Expression of Cytokine Genes in Adhesions on Uterine Tubes

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We studied expression of genes of interleukins, interferon-γ, tumor necrosis factor-α, and transforming growth factor-β_2 in adhesions on uterine tubes. In tubal adhesions the intensity of production of mRNA for proinflammatory cytokines, antiinflammatory cytokine interleukin-10, and regulator of cell proliferation surpassed that in normal tissues by 2.5-7.4, 2.2, and 50.2 times, respectively. Correlations were found between production of mRNA for tumor necrosis factor-α and transforming growth factor-β_2, interleukin-12, and interferon-γ and interleukin-12 and transforming growth factor-β_2. Our results suggest that expression of these genes during adhesion formation is regulated by the feedback mechanism.

Key Words: *mRNA; cytokines; tubal adhesions*

Tubal and peritoneal factors are the major causes of female infertility [8]. The formation of pelvic adhesions is associated with inflammation produced by surgery or sexually transmitted infections [3].

The mechanisms underlying regulation of the immune system during adhesion formation remain unknown. Changes in cytokine production in tubal adhesions were studied during adhesion formation in other abdominal organs. Published data show that proinflammatory cytokines are involved in the formation of peritoneal adhesions. Direct measurements of interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) concentrations in the peritoneal fluid from patients with adhesions on pelvic organs [7] and prevention of adhesion formation in the peritoneum of rats with neutralizing antibodies against IL-1 and TNF-α [10] indicate that these cytokines play a role in adhesion. In tissues of adhesions fibroblasts produce the antiinflammatory cytokine IL-10 more intensively than normal peritoneal fibroblasts [14]. The concentration of chemokine IL-8 in the peritoneal fluid increases by many times in patients with perforating appendicitis, which promotes migration of neutrophils into the focus of inflammatory adhesions [15]. There are contradictory data on the role of IL-6 and interferon-γ (IFN-γ) in adhesion formation [6,7,14]. The involvement of growth factors belonging to the family of transforming growth factor-β (TGF-β, particularly TGF-β_1) in adhesion formation was extensively studied. The increased local expression of TGF-β_1 is associated with increased incidence of peritoneal adhesion formation [5,6].

The immune system responds to bacterial infections by activation of the cytokine cascade. Intensive secretion of growth factors is necessary for the repair of tissue damage. Therefore, the search for new molecular markers is important for early evaluation of the risk for adhesion.

Her we compared expression of various cytokines in tubal adhesions (TA) and normal tissues.

MATERIALS AND METHODS

We examined tissues of TA obtained during laparoscopy for pelvic adhesions (group 1, n=15). Intact tubal tissues localized beyond the zone of adhesion and obtained during diagnostic laparoscopy from pa-

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tients without pelvic diseases served as the control (group 2, *n*=10). Samples (100-150 mg) were frozen in liquid nitrogen immediately after surgery.

Isolation, DNase treatment, reverse transcription (RT), and polymerase chain reaction (PCR) were performed simultaneously in all samples as described elsewhere [1]. RNA was isolated using Tri Reagent solution (Sigma). DNA was removed with DNase RQ1 (Promega). Reverse transcriptase MMLV (Promega), hexarandom, and oligo-dT primers were used for RT. PCR was performed in a Tertsik thermocycler (DNK-tekhnologiya). We assayed local expression of genes of proinflammatory cytokines IL-1β, TNF-α, IFN-γ, IL-6, and 35-kDa subunit of IL-12, antiinflammatory cytokine IL-10, growth and differentiation factors for T and B lymphocytes IL-2, IL-4, IL-15, and IL-18, cell proliferation regulator TGF-β_2, and chemokine IL-8.

Quantitative and statistical analyses were performed as described previously [2].

RESULTS

Formation of adhesion was accompanied by intensification of production of mRNA for proinflammatory cytokines IL-1β, TNF-α, IL-12, and IFN-γ by 2.5 (p=0.008), 2.7 (p=0.07), 7.4 (p=0.005), and 4.0 times (p=0.006), respectively (Fig. 1). However, secretion of the antiinflammatory cytokine IL-10 and TGF-b_2 was intensified by 2.2 (p=0.03) and 50.2 times (p=0.0001), respectively (Fig. 2).

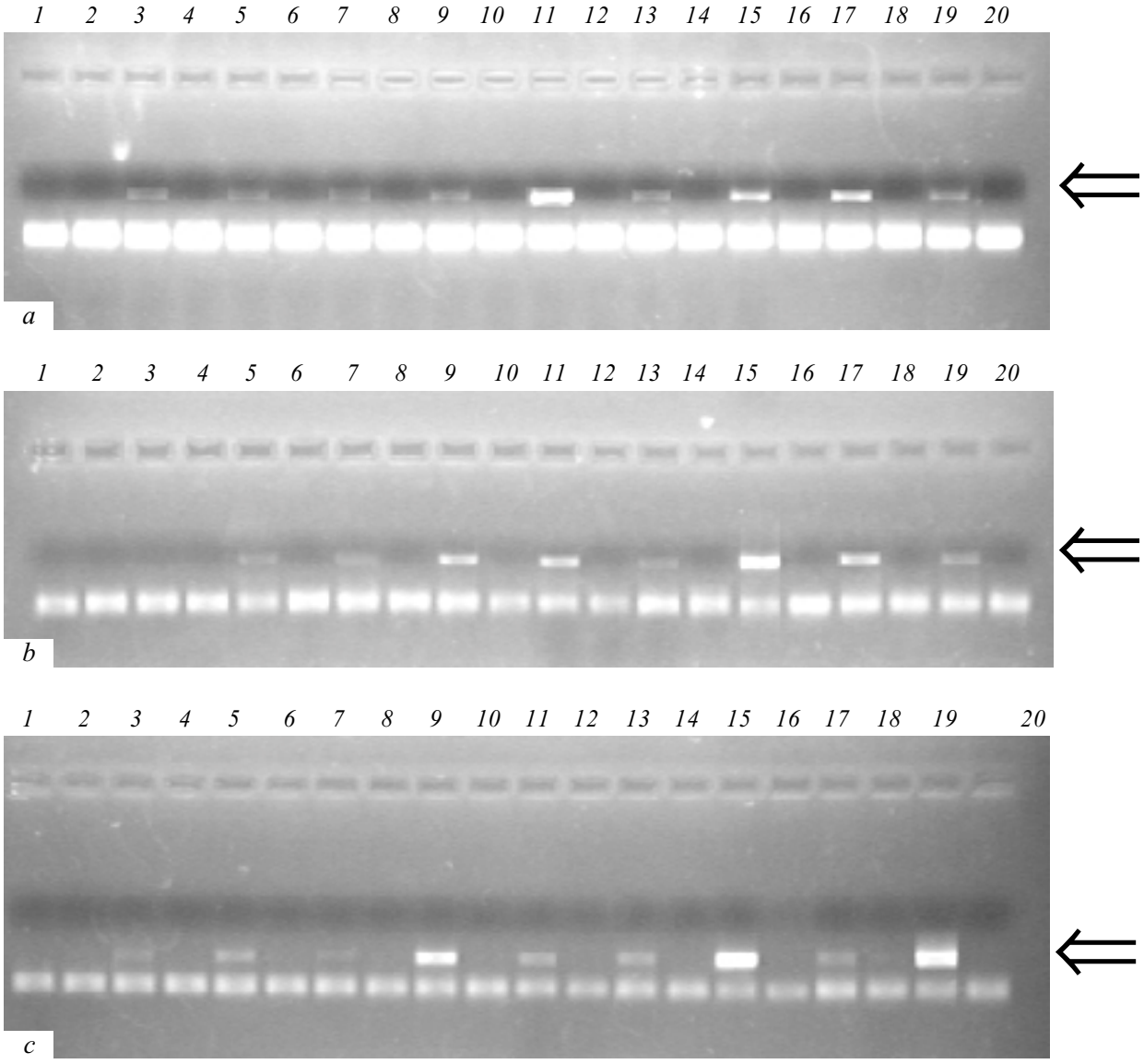

Fig. 1. Expression of IL-12 (*a*), IFN-γ (*b*), and TNF-α (*c*) genes in tubal adhesion samples. Electrophoretograms of RT-PCR in 2% agarose gel. Here and in Fig. 2: intact (*1-8*) and tubal adhesion samples (*9-20*). Even rows: negative control for reverse transcription. Arrows indicate localization of specific bands.

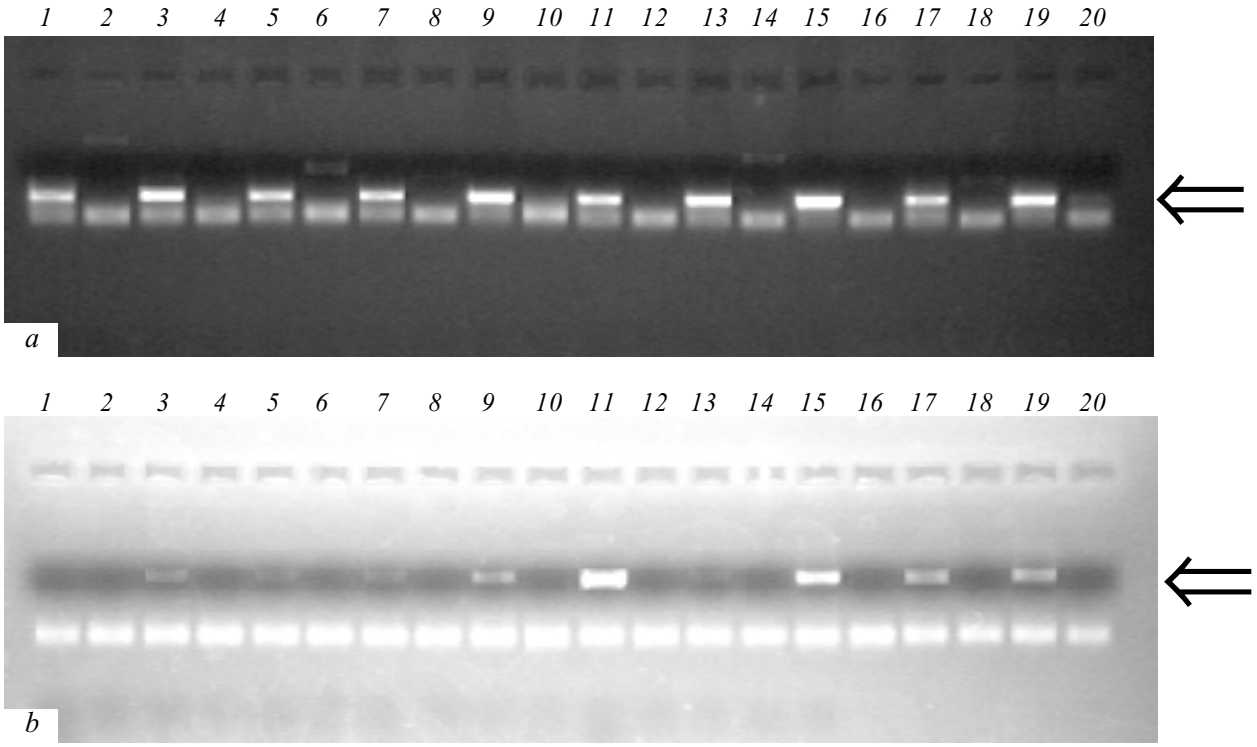

Fig. 2. Expression of IL-10 (*a*) and TGF-β_2 (*b*) genes in tubal adhesion samples. Electrophoretograms of RT-PCR products in 2% agarose gel.

Expression of IL-2 and IL-6 genes was not detected. The IL-8 gene was expressed only in 2 of 15 samples from adhesion tissues. Production of mRNA for IL-15 and IL-18 was insignificant in 25 test samples and did not differ between groups.

Changes in expression of genes of various cytokines in TA were interrelated. Most significant relationships were found between production of mRNA for the following factors: TNF-α and TGF-β_2 (r=0.88, p=0.0027), TNF-α and IFN-γ (r=0.51, p=0.026), TNF-α and IL-12 (r=0.62, p=0.014), IL-12 and TGF-β_2 (r=0.71, p=0.004). In the control group we revealed a correlation between expression of TNF-α and IL-12 (r=0.47, p=0.043).

Our results are consistent with published data that IL-1 and TNF-α play an important role in the formation of peritoneal adhesions [7,10]. The contribution of IL-12 and IFN-γ into this process is poorly understood. In our experiments the intensity of production of mRNA for these cytokines and expression of IL-β and TNF-α genes increased in TA. Moreover, we revealed a correlation between expression of TNF-α gene and amount of mRNA for IL-12 and IFN-γ. These data agree with general notions that IL-12 and IFN-γ activate production of Th1 cytokines IL-1, TNF-α, and IFN-γ. IL-12 and IFN-γ stimulate Th1 lymphocytes, but inhibit production of Th2 cytokines IL-4, IL-6, and IL-10. We found that expression of the IL-4 gene in TA did not differ from the control, while expression of IL-10 surpassed the normal. These results are consistent with published data [14]. TNF-α and IL-1 stimulate production of IL-10, which inhibits expression of genes of TNF-α, IL-1, and other proinflammatory cytokines. It was hypothesized that IL-10 act as the major factor preventing the inflammatory reaction [4]. In our experiments a positive correlation was found between the contents of IL-10 and IL-1β. Therefore, IL-10 production was most intensive in TA samples containing considerable amounts of IL-1β. These data suggest that the mechanisms of positive and negative regulation of inflammation are realized in TA.

Production of mRNA for TGF-β_2 in TA increased most significantly, which is consistent with published data [12]. Previous studies showed that cytokines belonging to the TGF-β family act as polyfunctional regulators of cell growth and differentiation and modulates the synthesis and hydrolysis of collagen and fibrin [9,13]. Therefore, TGF-β performs reparative function in tissue damage [4]. Probably, adhesion formation is the negative consequence of this process. We revealed positive correlations between production of mRNA for TGB-β_2 and TNF-α, TGB-β_2 and IL-12, which suggests that expression of these genes in interregulated. Induction of the inflammatory response and activation of connective tissue growth during adhesion formation are probably interrelated.

It is interesting that expression of the IL-8 gene in TA was low. The inflammatory reaction is usually accompanied by intensification of local production of chemokines. Previous studied showed that TGB-β_1 inhibits IL-8 production in epitheliocytes [11]. It can be hypothesized that TGB-β_2 performs similar functions in TA and prevents the release of neutrophil-derived IL-8 into the zone of adhesion formation.

Our results show that the intensity of production of mRNA for proinflammatory cytokines IL-1β, IL-12, IFN-γ, and TNF-α, antiinflammatory cytokine IL-10, and TGB-β_2 considerably increased in TA. The activation of expression of these genes can serve as a prognostic criterion for high risk of adhesion formation. Correlations between secretion of cytokines activating or inhibiting the inflammatory response and transforming growth of cells suggest that expression of these genes during adhesion formation is regulated via the feedback mechanism. These data indicate that it is possible to reduce the risk for adhesion formation and regulate this process at the molecular level (*e.g.*, by modulation of cytokine production).

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