

Materials/Methods: Samples fixed in Karnovsky solution were after osmication embedded in Epon 812. Routine electronmicroscopic evaluation was performed. Deepionized semithin sections were used for immunohistochemical processing with M30 CytoDeath detected by antibody labeled by fluorescein (FITC) or alkaline phosphatase (AIP). Biotinylated Annexin-V was applied on separately processed sections and detected with streptavidine labeled by FITC or AIP at the light microscopical level.

Results: The development of endometrium-like structures could be observed only in a minor portion (3 = 10%) of examined collection. Apoptotic markers were identified only sporadically on single cells. All these lesions exhibited structural details characterizing the proliferative phase of the cycle in corresponding manner as samples taken simultaneously from the endometrium. This fact could explain the singularity of signs of cellular degeneration. The majority (18 = 60%) of examined lesions consisted of clusters of cells performing no spatial organization resembling endometrial glands. High prevalence of apoptotic processes was determined especially in cells located in central parts of cell masses. In the rest of samples (9 = 30%) the massive hemorrhage substantially influenced the spatial arrangement and cellular structure of samples, complicating the evaluation of immunohistochemical tests. It was not possible to distinguish exactly if the massive destruction of tissue was caused by primary or secondary necrosis or apoptosis.

Conclusions: High prevalence of apoptotic process in endometriotic lesions is documented in our study. This process has been found both in the eutopic endometrium and in endometriotic lesions but in different prevalences. Findings of endometriotic lesions resembling the structure of typical endometrium favor the implantation theory of accidentally inoculated endometrial epithelium extra situm. The reason for different response to the same stimuli of eutopic endometrium and endometriotic lesions remains unexplained.

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Expression of apoptosis related genes in uterine endometrium from women with and without endometriosis. Jianchi Ding, Xiulong Xu, Mingxian Shen, Nasir Rana, Donald P. Braun, W. Paul Dmowski. Institute for the Study and Treatment of Endometriosis, Oak Brook, IL; Institute for the Study and Treatment of Endometriosis and Rush Medical Coll, Chicago, IL; Institute for the Study and Treatment of Endometriosis and Medical Coll of Ohio, Oak Brook, IL; Institute for the Study and Treatment of Endometriosis and Rush Medical Coll, Oak Brook, IL.

Objective: We have demonstrated previously that spontaneous apoptosis and immune-mediated lysis is reduced compared to normal in endometrial cells (EC) from women with endometriosis. We hypothesize that this condition favors EC survival outside of the uterine cavity. Nevertheless, reasons for the resistance of EC to apoptosis and immune-mediated lysis have yet to be elucidated. In the present study, we investigated expression of apoptosis related genes in eutopic EC from women with and without endometriosis.

Design: mRNA levels in uterine EC from women evaluated for endometriosis using RNase protection assay (RPA) to quantitate 11 apoptosis related genes.

Materials/Methods: Endometrial specimens were obtained from women with (n=11) and without (n=12) endometriosis at the time of laparoscopy. The populations were comparable with respect to phase of the menstrual cycle at which specimens were collected. Samples were treated with collagenase/DNase to obtain single cell suspensions, following which, EC were extracted with RNazol-B to obtain cellular RNA. The Multi-Probe RNase Protection Assay Kit, hAPO-3 from PharMingen, was used to detect expression of Caspase-8, Fas Ligand, Fas, FADD, DR3, FAP, FAF, TRAIL, TNF Receptor type-I, TRADD, and RIP. GAPDH and L32 were used as reference genes for semi-quantitative comparison.

Results: Values for individual genes varied amongst the 2 study populations and within each study population. There was no discernible pattern of expression for most genes attributable to either the presence of endometriosis or the phase of the menstrual cycle. Because the number of specimens in each group was limited, it was not surprising that statistically significant differences in gene expression were not seen when data were grouped. Nevertheless, it was noted that the expression of the gene for the cell death receptor, FAS, was decreased by about 20% in EC from women with endometriosis compared to the expression in EC from controls (range of values was 0.6%-3.5% for endometriosis vs 0.7%-6.1% for controls). Sim-

ilarly, the expression of the gene for the apoptosis-inducing protein, FAS-ligand, was increased by about 50% in EC from women with endometriosis compared to controls (range of values was 0.3%-3.4% for endometriosis vs 0.4%-1.1% for controls). Differences in expression of genes for other cell death-associated receptors were not apparent in these populations.

Conclusions: These results suggest that the expression of cell death associated receptors and ligands related to FAS may be differentially regulated on EC from women with and without endometriosis. The observed reduction of FAS gene expression and increase of FAS ligand gene expression in EC from some women with endometriosis is consistent with greater resistance to immunologically-mediated killing. This phenomenon might contribute to the resistance of ectopic EC from women with endometriosis to peritoneal macrophage-mediated cytolysis which we have reported previously. A similar observation has been made recently for tumor cells and has been invoked as 1 mechanism by which tumors escape immune surveillance. Demonstration of a comparable mechanism for escape of ectopic EC in endometriosis may provide guidance for development of immunologic approaches for management of this disease.

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Macrophage migration inhibitory factor expression in the human endometrium is cycle phase-dependent and markedly increased in women having endometriosis. Ali Akoum, Rouslan Kats, Christine Lawson, Christine Metz. Ctr de Research HSFA-CHUQ, Faculty of Medicine, Laval Univ, Quebec, QC, Canada; North Shore-LIJ Research Institute, Manhasset, NY.

Objective: Our previous studies have identified macrophage migration inhibitory factor (MIF) as one of the principal bioactive molecules involved in endothelial cell proliferation released by ectopic endometrial cells. We also found MIF to be produced locally within endometrial implants, particularly in those which are highly vascularized and representing the earliest and most active forms of the disease. This is consistent with recent data showing an important role for MIF in tumor growth-associated angiogenesis in vivo. The objective of the present study was to investigate MIF expression in the endometrial tissue of normal women throughout the menstrual cycle, and to assess whether any difference in MIF expression could be found between normal women and women suffering from endometriosis.

Design: Retrospective study using frozen endometrial tissue from women with and without endometriosis.

Materials/Methods: Endometrial tissue specimens were obtained from 45 women having endometriosis and 55 normal fertile women having no evidence of endometriosis at laparoscopy. MIF expression was assessed by immunohistochemistry, dual immunofluorescence, Western blot and ELISA for the protein, and by northern blot for the mRNA.

Results: Immunohistochemical and dual immunofluorescence analyses showed that MIF was expressed throughout endometrial tissue, particularly in the glands, and identified endothelial cells and macrophages as cells markedly expressing MIF in the stroma. Western blot analysis showed a single 12.5 kDa band corresponding to the known molecular weight of the molecule. MIF protein and mRNA expression followed a regulated cycle phase-dependent pattern. Being elevated in the late-proliferative/early secretory phase of the menstrual cycle, MIF expression decreased in the mid-secretory phase before augmenting markedly again during the late secretory phase. Furthermore, higher expression of MIF was found in women with endometriosis as compared to normal women (p < 0.0001).

Conclusions: In view of its potent proinflammatory and angiogenic properties, our findings make plausible the involvement of MIF in the cyclic physiological changes occurring in the endometrial tissue during the menstrual cycle, and point toward a significant role for this factor in the pathophysiology of endometriosis.

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Elevated leptin levels in the peritoneal fluid of women with endometriosis. Neal G. Mahutte, Ioannis M. Matalliotakis, Anastasia G. Goumenou, Aydin Arici. Yale Univ, New Haven, CT.