PRESERVATION OF CIRCADIAN RHYTHMS BY HUMAN LYMPHOCYTES IN VITRO

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KEY WORDS: human lymphocytes; E rosettes; circadian rhythm.

Regular fluctuations in the value of immunological parameters in man and animals during the 24-h period, such as the number of circulating blood lymphocytes, the level of their functional activity, and the plasma immunoglobulin concentration, have been firmly established [1, 3, 5-7, 15]. Circadian changes also have been found in the content of T-lymphocyte subpopulations in human blood [8-10, 12], including subpopulations of cells forming rosettes with sheep's erythrocytes (E rosettes) [2, 4]. Rhythmic fluctuations in the number of lymphocytes in the blood are usually attributed to altered influences from organs arising in vivo, although the presence of rhythms for certain types of mammalian cells also has been established in vitro [11].

The aim of this investigation was to determine whether rhythmic activity of human lymphocytes detected by the rosette-formation test is entirely determined by the organism as a whole and does not depend on any intrinsic rhythm of the cells.

EXPERIMENTAL METHOD

Circadian rhythms of E-rosette-forming ability of the lymphocytes were determined in 10 clinically healthy male blood donors aged 20-35 years, who were divided into three groups, with four donors in each group (two donors were common to groups 1 and 2). In group 1 blood samples were taken at 9 a.m., 1, 5, and 9 p.m., and 1 and 5 a.m., mononuclear cells were isolated without delay, and their rosette-forming ability determined. In group 2 all the blood was taken in one portion at 9 a.m. and kept in darkness at 20°C; samples for isolation of mononuclears and for setting up the rosette-formation test were taken from this total portion at the same time points during the 24-h period. In group 3, not blood but a suspension of mononuclear cells in medium 199, isolated from one 9 a.m. portion of blood, was preserved under the same conditions. On the eve of the experiment and on the day when it was performed, the donors did not take alcohol or medicines, and avoided hard physical exertion; otherwise they adhered to the normal program of work, sleep, and meals. Blood was taken from their cubital vein into a vessel containing heparin (20 U/ml) 6 times, in a volume of 2.5 ml each time, or 15 ml at one time. Mononuclear cells were isolated on a Ficoll–Verografin gradient ($d = 1.077$) and the rosette-formation test at each time point was carried out in three repetitions by the technique in [14], enabling active E-rosettes to be revealed. When the reaction was read 1000 cells in a preparation were counted (consequently, each point on the graph was the result of counting 3000 cells). The numerical results were subjected to statistical analysis by Student's test at the 0.05 level of significance.

EXPERIMENTAL RESULTS

Twelve circadian profiles were obtained by the study of 10 blood donors. It will be clear from Figs. 1 and 2a that the rosette-forming ability of lymphocytes from all four donors of group 1 exhibited regular circadian fluctuations with a maximum about noon and a minimum about midnight (differences between these points were statistically significant, $p < 0.05$). The amplitude of the fluctuations was quite considerable: the fraction of rosette-forming cells of some donors during the daytime was almost twice its level at night. When instead of lymphocytes taken directly from the donors, cells kept in vitro throughout the 24-h period of the

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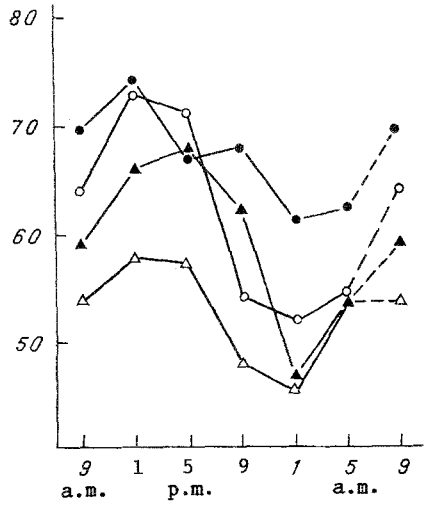

Fig. 1. Individual curves reflecting the circadian rhythms of rosette-forming ability of lymphocytes from four donors (1-4) during sixfold blood sampling. Abscissa, clock time; ordinate, fraction of E rosette-forming cells (in %). The value obtained at 9 a.m. also was used for the second time at 9 a.m.

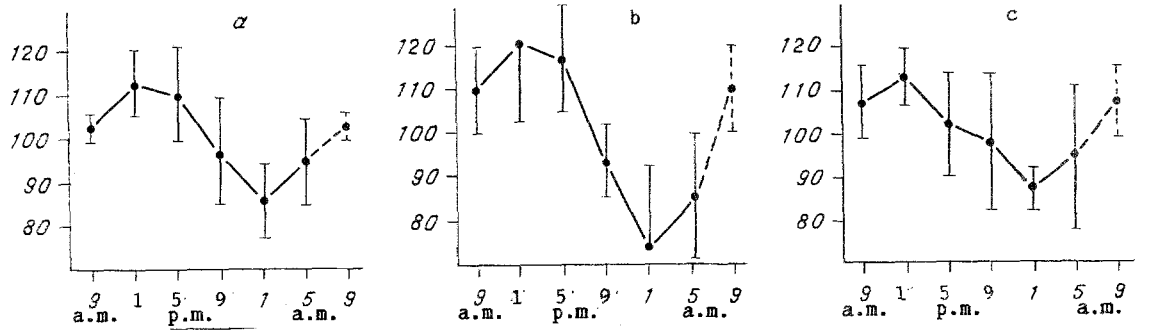

Fig. 2. Averaged circadian fluctuations of rosette-forming ability of lymphocytes in three groups each consisting of four donors. a) Sixfold blood sampling; b) single blood sampling (at 9 a.m.) followed by keeping for 24 h and isolation of cells from it every 4 h; c) single blood sampling (at 9 a.m.), extraction of cells from blood and keeping them for 24 h, until used for determination of rosette-formation every 4 h. Abscissa, clock time; ordinate, E rosette-formation (in % of average individual level for 24-h period, taken as 100, for each donor).

experiment (in whole blood or in lymphocyte suspension) were tested, the curves obtained (Fig. 2b, c) resembled the first (Fig. 2a). Consequently, the circadian rhythm of rosette-forming ability of lymphocytes is preserved in vitro. The range of the rhythmic fluctuations in the experiments with preserved blood (Fig. 2b) was actually wider than when freshly obtained samples were used (Fig. 2a). It will be noted that two donors were common to groups 1 and 2.

The data given in Figs. 1 and 2a agree with the results of one of the two investigations known to us, in which the circadian rhythm of rosette-forming ability of human lymphocytes was determined: in the experiments of Lozovoi and co-workers [2], the peak value of the number of T lymphocytes (E rosette-forming cells) in the blood also occurred in the morning or afternoon, and the minimum during the evening or night. However, Abo and co-workers [4] found the maximum of rosette formation at about midnight and the minimum during the morning. This disagreement may perhaps be due to the fact that these workers kept the cells isolated at night in a refrigerator, and carried out the rosette formation test next day. We found that lymphocytes isolated from blood continue to move along the circadian cycle not only at room temperature, at which they are well preserved [13], but also at 4°C. Thus the Japanese workers [4] must have obtained a rhythm in distorted form.

As regards the ability of human lymphocytes to preserve their rhythmic activity in vitro (in our experiments activity of this kind was found in a 4-day cell culture also), this is

evidently the first time that these data have been obtained. They indicate that the lymphocyte contains a natural oscillator (oscillators) which, in particular, control the circadian rhythm of activity of the E receptor. According to existing views, circadian variations in lymphocyte subpopulations contained in the blood (including cells forming E rosettes) are connected with circadian changes in the plasma cortisol level and with the dynamics of release of cells from the lymphoid tissues into the circulation [4, 10, 12]. In the light of our own data obtained with cells in vitro, rhythmic fluctuations in the subpopulation composition of the blood lymphocytes may depend on circadian changes in the cells themselves and, in particular, their receptors used for identifying subpopulations.

Circa-annual rhythms have a significant influence on the character of the circadian rhythms of E rosette-formation studied in this investigation: in summer circadian fluctuations of rosette formation were significantly reduced in amplitude. All the investigations described in this paper were therefore carried out in February and November.

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FLOATING CULTURES OBTAINED FROM HUMAN FETAL THYROID GLAND

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Experience with clinical transplantation of cultures of endocrine cells has accumulated in recent years. It can be regarded as one method of replacement therapy in the treatment of hormone disturbances. There is evidence that cultures can be obtained from human and animal thyroid glands [3, 6-10, 12]. Investigations [8] which showed that preliminary culture of thyroid gland tissue can considerably prolong its life in an allogeneic or xenogeneic host on subsequent transplantation are particularly interesting. According to Lafferty's hypothesis, this is connected with elimination of what are called "passenger leukocytes" — costimulator cells of the immune response. The problem of culture of thyroid gland cells is important in connection with their potential use for clinical transplantation into hypothyroid patients not responding to ordinary medication. Accordingly it was decided to study the possibility

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