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Apoptosis and expression of Bcl-2 and Bax in eutopic endometrium from women with endometriosis

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Objective: To evaluate and compare spontaneous apoptosis and Bcl-2 and Bax expression in eutopic endometrium from women with and without endometriosis.

Design: Apoptosis and Bcl-2 and Bax expression were examined in eutopic endometrium from women with and without endometriosis.

Setting: Instituto de Biología y Medicina Experimental-CONICET, Department of Gynecology and Department of Gynecological Pathology, Clínicas University Hospital, Buenos Aires, Argentina.

Patient(s): Women with untreated endometriosis (n = 14) and controls (n = 16).

Intervention(s): Collection of endometrial samples during diagnostic or therapeutic laparoscopy.

Main Outcome Measure(s): Apoptotic cells were detected with use of the dUTP nick-end labeling (TUNEL) assay; Bcl-2 and Bax expressions were assessed with use of immunohistochemical techniques.

Result(s): Spontaneous apoptosis was significantly lower in eutopic endometrium from patients with endometriosis, compared with healthy controls (2.26 ± 0.53 and 9.37 ± 1.69 apoptotic cells/field, respectively) and was independent of cycle phase. An increased expression of Bcl-2 protein was found in proliferative eutopic endometrium from patients with endometriosis. Bax expression was absent in proliferative endometrium, whereas there was an increase in its expression in secretory endometrium from both patients and controls.

Conclusion(s): Women with endometriosis show decreased number of apoptotic cells in eutopic endometrium. The abnormal survival of endometrial cells may result in their continuing growth into ectopic locations. (Fertil Steril® 2000;74:760–66. ©2000 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, eutopic endometrium, apoptosis, Bcl-2, Bax

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Endometriosis is characterized by the growth of endometrial tissue in locations other than the uterine cavity. The etiology and pathogenesis of endometriosis remain unclear. The most widely accepted hypothesis for the development of endometriosis is retrograde menstruation with subsequent implantation and growth of viable endometrium on pelvic structures (1). Despite the fact that this theory confers a crucial role in the pathogenesis of the disease to the eutopic endometrial tissue, little attention has been focused on the study of the intrauterine endometrium in endometriosis.

Growing evidence indicates that the endometrium of women with endometriosis is not normal. Wigfield et al. (2) found an increased number of proliferating cells in eutopic endometrium of women with endometriosis compared with controls and proposed that the endometrium of these women has an increased capacity to proliferate and, therefore, implant and grow in the peritoneal cavity.

Homeostatic control of cell number is thought to be the result of the dynamic balance between cell proliferation and cell death. Apoptosis, or programmed cell death, is an active process that plays a critical role in the maintenance of homeostasis in multicellular organisms (3). This type of regulation allows for the elimination of cells that have been produced in excess, that have developed improperly, or that have sustained genetic damage (4). Apoptosis

occurs in normal endometrial tissue throughout the menstrual cycle. In an earlier study, Hopwood and Levison (5) reported that some human endometrial cells appear apoptotic when observed by electron microscopy. More recently, specific nuclear DNA fragmentation related to the apoptotic process has been shown in human endometrium (6). In addition, several reports have indicated that apoptosis in the endometrium is affected by ovarian steroid hormones (7–10).

The B-cell lymphoma/leukemia-2 gene (Bcl-2) defines a new class of proto-oncogenes that block cell death without promoting cell proliferation (11). It is now clear that the action of Bcl-2 depends on the concentration of, and interaction with, a potential antagonist protein, Bax. Bax is a 21-kDa protein that shares homology with Bcl-2, heterodimerizes with Bcl-2, and homodimerizes with itself. When Bcl-2 was overexpressed, Bcl-2 heterodimerized with Bax and cell death was repressed (12). Thus, the ratio of Bcl-2 to Bax is important in determining susceptibility to apoptosis (13).

There have been several studies of normal endometrial Bcl-2 expression throughout the menstrual cycle (14–17). It is possible that the expression of apoptosis-related proteins is regulated by sex steroids (15). In this context, Critchley et al. (18) reported an increase in immunostaining for Bcl-2 protein in glandular and surface epithelium of antiprogestin-treated endometrium. These data indicate that the expression of this gene may be stimulated by estrogen and down-regulated by progesterone.

However, studies of apoptosis in endometrial tissue from patients with endometriosis resulted in conflicting conclusions. Recently, Gebel et al. (19) detected decreased apoptosis of endometrial cells in endometriosis. Jones et al. (20), however, reported that there was no statistically significant difference in apoptosis and Bcl-2 expression between endometrial tissue from control women and eutopic endometrium from patients with endometriosis.

The aim of the present study was to determine parameters related to apoptosis in eutopic endometrium from women, with and without endometriosis, throughout the menstrual cycle. The following factors were evaluated: [1] apoptosis by in situ end labeling of DNA fragments on paraffin sections and [2] Bcl-2 and Bax expression by a semiquantitative immunostaining technique.

MATERIALS AND METHODS

Patients

A total of 30 patients who underwent a laparoscopy participated in this study: 14 with endometriosis and 16 controls. Determination of the stage of the disease was performed according to the revised American Fertility Society Classification (21). Control subjects were infertile women without endometriosis undergoing diagnostic laparoscopy.

All the patients showed regular menstrual cycles and had not received any medical treatments. Biopsy specimens of eutopic endometrium were obtained from all subjects by means of a Novack cannula, as previously described (22). Cycle phase (late proliferative [LP] or late secretory [LS]) was assigned on the basis of histologic evaluation. This study was approved by an institutional review board, and all subjects included signed informed consent forms.

Apoptosis Detection System

Eutopic endometrial tissues were fixed in 10% buffered formaldehyde. For apoptosis quantification, tissue sections were processed for in situ immunocytochemical localization of nuclei exhibiting DNA fragmentation by the technique of terminal deoxynucleotidyl transferase (TdT)-mediated dUTP digoxigenin nick-end labeling (TUNEL) with use of an apoptosis detection kit (Oncor, Gaithersburg, MD). Sections were treated according to the manufacturer's instructions and as previously described (23).

Briefly, sections were deparaffinized and rehydrated with xylene and ethanol and permeabilized with 20 μg/mL of Proteinase K (GIBCO, Grand Island, NY). Endogenous peroxidase was inactivated by coating the samples with 3% H_2O_2. Sections were rinsed with phosphate-buffered saline (PBS) and then immersed 60 minutes in TdT buffer at 37°C. The appropriate dilution of this enzyme was determined in preliminary experiments. The sections were then incubated 30 minutes with the antidigoxygenin peroxidase conjugate, followed by the peroxidase substrate (3′-diaminobenzidine tetrahydrochloride [DAB]). Finally, sections were counterstained with 0.5% (wt/vol) methyl green.

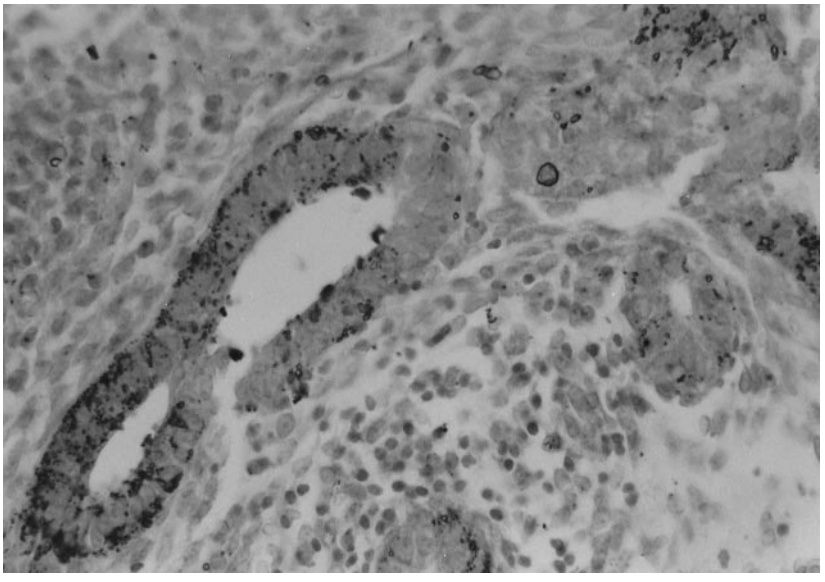
Sections of female rodent mammary gland obtained 3–5 days after weaning of pups were used as a positive control. As a negative control, a number of tissue samples were subjected to treatment without TdT. The numbers of apoptotic cells were determined by counting labeled cells in $\times$630 magnification randomly selected and homogeneous fields (20–30) and expressed as the apoptotic cell mean/field.

Statistical comparisons were performed by either two- or one-way analysis of variance (ANOVA), followed by Scheffé's multiple range test. Regardless of the statistical test, only a $P \leq 0.05$ was considered significant. In addition, the number of apoptotic cells were categorized as (−), <3 apoptotic cells/field; (+), 3–8 apoptotic cells/field; (++), >8 apoptotic cells/field, at $\times$630 magnification with use of an arbitrary scoring system comparable with that used by Watanabe et al. (24).

Also, apoptosis cells were identified by their characteristic morphological features in hematoxylin-eosin-stained LS endometrial sections. These included cell shrinkage and chromatin margination or chromatin condensation with formation of apoptotic bodies (23).

FIGURE 1

Representative photomicrograph of histologic section from eutopic endometrium from woman without endometriosis showing immunostaining of endometrial sections for apoptosis by the TUNEL technique. Note the presence of numerous immunostained apoptotic cells. Original magnification was ×400.



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Immunohistochemical Staining

Bcl-2 was studied from the same paraffin-embedded samples as Bax on serial sections with use of an immunohistochemical method.

Sections were deparaffinized in xylene and rehydrated through graded alcohols, followed by microwaving in 0.01 M of sodium citrate buffer for antigen retrieval. Endogenous peroxidase was blocked by treatment with 0.3% hydrogen peroxide for 30 minutes at room temperature, after which nonspecific binding was blocked by incubation with normal rabbit serum. Tissue sections were incubated 60 minutes with anti-human Bcl-2 mouse monoclonal antibody (Dako Ltd., Cambridge, United Kingdom) or Bax anti-human rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at 37°C after incubation for 60 minutes with anti-mouse-peroxidase conjugate or anti-rabbit-peroxidase conjugate (Dako Ltd.).

Binding was visualized by incubating sections with DAB and lightly counterstaining with hematoxylin before permanent mounting.

Tonsil tissue was included as a positive control for Bax, and lymph node tissue, as a positive control of Bcl-2. As a negative control, immunoglobulin of the same immunoglobulin class and concentration as the primary antibody was used. The negative control showed an absence of specific staining. Bcl-2- and Bax-positive cells were identified by the presence of brown nuclear reactivity.

The intensity of Bcl-2 and Bax staining was assessed in a blind fashion at ×400 magnification by two independent observers. The results were concordant. A semiquantitative assessment method was used, as previously described (14, 16). Each observer viewed multiple randomly selected fields (20–30) of each tissue specimen and scored their staining intensity by grades. The intensity grade was determined by averaging the scores for all the glands observed. The intensity of staining was scored as (−) negative, (+) weakly positive, and (++) strongly positive. The advantage of this method is its precise localization of the protein on the endometrial section, while at the same time it enables a correlation between the presence of apoptotic cells measured by the TUNEL method and Bcl-2/Bax expression.

RESULTS

Apoptosis in Eutopic Endometrium From Patients With Endometriosis and in Controls, Throughout the Menstrual Cycle

The apoptosis detection system revealed positive staining only in the glandular epithelium of the eutopic endometrium sections (Fig. 1).

A decreased apoptosis was detected in eutopic endometrium of women with endometriosis compared with controls (2.26 ± 0.53 vs. 9.37 ± 1.69, respectively; $P<.001$) (Fig. 2). Results were expressed as the number of apoptotic cells/field

FIGURE 2

Spontaneous apoptosis in eutopic endometria from patients with endometriosis (EDT) and control women (CONTROL). Histologic sections were immunostained by the TUNEL technique (see text). The numbers of apoptotic cells were determined by counting labeled cells in ×630 magnification randomly selected fields. Data were expressed as the apoptotic cell mean/field. □ = control; ■ EDT. *$P<.001$.

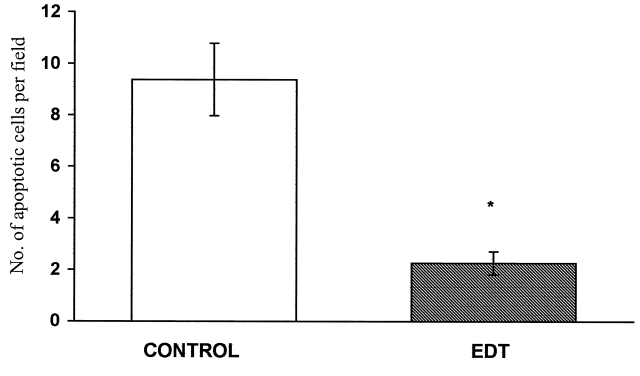

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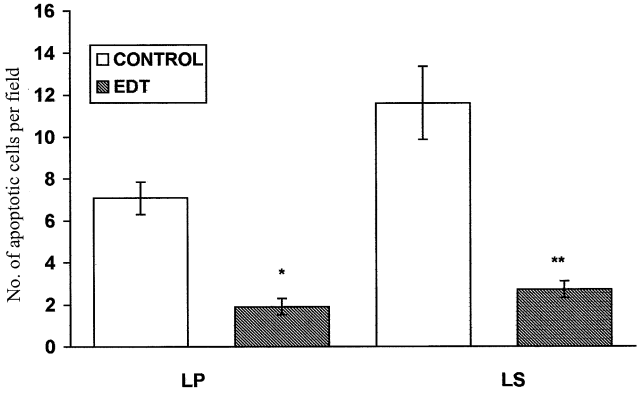
FIGURE 3

Spontaneous apoptosis in eutopic endometria from patients with endometriosis (EDT) and control women (CONTROL), in the late proliferative phase (LP) and late secretory phase (LS) of the menstrual cycle. Histologic sections were immunostained by the TUNEL technique (see text). The numbers of apoptotic cells were determined by counting labeled cells in ×630 magnification randomly selected fields. Data were expressed as the apoptotic cell mean/field. *LP: Control vs. endometriosis $P<.05$. **LS: Control vs, endometriosis $P<.001$.



Meresman. Apoptosis, Bcl-2, and Bax in eutopic endometrium. Fertil Steril 2000.

at ×630 magnification. Decreased apoptosis in eutopic endometrium from patients was observed independently of cycle phase (Fig. 3). Endometria from controls tended to show less spontaneous apoptosis at LP than the same tissue at LS. In this study, however, the differences among these two groups did not reach statistical significance (Table 1, Fig. 3).

Comparison of endometrial spontaneous apoptosis in patients with mild and severe endometriosis did not reach statistical significance (data not shown), suggesting no relationship between severity of the disease and susceptibility of eutopic endometrial cells to spontaneous apoptosis.

Bcl-2 and Bax Expression in Eutopic Endometrium From Patients With Endometriosis and in Controls, Throughout the Menstrual Cycle

Bcl-2 immunostaining was found predominantly in glandular cells (Fig. 4). In endometrial samples from patients with endometriosis, Bcl-2 expression peaked during the LP (Fig. 4A) and virtually disappeared during the LS, whereas in controls, Bcl-2 immunostaining was variable at LP and absent at LS (Table 2).

Bax expression in glandular cells was absent during the LP in most eutopic endometria from patients and controls (Table 2). Only a few glandular cells were Bax-immunopositive at LP, whereas secretory endometrium showed increased Bax expression.

Significant glandular staining for Bax was found in both eutopic and normal secretory endometrium from women with endometriosis and from controls (Fig. 4B).

In proliferative endometrium, there was correlation between the presence of apoptotic cells measured by the TUNEL method and Bcl-2/Bax expression. Decreased apoptosis was found in Bcl-2-immunopositive and Bax-immunonegative tissues; however, in secretory endometrium from patients with endometriosis, no significant correlation was found between the reduction in Bcl-2 expression and apoptotic cell distribution (Table 2).

TABLE 1

Spontaneous apoptosis in eutopic endometria from women with and without endometriosis.

	Late proliferative phase (LP)	Late secretory phase (LS)
Control	7.08 ± 0.92 (n = 10)	11.6 ± 1.74 (n = 6)
Endometriosis	1.9 ± 0.73[a] (n = 7)	2.7 ± 0.82[b] (n = 7)

Note: Values are means ± SEM.
[a] LP (Control group vs. endometriosis group) $P < .05$.
[b] LS (Control vs. endometriosis) $P < .001$.

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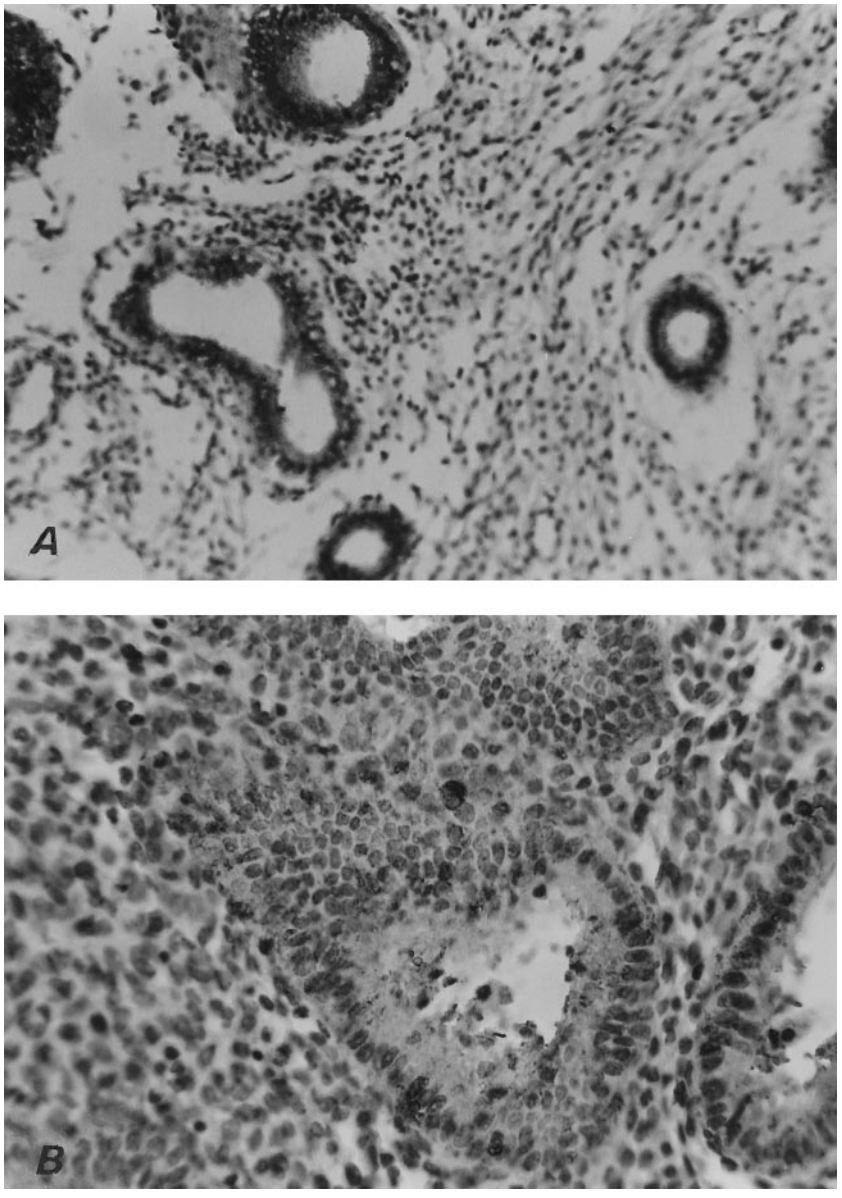
FIGURE 4

Sections immunostained for Bcl-2 and Bax protein expression. (**A**), Bcl-2 protein expression in proliferative endometrium from a patient with endometriosis (×200). (**B**), Bax protein expression in secretory endometrium from a control subject (×400). Note strong immunopositivity in endometrial sections. Bcl-2 and Bax staining predominated in glandular cells.



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DISCUSSION

Endometriosis is a disease characterized by the accumulation of cells in ectopic locations. Cell accumulation could result from either increased proliferation or the deficiency of cells to undergo apoptosis in response to appropriate stimuli. Some evidence suggests that the failure of cells to enter apoptosis might be involved in the pathogenesis of a number of human diseases, including cancer, autoimmune disorders, and AIDS (4). Recently, Dmowski et al. (25) proposed that survival of endometrial cells misplaced in ectopic locations may depend in part on the inherent ability of cells to undergo apoptosis.

The experiments reported in the present study show significantly decreased apoptosis in eutopic endometrium from women with endometriosis, compared with controls. This difference remained significant when proliferative and secre-

TABLE 2

Bcl-2 and Bax expression in eutopic endometria from women with and without endometriosis.

Cycle phase	Case no.	EDT/control	Bcl-2[a]	Bax[a]	Apoptosis (TUNEL)[b]
LP	1	EDT I	++	−	−
	2	EDT I	++	−	−
	3	EDT 1	++	−	−
	4	EDT I	++	−	−
	5	EDT I	++		+
	6	EDT IV	++	−	−
	7	EDT IV	++	−	−
	8	Control		−	+
	9	Control	−	−	++
	10	Control	−	−	+
	11	Control	+	−	−
	12	Control	−	+	++
	13	Control	+	−	−
	14	Control	−	++	+
	15	Control	++	−	−
	16	Control	++	−	−
	17	Control	+	−	−
LS	18	EDT I	−	+	−
	19	EDT I	+	−	−
	20	EDT I	−	−	−
	21	EDT I	−		−
	22	EDT IV	−	++	+
	23	EDT IV	−	−	−
	24	EDT IV	−	++	+
	25	Control	−	+	+
	26	Control	−	++	++
	27	Control	−	++	++
	28	Control	−	++	+
	29	Control	−	+	++
	30	Control	−	++	++

EDT = endometriosis; LP = late proliferative phase; LS = late secretory phase.

Bcl-2 and bax immunostaining was semiquantified by staining grade according to the scale: (−), negative, (+), weak positive, (++), positive.

TUNEL scored according to the scale: (−), <3 apoptotic cells/field; (+), 3–8 apoptotic cells/field; (++), >8 apoptotic cells/field, at ×630 magnification. Blank = no sample.

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tory phases were evaluated separately, indicating abnormal survival of eutopic endometrial cells from patients with endometriosis throughout the entire menstrual cycle.

The differences among endometria from patients with mild and severe endometriosis did not reach statistical significance, suggesting no relationship between the severity of the disease and susceptibility of eutopic endometrial cells to spontaneous apoptosis. The decreased apoptosis found in endometria from patients with endometriosis might explain why endometrial cells survive and grow in these patients in particular, whereas the dissemination of endometrial cells from the uterus into ectopic locations probably occurs monthly in all women.

In addition, normal secretory endometrium showed more spontaneous apoptosis than normal proliferative endometrium, suggesting that apoptosis helps maintain cellular homeostasis during the menstrual cycle in healthy women. The uterine endometrium begins to show regressive changes during its secretory phase, and apoptosis may be related to the paracrine effect of steroid hormones and cytokines (6, 26).

Autoradiographic analysis showed that there was little or no apoptosis in the mild to late proliferative endometrium, whereas DNA cleavage, characteristic of apoptosis, was found at the beginning of the late secretory phase (27). In our study, however, this difference did not reach statistical significance. It could be possible that no statistical significance can be found because of the relatively small control sample size.

One of the mechanisms that could explain the decreased incidence of apoptosis observed in endometria from patients with endometriosis is related to Bcl-2 and Bax expression. In a second series of experiments, the expression of Bcl-2 in eutopic endometrium was evaluated. Results support the findings by previous studies that Bcl-2 is expressed in glandular component of human endometrium (14, 16, 28). In the normal endometrium Tao et al. (28) found modest levels of Bax protein in proliferative phase that increased in secretory phase when apoptosis was prevalent. In contrast, Bcl-2 immunoreactivity was maximal in proliferative endometrium and decreased in secretory endometrium. In addition, Yamashita et al. (29) confirmed by Western blot analysis the immunohistochemical data, because the amount of Bcl-2 changed throughout the cycle, showing a peak during the late proliferative phase.

Our finding of maximal staining intensity during the proliferative phase is entirely consistent with these earlier studies. In this report we add critical information about endometrium from women with endometriosis. An increased expression of Bcl-2 protein was observed in the proliferative eutopic endometrium from patients with endometriosis compared with controls. We found a pattern of immunoreactivity for Bcl-2 in eutopic endometrium from patients that suggests regulation by ovarian steroids.

There is evidence that excess Bcl-2 prevents the accumulation and dominance of Bax homodimers, leading to protection from a range of apoptotic stimuli (30). However, in secretory endometrium from patients with endometriosis, no significant correlation was found between the reduction in Bcl-2 expression and apoptotic cell distribution. It can be speculated that the role of Bcl-2 in the endometrium is the impediment of apoptosis during the proliferative phase. Likewise, one must not forget that there are a number of other antiapoptotic-inducing pathways (31) that may also partially protect endometrial cells from patients with endometriosis from undergoing apoptosis in the secretory phase of the menstrual cycle.

In addition, the present study shows a different expression

pattern during the menstrual cycle of another member of the Bcl-2 family, the Bax protein. In normal proliferative endometrium, a few glandular epithelial cells were immunopositive for Bax protein, whereas proliferative endometrial cells from patients with endometriosis were immunonegative for this Bcl-2 antagonist. By comparison, Bax immunostaining was increased in secretory endometrium, fundamentally in the control group. In secretory eutopic endometria from patients with endometriosis, the pattern and intensity of staining were variable and associated, in most of the cases, with spontaneous apoptosis expression measured by the TUNEL method.

In situations in which Bax predominates over Bcl-2, the formation of the Bax homodimers predisposes the cell to undergo apoptosis (30). Thus, the results suggest that Bcl-2 is an important antiapoptotic factor in the human endometrium from patients with endometriosis, whereas Bax facilitates the increased normal endometrial cell death during the secretory phase that may be associated with menses.

In conclusion, the present study indicates that eutopic endometrium from patients with endometriosis is less susceptible to apoptosis than endometrium from controls. The augmented insensibility to apoptosis of these endometrial cells may result in their continuing growth into ectopic locations and would be fundamental to the pathophysiology of the disease.

References

1. Sampson JA. Benign and malignant endometrial implants in peritoneal cavity, and their relation to certain ovarian tumors. Surg Gynecol Obstet 1924;38:287–311.
2. Wigfield M, Macpherson A, Healy D, Rogers P. Cell proliferation is increased in the endometrium of women with endometriosis. Fertil Steril 1995;64:340–6.
3. Schwartzman RA, Cidlowski JA. Apoptosis: the biochemistry and molecular biology of programmed cell death. Endocr Rev 1993;14: 133–51.
4. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 1995;267:1456–62.
5. Hopwood D, Levinson DA. Atrophy and apoptosis in the cyclical human endometrium. J Pathol 1995;119:159–66.
6. Tabibzadeh S, Kong QF, Satyaswaroop PG. Distinct regional and menstrual cycle dependent distribution of apoptosis in human endometrium: potential regulatory role of T-cells and TNF-α. Endocrinol J 1994;2:87–95.
7. Nawaz S, Lynch MP, Galand P, Gerschenson LE. Hormonal regulation of cell death in rabbit uterine epithelium. Am J Pathol 1987;127:51–9.
8. Rotello RJ, Hocker MB, Gerschenson LE. Biochemical evidence for programmed cell death in rabbit uterine epithelium. Am J Pathol 1989;134:491–5.
9. Tabibzadeh S, Zupi E, Babaknia A. Site and menstrual cycle dependent expression of proteins of the TNF receptor family and Bcl-2 oncoprotein and phase specific production of TNFα in human endometrium. Hum Reprod 1995;10:277–86.
10. Harada M, Suganuma N, Furuhashi M, Nagasaka T, Nakashima N, Kikkawa F, et al. Detection of apoptosis in human endometriotic tissues. Mol Hum Reprod 1996;2:307–15.
11. Korsmeyer SJ. Bcl-2 initiates a new category of oncogenes: regulators of cell death. Blood 1992;80:879–86.
12. Oltvai ZN, Milliman CL, Korsmeyer ST. Bcl-2 heterodimerizes in vivo with a conserved homologue Bax that accelerates programmed cell death. Cell 1993;74:609–19.
13. Chao DT, Korsmeyer SJ. Bcl-2 family: regulators of cell death. Annu Rev Immunol 1998;16:395–419.
14. Gompel A, Sabourin JC, Martin A. Bcl-2 expression in normal endometrium during the menstrual cycle. Am J Pathol 1994 144:1195–202.
15. Otsuki Y, Misaki O, Sugimoto O, Ito Y. Cyclic Bcl-2 gene expression during the menstrual cycle. Lancet 1994;344:28–9.
16. Koh EAT, Illingworth PJ, Duncan WC, Critchley HOD. Immunolocalization of Bcl-2 protein in human endometrium in the menstrual cycle and stimulated early pregnancy. Hum Reprod 1995;10:1557–62.
17. McLaren J, Prentice A, Charnock-Jones AM. Immunolocalization of the apoptosis regulating proteins Bcl-2 and Bax in human endometrium and isolated peritoneal fluid macrophages in endometriosis. Hum Reprod 1997;12:146–52.
18. Critchley HOD, Tong S, Cameron ST, Drudy TA, Kelly RW, Baird DT. Regulation of Bcl-2 gene family members in human endometrium by antiprogestin administration in vivo. J Reprod Fertil 1999;115:389–95.
19. Gebel HM, Braun DP, Tambur A, Frame D, Rana N, Dmowski P. Spontaneous apoptosis of endometrial tissue is impaired in women with endometriosis. Fertil Steril 1998;69:1042–7.
20. Jones RK, Searle RF, Bulmer JN. Apoptosis and bcl-2 expression in normal human endometrium, endometriosis and adenomyosis. Hum Reprod 1998;13:3496–502.
21. The American Fertility Society. Revised American Fertility Society classification of endometriosis: 1985. Fertil Steril 1985;43:351–2.
22. Meresman GF, Barañao RI, Tenenbaum A, Singla JJ, Neuspiller NR, Rumi LS. Effect of peritoneal fluid from patients with minimal and severe endometriosis on endometrial stromal cell proliferation. Arch Gynecol Obstet 1997;259:109–15.
23. Andreu C, Parborell F, Vanzulli S, Chemes H, Tesone M. Regulation of follicular luteinization by a gonadotropin-releasing hormone agonist: relationship between steroidogenesis and apoptosis. Mol Reprod Dev 1998;51:287–94.
24. Watanabe H, Kanzaki H, Narukawa S, Inoue T, Katsuragawa H, Kaneko Y, et al. Bcl-2 and Fas expression in eutopic and ectopic endometrium during the menstrual cycle in relation to endometrial cell apoptosis. Am J Obstet Gynecol 1997;176:360–8.
25. Dmowski WP, Gebel H, Braun DP. Decreased apoptosis and sensitivity to macrophage mediated cytolysis of endometrial cells in endometriosis. Hum Reprod Update 1998;4:696–701.
26. Tabibzadeh S. The signals and molecular pathways involved in human menstruation, a unique process of tissue destruction and remodeling. Mol Hum Reprod 1996;2:77–92.
27. Kokawa K, Shikone T, Nakano R. Apoptosis in the human uterine endometrium during the menstrual cycle. J Clin Endocrinol Metab 1996;81:4144–7.
28. Tao XJ, Tilly KI, Maravei DV, Shifren JL, Krajewski S, Reed JC, et al. Differential expression of members of the Bcl-2 gene family in proliferative and secretory human endometrium: glandular epithelial cell apoptosis is associated with increased expression of bax. J Clin Endocrinol Metab 1997;82:2738–46.
29. Yamashita H, Otsuki Y, Ito Y, Matsumoto K, Ueki K, Ueki M. Fas ligand, Fas antigen and Bcl-2 expression in human endometrium during the menstrual cycle. Mol Hum Reprod 1999;5:358–64.
30. Oltvai ZN, Korsmeyer SJ. Checkpoints of dueling dimers foil death wishes. Cell 1994;79:189–92.
31. Vinatier D, Dufour P, Subtil D. Apoptosis: a programmed cell death involved in ovarian and uterine physiology. Eur J Obstet Gynaecol Reprod Biol 1996;67:85–102.