EXPERIMENTAL BIOLOGY

Proliferative Activity of Cells in Mouse Thymus and Spleen under Different Diurnal Regimens of Interleukin-2 Administration

I. G. Kovshik, A. N. Silkov, S. V. Sennikov**, A. V. Shurlygina, and V. A. Trufakin***

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Circadian variations in spontaneous and concanavalin A-stimulated proliferation of mouse thymocytes and splenocytes were studied after administration of recombinant interleukin-2 at different times of day. Differences were revealed in the effect of morning and evening treatment with the cytokine. The time of injection corresponded to various phases of the natural circadian rhythm of endogenous interleukin-2 production, which probably contributes to diurnal differences in the influence of this cytokine.

Key Words: *proliferation; interleukin-2; lymphocytes; circadian rhythm*

Cytokines act as specific regulators of the immune homeostasis and play a role in the pathogenesis of various immunopathological processes. Therefore, the evaluation of immunobiological activity of cytokines is an important problem. Published data show that the synthesis and secretion of all regulatory substances in the organism exhibit a rhythmic pattern [6]. Biological rhythms concern the expression of receptors for regulatory factors on target cells [3,8,9,11]. However, chronobiological aspects of cytokine immunoregulation are poorly understood. For example, the time dependence for the action of exogenous cytokines remains unknown. This is an important problem of clinical immunology. Cytokines are extensively used as potent immunocorrectors [2,7,10]. At the same time, cytokine therapy has several limitations. The search and development of optimized schemes for safe therapy with cytokines are of considerable importance. Chronotherapeutic studies would allow us to develop new diurnal regimens of treatment with immunocorrectors. This approach will increase the effectiveness of therapy, decrease the dosage, and reduce the severity of side effects [4].

Here we studied spontaneous and concanavalin A (ConA)-stimulated proliferation of mouse thymocytes and splenocytes under different diurnal regimens of interleukin-2 (IL-2) administration.

MATERIALS AND METHODS

Experiments were performed on male (CBA×C57Bl/6)F_1 (CBF1) mice aging 3-4 months and obtained from the nursery of laboratory animals of the Siberian Division of the Russian Academy of Medical Sciences (Nizhnyaya El'tsovka, Novosibirsk). The animals were maintained in a vivarium of the Institute of Clinical Immunology. They were housed in

Institute of Clinical and Experimental Lymphology; *Institute of Physiology; **Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** anna_v_s@mail.ru, a.v.shurlygina@iph.ma.nsc.ru. A. V. Shurlygina

plastic cages (Animark) under natural light/dark conditions and had free access to water and food. The communities of constant composition were presynchronized for at least 14 days.

The mice were divided into 3 groups. Control animals remained intact. Other mice received IL-2 in a single dose of 100 U in the morning (10.00) or evening time (20.00). The mice were killed under light ether anesthesia 3 days after cytokine administration. The study was conducted with 6-9 animals of each group at 10.00, 15.00, and 20.00. The thymus and spleen were isolated under sterile conditions.

The organs were homogenized. The cells were gently squeezed from tissue samples into a cold medium using a special pistil. The suspension was filtered to remove large stromal cells and washed 3 times in RPMI 1640 medium. Cell viability was estimated by incorporation of trypan blue or erythrosine B.

The cells (150 μl) were cultured in 96-well round-bottom plates. The final concentration was 0.1×10^6 cells per well. Culturing was performed at 37°C, 100% humidity, and 5% CO_2. The culture medium included RPMI 1640 medium containing 2 mmol/liter L-glutamine, 10% fetal bovine serum, 100 mg/liter ampicillin, and 50 mg/liter gentamicin.

The proliferative response was stimulated with ConA in a concentration of 5 μg/ml. The intensity of proliferation was evaluated by ^{3}H-thymidine incorporation into nucleoprotein cell fractions after 24 h. ^{3}H-Thymidine in a concentration of 1 μCi per well was added at the beginning of culturing. The cells were precipitated on fiberglass filters (Flow) using a Cell Harvester device (Flow Labs). Radioactivity of the sample was recorded on a SL-30 liquid scintillation counter (Intertechnic). The data are presented as the means of 3 identical cultures (cpm).

The results were analyzed by Statistica 5.0 and BIOSTAT 1.0 software. The significance of intergroup differences was evaluated by Kruskal—Wallis test (one-way ANOVA). Correlation study involved the Spearman correlation coefficient.

RESULTS

Administration of IL-2 in the morning and evening time had different effects on spontaneous (SpP) and mitogen-stimulated proliferation (StP) of thymocytes and splenocytes.

Evening treatment with the cytokine was followed by the appearance of significant circadian variations in thymocyte StP (Table 1). An increase in the proliferative response of thymocytes to ConA was revealed only after administration of IL-2 at 20.00 (Table 1). Probably, evening administration of IL-2 corresponded to the period of maximum IL-2 production by thymocytes. It should be emphasized that thymocytes practically do not produce IL-2 in the morning time [1]. The total content of IL-2 in animals receiving injection during the evening time is probably higher compared to mice with morning administration of the cytokine.

Circadian variations in StP of splenocytes disappeared after morning and evening administration of IL-2 (Table 2). Morning treatment with the cytokine was followed by a significant increase in SpP of splenocytes at 20.00 (Table 2).

These data show that exogenous IL-2 induces different reactions of splenocytes and thymocytes, which is probably associated with differences in the cellular composition of the thymus and spleen.

Exogenous IL-2 had a time-dependent effect on synchronous changes in proliferation of thymocytes and splenocytes over the period of study. The correlations typical of intact mice were preserved

TABLE 1. Circadian Variations in Thymocyte Proliferation under Different Diurnal Regimens of IL-2 Administration (cpm, *M*±*m*)

Group		Time of killing, h		
		10:00	15:00	20:00
Control	SpP	2372.13±814.77	1950.00±535.58	2088.61±626.73
	StP	3113.06±1234.03	2387.22±659.69	2218.61±692.86
Morning administration of IL-2				
	SpP	1170.28±240.43	1276.39±307.11	2027.22±600.09
	StP	2188.06±529.37	1662.04±342.54	2637.69±859.73
Evening administration of IL-2				
	SpP	1634.54±431.32	931.85±96.01$^+$	1722.31±420.51
	StP	2806.11±504.82	1373.43±190.12*	2205.46±475.54

Note. $p<0.05$: *compared to SpP; $^+$compared to morning administration.

TABLE 2. Circadian Variations in Splenocyte Proliferation under Different Diurnal Regimens of IL-2 Administration (cpm, *M*±*m*)

Group		Time of killing, h		
		10:00	15:00	20:00
Control	SpP	1002.31±110.02	895.19±153.48	835.19±144.12
	StP	6345.00±1774.42	3114.91±628.78*	1907.13±505.09*+
Morning administration of IL-2				
	SpP	1210.46±257.66	884.54±175.41	1506.76±246.44°
	StP	5697.87±1799.05	3407.04±1230.46	4203.33±1326.32
Evening administration of IL-2				
	SpP	1314.72±269.59	995.37±112.80	1786.67±527.38
	StP	4717.04±1658.51	3757.59±584.80	3690.09±1191.22

Note. $p<0.05$: *compared to 10.00; +compared to 15.00; °compared to the control.

after morning administration of the cytokine. SpP and StP of thymocytes positively correlated with SpP of splenocytes. The correlations between the proliferation parameters of thymocytes and splenocytes were similar in intact mice and animals receiving IL-2 during the evening time (Table 3).

Our results indicate that evening administration of IL-2 has no effect on synchronization of lymphoid cell proliferation over the morning—evening period. Probably, the time of cytokine administration coincides with its maximum production in the thymus [1]. This treatment "imitates" the natural circadian rhythm of IL-2 production by immune cells in mice.

Our previous studies showed that administration of IL-2 at different times of day modifies the subpopulation composition of thymocytes and splenocytes due to circadian variations in the expression of specific receptors [5]. Administration of the cytokine during the period corresponding to high number of IL-2 receptor-carrying cells in the thymus was accompanied by pronounced changes in the number of $CD8^+$ and $CD25^+$ lymphocytes. The time-dependent effect of IL-2 on thymocyte proliferation was associated with basal cytokine production by these cells, but not with the number of specific receptors. This cytokine was more effective in the period corresponding to high number or hyperactivity of IL-2-producing cells (*i.e.*, during the evening time) [1]. Moreover, this regimen of treatment did not impair the circadian rhythm of proliferative activity of thymocytes and splenocytes. These data indicate that the development of chronotherapeutic schemes for cytokine administration should be performed taking into account circadian rhythms of affected parameters.

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TABLE 3. Correlation Analysis for Proliferation of Thymocytes and Splenocytes over the Period of Study (10.00-20.00)

Pair of parameters	Correlation coefficient		
	Control	IL-2, 10.00	IL-2, 20.00
Thymocyte StP — splenocyte StP	0.41	0.51	0.48
Thymocyte SpP — splenocyte StP	0.39	0.37	0.47
Thymocyte SpP — thymocyte StP	0.96	0.89	0.90
Splenocyte SpP — splenocyte StP	0.65	0.66	0.51
Splenocyte StP — SI	0.82	0.85	0.68
Thymocyte SpP — splenocyte SpP		0.52	
Thymocyte StP — splenocyte SpP		0.47	

Note. SI, index of mitogen-stimulated proliferation of splenocytes. Only significant correlations ($p<0.05$) are shown.

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