ELSEVIER

Molecular and Cellular Endocrinology 165 (2000) 75–83

MCE
Molecular and Cellular Endocrinology

www.elsevier.com/locate/mce

Apoptosis and apoptosis-related proteins in human endometrium

T.E. Vaskivuo [a,b], F. Stenbäck [b], P. Karhumaa [c], J. Risteli [d], L. Dunkel [e], J.S. Tapanainen [a,b,*]

[a] *Department of Obstetrics and Gynecology, University of Oulu, FIN-90220 Oulu, Finland*
[b] *Department of Pathology, University of Oulu, FIN-90220 Oulu, Finland*
[c] *Department of Anatomy, University of Oulu, FIN-90220 Oulu, Finland*
[d] *Department of Clinical Chemistry, University of Oulu, FIN-90220 Oulu, Finland*
[e] *Hospital for Children and Adolescents, University of Helsinki, FIN-00029 Helsinki, Finland*

Received 1 December 1999; accepted 25 April 2000

Abstract

Apoptosis has been shown to be an important regulator of endometrium function. To study the regulation of apoptosis in the endometrium during the normal menstrual cycle, the expression of the apoptosis related proteins Bcl-2 and Bax and their correlation to serum estradiol and progesterone, as well as to a replication-related antigen Ki-67 were analyzed. Nine uterine tissue samples and thirty-nine endometrial biopsy specimens were studied. Apoptosis was studied quantitatively by Southern blot analysis of internucleosomal cleavage of genomic DNA, and qualitatively by using in situ 3'-end labeling of fragmented DNA, and Bcl-2, Bax and Ki-67 were detected and quantified immunohistochemically. Apoptotic cells were mostly detected in the glandular epithelium of late secretory and menstruating endometrium. Immunostaining of Ki-67 was detected predominantly in proliferative endometrium. The expression of Bcl-2 was high in proliferative endometrium and decreased in the secretory phase, being very low or absent in the mid- and late secretory and menstruatory phases. Similarly, Bax expression also decreased, but was still present throughout the secretory phase. In human endometrium, apoptosis occurs predominantly in the late secretory and menstrual phases, and is related to alterations in the expression of Bcl-2 and Bax. A decrease in the Bcl-2/Bax ratio in the early secretory phase precedes DNA fragmentation and may predispose the cells to apoptosis.

Keywords: Apoptosis; Endometrium; bcl-2; bax; ki-67

1. Introduction

Apoptosis is an active, regulated process of cell death. The characteristics of apoptosis; cell shrinkage, condensation of chromatin, and internucleosomal cleavage of DNA resulting in fragments which are multiples of approximately 180–200 base-pairs in size, differ distinctly from necrosis (Schwarzman and Cidlowski, 1993). Apoptosis has been shown to play a part in the development and maintenance of homeostasis in variety of tissues including the female reproductive tract (Behringer et al., 1994; Tilly 1996).

Apoptosis is regulated by extracellular signals in addition to an intracellular autonomous genetic program. One of the most studied proteins regulating apoptosis, Bcl-2, was first identified in human B-cell tumors (Tsujimoto et al., 1985). Bcl-2 protein has been shown to protect cells from apoptosis by regulating mitochondrial membrane function (Matsuyama et al., 1998). Another member of the Bcl-2 gene family, the Bax gene, encodes a protein that has high amino acid homology with Bcl-2. Bax protein increases the apoptotic susceptibility of cells in several organs (Brady and Gil-Gomez, 1998). A possible explanation for the apoptosis-promoting activity of Bax protein appears to be that it can counter the anti-apoptotic effects of Bcl-2 by forming heterodimers with Bcl-2 (Oltvai et al., 1993). This suggests that cell death susceptibility depends on the Bcl-2/Bax ratio. A high Bcl-2/Bax ratio makes cells

* Corresponding author. Tel.: +358-8-3153172; fax: +358-8-3154310.
E-mail address: juha.tapanainen@oulu.fi (J.S. Tapanainen).

0303-7207/00/$ - see front matter
PII: S0303-7207(00)00261-6

resistant to apoptotic stimuli, while a low ratio induces cell death (Hanada et al., 1994; Sedlak et al., 1995).

In human epithelial endometrium, expression of Bcl-2 protein has been shown to be active during the proliferative phase and to gradually disappear towards the secretory phase (Otsuki et al., 1994; Koh et al., 1995; Tao et al., 1997). Moderate expression of Bax in the proliferative endometrium and its increase in the secretory endometrium has been observed previously (Tao et al., 1997). To study the regulation of apoptosis in endometrium in more detail, we investigated the expression of the anti- and pro-apoptotic factors Bcl-2 and Bax during the menstrual cycle, and correlated the findings to serum estradiol and progesterone concentrations as well as to cell proliferation indicated by the detection of a replication-related antigen Ki-67.

2. Materials and methods

2.1. Subjects

Endometrial biopsies were collected from 39 healthy, premenopausal women undergoing laparoscopic sterilization. Uterine sections were obtained from nine premenopausal women who underwent hysterectomy as a result of benign conditions: myomas, endometriosis and ovarian cysts. Although these conditions hardly affect the endometrium, the analyses were based on the curettage samples from healthy subjects and the uterine sections were used to confirm the spatial localization of these findings. Blood samples for hormone measurements were taken before or during the procedure. The cycle phase was determined histologically (F.S.) excluding samples from the patients with abnormal serum estradiol or progesterone concentrations. This study was approved by the Ethics Committee of the Medical Faculty, University of Oulu, and written consent was obtained from each patient undergoing sterilization.

2.2. Hormone measurements

Serum progesterone and estradiol concentrations were measured using commercial RIA kits obtained from Wallac OY (Turku, Finland).

2.3. Tissue preparation

Endometrial tissue samples were divided into two parts. One part of each sample was fixed in 4% buffered formaldehyde for 24 h, embedded in paraffin and cut into 5 μm-thick sections before in situ analysis of apoptosis and immunohistochemistry. The other part of the sample was snap-frozen in liquid nitrogen and stored at −80°C for DNA fragmentation and western blot analysis. Only paraffin sections were used in the analysis of uterine tissue.

2.4. In situ 3′-end labeling of DNA in apoptotic cells

Paraffin sections were dewaxed with xylenol and rehydrated for 3′-end labeling. Tissue sections were incubated with Proteinase K (20 μg/ml) (Boehringer Mannheim GmbH, Mannheim, Germany) at room temperature for 15 min. The 3′-end labeling of apoptotic cell DNA was performed by using an ApopTag in situ apoptosis detection kit (Oncor, Gaithesburg, MD, USA) following the instructions of the manufacturer. Endogenous peroxidase activity was quenched with 2% hydrogen peroxidase in phosphate buffered-saline, pH 7.2 (PBS). Terminal transferase was used to catalyze the addition of digoxigenin-labeled nucleotides to the 3′-ends of fragmented DNA. Thereafter, anti-digoxigenin-peroxidase solution was applied to the slides. Diluted diaminobenzene-hydrogen peroxide was used to develop the color reaction. Specimens were lightly counterstained with hematoxylin. The number of apoptotic cells in glandular epithelium was quantified by counting 200 cells in light microscopy.

2.5. DNA fragmentation analysis

The specificity of the in situ 3′-end labeling technique was further validated by Southern blot analysis of fragmentation of genomic DNA. Total DNA was isolated from frozen endometrial tissue and quantified by absorbance at 260 nm. One microgram of DNA from each sample was labeled at 3′-ends with DIG-11-ddUTP (Boeringer–Mannheim), using 25 U of terminal transferase (Boehringer–Mannheim). The labeled DNA samples were loaded onto 2% agarose gels and separated by electrophoresis for 3 h at 50 V. After resolving, the gels were blotted on positively charged nylon membranes (Boehringer–Mannheim) and DNA was immobilized by UV irradiation. After blocking nonspecific binding, digoxigenin-bound nucleotides were identified by using sheep antidigoxigenin conjugated to alkaline phosphatase (Boehringer–Mannheim). The membranes were incubated in sealed hybridization bags for 5 min in 2 ml CSPD solution and exposed to Kodak Scientific Imaging Film.

2.6. Immunohistochemistry

Paraffin sections were deparaffinized in xylene and hydrated gradually through graded alcohols. The sections were incubated in 10 mM sodium citrate buffer (pH 6.0) at 60°C for 30 min. Endogenous peroxidase activity was prevented by incubating the samples in 225 ml of methanol with 25 ml of 30% hydrogen peroxidase. Nonspecific antibody binding was prevented with 1 ml of fetal calf serum and with 4 ml of PBS. Primary antibody was applied to the sample and incubated overnight. Bcl-2 was detected with monoclonal mouse

antihuman Bcl-2 oncoprotein (DAKO, Glostrup, Denmark), Bax with polyclonal rabbit antihuman Bax (Pharmingen, San Diego, CA), and Ki-67 with monoclonal mouse antihuman Ki-67 (DAKO). Dilution of 1:25 was used for Bcl-2 antibodies and 1:500 for Bcl-2 antibodies. Biotinylated rabbit antimouse immunoglobulins were used as secondary antibodies for monoclonals and biotinylated goat antirabbit immunoglobulins for polyclonals. A commercially available avidin-biotin immunoperoxidase system was used to visualize bound antibody (Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, CA). The color reaction was produced in diaminobenzene for 7 min. Counterstaining was carried out with hematoxylin.

Glandular epithelium and stroma of the specimens were evaluated separately. In both cell compartments the intensity of staining was estimated thus: $-$, negative; $\pm$, slightly positive; $+$, positive; $++$, strongly positive; and $+++$, very strongly positive. The staining of the epithelial cells was relatively uniform, while moderate variation in staining of the stromal cells was observed. The analysis of immunohistochemistry was done by two independent observers (T.E.V. and J.S.T.).

2.7. Western blot analysis

Frozen endometrial samples were sonicated in ice-cold 50 mmol/l Tris–SO_4, 150 mmol/l NaCl, 5 mmol/l EDTA buffer, pH 8.7 containing 1 mmol/l phenylmethylsuphonylfluoride (PMSF), 1 mmol/l benzamidine, and 1 mmol/l *o*-phenanthroline as protease inhibitors. Twenty five microlitre-aliquots of protein extract were separated by SDS-polyacrylamide gel electrophoresis using 13.5% acrylamide separating gel and transferred to PVDF membrane (Millipore Corporation, Bedford, MA) as previously described (Karhumaa et al. 2000). Primary antibody concentrations of 1:200 for bcl-2 and 1:1000 for bax were used. Alkaline phosphatase-conjugated rabbit anti-mouse and goat anti-rabbit were used as secondary antibodies to bcl-2 and bax, respectively. Visualization was carried out by chemiluminescence substrate (Bio–Rad Laboratories).

2.8. Statistics

The data were analyzed by ANOVA in appropriate statistical comparisons. $P < 0.05$ was considered significant.

3. Results

3.1. Hormone measurements

Serum estradiol and progesterone concentrations in the subjects were found to follow the normal cyclic changes of the menstrual cycle (Table 1).

3.2. Apoptosis

To study changes of DNA fragmentation in human endometrium, in situ 3′-end labeling with dig-ddUTP was carried out on endometrial and uterine sections. The findings of in situ 3′-end labeling assay were confirmed morphologically with hematoxylin and eosin staining (Fig. 1). Apoptotic cells were mainly detected in the glandular epithelium of the endometrium. Only very few positive cells were detected in the stroma at any stage of the cycle. In the late proliferative phase of the cycle apoptosis in the glands was scarce (Fig. 2). During the secretory phase, the abundance of apoptotic cells increased and reached a maximum at menstruation. At early proliferation apoptosis was still present but the number of apoptotic cells decreased towards late proliferation (Fig. 3).

DNA fragmentation analysis confirmed the results of in situ 3′-end labeling. Due to the abundance of blood cells present in the curettage samples the quality of analysis was not entirely satisfactory, but clearly shows that fragmentation was highest in the menstrual phase (Fig. 4).

3.3. Bcl-2

The intensity of Bcl-2 staining in glandular epithelial cells was highest in the late proliferative phase, although there was considerable variation in staining intensity between the samples. In the mid- and late secretory phase and in the menstruating phase, it was absent or scarce (Table 2, Fig. 5). In the stroma, Bcl-2 immunostaining was scarce throughout the cycle. Only a small decrease in the intensity of staining was detected during the secretory phase (Table 1). Western analysis of bcl-2 protein detected the expected 26 kDa protein in endometrium and a similar expression pattern as seen in immunohistochemistry was observed (Fig. 6).

3.4. Bax

Bax was expressed at all stages of the cycle. In glandular epithelium staining was most intense, but the protein was also expressed in the stroma (Fig. 4). In glandular epithelium, the expression of Bax was high during the late proliferative and early secretory phase. In secretory and menstruating endometrium, immunostaining of Bax decreased, but the relative decrease seemed to be less pronounced than that of Bcl-2 (Table 1). In the stroma Bax was also found at all phases of the cycle. In proliferative endometrium, stromal Bax immunostaining was more intense than in the secretory

or menstruating phases (Table 1). In western analysis of bax the expected 21 kDa protein was observed, and there was a decreasing tendency in the amount of protein detected towards menstruating endometrium (Fig. 6).

3.5. Ki-67

Cell proliferation in the endometrium was studied by immunostaining the endometrial samples for antigen Ki-67, which is a nuclear protein present in cells at the

Table 1
The serum concentrations (mean ± SE) of estradiol and progesterone at different stages of the menstrual cycle and summary of Bcl-2 and Bax immunostaining in endometrial epithelium and stroma[a]

Histology	CD	*S*-Estradiol concentrations	*S*-Progesterone concentrations	*n*	Glandular epithelium		Stroma	
					Bcl-2	Bax	Bcl-2	Bax
EP	3			2	++	+	+	±
	6	0.36 ± 0.23	0.9 ± 0.3	1	++	++	±	±
	9			2	++	++	+	+
LP	10			1	+	+++	+	+
	11			1	−	+++	±	++
	12	0.53 ± 0.08	1.7 ± 0.4	1	++	+++	±	+
	13			2	+++	+	−	+
	14			2	+	+++	+	±
ES	14			2	+/−	+++	+	+
	15	0.27 ± 0.08	18.1 ± 6.3	1	+/−	+++	±	+
	19			2	+/−	+++	±	++
MS	21			1	−	+/−	±	+
	22			1	−	+/−	−	+
	24	0.33 ± 0.07	21.3 ± 4.9	1	−	+	−	±
	25			2	−	+/−	−	±
	26			3	+/−	+	−	−
LS	27	0.24 ± 0.07	15.9 ± 6.9	5	+	++	−	±
	29			2	+/−	++	−	±
ME	2			1	+	++	−	−
	3	0.18 ± 0.04	3.2 ± 2.2	1	+	−	±	±
	4			2	+/−	++	±	±
	5			1	+	+	+	+

[a] The specimens are divided by histology to: early proliferative, EP; late proliferative endometrium, LP; early secretory, ES; middle secretory, MS; late secretory, LS; and menstruating endometrium, ME. CD represent cycle day. Intensity of the staining was assessed as follows: −, no staining; ±, slightly positive; +, positive; ++, strongly positive; and, +++ very strongly positive.

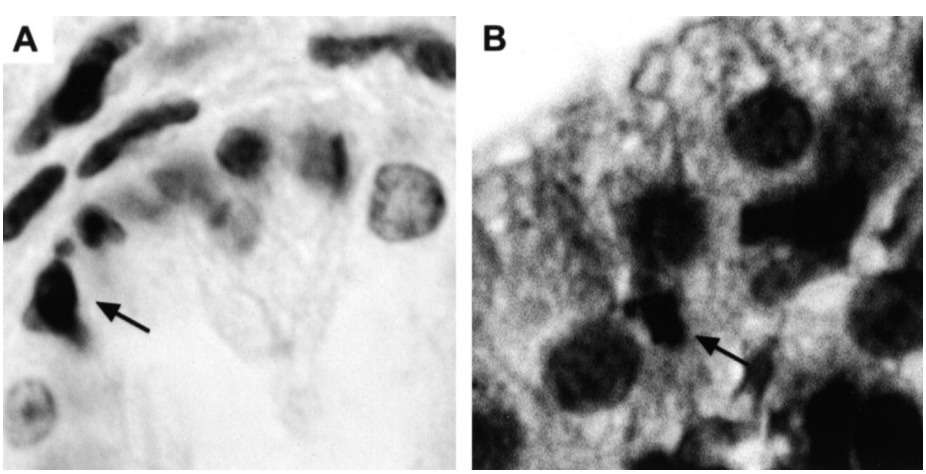

Fig. 1. Demonstration of apoptotic cells in late secretory endometrium using in situ 3′-end labeling technique (A) and hematoxylin and eosin staining (B).

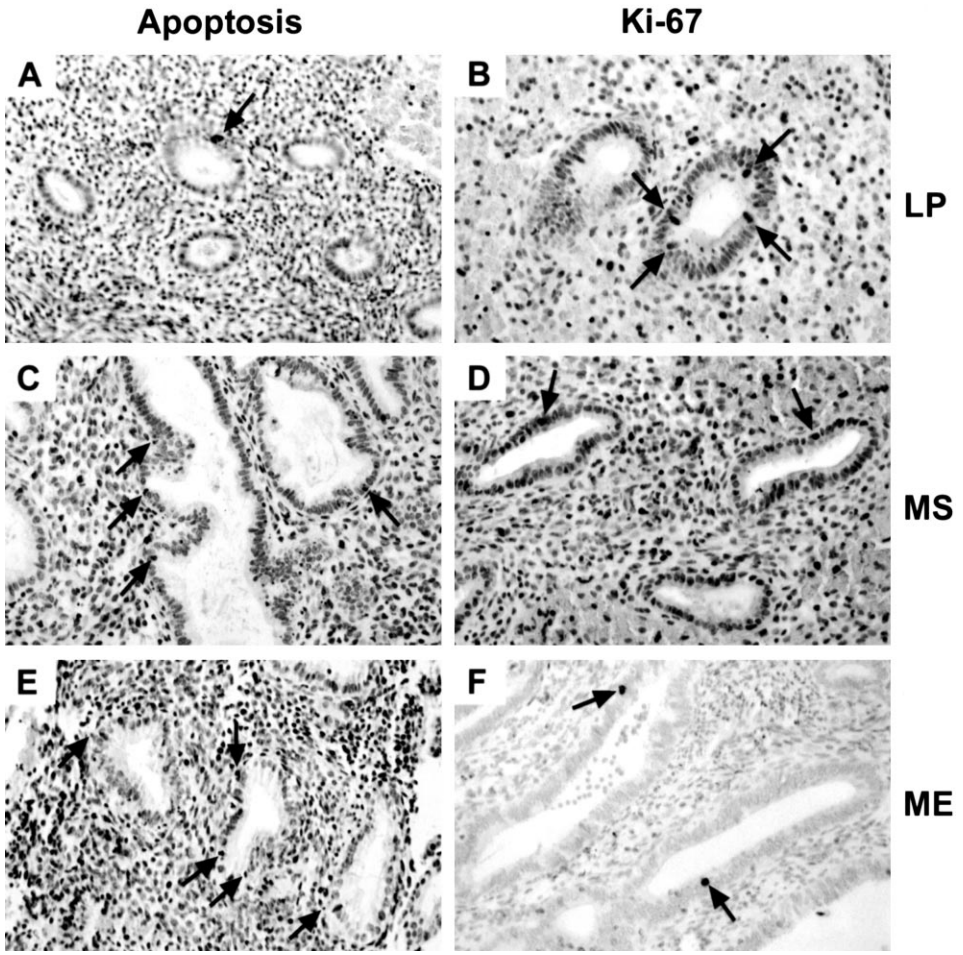

Fig. 2. Analysis of apoptosis by in situ 3′-end labeling and expression of Ki-67 in human endometrium. (A) Only very few apoptotic cells were detected in proliferative phase (arrow). (B) Immunohistochemistry of Ki-67 in proliferative phase demonstrated intense staining in endometrial epithelium and stroma. In secretory endometrium the number of apoptotic cells increased. Most of the apoptotic cells were in glandular epithelium (arrows), while only occasional apoptotic cells were observed in stroma (C). Expression of Ki-67 in the endometrial epithelium decreased clearly in the secretory phase. However, stromal cells remained immunopositive (D). In menstruating endometrium apoptosis was further increased in glandular cells (E) and the number of Ki-67 immunopositive cells was higher than in secretory endometrium but lower than in proliferatory endometrium (F).

replicating phase of the cell cycle. As expected, proliferative endometrium revealed intense staining of Ki-67. Positive cells were found mostly in the glandular epithelium, but also in the stroma (Fig. 2). The number of immunopositive cells diminished towards the secretory phase, and almost disappeared in the late secretory phase. During menstruation, the expression of Ki-67 was also low. The sections showing high intensity of Ki-67 staining clearly showed very little apoptosis (Fig. 3).

4. Discussion

In the present study we demonstrated the cyclic appearance of apoptotic cells in the endometrium by using qualitative in situ 3′-end labeling and DNA fragmentation analysis. Apoptosis was particularly 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. These findings confirm the cyclicity of apoptosis in the endometrium (Kokawa et al., 1996; Tao et al., 1997), and emphasize its possible role in regulating cell turnover and the number of glandular cells that survive to the next menstrual cycle.

Sex steroid hormones control cell proliferation and cell differentiation in endometrial epithelia, and removal of ovarian steroids, mainly demonstrated in animal studies, is known to induce apoptosis in the endometrium (Pecci et al., 1997). Furthermore, the proliferation of endometrial cells in the proliferative phase has been generally related to the action of estrogens, while progesterone is thought to direct the cells into the differentiation pathway, resulting in growth

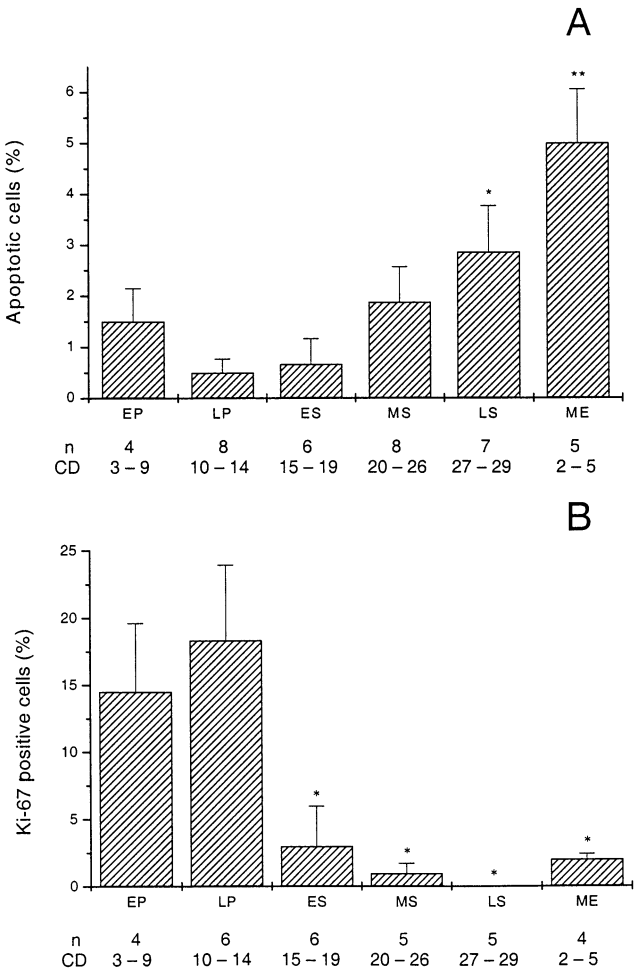

Fig. 3. (A) In situ 3′-end labeling analysis of DNA in glandular epithelium obtained by endometrial curettage. (B) Immunoreactive staining of Ki-67. * $P < 0.05$, ** $P < 0.01$ versus late proliferative phase.

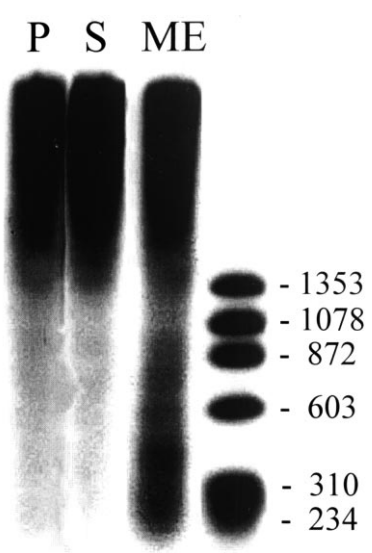

Fig. 4. Apoptotic DNA fragmentation analysis of proliferative (P), secretory (S) and menstrual (ME) endometrium.

arrest. In this study, the pattern of apoptosis and its negative correlation to serum estradiol concentrations in the proliferative phase indicated that, in contrast to many other cell types, proliferating endometrial cells do not undergo significant apoptosis. The signaling mechanisms involved in the regulation of apoptosis by sex steroids are largely unknown, but similar effects of sex steroids on cell survival have been recently reported in other tissues of the reproductive tract, e.g. in the testis (Erkkilä et al., 1997). Cell proliferation, indicated by the replication-related antigen Ki-67, was predominantly found in the proliferative phase. There have been reports concerning an association between Ki-67 expression and apoptosis, suggesting that cells in an active phase, entering the cell cycle, are susceptible to apoptosis. In the endometrium this seems not to be the case. High levels of apoptosis were seen in the menstrual phase, when only a few cells were positive for Ki-67, compared with the proliferative phase where Ki-67 was abundantly present and apoptosis was scarce. This supports the results of previous studies on human endometrium (Coates et al., 1996) and suggests that apoptosis in hormone-dependent cells may not be dependent on cell cycle entry.

The analysis of Bcl-2 and Bax expression demonstrated that changes in the amounts of these two proteins were associated with changes in the extent of apoptosis in glandular cells of the endometrium. As in previous studies (Koh et al., 1995; Tabibzdeh et al., 1995) we found that Bcl-2 is expressed during the proliferative phase but this expression decreases towards the secretory phase and is absent or negligible in midsecretory phase. The expression of Bax also decreased after the proliferative phase, but was still detectable during the entire secretory phase. Thus, the decrease in Bcl-2/Bax ratio especially in the early secretory endometrium preceded increase of apoptosis. The role of ovarian hormones, especially that of estrogens and progesterone, in regulating endometrial expression of Bcl-2 and Bax is not well understood. It has been suggested that estrogens might up-regulate while progesterone might down-regulate Bcl-2 expression (Koh et al., 1995). This is supported by observations in breast cancer cell lines (Formby and Wiley, 1998), in endometrial carcinomas (Saegusa and Okayasu, 1997a,b) and in the secretory endometrium expression of progesterone receptors have been found to negatively correlate with Bcl-2 expression (Dahmoun et al., 1999). Although our results are in line with this theory, more studies are needed to clarify the role of ovarian steroids in Bcl-2 and Bax expression in normal endometrium. Our results, however, support the notion that a decrease in the Bcl-2/Bax ratio would determine the susceptibility to apoptosis (Tilly et al., 1995). Tao et al. (1997) also reported a decrease in the Bcl-2/Bax ratio in the secretory phase of human endometrium. While their

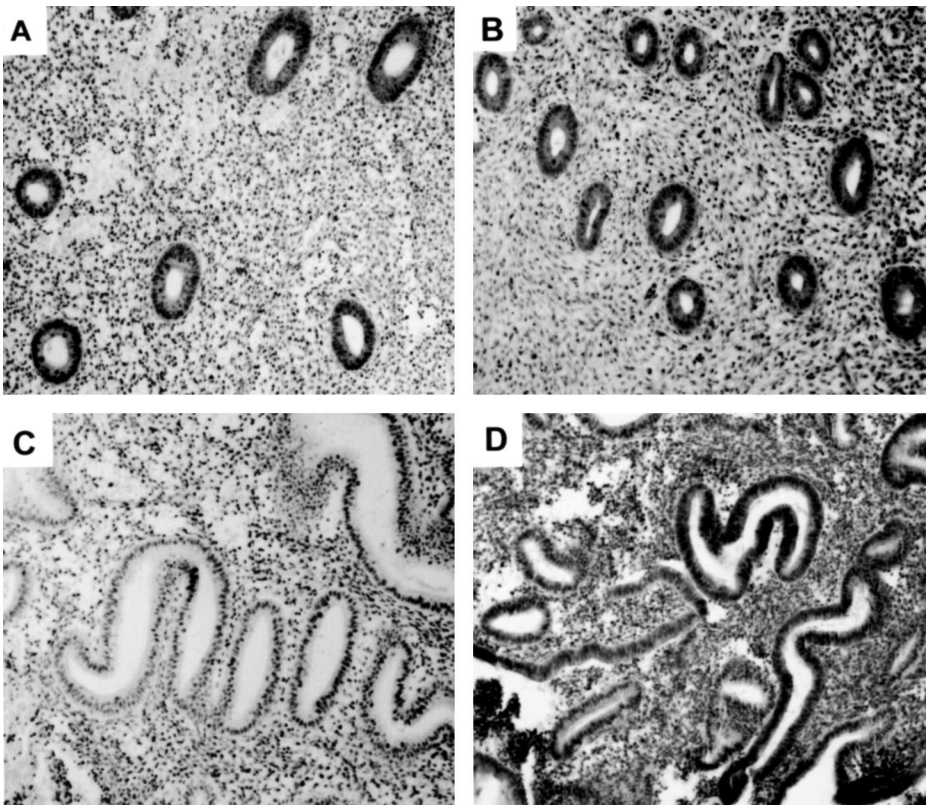

Fig. 5. Expression of apoptosis-related proteins Bcl-2 and Bax in human endometrium obtained from endometrial curettage. Immunostaining of both Bcl-2 (A) and Bax (B) was strong in the early proliferative endometrium and was concentrated predominantly in the glandular endometrium. The expression of Bcl-2 decreased in the early secretory endometrium (C), while the decrease of Bax expression was less prominent (D).

data show an increase in Bax expression during the secretory phase, we found that the immunoreactivity of Bax decreases, but the relative decrease is less prominent than that of Bcl-2. The finding that only a few stray apoptotic cells were observed in endometrial stroma emphasizes the specificity of DNA fragmentation and its hormonal control in glandular epithelium.

It has to be noted that no clear change in the Bcl-2/Bax ratio was observed in the transition from the secretory to the menstrual phase, where the most significant induction of apoptosis occurred. Hence, other members of the Bcl-2 family, such as Bcl-X, Bad and Bak (Yang et al., 1995), may regulate apoptosis and be complementary to the actions of Bcl-2. Bcl-X protein has been shown to be expressed in the endometrium, especially in the secretory phase (Tao et al., 1997). Bcl-X is expressed in two different splicing variants, Bcl-Xlong and Bcl-Xshort. The short form of Bcl-X promotes apoptosis but the long form acts as an anti-apoptotic factor (Boise et al., 1993). Bak protein is also present in the endometrium. It is a promoter of apoptosis and its expression has been shown to increase towards the secretory endometrium (Tao et al. 1998). While the Bcl-2/Bax ratio remains relatively unchanged from the late secretory phase to the menstrual phase, the alterations in Bcl-X and Bak expression might offer an explanation for the increased apoptosis in the menstrual phase. Similarly, the accumulation of Bak in the secretory endometrium offers a likely explantaion. It is also possible that when the threshold for apoptosis is lowered by a decrease in the Bcl-2/Bax ratio at the secretory phase, some other mechanisms can activate apoptotic events in the menstrual phase. Alternatively there are a number of pro- and anti-apoptotic pathways, which may influence the susceptibility of the cells to undergo apoptosis (Vinatier et al., 1996). For instance, cytokines such as tumor necrosis factor alpha (TNF-α) and Fas may play a part in switching on the apoptotic trigger. TNF receptor types I and II, which have been associated with apoptosis (Grell et al., 1994), are expressed in the human endometrium and also the production of TNF-α in human endometrium is phase-specific, with the highest amount of TNF-α being produced in the menstrual phase (Tabibzdeh et al., 1995). Furthermore Fas, a significant pro-apoptotic factor, is also expressed in the human glandular endometrium (Watanabe et al., 1997).

In conclusion, the present results confirm cyclic changes in the levels of Bcl-2 and Bax proteins and their relation to apoptosis of epithelial cells in the human endometrium. Although direct evidence is still lacking, the endometrial expression of Bcl-2 and Bax,

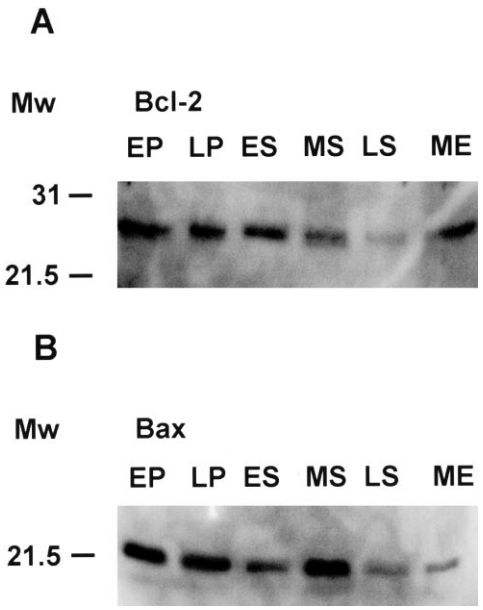

Fig. 6. Western blot analysis of Bcl-2 and Bax proteins in human early proliferatory (EP), late proliferatory (LP), early secretory (ES), mid secretory (MS), late secretory (LS) and menstruating (ME) endometrium. A. Anti-Bcl-2 antibody detected 26 kDa protein band with highest expression in the proliferative phase. B. Bax analysis confirmed that the 21 kDa protein was expressed throughout the cycle.

and the pattern of serum estradiol and progesterone, suggest that these apoptosis-related proteins may be controlled by ovarian hormones. The induction of apoptosis in the secretory phase coincided with a decrease of both Bcl-2 and Bax, but there was also that decrease in the Bcl-2/Bax ratio, which may facilitate endometrial cell turnover and apoptosis, and regulate the number of cells surviving to the next cycle. The low expression of the cell replication-related antigen Ki-67 in the secretory phase and its negative correlation to apoptosis indicate that endometrial cells undergo apoptosis even though they are not entering the cell proliferation cycle.

Acknowledgements

This work was supported by grants from the Sigrid Juselius Foundation and the Academy of Finland.

References

Behringer, R.R., Finegold, M.J., Cate, R.L., 1994. Mullerian-inhibiting substance function during mammalian sexual development. Cell 79, 415–425.

Boise, L.H., Gonzalez-Garcia, M., Postema, C.E., et al., 1993. bcl-x, a bcl-2 related gene that functions as dominant regulator of apoptotic cell death. Cell 74, 597–608.

Brady, H.J., Gil-Gomez, G., 1998. Bax: the pro-apoptotic Bcl-2 family member, Bax. Int. J. Biochem. Cell. Biol. 30, 647–650.

Coates, P.J., Hales, S.A., Hall, P.A., 1996. The association between cell proliferation and apoptosis: studies using the cell cycle-associated proteins Ki67 and DNA polymerase alpha. J. Pathol. 178, 71–77.

Dahmoun, M., Boman, K., Cajander, S., Westin, P., Backstrom, T., 1999. Apoptosis, proliferation, and sex hormone receptors in superficial parts of human endometrium at the end of secretory phase. J. Clin. Endocrinol. Metab. 84, 1737–1743.

Erkkilä, K., Henriksen, K., Hirvonen, V., et al., 1997. Testosterone regulates apoptosis in adult human seminiferous tubules in vitro. J. Clin. Endocrinol. Metab. 82, 2314–2321.

Formby, B., Wiley, T.S., 1998. Progesterone inhibits growth and induces apoptosis in breast cancer cells: inverse effects on Bcl-2 and p53. Ann. Clin. Lab. Sci. 28, 360–369.

Grell, M., Zimmermann, G., Hulser, D., Pfizenmaier, K., Scheurich, P., 1994. TNF receptors TR60 and TR80 can mediate apoptosis via induction of distinct signal pathways. J. Immunol. 153, 1963–1972.

Hanada, M., Aime-Sempe, C., Sato, T., Reed, J.C., 1994. J. Biol. Chem. 270, 11962–11969.

Karhumaa, P., Parkkila, S., Türeci, Ö., et al., 2000. Identification of carbonic anhydrase XII as the membrane isozyme expressed in the normal human endometrial epithelium. Mol. Hum. Reprod. 6, 68–74.

Koh, E.T.A., Illingworth, P.J., Duncan, W.C., Crithley, H.O.D., 1995. Immunolocalization of Bcl-2 protein in human endometrium in the menstrual cycle. Lancet 244, 28–29.

Kokawa, K., Shikone, T., Nakano, R., 1996. Apoptosis in the human uterine endometrium during the menstrual cycle. J. Clin. Endocrinol. Metab. 81, 4144–4147.

Matsuyama, S., Xu, Q., Velours, J., Reed, J.C., 1998. The Mitochondrial F0F1-ATPase proton pump is required for function of the proapoptotic protein Bax in yeast and mammalian cells. Mol. Cell. 1, 327–336.

Oltvai, Z.N., Milliman, C.L., Korsmeyer, S.J., 1993. Bcl-2 heterodimerizes in vivo with a conseved homolog, Bax, that accelerates programmed cell death. Cell 74, 597–608.

Otsuki, Y., Miaski, O., Sugimoto, O., Ito, Y., Tsujimoto, Y., Akao, Y., 1994. Cyclic bcl-2 gene expression in human uterine endometrium during menstrual cycle. Lancet 344, 28–29.

Pecci, A., Scholz, A., Pelster, D., Beato, M., 1997. Progestins prevent apoptosis in a rat endometrial cell line and increase the ratio of bcl-XL to bcl-XS. Biol. Chem. 272, 11791–11798.

Saegusa, M., Okayasu, I., 1997a. Down-regulation of bcl-2 expression is closely related to squamous differentiation and progesterone therapy in endometrial carcinomas. J. Pathol. 182, 429–436.

Saegusa, M., Okayasu, I., 1997b. Down-regulation of bcl-2 expression is closely related to squamous differentiation and progesterone therapy in endometrial carcinomas. J. Pathol. 182, 429–436.

Schwarzman, R.A., Cidlowski, J.A., 1993. Apoptosis the biochemistry and molecular biology of programmed cell death. Endocr. Rev. 14, 133–151.

Sedlak, T.W., Oltwai, Z.N., Yang, E., et al., 1995. Multiple Bcl-2 family members demonstrate selective dimerizations with Bax. Proc. Natl. Acad. Sci. USA 92, 7834–7838.

Tabibzdeh, S., Zupi, E., Bbaknia, A., Liu, R., Marconi, D., Romanini, C., 1995. Site and mentrual cycle-dependent expression of proteins of the tumour necrosis factor (TNF) receptor family, and BCL-2 oncoprotein and phase-spesific production of TNF-α in human endometrium. Hum. Reprod. 10, 277–286.

Tao, X.J., Sayehg, R.A., Tilly, J.L., Isaacson, K.B., 1998. Elevated expression of the proapoptotic BCL-2 family member, BAK, in human endometrium coicident with apoptosis during the secretory phase of the cycle. Fertil. Steril. 70, 338–343.

Tao, X.J., Tilly, K.I., Maravei, D.V., et al., 1997. Differential expression of membranes 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. 8, 2738–2746.

Tilly, J.L., Tilly, K.I., Kenton, M.L., Johnson, A.L., 1995. Expression of members of the bcl-2 gene family in the immature rat ovary: equine chorionic gonadotropin-mediated inhibition of granulosa cell apoptosis is associated with decreased bax and constitutive bcl-3 and bcl-xl messenger ribonucleic acid levels. Endocrinology 136, 232–241.

Tilly, J.L., 1996. Apoptosis and ovarian function. Rev. Reprod. 1, 162–172.

Tsujimoto, Y., Gorham, J., Cossman, J., Jaffe, E., Croce, C.M., 1985. The t(14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining. Science 229, 1390–1393.

Vinatier, D., Dufour, P., Subtil, D., 1996. Apoptosis: a programmed cell death involved in ovarian and uterine physiology. Eur. J. Obstet. Gynecol. Reprod. Biol. 67, 85–102.

Watanabe, H., Kanzaki, H., Narukawa, S., et al., 1997. Bcl-2 and Fas expression in eutopic and ectopic human endometrium during the menstrual cycle in relation to endometrial cell apoptosis. Am. J. Obstet. Gynecol. 176, 360–368.

Yang, E., Zha, J., Jockel, J., Boise, L.H., Thompson, C.B., Korsmeyer, S.J., 1995. Bad a heterodimeric partner for Bcl-xl and Bcl-2, displaces Bax and promotes cell death. Cell 80, 285–291.