be one of the critical events in the development of endometriosis. Under these observations, we could speculate that the expression levels of soluble/active FasL may be enhanced in shedding endometrial cells presenting in the peritoneal environment and protect endometrial cells from the immune effector cells of the peritoneal cavity.

Interleukin-8 (IL-8), a chemokine for neutrophils and a potent angiogenic agent, is elevated in the peritoneal fluid of women with endometriosis (Iwabe et al., 1998). IL-8 promotes proliferation of stromal cells derived from endometriotic tissues (Iwabe et al., 1998, 2000), suggesting that it may facilitate growth of endometriotic implants. Selam et al. (2002b) examined whether IL-8 may up-regulate FasL expression in endometrial cells and may be relevant for the development of a relative local immunotolerance in endometriosis. They demonstrated a concentration-dependent increase in the protein expression of FasL by IL-8 in endometrial stromal cells. The authors speculated that elevated peritoneal fluid IL-8 levels, via stimulation of FasL-induced apoptosis in activated T lymphocytes, contribute to an immune-privileged environment around the endometriosis implants, supporting their survival.

IL-8 exerts a chemotactic activity primarily on neutrophils and inhibits their apoptosis even in the presence of Fas engagement (Leuenroth et al., 1998). Kwak et al. (2002) investigated the effects of plasma and peritoneal fluid (PF) from patients with advanced endometriosis on apoptosis of neutrophils. Adding plasma and PF in neutrophil culture reduced spontaneous apoptosis. Neutralizing IL-8 antibody abrogated the delay of neutrophil apoptosis induced by PF, suggesting that IL-8 is one of the neutrophil survival factors in the PF of endometriosis patients. The impaired clearance of cells responsible for innate immunity in the peritoneal fluid of patients with endometriosis may be associated with the development of the disease (Kwak et al., 2002).

Apoptosis in peritoneal macrophages from patients with endometriosis

The peritoneal cavity, the commonest site of endometriosis (Jenkins et al., 1986), contains fluid whose major cellular constituents are macrophages (Eischen et al., 1994). In endometriosis, the number and secretory activity of these cells increase (Halme et al., 1983, 1987) and recent evidence suggests that these cells play an important role in development and maintenance of endometriosis (Ramsey, 1993; Harada et al., 2001).

The function of macrophages is also altered in several aspects. The cytotoxic power of peritoneal macrophages in endometriosis patients with respect to the endometrium is reduced (Halme et al., 1984b). The diminution of cytotoxicity of peritoneal macrophages could be more significant than that of the circulating macrophages (Braun et al., 1992). The reduced capacity of peritoneal macrophages from women with endometriosis to mediate lysis of endometrial cells together with the increased resistance of ectopic endometrial cells to macrophage-mediated cytolysis may promote survival of the endometrial cells in the peritoneal cavity of women with endometriosis (Braun et al., 1998).

McLaren et al. (1997) reported an increased percentage of Bcl-2-positive macrophages in the peritoneal fluid of women with endometriosis compared with the non-endometriotic group, resulting in an increased number of cells surviving the process of activation and thus delaying apoptosis. This may explain the increased numbers of macrophages found in the peritoneal fluid of patients with endometriosis.

Jones et al. (1998) noted a significantly increased BCL-2 expression in the endometrial stroma of normal and eutopic endometrium with a further increase during the late secretory phase. The authors demonstrated that most BCL-2-positive cells were leukocytes. The ectopic stroma contained significantly higher numbers of Bcl-2-positive cells than eutopic, only some of which were leukocytes.

Immunohistochemical staining revealed a population of BCL-2-positive, BAX-negative tissue macrophages present only in ectopic tissue during both phases of the menstrual cycle (McLaren et al., 1997). The expression of BCL-2 and the absence of BAX may confer on these macrophages a decreased susceptibility to apoptosis, given the known properties of BCL-2 and BAX, and may result in an extended life expectancy. The increased proportion of BCL-2-positive macrophages found in women with endometriosis may predispose these cells to resist apoptosis.

Apoptosis in pathophysiology of endometriosis

Accumulating evidence suggests that the endometrial cells from women with and without endometriosis have fundamental differences. Endometrial cells from women with endometriosis have enhanced proliferation and increased ability to implant and survive in ectopic locations. Impaired sensitivity of endometrial tissue to spontaneous apoptosis contributes to the abnormal implantation and growth of endometrium at ectopic sites. The inability of endometrial cells to transmit a ‘death’ signal or their ability to avoid cell death is associated with increased expression of anti-apoptotic factors (e.g. BCL-2) and decreased expression of pro-apoptotic factors (e.g. BAX) (Meresman et al., 2000). It remains unclear whether the abnormal apoptosis in the eutopic endometrium from patients with endometriosis is primary in origin or secondary after establishment of the pelvic endometriosis process. This could be attributed to the fact that at the time of clinical presentation and diagnosis, most women have already established disease, and therefore it is difficult to investigate the early developmental stages of the endometriosis.

Reflux of endometrial fragments during menstruation into the peritoneal cavity is a common phenomenon. Under normal conditions, cells that do not adhere to their extracellular matrix enter apoptosis as they receive different signals from their adhesion receptors (Aplin et al., 1998). However, in women with endometriosis these cells have the ability to adhere to mesothelial cells of peritoneum, to proliferate, and to produce neoangiogenesis resulting in the development of active endometriosis. The effect of MMP on apoptotic factors and their regulation by steroid hormones may provide a link between endometrial turnover and the invasive process necessary for the development of endometriosis.

Altered apoptosis in eutopic endometrium and endometriotic tissues is a fact that elucidates some aspects of the pathophysiology of endometriosis. However, it remains unclear if these
alterations are the cause or the result of the process for the development of the disease. Moreover, the underlying mechanisms that lead to the development and maintenance of endometriosis are still an enigma. Recent studies suggest that genetic factors are likely to influence individual susceptibility to endometriosis. Genetic alterations in somatic chromosomes (Kosugi et al., 1999) and DNA deletions that inactivate some tumour suppressor genes (PTEN) are likely to be involved for the initiation, persistence and progression of endometriosis (Jiang et al., 1998; Obata et al., 1998). These studies have also provided evidence that there is a common lineage between ovarian endometrioma and ovarian cancer (Campbell and Thomas, 2001).

Recently, cDNA microarray analysis has provided an interesting insight into altered gene expression profiles in patients with endometriosis. Using this method, Arimoto et al. (2003) found 97 up-regulated and 337 down-regulated genes in women with endometriosis. Genes related to apoptosis (GADD34, GADD45A, GADD45B, PIG11) and the tumour suppressor TP53 gene were down-regulated in endometriotic tissues. These findings are consistent with the decreased spontaneous apoptosis observed in eutopic endometrium from women with endometriosis (Imai et al., 2000; Meresman et al., 2003). The increase in apoptotic rate may be due to alterations in the expression of apoptosis-related genes after GnRH agonist administration. Treatment with GnRH agonists was found to affect the expression of a diverse range of genes, including those that encode apoptotic factors (Kakar et al., 2003). Sakamoto et al. (2003) compared the gene and protein expression of IL-8, an autocrine growth-promoting factor, in endometriotic stromal cells of patients treated with GnRH agonist and those of patients without treatment before laparoscopic surgery. They showed that GnRH agonist treatment attenuated the expression of IL-8 by reducing TNF-α-induced NF-κB activation.

Combined oral contraceptives (OC) can be administered to women with endometriosis in order to maintain the status quo and to prevent progression or recurrence of the disease (Lessey, 2000). In histological studies, there was an arrest in endometrial gland proliferation resulting in progressive atrophy of the endometrium after long-term use of OC (Koh et al., 1995). Meresman et al. (2002) have demonstrated that OC can enhance programmed cell death (decreased BCL-2/BAX expression ratio) in the eutopic endometrium of women with endometriosis. Another study has confirmed the inhibitory effects of progestogens on endometrial proliferation and the authors proposed that these compounds enhance apoptosis in the endometrium (Critchley et al., 1999). Clinically, the use of progestins or OC was also suggested as efficacious treatment for endometriosis (Moghissi, 1988; Kettel et al., 1998; Fedele et al., 2001).

Although nowadays the medical management of endometriosis is almost exclusively accomplished through the use of GnRH agonists or steroidogenic compounds, these treatments are not ideal. There is definitely room for improvement of medical treatment of endometriosis with respect to the desire to avoid the adverse side-effects associated with the hypo-estrogenic environment induced by the current GnRH agonist therapies. Recently, compounds that can regulate apoptosis in endometrial cells were examined in experimental studies. Apoptosis of endometrial stromal cells in culture was elevated after exposure to κ-opioid agonists (Chatzaki et al., 2001). κ-Opioid caused a rapid but transient up-regulation of Fas protein, suggesting that its effect on apoptosis is mediated by activation of the Fas/FasL apoptotic pathway.

**Apoptosis in endometriosis and gynaecological tumours**

Endometriosis and cancer are similar in several aspects such as cell invasion, unrestrained growth, development of new blood vessels and a decrease in the number of cells undergoing apoptosis. Abnormalities in the control of proliferation and apoptosis in the normal endometrium may contribute to the development of neoplasia. Highly metastatic cancers exhibit a higher resistance to apoptotic cell death compared with low metastatic forms (Glinsky and Glinsky, 1996). Similarly, endometriotic cells present with lower susceptibility to apoptosis. Endometriotic tissues showed high survivin gene expression (Ueda et al., 2002). Survivin may antagonize caspase-3-mediated apoptosis, and subsequently promote the development of endometriosis and additionally may correlate with a more aggressive phenotype of cancer cells. However, additional studies are needed to clarify the molecular events that regulate the expression of surviving genes in endometriosis and gynaecological tumour cells.

Peiro et al. (2001) found higher expression of pre-apoptotic proteins and genes [CAS (cellular apoptosis susceptibility gene), Bax and caspase-3] and lower expression of the anti-apoptotic protein BCL-2 in endometriotic carcinoma tissues compared with normal endometrium. In addition, increased levels of CAS and caspase-3 were associated with more aggressive behaviour and shorter survival of the patients (Peiro et al., 2001).

A decrease in BCL-2 expression from benign to borderline and malignant ovarian tumours has also been reported (Ben-Hur et al., 1999; Chan et al., 2000; Zusman et al., 2001). Nezhat et al. (2002) reported a less frequent BCL-2 expression in benign endometriotic cysts than endometrioid, clear cell and serous papillary carcinomas, suggesting that BCL-2 expression pattern differs according to histological type of epithelial ovarian tumours. In contrast, Fauvet et al. (2003) found no differences in BCL-2 expression among endometriomas, benign and malignant ovarian tumours. It becomes apparent that the expression of apoptotic proteins and genes in gynaecological tumours remains controversial. The differences observed in these studies may be attributed to a different distribution of BCL-2 in the tumour cells, to the histological grade of the tumour, or to the different methodologies performed for the identification of apoptotic factors.
The tumour suppressor protein p53, another potent inducer of apoptosis, seems to be implicated in the malignant transformation of endometriosis. Activation of p53 can promote apoptosis of damaged or aged cells serving as an effective tumour suppressor (Oren et al., 1992). The p53 gene, which is located on the short arm of chromosome 17, encodes a nuclear protein that normally acts to restrain inappropriate cellular proliferation and negatively regulates the cell division (Kohler et al., 1993). Nezhat et al. (2002) speculated that alterations in p53 may be associated with the malignant transformation of endometriotic cysts. In that study, p53 staining was negative in benign endometriotic cysts and positive in 35–55% in malignant cysts. In contrast, in a study by Fauvet et al. (2003), p53 expression was increased in endometriomas compared with benign tumours, but no difference was observed between endometriomas and malignant ovarian lesions.

Apoptosis represents one of the central points for the development of carcinoma in endometriotic patients. However, more studies are needed to clarify the role of the apoptotic factors in malignant transformation of endometriotic lesions. In the near future, it may be possible that the investigation of apoptotic factors could identify those women with endometriosis who are at high risk for tumour development.

Conclusions

We consider the data presented in this review as a beginning in exploring the role of apoptosis in the pathophysiology of endometriosis. Apoptosis may play an important role in the development of the disease. Manipulation of cell death processes could be used to treat endometriosis. Advances in molecular biology and genetics will help us to understand these issues and may yield prevention and treatment modalities for endometriosis in the near future.

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