

Apoptosis in endometrial glandular and stromal cells in women with and without endometriosis

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BACKGROUND: The aetiology of endometriosis is unknown. Ectopic dissemination of the endometrial cells gives origin to endometriotic lesions, but occurs in women with and without endometriosis. It has been suggested that increased ectopic cell survival facilitates their implantation. The objectives of this study were to evaluate endometrial apoptosis in women with endometriosis according to: (i) cyclic changes, (ii) glandular and stromal contribution, and (iii) stage of the disease. **METHODS:** The subjects were women undergoing diagnostic laparoscopy and endometrial biopsies for suspected endometriosis. Spontaneous apoptosis was evaluated using TdT-mediated dUTP-biotin nick end-labelling (TUNEL) assay. Apoptotic cells per 10 mm² (apoptotic index) in an area of 10–50 mm² in 5 µm endometrial tissue sections were counted and location of these cells was recorded. **RESULTS:** The apoptotic index in glandular epithelium was lower in endometriosis than controls (26.0 ± 5.5 versus 51.2 ± 9.7, *P* = 0.03) but not in the stroma (36.3 ± 6.4 versus 48.4 ± 11.3, NS). In controls, apoptosis was highest during the late secretory/menstrual and early proliferative phases and cyclic variability was apparent. In endometriosis, this cyclic variability was lost. There was a trend toward decreased apoptosis with increasing stage of the disease, but the differences lacked statistical significance. **CONCLUSIONS:** Spontaneous apoptosis is decreased in the endometrial glands in women with endometriosis, especially during late secretory/menstrual and early proliferative phases of the cycle. This may indicate increased viability of endometrial cells shed during menses, facilitating their ectopic survival and implantation.

Key words: apoptosis/endometrial glands/endometriosis/stroma

Introduction

Endometriosis, a disease affecting ~10% of women of reproductive age, is characterized by the ectopic (extrauterine) growth of the endometrial tissue. Outside their physiological location, endometrial cells respond to the ovarian hormones and undergo the same cyclical changes as the uterine endometrium, including cyclical bleeding and shedding. The aetiopathogenesis of endometriosis is unknown, even though several theories have been proposed over the years. The most widely acceptable has become the theory of Sampson (Sampson, 1925). According to this concept, uterine endometrial cells in some women are disseminated during menses in a retrograde fashion through Fallopian tubes, into the peritoneal cavity, where they implant and give origin to endometriotic lesions. Retrograde dissemination of the endometrial cells has been demonstrated repeatedly in a number of clinical studies (Koninckx *et al.*, 1980; Halme *et al.*, 1984; Bartosik *et al.*, 1986). However, this event appears to be a physiological phenomenon, which occurs in all women, regardless whether endometriosis is or is not present (Koninckx *et al.*, 1980; Halme *et al.*, 1984;

Bartosik *et al.*, 1986; Kruitwagen *et al.*, 1991a). For some reason, misplaced endometrial cells in healthy women do not implant and do not develop into endometriotic lesions. The factor(s), which facilitate(s) survival and implantation of misplaced endometrial cells, may contribute to the development of endometriosis.

We have demonstrated previously that in women with endometriosis, proliferation of the endometrial cells in an in-vitro co-culture system is stimulated by autologous peripheral blood monocytes (Braun *et al.*, 1994). In women without endometriosis, in the same coculture system, peripheral blood monocytes suppress proliferation of the autologous endometrial cells. This differential effect of monocytes on endometrial cells depends on the presence or absence of endometriosis, and can also be duplicated with the supernatant from the monocyte/macrophage culture, or with tumour necrosis factor alpha (TNFα), which is one of the major cytokines produced by the monocytes/macrophages (Braun and Dmowski, 1998b; 1999). Interesting in this respect are reports indicating that in some in-vitro cell culture systems, inflammatory cytokines, including TNFα, can stimulate through the sphingomye-

lin pathway, either inflammation and cell proliferation or programmed cell death (apoptosis), depending on specific conditions (Pena *et al.*, 1997).

Apoptosis is a fundamental physiological process responsible for maintaining homeostasis in multicellular organisms (Stellar, 1995). The orderly progression of events during apoptosis results in cell death without the leakage of protease enzymes and cellular contents from dying cells, thereby reducing the likelihood of an inflammatory response (Wyllie *et al.*, 1980). Accumulating evidence suggests that apoptosis is directly involved in the regulation of the menstrual cycle, through elimination of senescent cells from the functional layer of the uterine endometrium during the late-secretory and menstrual phases (Hopwood and Levison, 1976; Tabibzadeh *et al.*, 1994; Kokawa *et al.*, 1996; Shikone *et al.*, 1996). This is followed by proliferation of new cells from the basal layer during the proliferative phase of the following cycle.

Recent studies from our laboratories using a cell death detection ELISA assay (Dmowski *et al.*, 1998; Gebel *et al.*, 1998) demonstrated that endometrial apoptosis in the eutopic endometrium is lower in women with endometriosis than controls and is further decreased in the ectopic endometrium. However, the design of these studies did not allow identification of the apoptotic cells and the pattern of apoptosis was not studied during different phases of the cycle. The objective of the present study was to evaluate further spontaneous apoptosis in the uterine endometrium of women with and without endometriosis using a TUNEL assay and specifically: (i) to determine spontaneous apoptosis during different phases of the menstrual cycle, (ii) to determine the location of the apoptotic cells in endometrial glands and stroma, and (iii) to evaluate the degree of spontaneous apoptosis according to the stage of the disease.

Materials and methods

Study population

Paraffin blocks of the uterine endometrial specimens from 51 endometriosis and 24 non-endometriosis (control) women were retrieved for the study from the pathology laboratory repository. The women were of reproductive age, had regular menstrual cycles, and underwent laparoscopy by the senior author as a part of infertility evaluation between 1996 and 1998. The subjects participated in a clinical study approved by the Institutional Review Board in which portions of the eutopic and ectopic endometrial specimens were evaluated using functional immune assays. No hormonal medications were used during the cycle. At the time of laparoscopy, pelvic organs were examined for the presence and extent of endometriosis. If there was no evidence of endometriosis, the subject was included into the control group. If endometriosis was present, staging of the disease was performed according to the revised AFS classification (American Society for Reproductive Medicine, 1996) and accordingly, 18, 19, 8 and 6 patients were stage 1, 2, 3 and 4 respectively. Women with pelvic diseases other than endometriosis and adhesions were not included in the study. During the laparoscopic procedure, samples of the uterine endometrium were obtained from the uterine fundus with the Novak's curette. Part of each specimen was fixed immediately in 4% formaldehyde and transferred to the pathology laboratory.

Table I. Histological phases of the endometrial cycle in relation to the days of the menstrual cycle and sample distribution

Endometrial phase	Days of cycle	No. biopsies	
		Endometriosis	Controls
Early proliferative (EP)	4-7	6	6
Mid-proliferative (MP)	8-11	9	3
Late-proliferative (LP)	12-15	14	5
Early-secretory (ES)	16-19	9	1
Mid-secretory (MS)	20-23	6	2
Late secretory (LS)	24-28	5	5
Menstrual phase (M)	1-3	2	2
Total		51	24

Identification of endometrial phases and apoptosis analysis

In the pathology laboratory, endometrial specimens were dehydrated and embedded in paraffin. Two cases of normal spleen, thymus, bowel, and embryonic kidney were selected as positive controls of apoptosis. For the purpose of this study, paraffin blocks were retrieved, sectioned (5 µm), and mounted on positive charged microscopic glass slides. The slides were then coded and sent to the Pathology laboratory at another institution for a blind analysis. One set of slides was stained with haematoxylin-eosin and examined microscopically for the endometrial phase of the cycle. Endometrium was classified as early-proliferative (EP), mid-proliferative (MP), late-proliferative (LP), early-secretory (ES), mid-secretory (MS), late-secretory (LS), and menstrual (M) according to its histological appearance (Noyes *et al.*, 1950). The distribution of the samples according to the endometrial phases and corresponding days of the menstrual cycle is presented in Table I.

Another set of slides was stained with the terminal deoxynucleotide transferase mediated dUTP nick-end labelling (TUNEL) as described by Gavrieli *et al.* (Gavrieli *et al.*, 1992) with minimal modification to identify the apoptotic cells. Briefly, one set of sections was deparaffinized and digested with 20 µg/ml protease K (Sigma, St Louis, MO, USA) for 15 min at room temperature. The endogenous peroxidase was blocked with 2% (v/v) hydrogen peroxide [diluted from 30% H₂O₂ in water (w/w), Sigma] for 5 min. After briefly immersing in TdT buffer (30 mmol/l Trizma, 140 mmol/l sodium cacodylate and 1 mmol/l cobalt chloride, pH 7.4), the slides were incubated in TdT reaction solution containing 0.3 IU/µl TdT and 0.005 mmol/l biotin-d-UTP (both from Boehringer Mannheim, Indianapolis, IN, USA) in TdT buffer for 90 min at 37°C. The reaction was terminated by incubating slides in TB buffer (300 mmol/l sodium chloride and 30 mmol/l sodium citrate) for 15 min. Afterwards, slides were incubated in 2% bovine serum albumin (BSA, Sigma) for 10 min and then in 0.5% HRP-streptavidin (Zymed, S. San Francisco, CA, USA) for 30 min. The TUNEL was developed with 0.05% 3'-3'-diaminobenzidine (DAB) and counter stained with Mayer's haematoxylin (Sigma). For each batch of TUNEL staining, positive and negative controls were run in parallel. Negative controls were processed by omitting the TdT from TdT reaction solution of the same TUNEL procedure.

The following criteria for apoptotic cells were applied in this study: TUNEL positive stained nucleus with nuclear morphological features of an apoptotic cell, i.e. shrinkage of the nucleus with condensed chromatin and/or densely aggregated marginal chromatin or dot-like or drop-like condensed nuclear fragments (Figure 1). TUNEL stained swollen nuclei were considered as degenerated necrotic cells and were excluded from the apoptotic cell population. Quantitative analysis of the apoptotic cells was performed with a cytometer under ×400

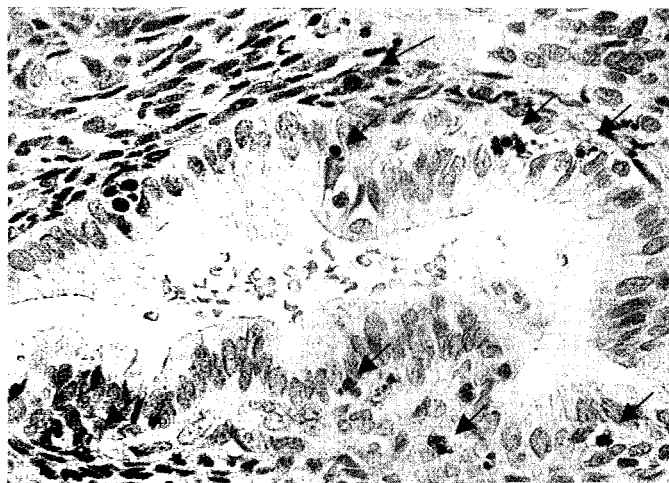


Figure 1. A photomicrograph of late secretory phase endometrium stained with TUNEL ($\times 200$) from a control patient, showing typical nuclear morphology of apoptotic cells (arrows).

magnification using Olympus (model BX50) microscope equipped with super-wide eyepieces. The functional layer of the endometrium in the entire tissue section was counted for the apoptotic cells. That area varied from 10 to 50 mm² (corresponding to $\times 15$ –75 400 fields) depending on the size of the tissue sample. The numbers of apoptotic cells in the endometrial glandular epithelium and stroma were counted separately. The apoptotic index was defined as the number of apoptotic cells per 10 mm² unit area.

Statistical analysis

The data were subjected to the analysis of variance (ANOVA). For the comparative analysis of apoptosis in endometrial glands, stroma, and total endometrium between endometriosis and controls, and for comparisons of cycle phases within and between the groups, the variables included in the ANOVA model were endometriosis (controls versus endometriosis), cycle phase, and endometriosis-by-cycle phase interaction. For comparisons of differences in apoptosis between endometriosis stages (control, stage 1, 2 and 3/4 endometriosis), the variables included in the ANOVA model were endometriosis stage and cycle phase. Statistical analysis was carried out using the general linear model procedures of the Statistical Analysis System (SAS, Release 6.12, SAS Institute, Cary, NC, USA). Multiple comparisons were made using the least-significant differences (LSD) test of SAS. Statistically significant difference was declared when P value was < 0.05 . The data were presented as the least squares mean (LSM) \pm SE of LSM.

Results

Apoptotic cells were clearly identifiable after TUNEL staining of the endometrium in both patients and controls during all phases of the cycle. Photomicrographs (Figure 2) and bar graphs (Figure 3) demonstrate the pattern of spontaneous endometrial apoptosis in both patients and controls according to the endometrial phase. In control subjects the apoptotic index was high during the EP phase. It decreased by $>50\%$ during MP (EP versus MP, $P = 0.02$), decreased further during LP (EP versus LP, $P = 0.001$), and reached the lowest level during ES and MS phases. A dramatic increase was then noted during the LS/M phase ($P < 0.01$ for LS/M versus MP, LS/M versus LP, LS/M versus ES and LS/M versus MS, but

$P = \text{NS}$ for LS/M versus EP). According to the analysis of variance, changes in the apoptotic index during the menstrual cycle for control subjects were significant at $P < 0.05$. In women with endometriosis differences in the apoptotic index between different phases of the cycle were much less pronounced. The values for EP and LS/M were higher than for MP, LP, ES, and MS, suggesting a similar trend as in controls, but the differences were not statistically significant, even though the number of samples for each subgroup was larger in endometriosis than in controls. The apoptotic index was significantly lower in endometriosis than in controls during EP and LS/M phases ($P < 0.05$). A slight, non-significant decrease in the apoptotic index was noted in the endometriosis group during MP and LP phases, and a slight non-significant increase during ES and MS phases, when compared with the control group.

When analysed separately for the endometrial glands and stroma, the pattern of changes in the apoptotic index in relation to the endometrial phase was similar to the combined data for both endometriosis and controls (Figure 3b and c). However, the difference between endometriosis and controls was much higher in the endometrial glands than stroma with statistical significance reached only for the EP phase in the stroma. A cumulative analysis, adjusted for the cycle phase, demonstrated a significant decrease in the apoptotic index in endometriosis than controls in the endometrial glands (51.2 ± 9.7 versus 26.0 ± 5.5 , $P < 0.03$), but not in the endometrial stroma (48.4 ± 11.3 versus 36.3 ± 6.4 , $P = \text{NS}$, Figure 4).

The effect of the endometriosis stage on endometrial apoptosis is demonstrated in Figure 5. When adjusted for the cycle phase, the apoptotic index in controls was significantly higher than that of stages 1, 2, and 3/4 ($P < 0.03$). There was no significant difference between the stages, although a trend to lower apoptosis with increasing stage of the disease was apparent.

Discussion

The intensity of spontaneous apoptosis in the human endometrium varies during the cycle, and is the highest during late secretory and early-proliferative phases (Richart and Ferenczy, 1974; Hopwood and Levison, 1976; Verma, 1983; Tabibzadeh *et al.*, 1994; Kokawa *et al.*, 1996; Spencer *et al.*, 1996; Dahmoun *et al.*, 1999; Vaskivuo, 2000). Apoptotic cells have been identified in the basal and functional endometrium and in both glands and stroma, but there is no agreement as to their frequency and changes during the cycle. Some reports indicate predominance of apoptotic cells in the functional layer (Kokawa *et al.*, 1996) while others in the basalis with a progressive decrease towards the surface epithelium (Tabibzadeh *et al.*, 1994). A comparable percentage of apoptotic cells and similar cyclic variability in the epithelium and stroma were reported previously (Kokawa *et al.*, 1996) while according to others (Tabibzadeh *et al.*, 1994; Vaskivuo *et al.*, 2000), the majority of apoptotic cells were of epithelial origin and much less apoptosis was present in the stroma. Dahmoun *et al.* reported a somewhat lower apoptotic index in the stroma and a rapid increase in apoptosis in both epithelium

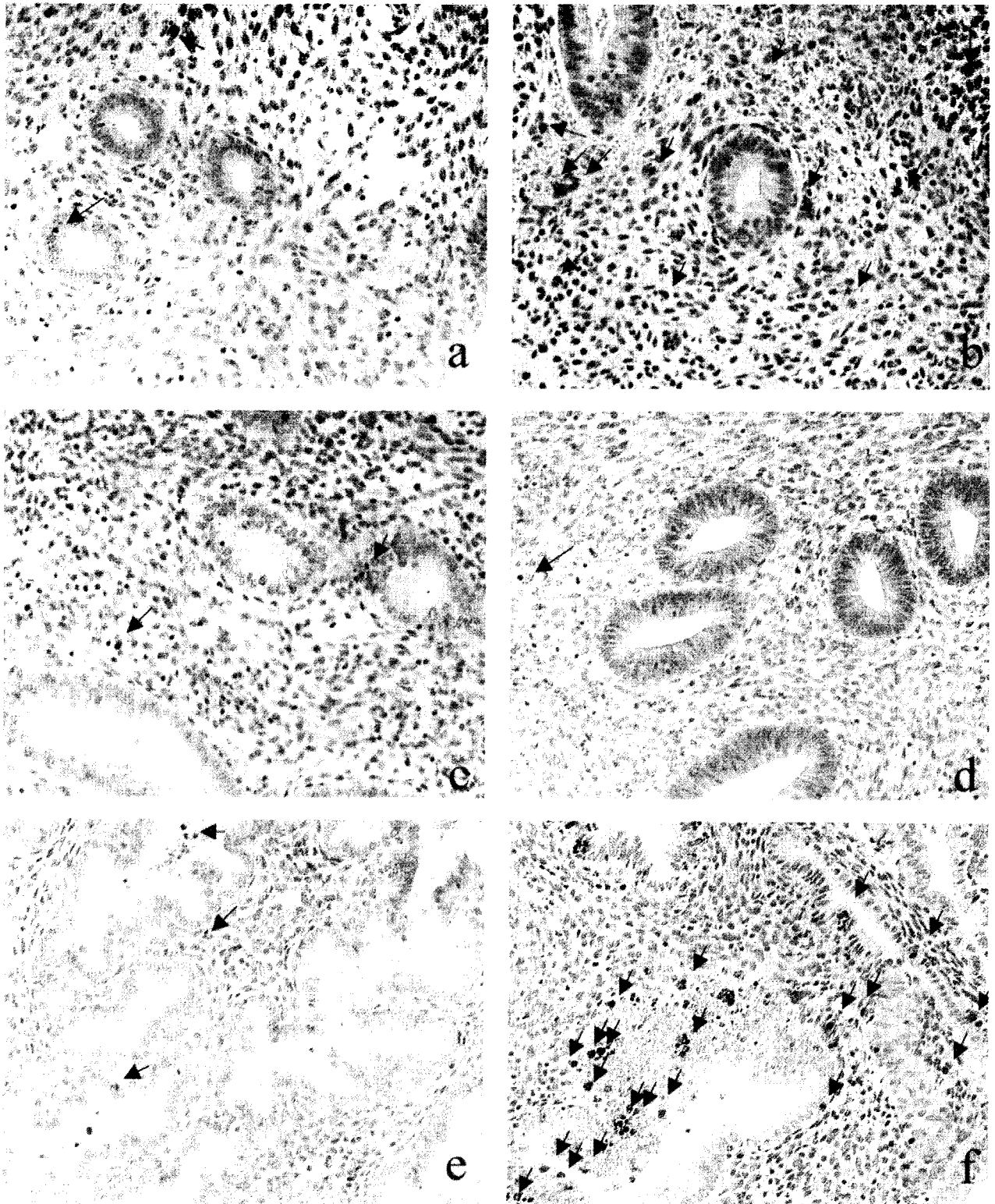


Figure 2. Photomicrographs of uterine endometrial sections stained with TUNEL ($\times 200$), showing apoptotic cells (arrows). (a, c, e) endometrium from women with endometriosis at early-proliferative, late proliferative and late-secretory phases respectively, and (b, d, f) endometrium from women without endometriosis during corresponding phases of the cycle.

and stroma during the last few days of the cycle (Dahmoun *et al.*, 1999). It is possible, as suggested by von Rango *et al.*, that apoptosis is a dynamic process that begins in the basal glands in the early secretory phase, then spreads through the

functionalis into the stroma and that appearance of apoptotic bodies is restricted to the time window between the beginning of DNA fragmentation and phagocytosis of apoptotic cells (von Rango *et al.*, 1998). Thus, individual results may only

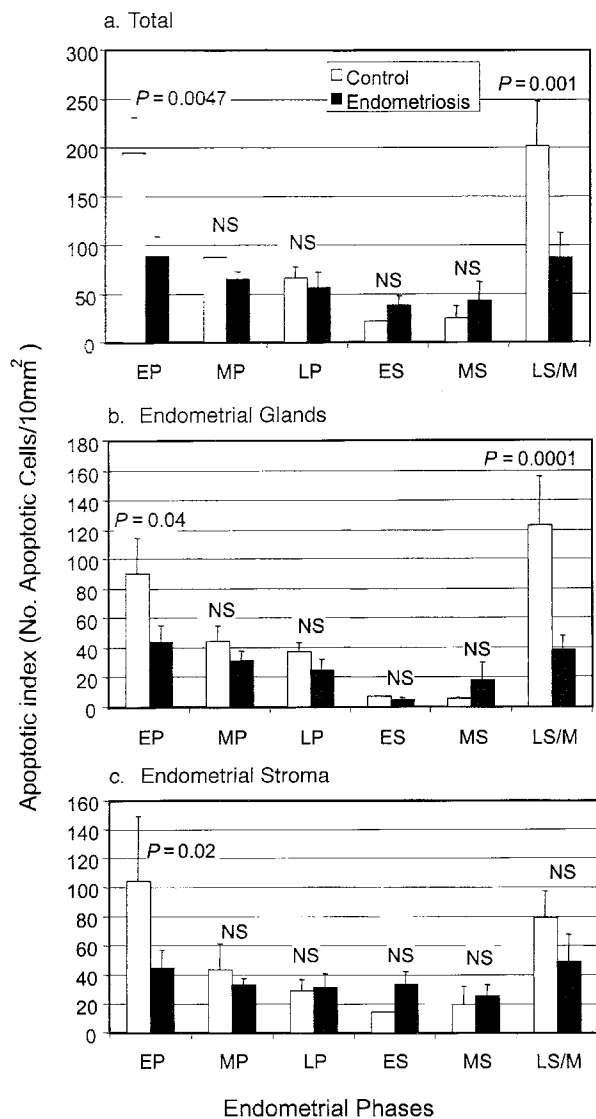


Figure 3. Endometrial apoptosis in endometrial glands and stroma according to the endometrial phase (early-proliferative = EP, mid-proliferative = MP, late-proliferative = LP, early-secretory = ES, mid-secretory = MS, and late-secretory = LS) in women with endometriosis and controls expressed as apoptotic index (LSM \pm SE). NS: non-significant.

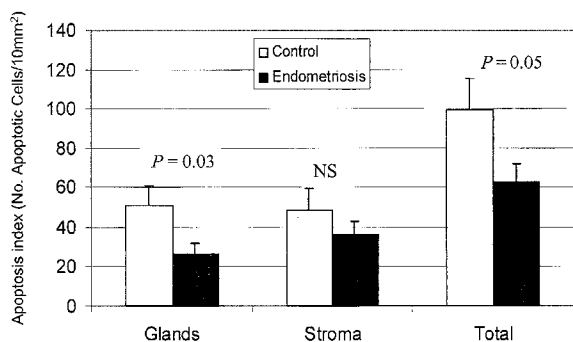


Figure 4. Endometrial apoptosis (glands, stroma and total) in women with endometriosis and controls expressed as apoptotic index (LSM \pm SE). NS: non-significant.

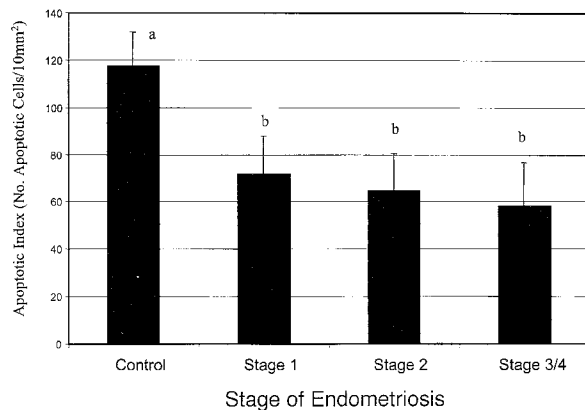


Figure 5. Endometrial apoptosis according to the stage of endometriosis expressed as apoptotic index (LSM \pm SE). Different letters represent significant differences ($P < 0.05$).

represent 'snapshots' of the specific day of the cycle and careful histological dating may be of major importance for any comparative studies.

Endometrial biopsies in our patients were obtained from the functional endometrial layer, which is primarily under the hormonal control and where cyclic events take place. All specimens in both patients and controls were carefully dated histologically and all comparisons were made according to the cycle phase. In controls, we observed a similar pattern of apoptosis during the menstrual cycle as previously reported (Tabibzadeh *et al.*, 1994; Tabibzadeh, 1995; Kokawa *et al.*, 1996; von Rango, *et al.*, 1998; Dahmoun, *et al.*, 1999; Vaskivuo, *et al.*, 2000). The absolute numbers of apoptotic cells in our study were lower when compared with Kokawa *et al.* (Kokawa *et al.*, 1996), who did not use morphological criteria, but similar to those of (Dahmoun *et al.*, 1999), who did. A recent study (Vaskivuo *et al.*, 2000) correlated the degree of apoptosis as determined by 3'-end labelling, DNA fragmentation analysis and expression of apoptosis-related proteins Bcl-2 and Bax with cyclic changes in serum oestradiol and progesterone concentrations. Endometrial apoptosis was negatively correlated with serum oestradiol concentrations and was most pronounced during oestradiol and progesterone withdrawal in agreement with the prior data (Koh *et al.*, 1995; Pecci *et al.*, 1997). Altogether, these studies indicate that during the late secretory phase of the cycle, functional endometrium undergoes extensive apoptosis, the peak of which appears to be associated with endometrial shedding during menstruation. A decline in oestradiol and progesterone concentrations at the end of the cycle is associated with prolonged and intense vasoconstriction of the coiled arterioles leading to endometrial ischaemia and necrosis, which coincide with the peak of apoptosis. Early during menstruation, both apoptotic and necrotic cells can be identified in the endometrial glands and stroma of the functional layer (Dahmoun *et al.*, 1999). This suggests that ovarian steroid-controlled endometrial cell necrosis and apoptosis may be the mechanisms involved in the regulation of the endometrial cyclicality and menstruation.

Endometrium shed during menses is typically expelled with the menstrual flow. However, endometrial cells and tissue

fragments can be identified during menses in the lumen of the Fallopian tubes and in the peritoneal cavity (Ridley, 1968; Koninckx *et al.*, 1980; Halme *et al.*, 1984; Bartosik *et al.*, 1986; Kruitwagen *et al.*, 1991a). This phenomenon seems to occur with equal frequency in women with and without endometriosis (Koninckx *et al.*, 1980; Halme *et al.*, 1984; Bartosik *et al.*, 1986; Kruitwagen *et al.*, 1991a). Clinical observations and in-vitro studies further suggest that in women with endometriosis misplaced endometrial cells implant in ectopic locations giving origin to endometriotic lesions (Evers and Willebrand, 1987; Kruitwagen *et al.*, 1991b; Evers, 1996; Koks *et al.*, 2000), while in healthy women such implantation does not take place. The factor(s) which protect(s) healthy women from the ectopic implantation of misplaced endometrial cells and tissue fragments has been puzzling to us as well as to other investigators.

Our recent studies demonstrated that spontaneous apoptosis when measured using a cell death detection ELISA assay was significantly reduced in the uterine endometrium of women with endometriosis as compared to normal controls (Dmowski *et al.*, 1998; Gebel *et al.*, 1998). These results are in agreement with the present study and suggest that in healthy women, endometrial cells and tissue fragments expelled during menses, do not survive in ectopic locations because of programmed cell death, while decreased apoptosis may lead to the ectopic survival and implantation of these cells and development of endometriosis. When paired samples of eutopic and ectopic endometria were compared, the level of apoptosis was significantly lower in the ectopic samples suggesting ectopic preselection of apoptosis-resistant cells (Gebel *et al.*, 1998). In the present study, we did not evaluate apoptosis in the ectopic endometrium. Apoptosis of the endometrial cells appears to be under the control of endometrial monocytes/macrophages and their secretory products. Immune cell-, and especially monocyte/macrophage-derived cytokines control proliferation versus apoptosis in the eutopic endometrial cells and may also do the same in the ectopic cells, determining thereby development of endometriosis versus normal health (Braun *et al.*, 1994; Tabibzadeh, *et al.*, 1994; Braun and Dmowski, 1998b; Braun *et al.*, 1999).

The hypothesis that decreased apoptosis in the endometrial or immune cells of the reproductive system may contribute to the pathogenesis of endometriosis has been considered and studied by several investigators with mixed results (Harada *et al.*, 1996; McLaren *et al.*, 1997; Sukanuma *et al.*, 1997; Dmowski *et al.*, 1998; Gebel *et al.*, 1998; Jones *et al.*, 1998; Matsumoto *et al.*, 1999; Meresman *et al.*, 2000). In agreement with this report, apoptotic cells were observed in adenomyotic and ovarian endometriotic tissues, without apparent cyclic pattern and without correlation between the intensity of apoptosis and the phase of the menstrual cycle (Harada *et al.*, 1996; Sukanuma *et al.*, 1997; Matsumoto *et al.*, 1999). The assays used in these studies were semiquantitative and there was no comparative evaluation of normal healthy controls. Jones *et al.*, who also used a semiquantitative TUNEL assay, reported only rare apoptotic stromal or epithelial cells without apparent difference between normal, eutopic, ectopic, or adenomyotic endometrium (Jones *et al.*, 1998). These

authors, 'only rarely' identified apoptosis in the normal endometrium and it is unclear how carefully did they match endometrial phases between patients and controls. Only one recent study compared the frequency of endometrial apoptosis in normal controls and women with endometriosis according to the phase of the cycle (Meresman *et al.*, 2000). The authors used a similar patient population as in our study, had a similar study design, and evaluated apoptosis with the TUNEL assay. Although the number of subjects was smaller than in our study, and only two endometrial phases were compared, the results were similar.

It has been 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). Meresman *et al.* noted increased *Bcl-2* and absent *Bax* expression in the late proliferative eutopic as compared to normal endometrium (Meresman *et al.*, 2000). In the late secretory eutopic endometrium there was a significant decrease in *Bax* expression. Decreased apoptosis was found in *Bcl-2* immunopositive and *Bax*-immunonegative tissues. The authors suggested that in women with endometriosis increased *Bcl-2* and decreased *Bax* expression are the anti-apoptotic factors. However, McLaren *et al.* reported essentially similar patterns of *Bcl-2* and *Bax* expression in the glandular cells of normal, eutopic, and ectopic endometrium during the proliferative and secretory phases (McLaren *et al.*, 1997). These authors also reported an increased percentage of *Bcl-2*⁺ macrophages in the peritoneal fluid from women with endometriosis, and concluded that *Bcl-2*⁺ macrophages may predispose endometriotic cells to resist apoptosis. Interestingly, Jones *et al.* noted a significant *Bcl-2* expression in the endometrial stroma in normal and eutopic endometrium with further increase during the late secretory phase (Jones *et al.*, 1998). Using double labelling, these authors demonstrated that most *Bcl-2*⁺ cells were leukocytes. The ectopic stroma contained significantly higher numbers of *Bcl-2*⁺ cells than eutopic, only some of which were leukocytic. Altogether, these reports indicate that endometrial apoptosis in both eutopic and ectopic endometrium in women with endometriosis may be quantitatively different than in normal healthy women and that abnormal expression of apoptosis controlling proteins *Bcl-2* and *Bax* in the endometrial cells, as well as leukocytes, may play a role in this phenomenon.

In the present study, the apoptotic index was significantly lower in women with endometriosis than in controls. The difference was caused primarily by a significant decrease in apoptosis during the late secretory/menstrual and early proliferative phases. Interestingly, the decrease in the apoptotic index between endometriosis and controls was much higher in the glandular epithelium than in the stroma, indicating that these two cell types may not contribute equally to the subsequent development of the disease. The above seems to be consistent with the histological appearance of endometriotic lesions.

If the decrease in programmed cell death facilitates ectopic survival and implantation of the endometrial cells, one might expect an inverse relationship between the level of apoptosis and the severity of the disease. To test this hypothesis, we

analysed our data according to the stage of endometriosis. Disappointingly, we were unable to demonstrate statistically significant differences in apoptosis between different stages, although the trend was apparent. It is quite likely that apoptosis is only one of the mechanisms that control development and progression of endometriosis. Growth autonomy of endometriotic cells and immune inflammatory reaction within the peritoneal cavity are some of the other factors involved (Braun and Dmowski, 1998a). Local and systemic immune response may lead to spontaneous resorption of old endometriotic lesions, while retrograde dissemination to the development of new ones. The balance between these two events may determine the progression or spontaneous regression of the disease and may explain the existence of microscopic endometriosis. Future studies should correlate the stage of endometriosis with the intensity of eutopic and ectopic endometrial apoptosis, taking into consideration the local and systemic immune response, as well as the effect of the cycle and serum hormone concentrations.

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