EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Local Expression of Cytokine Genes in Uterine Adnexa and Endometrium of Women with Pyoinflammatory Adnexal Diseases

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Changes in the local expression of IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-15, IL-18, TNF-α, IFN-γ- and TGF-β_2 genes in the uterine adnexa and endometrium were studied in women with pyoinflammatory adnexal diseases. Examination of tissue specimens from the uterine adnexa involved in inflammation revealed a direct correlation in the levels of mRNA production between IL-6 and IL-10 (r=0.93, p<0.1), IL-6 and IL-4 (r=0.96, p<0.01), IL-10 and IL-4 (r=0.91, p<0.01), IL-12 and IFN-γ (r=0.98, p<0.01). Expression of IL-4 gene increased 5.1-fold (p=0.001), IL-6 2-fold (p=0.007), IL-8 90.2-fold (p=0.009), IL-10 2.9-fold (p=0.008), IL-12 2.3-fold (p=0.3), and TGF-β_2 gene 10.3-fold (p=0.1). In the endometrium of women with pyoinflammatory adnexal diseases only IL-10 gene expression increased (15.6-fold, p=0.007).

Key Words: *mRNA; cytokines; inflammation of uterine adnexa; endometrium*

Pyoinflammatory diseases of the uterine adnexa (PIDUA) are a prevalent cause of disorders in the female reproductive function [4]. The intensity of local production of cytokines in PIDUA is poorly studied, and we can only hypothesize it from the data on cytokine gene expression in inflammatory diseases of other viscera. It is known that acute inflammation in abdominal organs is associated with increased production of IL-1 by polymorphonuclear lymphocytes [13]. Cytokines IL-1 and TNF-α induce production of chemokines IL-8, MCP-1, and RANTES by mesothelial peritoneal cells, which can promote leukocyte migration into the abdominal cavity [10]. A protective role of IL-10 in the development of peritoneal inflammation in rats was reported [12].

When inflammation is life threatening, the uterine adnexa are removed. However the technology of extracorporeal fertilization allows childbirth in women with a history of adnexectomy. The readiness of the endometrium to implantation of a fertilized oocyte is an obligatory condition for normal gestation. Expression of cytokine genes plays an important role in this process. The prerequisites of effective implantation and development of the embryo are enhanced production of proinflammatory cytokines (IL-10) causing local immunosuppression and of Th cytokines (IL-4, IL-6) promoting production of specific immunoglobulins masking the trophoblast antigens and preventing their recognition by type 1 T helpers (Th1) [11]. The effects of PIDUA on expression of cytokine genes

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in the endometrium remain little studied. Investigation of cytokine profiles in patients with PIDUA can help to evaluate the possibility of recovery of reproductive function after removal of the fallopian tubes.

Here we studied expression of some pro- and anti-inflammatory cytokines and factors of cell growth, differentiation, and chemotaxis in the fimbrial compartment of the fallopian tube (FCFT) and endometrium of patients with PIDUA.

MATERIALS AND METHODS

Local expression of cytokine genes was evaluated in 7 patients with PIDUA (main group, mean age 33.7±4.1 years). Control group consisted of 7 women without clinical signs of inflammatory adnexal diseases (mean age 32.5±3.6 years). Fragments of FCUT and endometrium (100-1560 mg) collected during surgery were immediately frozen in liquid nitrogen.

RNA isolation and DNAse treatment, reverse transcription and polymerase chain reaction were carried out as described previously [1] using TRI REAGENTd (Sigma), DNAse RQ1 (Promega), MMLV reverse transcriptase (Promega), hexarandome- and oligo-dT primers. PCR was carried out in PCR Express (Hybaid) and Tertsik thermocyclers (DNA-Tekhnologiya). PCR products were analyzed by electrophoresis and documented using Gel Doc 1000 system (Bio-Rad).

Quantitative analysis and statistical processing of the results were carried out as described previously [3].

RESULTS

According to gel electrophoresis of PCR products, the increase in the expression of IL-8 and TGF-β_2 cDNA in FCUT involved in inflammation was most pronounced (Fig. 1, *a*, *b*). Local expression of the following cytokine genes significantly increased in comparison

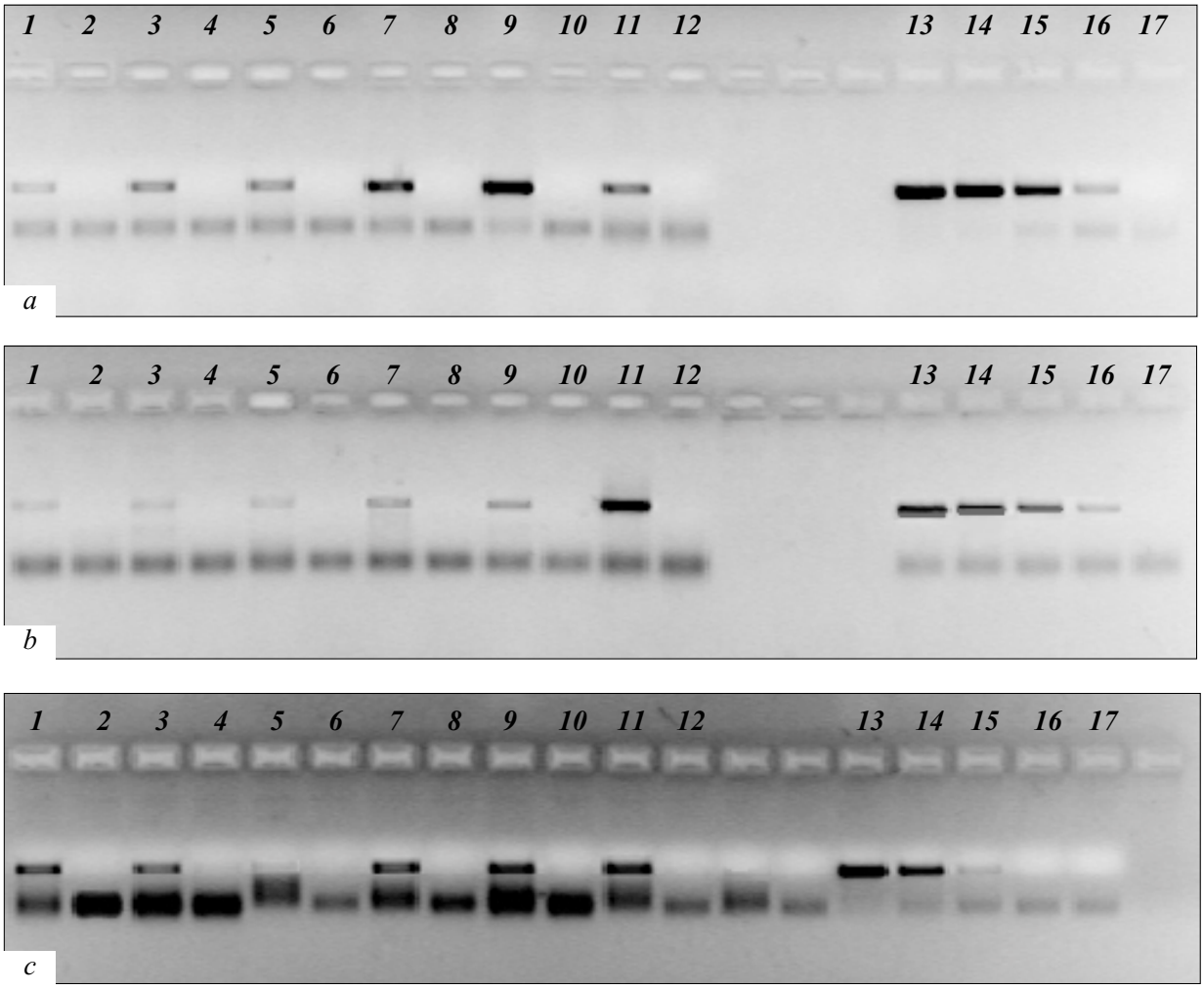

Fig. 1. Local expression of IL-8 (*a*), TGF-β_2 (*b*), and IL-10 (*c*) genes in pyoinflammatory diseases of the uterine adnexa (electrophoregram of PCR products). *a*, *b*) fimbrial compartment of uterine tubes; *c*) endometrium. *1-6*) control; *7-12*) patients with pyoinflammatory diseases of uterine tubes (PIDUA); even numbers: negative control for reverse transcriptase; *13-17*) calibration dilutions of control RNA.

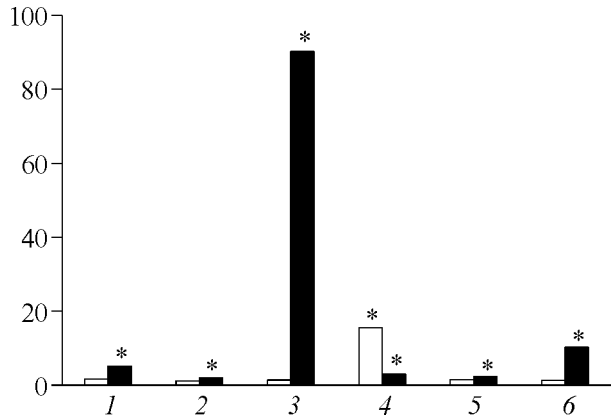

Fig. 2. Expression of cytokine genes in uterine adnexa and endometrium of patients with PIDUA. Light bars: endometrium; dark bars: uterine adnexa. *1*) IL-4; *2*) IL-6; *3*) IL-8; *4*) IL-10; *5*) IL-12; *6*) TGF-β_2. **p*<0.05 compared to normal.

with the control: IL-4 (5.1-fold p=0.001), IL-6 (2-fold, p=0.007), IL-8 (90.2-fold, p=0.009), IL-10 (2.9-fold, p=0.008), IL-12 (2.3-fold, p=0.3), TGF-β_2 (10.3-fold p=0.1) (Fig. 2). The levels of IL-1β, TNF-α, and IFN-γ mRNA in the studied samples tended to increase; the content of IL-2 and IL-18 mRNA did not differ from normal.

Analysis of endometrial samples from patients with PIDUA showed a significant increase in IL-10 mRNA production (15.6-fold, p=0.007) (Fig. 1, *c*; Fig. 2) and slightly increased expression of IL-1β, IL-6, and IL-8 genes. The levels of IL-2, IL-4, IL-12, IL-18, TNF-α, IFN-γ, and TGF-β_2 did not differ from normal.

Analysis of correlations revealed a relationship between changes in the expression of some cytokine genes in FCFT. Significant relationships between the levels of IL-6 and IL-10 (r=0.93, p<0.1), IL-6 and IL-4 (r=0.96, p<0.01), IL-10 and IL-4 (r=0.91, p<0.01), IL-12 and IFN-γ mRNA production (r=0.98, p<0.01) were detected. A trend to inverse correlation between the levels of mRNA production was observed for IL-8 and IL-12 (r=-0.52) and IL-8 and TGF-β_2 (r=-0.53).

These findings suggest that acute inflammation in the uterine adnexa is associated with increased production of Th2 cytokines (IL-4, IL-6, IL-10), while expression of Th1 cytokine genes (TNF-α, IL-1β, IFN-γ) did not differ from normal. At the same time, production of IL-12 mRNA (inductor of Th1 cytokine gene expression) increased in inflamed adnexa. Presumably, the role of this cytokine in activation of Th1 component of the cytokine cascade is leveled by the effects of IL-10 and IL-4 [8], because IL-12 stimulates production of antiinflammatory cytokine IL-10, which suppresses its own synthesis and the synthesis of Th1 cytokines, while IL-4 activates the expression of Th2 cytokine genes [14].

The expression of TGF-β_2 gene is essential for the development of PIDUA. According to published reports, TGF-β cytokines realize the reparative function during inflammation or other tissue injuries [5], which leads, among other things, to the formation of adhesions. We previously showed that expression of TGF-β_2 increased several times in tubal adhesions in comparison with intact tissues [2]. Considerably increased production of TGF-β_2 mRNA in PIDUA attests to activation of repair processes associated with acute inflammation in FCFT. In addition, TGF-β exhibits immunosuppressive activity [8] and activation of its production, together with increased expression of IL-10 gene, can be an element of local antiinflammatory reaction of the immune system.

Neutrophil attraction factor IL-8 is a proinflammatory cytokine, whose expression can be negatively regulated by TGF-β_2 and IL-10 [6,15]. However production of IL-8 is not inhibited (according to our data, the content of IL-8 mRNA in inflamed FCFT 90-fold surpassed the normal).

The increase in local expression of IL-10 gene in the endometrium in PIDUA can be regarded as a preventive reaction protecting the endometrium from inflammation and maintaining its readiness to implantation of a fertilized oocyte. However, increased production of IL-1β, IL-6, and IL-8 mRNA indicates that the immunological balance in the endometrium is unstable.

Hence, suppurative inflammation of the uterine adnexa is associated with migration of neutrophils into the inflammation focus, activation of local production of Th2 cytokines and reparative processes in damaged tissue. Acute inflammation in the uterine adnexa affects the endometrium: the expression of antiinflammatory IL-10 gene in the endometrium markedly increased, presumably in order to prevent inflammatory changes in the endometrium. It seems that normalization of cytokine production and realization of the reproductive function require elimination of the focus of suppurative inflammation in the uterine adnexa.

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