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Chronodependent Effect of Interleukin-2 on Mouse Spleen Cells in the Model of Cyclophosphamide Immunosuppression

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We studied the chronodependent effect of IL-2 in the experimental model of immunodeficiency, cyclophosphamide-induced immunosuppression in mice. IL-2 in a dose of 100 U/mouse was administered at 10.00 and 16.00 for 3 days after injection of cyclophosphamide. In contrast to the morning treatment with the cytokine, evening administration produced antiapoptotic effect on splenocytes and stimulated proliferation to a greater extent. This was accompanied by an increase in the number of $CD4^+$, $CD25^+$ and $CD4^+25^+$ cells in the spleen to a level of intact mice. More pronounced effect of the evening mode of IL-2 administration on the proliferation and subpopulation composition of mouse spleen cells in the studied model can be associated with high blood level of $CD25^+$ cells at this time of the day.

Key Words: *interleukin-2; cyclophosphamide; biorhythms; spleen cells*

The use of cytokines as effective immunocorrectors is now extensively studied. However apart from high efficiency, cytokine therapy of various immunopathologies has some negative effects including high risk of adverse events, unpredictable delayed effects, and difficulty of predicting and controlling organism's response to cytokine therapy [1]. Thus, the search and development of more effective and safe cytokine therapies are in progress. Chronotherapeutic approach can contribute to the solution of this problem, because is allows the choice of time regimens for drug application considering biorhythms of endogenous production of a particular cytokine and the presence of its receptors on the target cells. Benefits of chronotherapy are shown for a wide range of treatment modalities, namely reduction (or increase when necessary) of the dosage, improvement of the efficiency, and minimization of side effects [2,4,6,10,11]. However, chronocorrection of functional disorders in immune cells is little studied.

IL-2 is widely used in clinical practice for a variety of diseases. It has been successfully used in oncology, infectious and inflammatory diseases, and autoimmune processes. However, along with high efficiency, clinical studies have shown a dose-dependent and multiorgan toxicity of this cytokine [8]. This necessitates the development of optimized chronoherapeutic regimens of IL-2 treatment that would enable dose reduction, increase the effectiveness of treatment, and prevent adverse reactions. The existence of diurnal variations in the subpopulation composition of immunocompetent cell pool and in the number of lymphocytes expressing receptor for IL-2 indicates that cytokine administered in different phases of the circadian cycle can lead to different effects [5].

We have previously shown that the effect IL-2 on the immune system of intact mice depended on the time of its application [2,7]. However, there are no data on chronoefficiency of IL-2 in immunopathological states under experimental and clinical conditions.

Here we studied the chronodependent effect of IL-2 under conditions of cyclophosphamide-induced immunosuppression, which is, according to published data, an adequate model for evaluation of the effectiveness of immunomodulatory effects [12].

MATERIALS AND METHODS

The work was carried out on 3-4-month-old male CBA mice kept in the vivarium of the Research Institute of

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Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences. The animals were housed in plastic cages (Animark) with free access to water and food under natural light/dark conditions. The communities of constant composition were presynchronized for at least 14 days. The experiments were carried out in the fall and winter and were repeated three times.

In each experiment, the mice were divided into 6 groups (5 animals each) as follows: group 1, intact controls; group 2, days 2 to 4 after injection of cyclophosphamide (CP); group 3, IL-2 introduction at 10.00 to mice treated with CP; group 4, IL-2 introduction at 16.00 to mice treated with CP; group 5, day 2 after CP administration (10.00); group 6, day 2 after CP administration (16.00). CP was intraperitoneally injected once in the dose of 200 mg/kg in the morning. On the next day after CP injection, the mice of groups 3 and 4 received intraperitoneal injection of recombinant murine IL-2 (Sigma) in a dose of 100 U/mouse for 3 days. The animals were decapitated under light ether anesthesia on the next day (groups 5 and 6) and on day 4 (group 2) after CP administration and on the next day after the 3rd injection of IL-2 to mice pretreated with CP (groups 3 and 4). The blood was collected from the decapitation wound. The spleen was gently crushed in a glass pestle homogenizer and the cell suspension was prepared.

To determine lymphocyte subpopulations, the cells were treated with FITC-labeled monoclonal antibodies to lymphocyte surface antigens CD3, CD4 and phycoerythrin-labeled monoclonal antibodies to CD25 and CD8 (BD Pharmingen). To determine the ratio of cells in different phases of the cell cycle in the organ, the suspension was washed twice with cold PBS (pH 7.4) and fixed in 70% ethanol. The samples were centrifuged after 1 h and the alcohol was removed. Fixed cells were incubated with 1 ml propidium iodide (10 μg/ml in PBS) and RNase (0.2 μg/ml) for 15 min at room temperature. The samples were assayed using a flow cytofluorimeter FACSCalibur (Becton Dickinson).

The data were statistically processed using Statistica 6.0 software. The arithmetic mean (*M*) and standard error (*SE*) was determined by methods of descriptive statistics. The results were compared using nonparametric statistics, because the data were not normally distributed. The means of the three experiments are presented. The significance of intergroup differences was evaluated using non-parametric Mann–Whitney *U* test. Differences were significant at 95% confidence level.

RESULTS

After IL-2 administration at 10.00, the percentage of hypodiploid cells (apoptosis) in the spleen remained

TABLE 1. Relative (%) and Absolute Number of Cells in Different Phases of the Cell Cycle in Mouse Spleen after Administration of IL-2 at Different Time of the Day (n=15; $M\pm SE$)

Group	Hypodiploid splenocytes		G0/G1-phase splenocytes		G2/M-phase splenocyte		S-phase splenocytes	
	%	10^6	%	10^6-	%	10^6	%	10^6
Intact mice (group 1)	0.21±0.04	0.46±0.11	97.35±0.46	197.72±23.69	1.20±0.34	3.29±1.19	1.24±0.15	2.71±0.58
CP (day 4 after injection, group 2)	0.56±0.18*	0.17±0.03*	96.47±0.61*	49.94±8.40*	1.08±0.33	0.37±0.10*	1.90±0.24	0.85±0.18*
CP+IL-2 at 10.00 h (group 3)	0.62±0.23*	0.56±0.30	95.07±0.71*+	71.63±15.00*	1.67±0.45	0.63±0.12	2.64±0.47*	1.64±0.46
CP+IL-2 at 16.00 h (group 4)	0.34±0.09	0.13±0.03*+	94.84±0.69*+	75.84±17.17*	1.52±0.48	0.46±0.08	3.30±0.40	2.38±0.61+

Note. $p<0.05$ in comparison with *intact animals, +CP.

TABLE 2. Effect of Different Schedules of IL-2 Administration on the Relative (%) and Absolute Number of Cell Subpopulations in Mouse Spleen (n=15; $M\pm SE$)

Parameter	Intact mice (group 1)	CP (day 4 after injection, group 2)	CP+IL-2 at 10.00 h (group 3)	CP+IL-2 at 16.00 h (group 4)
$CD4^+$, %	34.88±1.35	52.12±1.81*	47.25±2.07*	46.18±1.36*
$CD8^+$, %	14.68±0.55	24.61±1.28*	23.38±1.56*	24.14±1.56*
$CD4^+25^+$, %	2.45±0.22	2.79±0.43	2.09±0.27	2.62±0.17
$CD25^+$, %	2.84±0.24	3.54±0.43	3.04±0.32	3.56±0.27
$CD4^+25^+$, 10^6	4.87±0.65	1.65±0.42*	1.62±0.54*	2.50±0.76
$CD4^+$, 10^6	73.36±11.69	27.64±5.82*	32.72±11.10*	39.41±11.03
$CD8^+$, 10^6	29.20±3.07	14.81±3.80*	18.08±6.50	19.65±5.43
$CD25^+$, 10^6	5.65±0.68	2.20±0.54*	2.54±0.95*	3.61±1.20

Note. *p<0.05 in comparison with intact mice.

elevated in comparison with intact controls similar to group 2 treated only with CP. Their absolute number was increased in comparison with group 2 to the level of the intact control. The percentage of S-phase splenocytes was raised compared to intact controls, whereas in group 2 this parameter did not differ from that in intact animals. The absolute number of G2/M-cells and S-phase cells returned to normal level. The percentage of G0/G1-phase spleen cells decreased in comparison with group 2 (Table 1).

After evening IL-2 administration, the percentage of apoptotic splenocytes returned to the normal level and their absolute number decreased in comparison with group 2 remaining below the level in intact controls. The absolute number of S-phase splenocytes decreased in comparison with group 2 to the level of the intact control (Table 1).

Application of IL-2 in different time regimens had different effects on splenocyte subpopulations. Administration of IL-2 at 10.00 normalized the absolute number of $CD8^+$ splenocytes and administration of IL-2 at 16.00 normalized the absolute number of $CD4^+$, $CD4^+25^+$, and $CD25^+$ splenocytes. The percentage of cell subpopulations in the spleen did not change after IL-2 administration in both daily modes (Table 2).

Thus, the evening mode of cytokine administration produced an antiapoptotic effects on splenocytes and stimulated proliferation to a greater extent. This was accompanied by an increase in the absolute number of $CD25^+$ spleen cells to the level of intact control indicating enhanced activation of splenocytes under the influence of the cytokine. Noteworthy, the absolute number of regulatory T cells in the spleen ($CD4^+25^+$) increased to the level of intact control, which was not observed after administration of the cytokine at 10.00. This may help to prevent the development of autoimmune reactions, often observed as a result of intensive lymphocyte proliferation, in particular, during regeneration after cytostatic treatment [9].

On day 2 after CP treatment (*i.e.* before IL-2 administration), the absolute number of cells with IL-2 receptor ($CD25^+$) was significantly higher at 16.00 (Table 3). Thus, the more pronounced effect of the evening mode of IL-2 administration on the proliferation and subpopulation composition of mouse spleen cells in the examined model can be associated with high blood level of $CD25^+$ cells at this time of day. We cannot exclude the fact that the production of endogenous IL-2 in intact mice was enhanced in the evening, therefore evening administration of the cytokine could be more effective due to its imitative character echoing the normal circadian rhythm of endogenous IL-2 [3].

Further study of chronobiological patterns of the effects of cytokines will provide more efficient and safe therapies aimed at correction of immunopathological states.

TABLE 3. Daily Variations in $CD25^+$ Cell Blood Levels in Mice on Day 2 after CP Administration (n=15; $M\pm SE$)

Parameter	Time of the day	
	10.00 (group 5)	16.00 (group 6)
$CD4^+25^+$ cells from peripheral blood, 10^6/ml	0.030±0.004	0.15±0.04*
$CD4^+25^{high}$ cells from peripheral blood, 10^6/ml	0.010±0.002	0.04±0.02*
$CD25^+$ cells from peripheral blood, 10^6/ml	0.040±0.005	0.16±0.04*

Note. *p<0.05 in comparison with 10.00.

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