EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Expression of Cytokine Genes in Blood Mononuclear Cells in Women with Pyoinflammatory Diseases of Adnexa Uteri

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 11, pp. 555-559, November, 2002
Original article submitted April 19, 2002

Production of interleukin, interferon-γ, and transforming growth β_2-factor mRNA in peripheral blood mononuclear cells was studied in women with pyoinflammatory diseases of adnexa uteri. Predominant expression of Th1 proinflammatory cytokine genes in patients was demonstrated. Production of interleukins 4 and 8 mRNA decreased 200- and 60-fold, respectively, in comparison with that in healthy women. One month and a half after extirpation of fallopian tubes the cytokine production in blood mononuclear cells shifted towards antiinflammatory cytokines, expression of interleukin-8 gene returned to normal, and expression of interleukin-4 gene increased.

Key Words: *inflammation of adnexa uteri; cytokines; mRNA*

Inflammations of fallopian tubes are a prevalent cause of reproductive dysfunction. Every fourth woman with a history of genital inflammations suffers from complications [1,4], the severity of these complications depends on the type of the inflammatory process determined by factors the immune system, in particular, cytokines.

Acute inflammations of abdominal organs often lead to pronounced changes in the immunity not only at the local [6,12,14], but even at the system level, which manifests in increased production of proinflammatory cytokines, *e. g.* interferon-γ (IFN-γ) [5]. The data on the regulatory role of antiinflammatory cytokine interleukin-10 (IL-10) are contradictory, but it can be hypothesized that decreased production of this cytokine leads to exacerbation and chronization of the inflammatory process [8,10].

Changes in cytokine production in peripheral blood cells in patients with salpingo-oophoritis, tubo-ovarian abscess, and pyosalpinx are poorly studied. Here we studied gene expression of proinflammatory cytokines IFN-γ, IL-6, and IL-12 35 kDa subunit (referred to as IL-12 below), antiinflammatory cytokine IL-10, T and B lymphocyte growth and differentiation factors IL-2, IL-4, IL-15, IL-18, cell proliferation regulator transforming growth factor β_2 (TGF-β_2), and chemokine IL-8 in blood mononuclear cells (MNC) in women with pyoinflammatory diseases of the adnexa uteri.

MATERIALS AND METHODS

Expression of cytokine genes was evaluated in 25 women with pyoinflammatory diseases of the adnexa uteri before and 1.5 months after treatment. Group 1 (mean age 34.1±3.8 years) consisted of 12 patients

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0007-4888/02/1345-0477$27.00

with salpingo-oophoritis, 8 with ovarian abscess, and 5 with pyosalpinx, group 2 consisted of 9 women without inflammatory diseases and complaints (mean age 33.4±4.1 years). Peripheral blood MNC were studied. The patients were subjected to extirpation of the uterus with adnexa or without adnexa or extirpation of fallopian tubes.

Peripheral blood MNC were isolated on a Ficoll-Paque density gradient (Flow Laboratories). Tri Reagent solution (1 ml, Sigma) was added to 5×10^6 cells and homogenized using RiboLyzer (Hybaid). Experiments were carried out as described previously [2] with minor modifications. During precipitation, 2.5 μg co-precipitate LPA (Supelco) was added to RNA. The quality of isolated RNA was evaluated by denaturing electrophoresis in 1% agarose gel, RNA concentration was measured spectrofluorometrically at λ=260 nm. DNA was cleaved with DNase RQ1 (2 U, Promega). PCR was carried out with 0.2 μM primers (Table 1), a total of 25-35 cycles (15 sec at 95°C, 20 sec at 58-62°C, and 15 sec at 72°C) were performed.

RNA from 10^6 MNC stimulated with LPS and phytohemagglutinin (both from Sigma) for 12 h at 37°C in RPMI-1640 (Flow Laboratories) served as the positive control.

The Gel-Doc 1000 videosystem (Bio-Rad) parameters used in our study allowed detection of products of amplification of 10^3 and more cDNA copies. Quantitative analysis was carried out using calibration curves plotted after amplification of cDNA of positive control (5 serial 10-fold dilutions). The calibration curves were plotted on the basis of electrophoresis data for each gene separately (5 points); logarithms of reciprocal cDNA dilution were plotted along the *X* axis and the corresponding signal intensity along the *Y* axis.

The significance of differences between the groups was evaluated using nonparametric Mann—Whitney *U* test, Wald—Wolfowitz test for independent samples, and Wilcoxon test for bound samples; the differences were considered significant at $p<0.05$.

RESULTS

The most pronounced differences were observed for expression of IL-4 (Th2 cytokine) and IL-8 (neutrophil

TABLE 1. Primers Used in the Study

mRNA	Nucleotide sequence	Amplicon length, b.p.
β-actin	C: 5'-AGGCCAACCGCGAGAAGATGAC	
	A: 5'-TCGGCCGTGGTGGTGAAGC	278
TGF-β_2	C: 5'-GCGTGTCCCAAGATTTAGAACC	
	A: 5'-TCAAGTGAGGCGCGG-GATAGG	515
IFN-γ	C: 5'-GCAGGTCATTCAGATGTAGCGGA	
	A: 5'-ACCTCGAAACAGCATCTGACTCCT	366
IL-2	C: 5'-GCCCAAGAAGGCCACAGAACTGA	
	A: 5'-GGCCTGATATGTTTTAAGTGGGAAGCA	309
IL-4	C: 5'-CTGCTTCCCCCTCTGTTCTTCCTG	
	A: 5'-ACGTACTCTGGTTGGCTTCCTTCACA	369
IL-6	C: 5'-GATTCCAAAGATGTAGCCGCCCCACA	
	A: 5'-CATTTGTGGTTGGGTCAGGGGTGGT	415
IL-8*	C: 5'- AAACATGACTTCCAAGCTGGC	
	A: 5'- GCTTGAAGTTTCACTGGCATCT	348
IL-10	C: 5'-GTGGAGCAGGTGAAGAATGCCTT	
	A: 5'-TATCCCAGAGCCCCAGATCCGAT	201
IL-12, 35 kDa	C: 5'-CCAGGTGGAGTTCAAGACCATGAATG	
	A: 5'-TCATGTGGATGTAATAGTCCCATCCT	381
IL-15*	C: 5'-CATGTCTTCATTTTGGGCTGT	
	A: 5'-GAAGTGTTGATGAACATTTGGAC	392
IL-18*	C: 5'-G(G/A)AAT(T/A)T(G/A)AATGACCAAGTTCTC	
	A: 5'-GG(A/G)ACAC(T/G)T(T/C)TCTGAAAGAATATG	283

Note. *These primers were a gift from Dr. B. N. Pestov, Cand. Biol. Sci, from M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry.

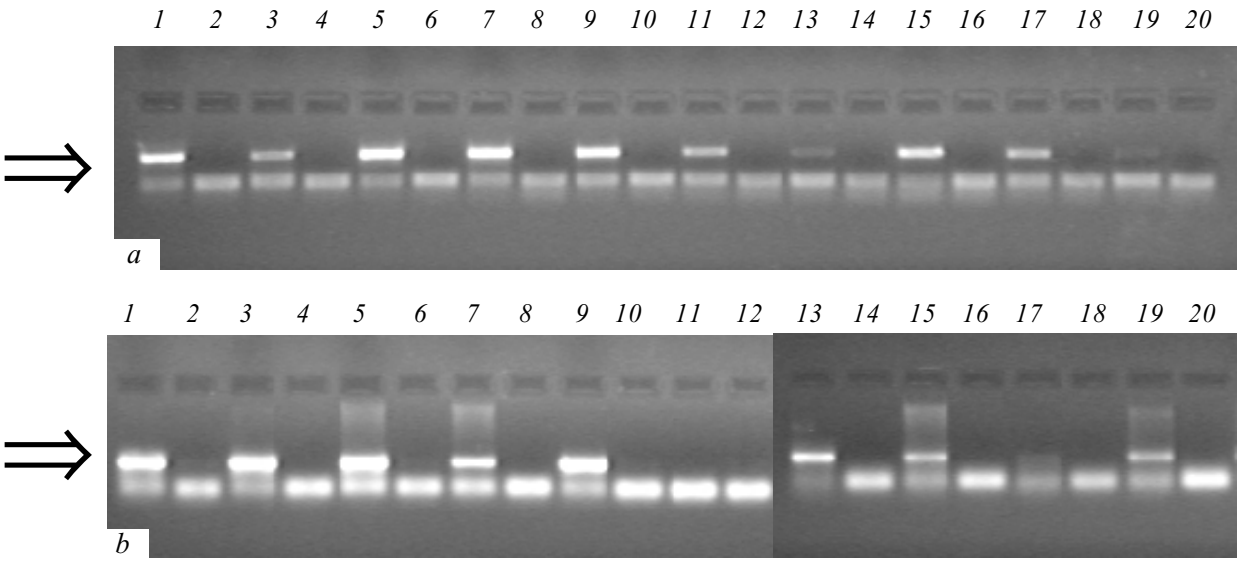

Fig. 1. Expression of IL-4 (*a*) and IL-8 (*b*) genes by blood mononuclear cells in healthy women (*1-10*) and patients with inflammations of adnexa uteri before therapy (*11-20*). Electrophoregram of reverse transcription-PCR results. Here and in Fig. 2: even rows correspond to negative control of reverse transcription. Arrows show the mobility of specific band.

chemotaxis factor) genes (Fig. 1). The expression of IL-8 and IL-4 genes in patients was 1.5% and 0.4% of normal, respectively (Table 2).

Significant differences in the content of IFN-γ and IL-10 mRNA in MNC were detected in women of groups 1 and 2 (Fig. 2). Expression of IFN-γ gene (proinflammatory Th1 cytokine) in group 1 2.5-fold surpassed that in group 2 (p=0.0007). By contrast, production of IL-10 mRNA (antiinflammatory Th2 cytokine) in patients was only 50% of normal (Table 2).

Expression of IL-12 (activator of Th1 lymphocytes) and IL-2 genes tended to increase (by 1.3 times, p=0.43, and by 1.2 times, p=0.29, respectively). The content of IL-6 and IL-18 mRNA was the same in the peripheral blood MNC of patients and healthy women; no TGF-β_2 mRNA was detected.

One month and a half after extirpation of fallopian tubes the content of IL-4, IL-8, and IL-15 mRNA in MNC increased significantly, while the content of IFN-γ and IL-12 mRNA decreased (Table 2). Production of IL-8 and IL-15 mRNA returned to normal. IFN-γ gene expression after treatment decreased, but still 2-fold surpassed the normal (Table 2). Production of IL-10, IL-2, IL-6, IL-18, and TGF-β_2 mRNA did not change after therapy.

Analysis of cytokine mRNA production after therapy revealed a shift from proinflammatory to moderate proinflammatory pattern. The ratio of IFN-γ/IL-10 gene expression decreased 2.2 times, but still differed from the corresponding parameter in healthy women (Table 3). IL-12/IL-10 ratio in MNC from group 1 women significantly surpassed the normal, while after surgery this parameter tended to decrease (Table 3).

A decrease in IFN-γ/IL-10 and IL-12/IL-10 ratios and drastic decrease in IFN-γ/IL-4, IL-2/IL-4, and IL-12/IL-4 ratios (primarily due to increased production of IL-4 mRNA) attested to suppression of Th1 lymphocytes and activation of Th2 lymphocytes (Table 3).

TABLE 2. Expression (%) of Cytokine Genes in Inflammatory Diseases of the Adnexa Uteri before and after Therapy (n=25)

Cytokines	Mean value		25-75% quartiles		p compared to normal	
	before therapy	after therapy	before therapy	after therapy	before therapy	after therapy
IFN-γ	251.2	199.5*	199.5-302.3	141.3-241.2	0.0007	0.027
IL-12	128.5	89.7*	112.1-146.4	71.3-106.5	0.12	0.24
IL-10	50.1	56.2	34.0-63.1	22.4-83.0	0.002	0.015
IL-15	22.5	93.6*	15.9-25.1	76.3-112.7	0.00001	0.75
IL-8	1.6	53.9*	0.9-2.8	39.5-82.6	0.00002	0.09
IL-4	0.4	7.9*	0.3-1.0	2.8-23.8	0.00001	0.02

Note. *Significant difference from the pretreatment level (p<0.05).

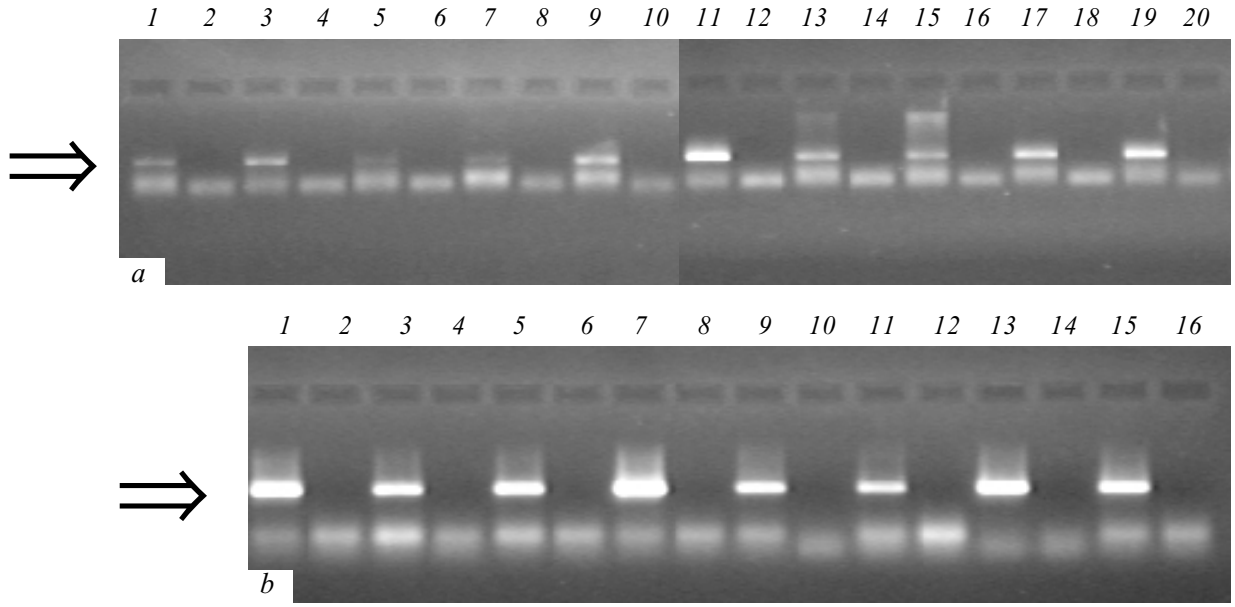

Fig. 2. Expression of IFN-γ (*a*) and IL-10 (*b*) genes by blood mononuclear cells of healthy women (*1-10*, *a*; *1-8*, *b*) and patients with inflammation of adnexa uteri before treatment (*11-20*, *a*; *9-16*, *b*). Electrophoregram of reverse transcription-PCR results.

Exogenous IL-4 produces a therapeutic effect in acute enteric inflammation in rats [5], while IL-10 is involved in termination of the inflammatory reaction in abdominal organs [13] via suppression of the production of a wide spectrum of proinflammatory cytokines and stimulation of the production of their inhibitors [9]. Decreased expression of IL-4 and IL-10 genes in MNC promotes the development of acute inflammation in fallopian tubes (which is confirmed by increased production of IFN-γ mRNA). Increased expression of IL-4 gene in MNC after therapy suppressed Th1 lymphocytes, producers of proinflammatory cytokines [9] and activated Th2 lymphocytes.

It was previously demonstrated that local increase of IL-8 production promotes the development of inflammatory reaction [15], while increased blood content of this cytokine can inhibit local inflammation [7]. Sharp inhibition of IL-8 gene expression in MNC in pyoinflammatory diseases of the adnexa uteri creates a gradient of this chemokine essential for attraction of neutrophils. After removal of the focus of inflammation IL-8 production in blood cells returned to normal.

Hence, we detected considerable changes in gene expression of Th1 and Th2 pro- and antiinflammatory cytokines in peripheral blood MNC in patients with pyoinflammatory diseases of the adnexa uteri and normalization of these processes or a tendency to normalization after treatment. These results confirm usefulness of analysis of cytokine expression in these patients for prevention of deep disturbances of the immune homeostasis and correction of the balance between pro- and antiinflammatory activities of the immune system.

TABLE 3. Ratios of Cytokine Gene Expression (Median/Quartile Range) in MNC of Patients with Inflammation of Adnexa Uteri before and after Therapy

Cytokines	Before therapy	After therapy	p^+
IFN-γ/IL-10	4.5/7.4*	2.0/1.9*	0.038
IL-12/IL-10	2.8/2.9**	1.7/1.7	0.16
IFN-γ/IL-4	562/128*	12.6/13.6*	<0.00001
IL-2/IL-4	97.7/112.4*	5.6/5.7*	<0.00001
IL-12/IL-4	199.5/76.7*	7.1/7.7*	<0.00001

Note. *p<0.01, **p<0.05 compared to normal; $^+$compared to the corresponding parameter before treatment.

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