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Regulation of Reactive Oxygen Species Production by Peripheral Blood Neutrophils from Women with Postpartum Endometritis

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Production of reactive oxygen species in unfractionated peripheral blood increased in parturient women without postpartum infectious complications and patients with postpartum endometritis. The control group included nonpregnant women with normal reproductive function. Intergroup differences were revealed in the degree of respiratory burst activation with opsonized zymosan and response of isolated granulocytes to chemotactic peptide N-formyl-Met-Leu-Phen (1 μM). Production of reactive oxygen species tended to normal after therapy. We studied the effects of a specific mitogen-activated protein kinase p38MAPK inhibitor and inhibitors of tyrosine protein phosphatases and phosphatidylinositol-3-kinase. The role of p38MAPK in reactive oxygen species generation by cells changes significantly in parturient women.

Key Words: *granulocyte; reactive oxygen species; intracellular signaling; postpartum endometritis*

Activation of phagocytes is a key mechanism of inflammation [4]. Polymorphonuclear neutrophilic granulocytes (neutrophils) are most rapidly mobilized during the inflammatory response. The cytotoxic potential of these cells depends on the content of reactive oxygen species (ROS). ROS generation by neutrophils is determined by activity of the membrane enzyme NADPH oxidase, which is regulated by intracellular signal systems [6]. They are considered as a target for medicinal drugs [8]. Various diseases are accompanied by changes in the intensity of ROS generation by neutrophils [11,13]. However, the mechanisms of regulation of NADPH oxidase activity remain unclear. It is important to study not only cells present in the inflammatory focus, but also those circulating in the vascular bed. This approach allows us to perform diagnostics and monitoring of therapy with considering characteristics of peripheral blood cells.

The presence of neutrophils in the endometrial epithelium is considered as the major histological sign of endometritis [5]. Previous studies revealed changes in functional activity of neutrophils and macrophages in the cervical and endometrial fluid from patients with endometritis and salpingoophoritis [3]. The presence of neutrophils in the upper part of the reproductive tract is of considerable prognostic importance for the diagnostics of infections [15]. Neutrophils were revealed in the uterine fluid during endometritis [10]. Increased content of bactericidal peptides from neutro-

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phil granules (defensins), but not neutrophils in the vagina of women infected with *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, or *Chlamydia trachomatis* is a manifestation of endometritis [14]. Activation of phagocytosis serves as an integral criterion for the effectiveness of immunocorrection (*e.g.*, during endometritis) [1].

Here we studied ROS generation by peripheral blood neutrophils and evaluated the mechanisms of regulation of the respiratory burst in granulocytes from patients with postpartum endometritis.

MATERIALS AND METHODS

Experiments were performed with unfractionated samples of the peripheral blood and blood granulocytes from patients with postpartum endometritis isolated before (n=22) and after therapy (n=11). The reference group included parturient women without postpartum infectious complications (n=21). The control group included healthy nonpregnant women with normal reproductive function (n=20). ROS generation was assayed 5-6 days after labor. Study of ROS generation in parturient women with postpartum endometritis was performed on days 7-10 and after 1-day treatment (antibacterial drugs, infusion therapy, and vacuum aspiration).

The intensity of ROS generation was determined by luminol-dependent chemiluminescence (CL) on a Khemiljum-2001 chemiluminometer (Institute of Cell Biophysics). Sampling and isolation of granulocytes were performed as described elsewhere [2]. Spontaneous and opsonized zymosan-induced (OZ, 0.5 mg/ml) ROS generation was measured in unfractionated blood samples.

The cells were maintained in a Ca^{2+}-free medium at 4°C and studied 1 h after isolation. Intact and inhibitor-treated cells were placed in a well (10^6 cells/ml) and incubated at 37°C for 30 min. We used a tyrosine phosphatase inhibitor sodium orthovanadate (0.1 mM), mitogen-activated protein kinase p38MAPK inhibitor SB203580 (1 μM), and phosphatidylinositol-3-kinase inhibitor wortmannin (0.1 μM). The cells were activated with 1 μM N-formyl-Met-Leu-Phen (FMLP). We calculated the ratio between the intensity of ROS production by cells treated with one of the inhibitors and intact cells. Intergroup differences were evaluated by Student's t test.

RESULTS

Basal production of ROS in women of the reference group and patients with postpartum endometritis was much higher than in controls (Fig. 1). In blood samples from patients with postpartum endometritis the amplitude of the response to OZ increased by more than 3-fold. This parameter practically did not differ in women of the reference and control groups. The activation index in parturient women was lower than in the control (Table 1). A decrease in the ratio between spontaneous and OZ-induced ROS generation by blood cells (activation index) can be related to an imbalance between the systems for generation and elimination of ROS. These changes are considered as a criterion for oxidative stress [7]. In various diseases accompanied by oxidative stress neutrophils serve as the target of ROS, which modulates their functional activity [12]. The study of total ROS generation revealed overproduction of ROS by blood cells from patients with postpartum endometritis (Table 1). The data indicate that neutrophils from patients with postpartum endometritis cannot perform protective function. On day 2 after therapy this parameter significantly decreased, while the activation index increased. However, the test parameters did not reach the control level (Fig. 1, *b*, *c*, Table 1).

After treatment with OZ the main pool of ROS was generated by phagocytes. Binding of OZ to phagocytes is followed by activation of receptors for blood plasma opsonins and triggers the corresponding intracellular pathways of signal transduction from these receptors. However, it is difficult to evaluate the role of individual components in transduction of a complex signal from receptors to NADPH oxidase.

We studied the response of blood granulocytes from women of various groups to chemotactic peptide FMLP, which has specific G_i protein-coupled 7TM receptors on the surface of neutrophils [6]. The structure of receptors and pathways of signal transduction

TABLE 1. Generation of ROS by Unfractionated Blood Cells ($M \pm m$)

Parameter	Control	Reference group	Endometritis	
			before therapy	after therapy
Activation index	124.5±21.6	9.0±1.8*	17.9±3.6*+	38.5±11.8°
ROS generation, arb. units	543±55	351±63*	1352±185*+	850±91°

Note. Activation index is calculated as the ratio between the amplitude of the OZ-induced response and basal level. ROS generation is determined by the integral of the time dependence for CL. p<0.001 *compared to the control, +compared to reference group, °compared to endometritis.

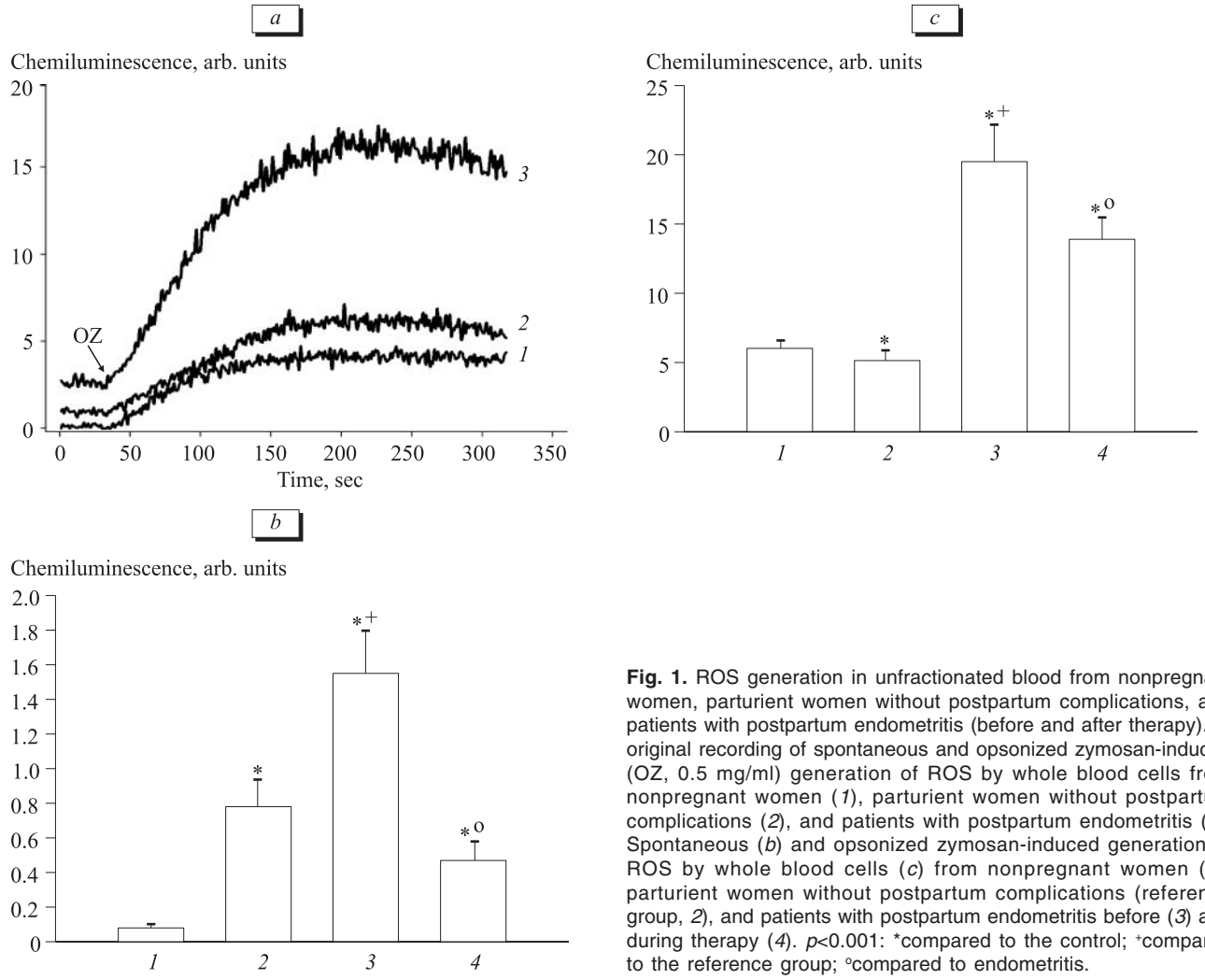

Fig. 1. ROS generation in unfractionated blood from nonpregnant women, parturient women without postpartum complications, and patients with postpartum endometritis (before and after therapy). *a*: original recording of spontaneous and opsonized zymosan-induced (OZ, 0.5 mg/ml) generation of ROS by whole blood cells from nonpregnant women (*1*), parturient women without postpartum complications (*2*), and patients with postpartum endometritis (*3*). Spontaneous (*b*) and opsonized zymosan-induced generation of ROS by whole blood cells (*c*) from nonpregnant women (*1*), parturient women without postpartum complications (reference group, *2*), and patients with postpartum endometritis before (*3*) and during therapy (*4*). $p<0.001$: *compared to the control; +compared to the reference group; °compared to endometritis.

from the receptor to NADPH oxidase are extensively studied [9].

The amplitude of the response of blood granulocytes from women of the reference and control groups and patients with endometritis to 1 μM FMLP was 58.1±19.0, 34.6±10.3, and 46.4±13.4, respectively (n=10). Binding of FMLP to the receptor activates the signal cascades involving mitogen-activated protein kinases (MAPK), tyrosine protein kinases and phosphatases, and phosphatidylinositol-3-kinase (PI3K) [6,9]. Specific inhibitors were used to study signal systems that play a role in signal transduction from the FMLP receptor to NADPH oxidase (Table 2). After treatment with p38MAPK inhibitor SB203580 (1 μM) the neutrophil respiratory burst induced by 1 μM FMLP increased in control women, significantly decreased in women of the reference group, and remained unchanged in patients with endometritis. The effect of sodium orthovanadate (0.1 mM) on the respiratory burst in cells from patients with postpartum endometritis was lower than in women of the reference and control groups. Wortmannin (0.1 μM) inhibited ROS generation by granulocytes from women of different groups. This effect was most pronounced in control women.

Our results indicate that postpartum endometritis is accompanied by increased production of ROS in peripheral blood cells under basal conditions and during activation with a specific agent. Parturient women without postpartum complications and patients with endometritis were characterized by changes in inhibition of intracellular signal systems involved in signal transduction from the FMLP receptor.

Activation of ROS generation by blood cells from patients with postpartum endometritis is a sign of acute inflammation. Previous observations revealed increased spontaneous and induced CL of the blood from patients with bacterial infection of the urogenital tract in the third trimester of pregnancy. The study of ROS generation by blood cells (high spontaneous and induced production, low activation index, and increased total production) showed that phagocytes serve as a major source of ROS in the blood, which can con-

TABLE 2. Effect of Inhibitors on the Respiratory Burst in Blood Granulocytes Induced by 1 μM FMLP (*M*±*m*)

Inhibitor	Control	Reference group	Endometritis
SB203580, 1 μM	1.21±0.09 *n*=19	0.72±0.11* *n*=10	0.98±0.06*+ *n*=9
Sodium orthovanadate, 0.1 mM	1.46±0.11 *n*=14	1.43±0.09 *n*=7	1.27±0.17**++ *n*=8
Wortmannin, 0.1 μM	0.27±0.045 *n*=9	0.35±0.03* *n*=11	0.41±0.10*** *n*=8

Note. Ratio between study parameters for inhibitor-treated and intact cells (1, rel. units). *p<0.001, **p<0.02, and ***p<0.01 compared to the control; +p<0.01 and ++p<0.02 compared to reference group.

tribute to damage in endometritis (overproduction of ROS). The test parameters tended to normal after therapy, which provide grounds for using these parameters in the monitoring of treatment for endometritis.

The observed differences are probably associated with changes in the regulation of NADPH oxidase activity. Most significant differences were revealed in the effects of p38MAPK inhibitor. This inhibitor produced an opposite effect on cells from women of the control and reference groups, but was ineffective in patients with endometritis (Table 2). Under normal conditions p38MAPK can decrease or increase NADPH oxidase activity, respectively. These data indicate that the regulation of NADPH oxidase in blood granulocytes is modified after labor. This enzyme either plays minor regulatory role or undergoes significant activation in patients with endometritis. This problem requires further investigations.

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