EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Several-Day Rhythmic Changes in Lymphocyte Subpopulation Composition and Peripheral Blood Levels of Interleukin-2 and Hydrocortisone in Donors

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Several-day rhythmic changes in the lymphocyte subpopulation composition and peripheral blood levels of IL-2 and hydrocortisone were studied in healthy men. The samples were collected daily at 8.00. A 4-day rhythm of hydrocortisone secretion manifested synchronously in different individuals. Rhythmic changes in IL-2 level, T-helper/T-suppressor cytotoxic lymphocyte index, and percentage of NK cells in the peripheral blood related to the hormone levels were detected. Rhythmic changes in these parameters should be taken into consideration when evaluating the hormonal profiles and immunological status of healthy subjects and development of methods for correction of abnormalities.

Key Words: *hydrocortisone; infradian rhythm; cytokines; lymphocytes*

Chronobiological approach is now effectively used in various spheres of medicine for detection of states preceding the clinical manifestation of disease and for disease treatment. Circadian rhythms in the functioning of the immune and endocrine systems are well known: for example, morning elevation of steroid hormone levels in the blood, morning increase in the count and activity of NK cells in circulating blood, nocturnal elevation of T-helper/T-suppressor cytotoxic lymphocyte (Th/Tc) index, elevation of proinflammatory cytokine levels in the night and morning hours, *etc*. [12,16]. In addition to circadian rhythms, several-day periodicities are known. For example, a 4-day rhythm of changes in the peripheral blood leukocyte count and 6-7-day periodicity of thymus weight changes in laboratory mice [4] have been described. Four-day rhythmic activity of steroid hormone production has been described for male Wistar rats separated from females.

The cytokine levels in the circulating blood of animals and humans are dynamically balanced with body functions. The cytokine secretion is to a certain measure determined by the hormonal profile [9,10].

Elevation of IL-2 level causes immune response polarization by the Th1 type. It is known that IL-2 stimulates the synthesis and secretion of proinflammatory cytokines IL-6 and TNF-α [3]. Therefore, the data on normal dynamic fluctuations in IL-2 levels are essential for understanding of physiological changes in the Th1 and Th2 immune response balance.

Circadian rhythms of cytokine levels and subpopulation composition of the peripheral blood lymphocytes have been described [15], but not several-day rhythms of these immunological parameters.

We studied several-day rhythms of lymphocyte subpopulation composition, IL-2 level in the periphe-

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ral blood, and the relationships of these parameters with hydrocortisone levels in donors.

MATERIALS AND METHODS

The study was carried out in 8 men aged 24-30 years, sleeping from 22.30 till 7.00 and not experiencing distress throughout the study. Blood was collected daily from June 2 to 22, 2010, at 8.00 (local natural time, estimated from the moment of the Sun position in the lower culmination). Serum specimens were stored at -40°C for no longer than 1 month.

Serum hydrocortisone was measured by EIA using DRG kits. The concentration of IL-2 was measured by EIA with Bender MedSystems kits. The results were recorded using ANTOS-2020 microplate EIA analyzer.

The percentage of NK cells and Th/Tc lymphocyte index in the peripheral blood were evaluated on a Cytomics FC500 flow cytofluorometer (Beckman Coulter) using 4 color labels. The significance of differences between the parameters was evaluated by Mann–Whitney *U* test.

RESULTS

Synchronous 4-day fluctuations of hydrocortisone levels were found in 6 of 8 volunteers. Significant differences in hydrocortisone levels were seen on different days of the study (Fig. 1, *a*). In one volunteer, hydrocortisone levels exhibited an 8-day periodicity with peaks on the days corresponding to hydrocortisone peaks in the main group of donors. In the other volunteer just negligible fluctuations of hydrocortisone level were detected.

Hydrocortisone levels depend on the geomagnetic storms [13]. The geomagnetic situation during the study was uneventful, while the amplitude of hydrocortisone fluctuations was about 40%.

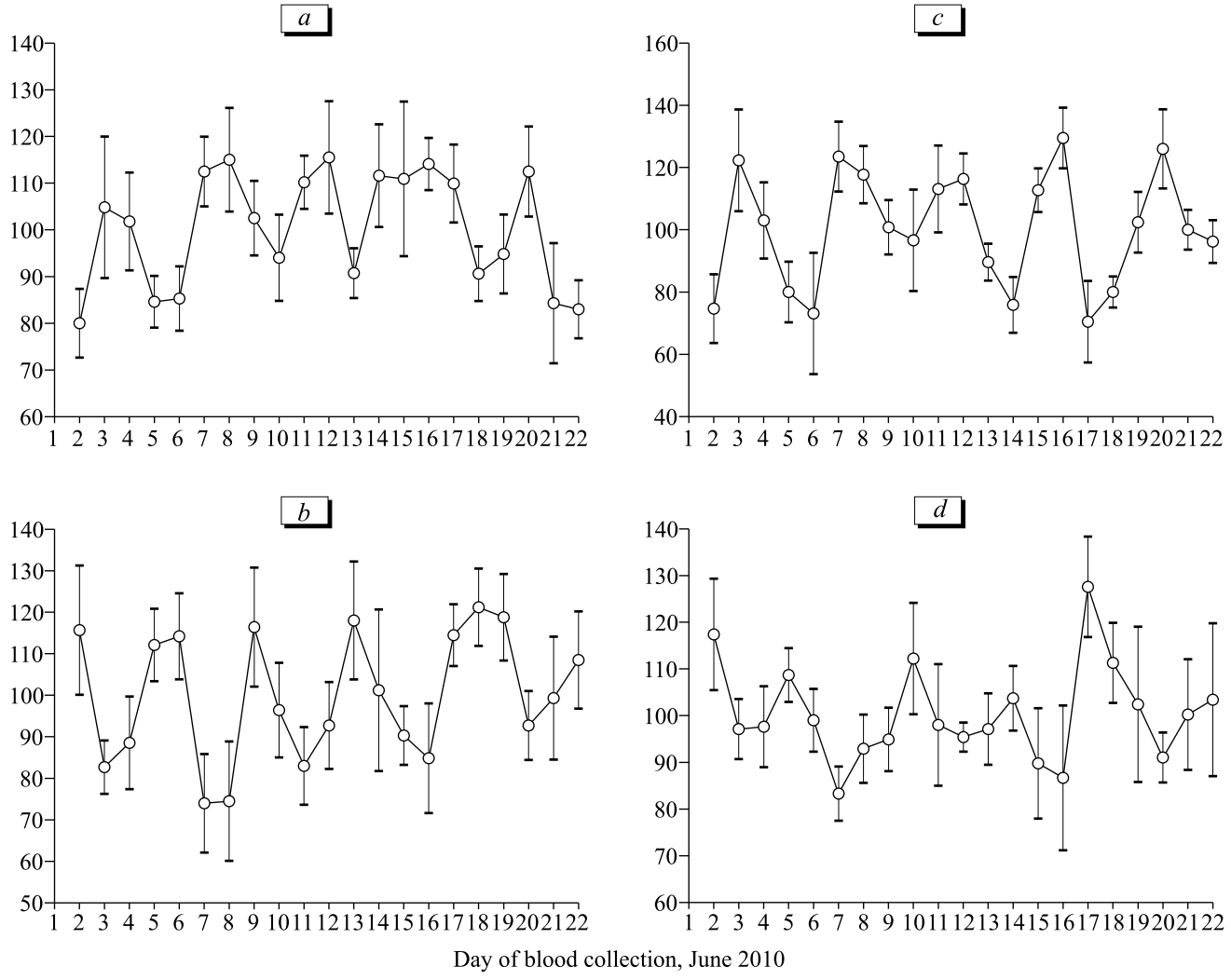

Fig. 1. Dynamics of hydrocortisone (*a*) and IL-2 (*b*) content, NK-cell percentage (*c*), and Th/Tc index (*d*) in the peripheral blood of donors (*n*=6). Ordinates: % of mean value.

A 3-4-day periodicity of hydrocortisone levels in the salivary specimens was detected in first-year schoolchildren adapting to school [1], which was in line with our data.

A 4-day period was clearly seen in the time course of serum IL-2 fluctuations in donors with manifest hydrocortisone fluctuations (Fig. 1, *b*). The IL-2 level was in the antiphase to hydrocortisone level. The correlation coefficient (r) between these parameters was -0.57. Antiphase 8-day rhythm of IL-2 level was found in volunteers with 8-day hydrocortisone rhythm (r= -0.51). No rhythmic fluctuations in the level of IL-2 were detected in the donor with insignificant fluctuations of hydrocortisone concentrations; IL-2 level in this volunteer 2-fold surpassed the normal, and hence, we excluded him from the group of healthy volunteers. According to some data [5], dyschronosis and loss of rhythmic changes in physiological parameters are characteristic of disease and are often detected before clinical manifestation of the illness.

Glucocorticoid hormones inhibit synthesis of proinflammatory cytokines at the transcription and post-transcription levels [7]. After binding to cytosolic receptor glucocorticoids are translocated into the nucleus and there bind to the promotor region of genes responsible for cytokine synthesis. In addition to direct DNA binding, glucocorticoids inhibit the NF-κB and AP-1 transcription factors, which leads to suppression of cytokine synthesis [14]. Glucocorticoid hormones stimulate the differentiation of Th2 lymphocytes and polarize the immune response by the Th2 type [6]. Moreover, glucocorticoids stimulate the expression of TGF-β (immunosuppressive cytokine inhibiting production of proinflammatory cytokines [14]).

The percentage of NK cells in the peripheral blood of donors exhibited a clear-cut 4-day periodicity coinciding by phase with hydrocortisone level (r=0.62; Fig. 1, *c*). The Th/Tc index also exhibited a 4-day periodicity and was in antiphase with hydrocortisone level (r=0.47; Fig. 1, *d*).

Corticosteroids activate NK cells. Normally hydrocortisone positively correlates with NK cell count in circulating blood with a 2-h lag period [11]. The relationship between these parameters is proven experimentally: hydrocortisone treatment of monkeys led to a significant increase in NK cell percentage in the peripheral blood [17]. Injection of dexamethasone to volunteers also led to an increase in blood counts of NK cells [8].

NK cells are an important part of immune defense. Significant 4-day fluctuations in their peripheral blood levels can determine, for example, the temporary resistance to viral diseases.

Th/Tc lymphocyte ratio in the peripheral blood is the key parameter for evaluation of the immune status. Reduction of T-suppressor function leads to predominance of the stimulatory effect of Th determining hyperergic reactions of the immune system. By contrast, reduction of the Th/Tc index leads to suppression of cellular immunity reactions [2].

Hence, fluctuations in blood levels of hydrocortisone in donors are characterized by a 4-day periodicity. They are synchronous in different individuals and modify the peripheral blood IL-2 concentration, Th/Tc index, and NK cell percentage. These dynamic regularities in hydrocortisone levels should be taken into consideration in evaluation of the immune status of normal subjects and development of methods for correction of its disorders.

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