Ratio between the Number of Th1 and Th2 Lymphocytes in the Peripheral Blood and Concentration of Proinflammatory Cytokines in Lochia of Women with Postpartum Endometritis

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We estimated the percentage of $CD4^+$ lymphocytes synthesizing IFN-γ and IL-4 in the peripheral blood and measured the concentrations of IL-1, IL-6, and TNF-α in lochia of patients with postpartum endometritis. T lymphocytes with intracellular IFN-γ prevailed in women with physiological course of the postpartum period. The number of cells containing IL-4 increased in patients with postpartum endometritis. On days 5-7 after childbirth the concentrations of proinflammatory cytokines IL-1β and TNF-α in lochia of these patients were higher than in healthy postpartum women (by 1.4 and 2.7 times, respectively). The concentrations of cytokines IL-1β, TNF-α, and IL-6 increased most significantly in patients with postpartum endometritis and active viral infection (by 3.4, 4.2, and 1.6 times, respectively).

Key Words: *cytokines; T lymphocytes; postpartum endometritis*

Cytokines serve as mediators of cell-cell interactions and regulate the inflammatory response. Elimination of viruses and bacteria from human organism depends on the immune response regulated by $CD4^+$ T lymphocytes. The immune response to intracellular pathogens is mediated by Th1 cells. The immune response to extracellular pathogens mainly depends on the function of Th2 cells, which is associated with production of the corresponding cytokines [5]. In the peripheral blood from women with viral infection the number of $CD4^+$ T cells synthesizing IFN-γ is higher than the count of $CD4^+$ T cells with IL-4 [4]. Bacterial infection triggers the cytokine cascade. The process includes production of proinflammatory cytokines and is followed by an increase in the concentration of antiinflammatory cytokines and growth factors. These changes play a role in the prevention of inflammation and reparation of damaged tissues [2].

Study of purulent and inflammatory diseases in postpartum women is an urgent obstetric problem [3]. The development and course of puerperal infectious complications depend on the immune status of patients. Study of the peripheral blood from women on days 2-3 after cesarean section revealed a correlation between the concentrations of proinflammatory cytokines IL-8 and TNF-α and severity of uterine inflammation. Hence, monitoring of these cytokines is used for early diagnostics of endometritis [1]. Published data show that IL-6 concentration in the cervical canal of patients with endometritis is much higher than in healthy women [6].

Here we estimated the ratio between the number of $CD4^+$ lymphocytes with intracellular cyto-

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kines IFN-γ and IL-4 (Th1/Th2) in the peripheral blood and measured the concentrations of proinflammatory cytokines (IL-1β, IL-6, and TNF-α) in lochia of patients with postpartum endometritis.

MATERIALS AND METHODS

Seventy postpartum women were examined after natural childbirth and abdominal delivery. Thirty-five conventionally healthy postpartum women entered the control group. The main group consisted of 35 patients with postpartum endometritis. Postpartum women of the main group were divided into subgroups depending on the course of the disease. Subgroup 1 included 20 patients with moderate postpartum endometritis. Subgroup 2 included 15 patients with severe postpartum endometritis and active viral infection (genital herpes, cytomegaly).

The percentage of peripheral blood cells synthesizing IL-4 and IFN-γ was estimated using phycoerythrin-conjugated monoclonal antibodies against intracellular IL-4 and IFN-γ and fluorescein isothiocyanate-conjugated antibodies against CD4 membrane antigen. Flow cytometry was performed on a Bryte HS cytofluorometer (Bio-Rad). Lochia were sampled from the uterine cavity using a disposable sterile polyethylene catheter and syringe on days 5-7 after labor to measure the concentrations of proinflammatory cytokines IL-1β, IL-6, and TNF-α. The concentrations of cytokines were determined by enzyme immunoassay according to manufacturer's instructions. Experiments were performed with Cytelisa kits. The data were recorded on a Biorad M.550 photometer at 450 nm.

The results were analyzed by means of Excel and Statistica softwares. The differences were significant at $p<0.05$.

RESULTS

The percentage of IFN-γ-synthesizing $CD4^+$ cells in subgroup 1 patients with postpartum endometritis was lower than in women of the control group (8.7±1.5 and 11.6±3.3%, respectively, $p<0.05$). The percentage of IFN-γ-synthesizing cells in women with postpartum endometritis and active viral infection was higher than in subgroup 1 patients (12.9±3.1%, $p<0.05$, Table 1).

IFN-γ stimulates production of Th1-type cytokines. IFN-γ not only activates Th1 cells, but also suppresses production of Th2-type cytokines, *e.g.* IL-4 stimulating antibody synthesis and suppressing cellular immune response.

Our results showed that the percentage of $CD4^+$ lymphocytes synthesizing IL-4 in patients with postpartum endometritis was higher than in women of the control group (12.2±3.5%, $p<0.05$). The decrease in the IFN-γ/IL-4 ratio was mainly related to an increase in the number of IL-4-producing cells. These changes reflected activation of Th2 lymphocytes. The development of endometritis was accom-

TABLE 1. Number of $CD4^+$ Lymphocytes Synthesizing IFN-γ and IL-4 in Patients with Postpartum Endometritis and Women with Physiological Course of the Postpartum Period

Parameter	Control group	Main group	
		subgroup 1	subgroup 2
IFN-γ, %	11.6±3.3	8.7±1.5*	12.9±3.1$^+$
IL-4, %	5.3±1.7	12.2±3.5*	11.0±3.5*
IFN-γ/IL-4	2.18	0.71	1.17

Note. $p<0.05$: *compared to the control group; $^+$compared to subgroup 1.

TABLE 2. Concentration of Proinflammatory Cytokines in Lochia of Conventionally Healthy Postpartum Women and Patients with Endometritis ($M±m$, pg/ml)

Cytokines	Control group	Main group	
		subgroup 1	subgroup 2
IL-1β	131.1±16.2	182.2±40.4***	447.1±49.7**$^{+++}$
IL-6	110.4±19.8	118.6±23.7	176.1±13.2**$^{++}$
TNF-α	23.8±4.7	65.2±14.1**	99.6±18.1*$^+$

Note. *$p<0.001$, **$p<0.005$, and ***$p<0.05$ compared to the control; $^+p<0.001$, $^{++}p<0.005$, and $^{+++}p<0.05$ compared to subgroup 1.

panied by a systemic increase in the percentage of lymphocytes preventing uterine inflammation. The percentage of IL-4-synthesizing cells did not differ in patients of subgroups 1 and 2 ($p>0.05$).

Study of the ratio between cells synthesizing IFN-γ and IL-4 revealed predominance of Th1 and Th2 lymphocytes in women with normal course of the postpartum period and in patients with postpartum endometritis, respectively. This proportion changed in exacerbation of herpes infection, which was associated with an increase in the percentage of IFN-γ-synthesizing cells.

The mean concentration of proinflammatory cytokines was measured in lochia of women on days 5-7 after labor (Table 2).

The concentrations of IL-1β and TNF-α in patients with postpartum endometritis were higher than in women of the control group (by 1.4 and 2.7 times, respectively). No differences were revealed in IL-6 concentration in patients with postpartum endometritis and women of the control group.

The concentrations of IL-1β, TNF-α, and IL-6 increased most significantly in patients of subgroup 2 (by 3.4, 4.2, and 1.6 times, respectively, compared to healthy women).

Our results indicate that patients with postpartum endometritis are characterized by a significant change in the concentrations of IL-1β and TNF-α in lochia. The observed changes were statistically significant. The increase in the concentration of proinflammatory cytokines IL-1β and TNF-α is of high diagnostic importance; it reflects the severity of uterine inflammation and can serve as a criterion for inflammatory complications. Therefore, postpartum endometritis is the infectious processes accompanied by an increase in local production of proinflammatory cytokines IL-1β and TNF-α.

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