Rhythmicity of Specific and Nonspecific Resistance in Adult Women

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We studied biological rhythms of immunological parameters and absorption and digestive activities of neutrophils and monocytes in women during the menstrual cycle. Circadian rhythms of phagocytosis were evaluated. The phagocytic system was characterized by highest activity during ovulation and early luteal phase of the menstrual cycle accompanied by physiological immunosuppression. It was shown that macrophages determine the spatial and temporal organization of specific and nonspecific resistance in women. We evaluated phases of the menstrual cycle optimal for studying rhythms of the immune system and phagocytosis.

Key Words: *biorhythms; lymphocytes; neutrophils; monocytes; menstrual cycle*

Near-monthly rhythms of hormonal regulation and autonomic reactions during menstrual cycle (MC) ensure reproductive function and modulate the state of somatic systems in women. Immunocompetent cells carry receptors for sex steroids and pituitary peptide hormones [12,13], which determines sexual dimorphism in the immune response [6] and changes in specific and nonspecific resistance during MC.

There is evidence on variations in the total and differential lymphocyte count and functional and metabolic activities of these cells during MC [1,2,4,7]. However, temporal relationships between specific and nonspecific resistance in women during various phases of MC are poorly understood.

Here we studied the spatial and temporal organization of specific and nonspecific resistance during normal MC in women of reproductive age and determined MC phases optimal for evaluation of the immune status.

MATERIALS AND METHODS

Healthy nonpregnant women (19-25 years, n=148) were examined 2 times during MC. For verification of MC phase buccal scrapes were examined and plasma progesterone was measured by radioimmunoassay (IRMA ferritin). The blood was taken from the cubital vein. Heparin (1 ml, 500 U) dissolved in 5 ml physiological saline served as the anticoagulant. Test parameters were measured 4 times a day at 6.00, 12.00, 18.00, and 24.00.

The total and differential leukocyte count were estimated by routine laboratory tests. Lymphocyte subsets were analyzed by the method of rosette formation: the count of T and B cells was estimated using sheep and mouse erythrocytes, respectively; the number of T helper cells was determined after incubation with theophylline using sheep erythrocytes. The contents of IgA, IgM, and IgG were measured by radial immunodiffusion [9].

Adsorption activity of peripheral blood neutrophils and monocytes was evaluated by phagocytosis of latex particles (diameter 0.9 m). Digestive activity of cells was determined using nitroblue tetrazolium (NBT test). The cells were stimulated with pyrogenal. The phagocytic index (PI), phagocytic number (PN), and counts of NBT-positive cells and formazan granules were estimated by examining blood smears (50 neutrophils and 10 monocytes). The reserve capacity of phagocytosis was evaluated by activation coefficients (ratio between stimulated and spontaneous parameters).

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The results were analyzed by Student's *t* test.

RESULTS

Studies of specific resistance sowed that the absolute count of lymphocytes and T and B cells underwent simultaneous daily changes at the beginning of MC (Table 1). During ovulation the mean daily count of lymphocytes and B cells decreased, while the number of 0 cells increased. Variations in the count of T lymphocytes were less pronounced in this period. These changes determine impaired specific resistance. Desynchronization in this system was also observed after ovulation.

Functional activity of phagocytes in healthy women increased during and after ovulation accompanied by physiological immunosuppression. The absolute count of phagocytosing neutrophils and PN increased in the mid-MC period. Digestive activity of these cells increased in the early luteal phase (Fig. 1). Similar mean daily values of PI and count of NBT-positive neutrophils, synchronization of their daily dynamics, circadian rhythms of neutrophil PI and PN, and PN and digestive activity of cells, and inverse relationship between rhythms of spontaneous cell activity and activation coefficients indicate that all stages of neutrophil-mediated phagocytosis were synchronized (Fig. 1). In this period estrogens act as synchronizers of the phagocytic system [3].

The response of neutrophils to high estrogen titers is realized during ovulation, while monocyte response was delayed. This is probably associated with maximum titers of estrogens [5], glucocorticoids, and testosterone [8,11], to which macrophages are highly sensitive. The absolute number of neutrophils did not change in MC, while the mean daily count of peripheral blood monocytes decreased before and during ovulation. However, during ovulation we revealed temporal relationships between the activity of monocytes and other components of the system. The absolute count of latex-binding and NBT-positive cells and PN increased in this period (Fig. 2). Temporal relationships between parameters reflecting phagocytic activity of monocytes were observed in the early luteal phase, when the absolute count of macrophages and their PI increased. The phagocytic system was characterized by physiological desynchronization in the initial and final stages of MC (Figs. 1, 2).

Since macrophages play a key role in the immune response, it can be hypothesized that they potentiate specific immune reactions during suppression of cellular immunity in ovulation. In this period phagocytes probably stimulate humoral immunity, which is accompanied by an increase in the amplitude of rhythmic changes in immunoglobulin content and decrease in the mean daily count of B lymphocytes.

Temporal relationships between lymphocyte populations and functional activity of monocytes in healthy women indicate that macrophages play an organizing role in the immune response. During MC we found positive correlations between changes in the absolute number of T cells and monocyte PI (r=0.75, p<0.05) and between near-monthly rhythms of T lymphocyte and NBT-positive cell counts (r=0.85, p<0.05). The relationships between circadian rhythms of macrophages and lymphocytes in healthy women were most significant in the early luteal phase of MC. There was no significant correlation between the total counts

TABLE 1. Daily Dynamics of the Absolute Count of T, B, and 0 Lymphocytes in Healthy Women during Various Phases of MC (10^9/liter, $M\pm m$)

Cells; cycle phase	Time, h					Mean daily interval
	0.00	6.00	12.00	18.00	24.00	
T lymphocytes						
early follicular	1.52±0.00	0.96±0.06	1.46±0.10	1.16±0.18	1.52±0.03	1.37±1.18
ovulatory	1.09±0.00	0.92±0.15	0.75±0.11	1.14±0.25	1.09±0.15	1.14±0.81
early luteal	1.36±0.00	0.55±0.11	0.94±0.12	1.15±0.12	1.36±0.20	1.14±0.86
B lymphocytes						
early follicular	0.4±0.0	0.24±0.01	0.32±0.02	0.23±0.01	0.40±0.02	0.31±0.28
ovulatory	0.35±0.00	0.19±0.01	0.22±0.02	0.27±0.01	0.35±0.02	0.27±0.24
early luteal	0.44±0.00	0.17±0.02	0.28±0.02	0.33±0.02	0.44±0.02	0.33±0.29
0 lymphocytes						
early follicular	0.78±0.00	0.53±0.06	0.81±0.07	0.41±0.03	0.78±0.05	0.68±0.58
ovulatory	0.93±0.00	1.07±0.10	0.85±0.14	1.11±0.20	0.93±0.10	1.12±0.85
early luteal	0.56±0.00	0.51±0.15	0.55±0.06	0.73±0.07	0.56±0.16	0.70±0.48

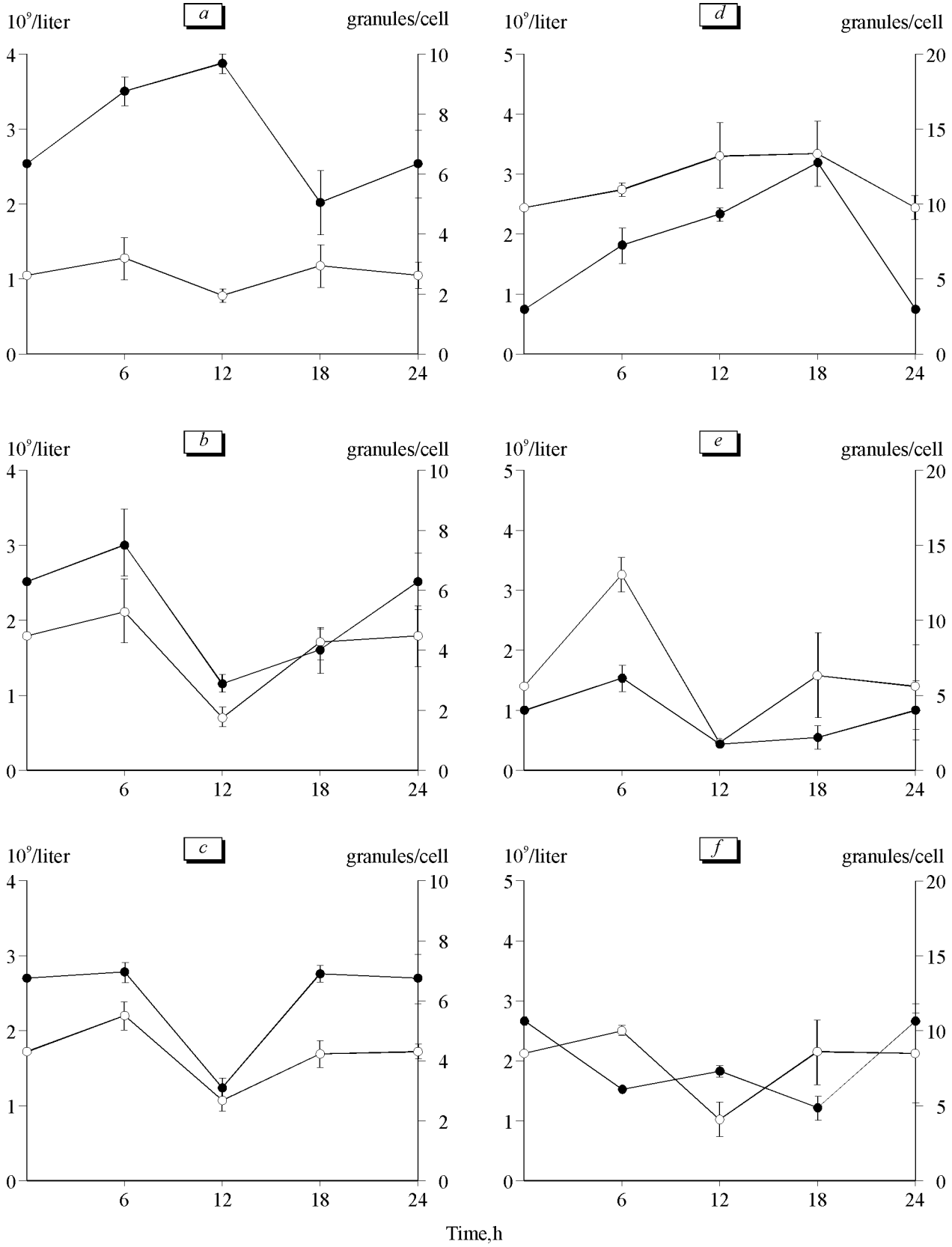

Fig. 1. Daily dynamics of neutrophil activity in healthy women during the early follicular (*a*, *d*), ovulatory (*b*, *e*), and early luteal phases (*c*, *f*) of the menstrual cycle. Here and in Fig. 2: *a-c*) absolute values of the phagocytic index (*1*, left ordinate) and phagocytic number (*2*, right ordinate); *d-f*) count of NBT-positive cells (*1*, left ordinate) and formazan granules (*2*, right ordinate).

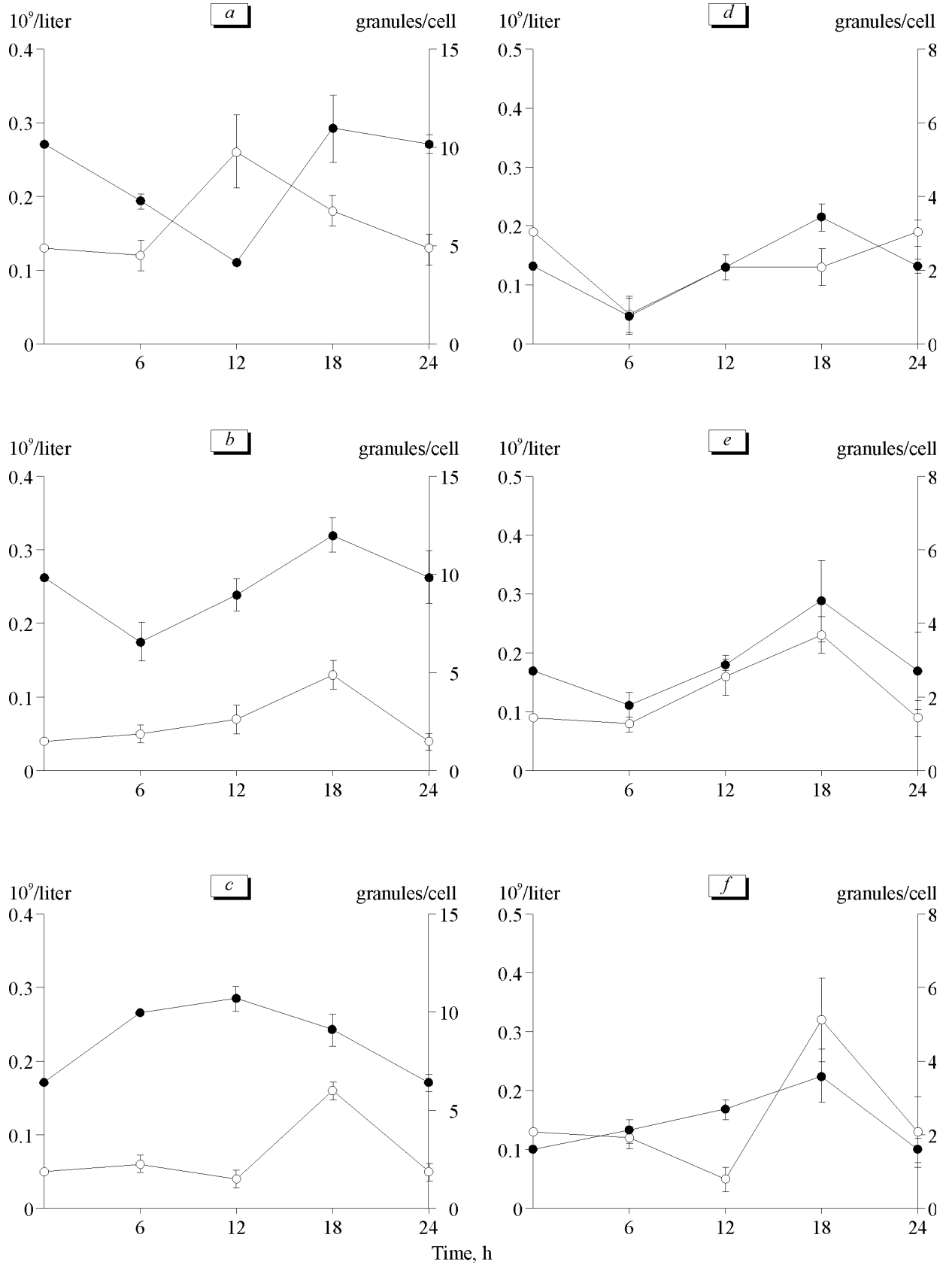

Fig. 2. Daily dynamics of monocyte activity in healthy women during the early follicular (*a*, *d*), ovulatory (*b*, *e*), and early luteal phases (*c*, *f*) of the menstrual cycle.

of peripheral blood monocytes and T lymphocytes (r=-0.43, p>0.05). The increase in the number of functionally active macrophages coincided with the increase in T (due to t helpers) and B cell counts (r=0.79 and 0.72, respectively, p<0.05).

Our results indicate that phagocytic activity of neutrophils and monocytes in adult women should be estimated during ovulation and early luteal phase of MC. The first half of MC is the optimal period for studying parameters of specific resistance.

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