IMMUNOLOGY AND MICROBIOLOGY

Apoptosis in Fimbriae of Fallopian Tubes and Endometrium in Pyoinflammatory Adnexal Diseases

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In patients with pyoinflammatory adnexal diseases moderate or intense DNA degradation was observed in the majority of epithelial, stromal, and inflammatory infiltration cell in inflammatory foci in the fimbrial compartment of fallopian tubes without signs of tissue destruction. Expression of *TNFR1* gene increased 2.7-fold and expression of *Fas* gene decreased 3.1-fold compared to intact endosalpinx, which indicates induction of apoptosis triggered by tumor necrosis factor-α and inhibition of the *Fas*-dependent pathway. No signs of apoptosis were detected in the endometrium. Generalized apoptosis in the fimbrial compartment of the tubes can be a mechanism limiting the inflammatory process.

Key Words: *apoptosis; inflammation of uterine appendages; cytokines; apoptosis inductors*

Apoptosis is involved in many physiological and pathophysiological processes associated with embryogenesis, atrophy, and immune reactions. Apoptosis during cell growth transformation and inflammation is regulated by numerous factors, *e.g.* cytokines and growth factors. The effect of cytokines is tissue-specific [8-10,13].

Apoptosis of epitheliocytes, lymphocytes, macrophages, and other cells involved in acute local inflammation is induced by cytokines with different properties: antiinflammatory tumor necrosis factor-α (TNF-α), neutrophil chemotaxis factor interleukin-8 (IL-8), type Th2 lymphocyte activator IL-4, transforming growth factor-β (TGF-β), and antiinflammatory IL-10 [3,5,7,8,12,13,15]. The interactions of TNF-α and Fas ligand with molecules of the TNF receptor family (TNFR, including TNFR1, TNFR2, Fas) directly triggers the cascade of intracellular reactions leading to death and elimination of different types of cells, including epithelial and inflammatory infiltration cells [3,11,14].

Disorders in the female reproductive function in pyoinflammatory adnexal diseases are largely determined by the severity of inflammation and the possibility of its localization, in particular through apoptosis of defective and activated cells. We previously demonstrated enhanced expression of IL-4, IL-6, IL-8, and TNF-β_2 genes in specimens from the fimbrial compartment of fallopian tubes (FCFT) and of IL-10 gene in the endometrium in patients with adnexal diseases [1]. Here we studied the intensity and role of apoptosis in the development of these diseases and evaluated local expression of genes encoding apoptosis inductors Fas, Fas ligand, TNFR1, and TNFR2.

MATERIALS AND METHODS

Apoptosis in FCFT and endometrium was evaluated in 14 patients (5 with tubo-ovarian tumors, 4 with ovarian abscesses, and 5 with pyosalpinx, mean age 32.3±3.8 years). The reference group (7 patients, mean

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age 35.7±4.1 years) included patients with uterine myoma. Extirpation of the uterus with or without tubes, supravaginal amputation of the uterus with or without tubes, or removal of the adnexa were carried out. Tissue fragments (100-150 mg) were collected in the middle of the proliferative stage of the menstrual cycle.

The specimens were fixed in 4% formaldehyde buffered with phosphate saline for 24 h and embedded in histoplast (Shandon); 5-μ sections were fixed on slides treated with 0.1% polylysine (Sigma).

Gene expression in FCFT was evaluated in 9 patients. FCFT fragments (50-100 mg) were frozen in liquid nitrogen immediately after surgery and stored for up to 6 months.

The TUNEL method was previously described in detail [2]. In this study we used this method with minor modifications.

Deparaffinated sections were treated with proteinase K (Amresco, 25 μg/ml) for 30 min at 20°C. After inhibition of endogenous peroxidases and biotin, DNA was labeled with terminal deoxynucleotidil transferase (8 U, Sigma) and 0.15 nmol biotin-16-dATP (Sigma). The sections were incubated with avidin-horseradish peroxidase conjugate (Sigma, 0.5 μg/ml) and stained with diaminobenzidine (DAKO) in phosphate saline.

The results were analyzed under an Axiovert 100 microscope (Zeiss). The staining of the nuclei was estimated as weak, moderate, or intense.

Isolation and DNAse treatment, reverse transcription, and PCR were carried out as described previously [1] using TRI REAGENTd (Sigma), DNAse RQ1 (Promega), MMLV reverse transcriptase (Promega), hexarandom- and oligo-dT primers. PCR was carried out in the Tertsik thermocycler (DNA Tekhnologiya) with primers presented in Table 1. The results of PCR were analyzed using a Gel Doc 1000 system (BioRad) [1].

RESULTS

Exacerbation of chronic inflammatory process in the adnexa uteri (*e.g.* tubo-ovarian abscess or pyosalpinx) produces considerable damages to the studied tissues. Analysis of tissue specimens showed deep morphological changes, pronounced lymphoid infiltration with admixture of neutrophilic leukocytes. The stain was found not only in cell nuclei (50-100% cells were stained with different intensity), but was distributed diffusely, which indicated the presence of genome DNA fragments outside the nuclei in fibrous and muscular tissues. We could not regard these findings as an evidence of apoptosis in foci of tissue destruction; moreover, these changes rather looked like necrosis. It was therefore interesting to evaluate the intensity of apoptosis in inflammation foci in FCFT without signs of tissue degradation.

The intensity of apoptosis in these foci was very high (Fig. 1). Up to 100% cells in the endosalpinx epithelium and stroma, including cells of the inflammatory infiltration, had moderately or intensely stained nuclei, which indicated activation of programmed cell death in the inflammation focus. No signs of apoptosis were seen in the endometrium.

Quantitative processing of RT-PCR results showed that expression of *TNFR1* gene in inflammation foci in FCFT increased 2.7-fold compared to normal ($p<0.01$), expression of Fas gene decreased 3.1-fold ($p<0.01$; Fig. 2). Expression of *TNFR2* and *FasL* genes was low in both the main and reference groups.

The processes of inflammation and apoptosis are closely related, and many factors playing the key role in the development of inflammatory reaction (IL-4, IL-6, IL-8, IL-10, TGF-β) are involved in the regulation of apoptosis in lymphocytes, fibroblasts, neutrophils, and epitheliocytes [8,12,13,15]. Induction of apoptosis by cytokines is tissue-specific [11]: IL-10 and TGF-β activate apoptosis in the gastrointestinal, endometrial, and mammary gland epithelium [5,15], IL-8 and IL-10 are positive apoptosis regulators for infiltration lymphocytes [12,13].

Generalized apoptosis observed in tissues adjacent to the zone of destructive changes involved not only epithelial and stromal cells, but also infiltration cells (macrophages, granulocytes, lymphocytes). Our previous findings indicate enhanced expression of

TABLE 1. Primers

mRNA	Nucleotide sequence	Fragment length, b. p.
TNFR1	5'-TCTAAGGACCGTCCTGCGAGATCGCCTT	
	5'-AAACGGGCATGAGGCATAGCGTCCCTCA	247
TNFR2	5'-GCTCCTGGAAAGGCTCAGTCTCAG	
	5'-CTGCCCTGTGATGCCAAGGAAGCC	479
FasL	5'-CACCACTGCCTCCACTACCGCTG	
	5'-CAGAGGCATGGACCTTGAGTTGGAC	299
Fas	5'-AGACTGCGTGCCCTGCCAAG	
	5'-AGAAGACAAAGCCACCCCAAGTTAGA	191

TGF-β, IL-4, IL-6, IL-8, and IL-10 genes in the focus of inflammation [1], and intense DNA fragmentation in FCFT tissues could be due to enhanced expression of IL-10, IL-8 genes (apoptosis of inflammatory infiltration cells) and TGF-β_2 gene (epithelial cell apoptosis). Expression of TNF-α, a Th1 proinflammatory cytokine and a possible candidate for apoptosis inductor during the development of inflammatory process, did not differ from normal. It is known that the interaction of TNF-α with TNFR1 is a signal directly triggering apoptosis due to the presence of the "death domain" in the receptor. Activation of TNFR1 is a key event triggering apoptosis in many cell types, including epithelial and immune cells. The intensity of apoptosis can be regulated by modulation of TNF-α receptor gene expression [3,14]. Moreover, secondary induction of apoptosis through TNFR1 was demonstrated: interaction between TNF-α and TNFR1 can lead to increase of Fas ligand production in epithelial cells and hence, to activation of Fas-mediated programmed cells death [11].

We hypothesized that high level of apoptosis in FCUT can be associated with changes in expression of TNFR1, TNFR2, Fas ligand, and Fas. The detected regularities indicate induction of TNF-α-dependent pathway of apoptosis in inflammation foci in FCFT, while Fas-dependent pathway of cell elimination is inhibited. IL-4, IL-6, and IL-8 inhibit apoptosis in epithelial and tumor cells and thus exhibit a cytoprotective effect [9,10,13]; our previous studies demonstrated enhanced local expression of these cytokines in FCFT [1]. The role of these cytokines in pyoinflammatory adnexal diseases consists, among other things, in limitation of the apoptosis zone. Interestingly, the interaction between TNF-α and receptors not only triggers apoptosis, but also promotes production of IL-6 and IL-8 inhibiting apoptosis in epithelial cells [6].

Hence, changes in the expression of gene encoding cytokines and other factors involved in the regulation of apoptosis can promote the development of generalized apoptosis in the focus of inflammation, on the one hand, and restrict this process, on the other.

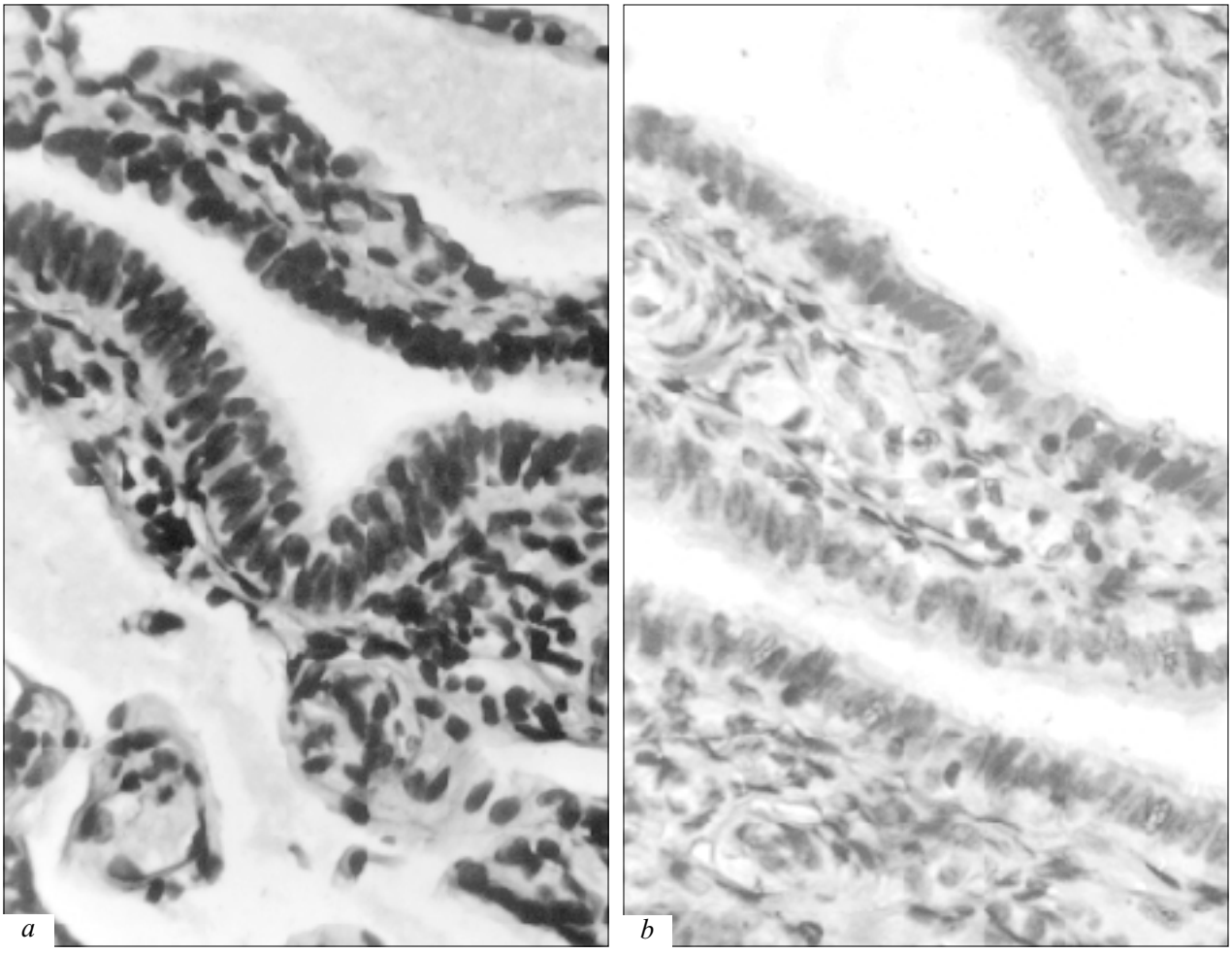

Fig. 1. Activation of programmed cell death in fimbrial compartment of the uterine tubes during inflammation (staining of the nuclei with fragmented DNA by the TUNEL method). *a*) moderate and intense staining of nuclei in virtually all cells; *b*) negative control.

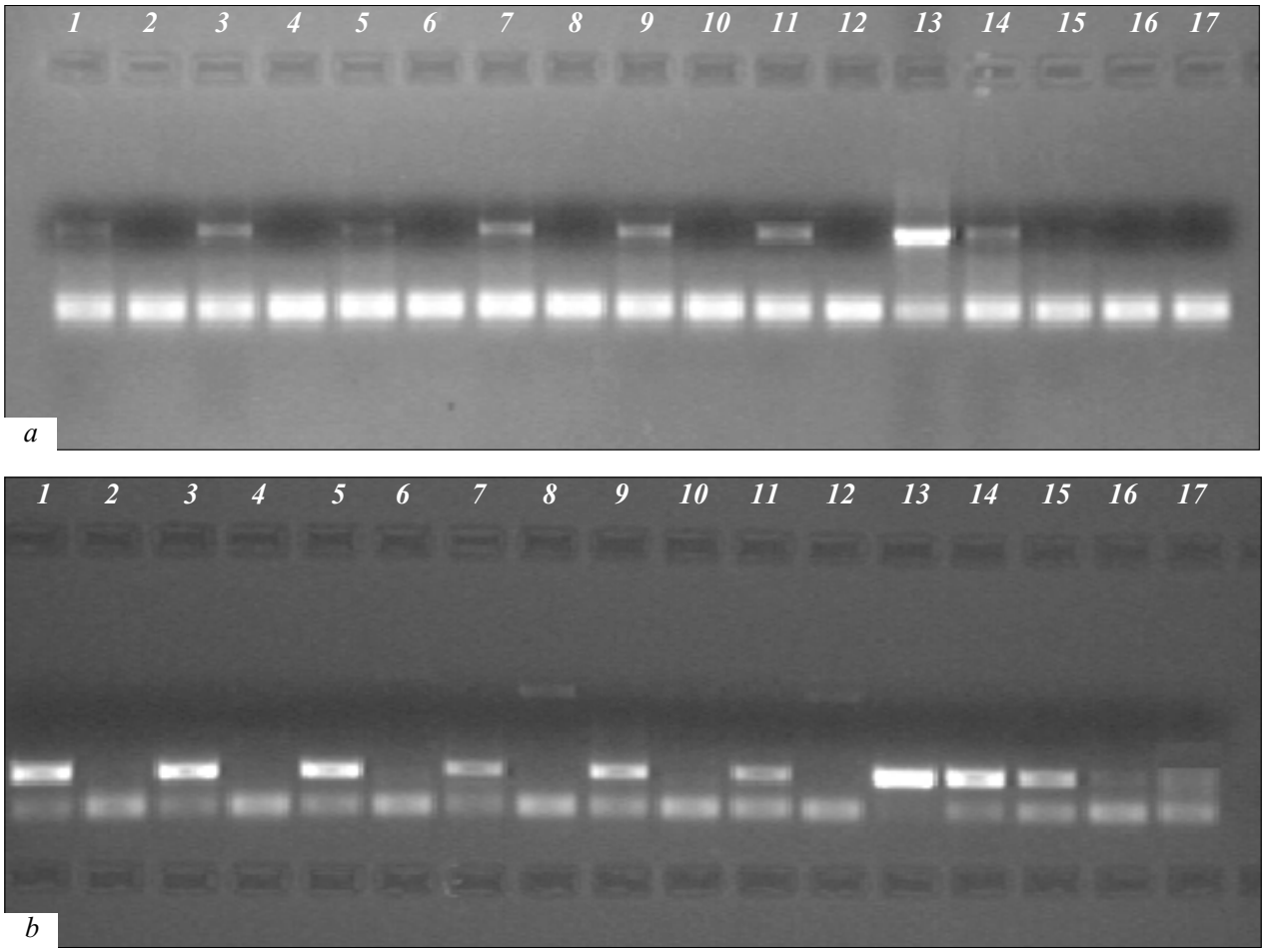

Fig. 2. Local expression of *TNFR1* (*a*) and *Fas* (*b*) genes in pyoinflammatory diseases of uterine adnexa (electrophoregram of PCR products). *1-6*) reference group; *7-12*) patients; even numbers: negative control of reverse transcriptase; *13-17*) calibration 10-fold dilutions of control RNA.

Due to multicomponent regulation of apoptosis the organism can use it as a fine instrument preventing excessive damage to tissues.

REFERENCES

1. A. V. Zhdanov, L. Yu. Sosulina, D. F. Kurbanova, *et al.*, *Byull. Eksp. Biol. Med.*, **134**, No. 11, 555-559 (2002).
2. S. I. Rogovskaya, G. T. Sukhikh, A. V. Zhdanov, *et al.*, *Ibid.*, **131**, No. 6, 576-582 (2001).
3. M. P. de Miguel, M. Royuela, F. R. Bethencourt, *et al.*, *Cytokine*, **12**, No. 5, 535-538 (2000).
4. N. Dunker and G. Aumuller, *J. Anat.*, **201**, Pt. 2, 173-183 (2002).
5. N. Dunker, K. Schmitt, N. Schuster, and K. Krieglstein, *Gastroenterology*, **122**, No. 5, 1364-1375 (2002).
6. N. J. Gallagher, A. G. Eliopoulos, A. Agathangelo, *et al.*, *Mol. Pathol.*, **55**, No. 2, 110-120 (2002).
7. P. Golstein, D. M. Ojcius, and J. D. Young, *Immunol. Rev.*, **121**, 29-65 (1991).
8. Y. W. Lee, H. Kuhn, B. Hennig, and M. Toborek, *FEBS Lett.*, **485**, Nos. 2-3, 122-126 (2000).
9. M. T. Lin, C. Y. Juan, K. J. Chang, *et al.*, *Carcinogenesis*, **22**, No. 12, 1947-1953 (2001).
10. S. K. Manna and B. B. Agarwal, *J. Biol. Chem.*, **273**, No. 50, 33,333-33,341 (1998).
11. M. J. Pinkoski, N. M. Droin, and D. R. Green, *Ibid.*, **277**, No. 44, 42,380-42,385 (2002).
12. I. Saito, *Crit. Rev. Immunol.*, **20**, No. 2, 153-165 (2000).
13. B. Selam, U. A. Kayisli, J. A. Garcia-Velasco, *et al.*, *J. Clin. Endocrinol. Metab.*, **87**, No. 8, 3921-3927 (2002).
14. K. Smolnikar, S. Loffek, T. Schultz, *et al.*, *Breast Cancer Res. Treat.*, **63**, No. 3, 249-259 (2000).
15. B. H. Sohn, H. B. Moon, T. Y. Kim, *et al.*, *Immunology*, **169**, No. 8, 4288-4297 (2002).