Circadian Dynamics of Cell Composition of the Thymus and Lymph Nodes in Mice Normally, under Conditions of Permanent Illumination, and after Melatonin Injection

G. I. Litvinenko, A. V. Shurlygina, L. V. Verbitskaya, N. G. Panteleeva, E. V. Mel'nikova, and V. A. Trufakin*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 8, pp. 181-185, August, 2005
Original article submitted January 20, 2005

The effect of melatonin on disturbed circadian variations in the lymphocyte subpopulation composition of the thymus and inguinal lymph nodes was studied in CBA mice exposed to constant illumination for 14 days. The desynchronizing effect of permanent illumination on the thymus consisted in disappearance of circadian variations in the total number of thymocytes, absolute count of thymocytes, absolute counts of $CD8^+$ and $CD4^+$ cells, and in inversion of changes in the absolute counts of $CD4^+8^+$ cells from 15.00 to 20.00. In lymph nodes circadian variations in the percentage of $CD4^+$ lymphocytes disappeared, while absolute counts of $CD4^+8^+$ and $CD8^+$ cells changed from 15.00 to 20.00. Melatonin restored circadian dynamics of some parameters mainly in the thymus.

Key Words: *desynchronosis; lymphocytes; melatonin*

The optimal level of functioning of all live systems, specifically, the immune system, is determined by the realization of a periodical program [4] maintaining necessary sequence of physiological, metabolic, and biochemical processes and the needed instant ratio of its parameters. Circadian periodical program of the immune system is presented by a complex of biorhythms, which are in a certain phase relationship with each other [5]. The function of the immune system, metabolic status of immunocompetent cells, subpopulation composition of cells in the central and peripheral lymphoid organs change over 24 h [5,10,12]. Light/darkness regimen is a potent synchronizers of circadian biological rhythms in mammals. Its disturbances cause desynchronosis (temporal dissociation of physiological processes) and distortion of the periodical program [4]. Modification of light/darkness alternation rhythm produces a desynchronizing effect on the immune system, changing the circadian rhythm of cell composition of lymphoid organs and blood [2,5] and inducing the development of immunodeficiency [13].

Melatonin (MT), a pineal gland hormone, is a synchronizer of circadian rhythms [1,3]. Its chronotropic activity predisposes to optimization of the immunomodulating role of MT. The hormone is a universal mediator for various physiological functions with circadian rhythm, including the immune system [6]. Experiments on animals with induced desynchronosis showed that regular injections of MT at a certain time of the day restored lost periodicity of fluctuations [7]. Moreover, MT exhibited immunoregulatory activity during the development of secondary immunodeficiency [9,11].

We studied the effect of MT on cell composition of the thymus and inguinal lymph nodes in mice exposed to constant illumination for 14 days.

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

0007-4888/05/1402-0213

MATERIALS AND METHODS

Experiments were carried out on 4-month-old male CBA mice (n=60). The animals were divided into 4 groups. Group 1 animals were kept under conditions of standard illumination regimen for 14 days, group 2 mice were exposed to permanent illumination (PI) for 14 days and then transferred to standard light/darkness regimen for the next 3 days. Group 3 animals were exposed to PI for 14 days and then transferred to standard day/night regimen with daily injections of 0.5 ml normal saline (NS) intraperitoneally at 18.00 for 3 days. Group 4 animals received the same exposure as group 3, but after PI received 3 injections of MT (0.02 mg) intraperitoneally in 0.05 ml NS once daily at 18.00. The next day after the last injections of MT and NS the animals were sacrificed at 10.00, 15.00, and 20.00. The thymus and inguinal lymph nodes were collected for analysis. Total cell counts, subpopulation composition of lymphocytes, and their absolute counts ($CD4^+$, $CD8^+$, $CD4^+8^+$) and $CD4^+/CD8^+$ ratio were evaluated. Lymphocyte subpopulations were evaluated using monoclonal antibodies labeled with FITC and PE (Pharmingen) on a FACSCalibur flow cytometer (Becton Dickinson). The data were statistically processed using Mann—Whitney nonparametric test.

RESULTS

In group 1 (intact control) the total number of cells in the thymus and absolute counts of $CD4^+$, $CD8^+$, and $CD4^+8^+$ thymocytes increased from 10.00 to 15.00 and still more to 20.00 (Table 1). In group 2 cytosis of the thymus and absolute counts of $CD8^+$ lymphocytes increased from 10.00 to 15.00 and then did not change, the absolute count of $CD4^+8^+$ cells increased from 10.00 to 15.00, but, in contrast to group 1, decreased by 20.00. The absolute counts of $CD4^+$ thymocytes in group 2 animals was higher at 15.00 and 20.00 than at 10.00. A special feature of this group in comparison with intact control was the increase of the percentage of $CD4^+$ thymocytes from 10.00 to 20.00, while in group 1 this parameter did not change. A standard characteristic of groups 1 and 2 is circadian dynamics of the absolute count of $CD4^+$ cells in the thymus: it increased from 10.00 to 20.00 (Table 1).

TABLE 1. Circadian Fluctuations in Lymphocyte Subpopulation Composition in the Thymus under Conditions of Normal and Permanent Illumination ($M\pm m$)

Cell subpopulation	Standard light/darkness regimen, h			PI, h		
	10:00	15:00	20:00	10:00	15:00	20:00
$CD4^+$, %	18.6±3.4	21.8±2.5	29.0±2.6	17.5±1.1	26.7±1.9	27.3±4.1$^+$
$CD4^+8^+$, %	51.8±1.9	61.2±4.9	49.4±2.8	60.8±2.2*	53.0±2.2	48.7±0.5$^+$
$CD8^+$, %	15.5±3.02	8.6±1.34	12.9±1.20	10.1±0.9	7.6±0.7	11.8±3.5
Total cell count, $\times10^6$	5.4±1.5	25.2±2.9*	37.2±6.6*	10.0±3.4	30.1±4.9+	23.5±3.3
Absolute count of $CD4^+$, $\times10^6$	1.1±0.4	4.0±1.1	12.3±1.5*^{o}	1.7±0.4*	7.7±0.7$^+$	5.5±0.4$^+$
Absolute count of $CD4^+8^+$, $\times10^6$	2.8±0.8+	14.9±1.6	20.6±2.6*	6.1±2.0	16.3±3.2$^+$	10.2±2.3
Absolute count of $CD8^+$, $\times10^6$	0.8±0.2	1.9±0.3*	5.4±0.7*^{o}	0.9±0.33$^+$	2.2±0.3$^+$	2.6±1.3

Note. p<0.05 compared to *10.00 at standard light/darkness regimen, ^{o}15.00, $^+$PI, 10.00.

TABLE 2. Circadian Fluctuations in Lymphocyte Subpopulation Composition in the Thymus after Injection of NS or MT ($M\pm m$)

Cell subpopulation	PI+NS, h			PI+MT, h		
	10:00	15:00	20:00	10:00	15:00	20:00
$CD4^+$, %	13.3±3.5	16.4±6.7	13.8±2.3	23.4±8.7*	12.7±3.3	16.8±3.3
$CD4^+8^+$, %	77.9±2.1	72.9±3.44	72.9±2.8	64.4±6.1*	79.4±2.6	69.1±2.1
Total cell count, $\times10^6$	46.4±9.8	53.9±9.5	22.4±4.6*^{o}	60.1±10.2	12.9±2.9^{+o}	18.3±3.5^o
Absolute count of $CD4^+$, $\times10^6$	6.1±1.0	9.6±3.2	3.1±0.9	13.8±2.9*	1.8±0.5$^+$	2.9±0.7$^+$
Absolute count of $CD4^+8^+$, $\times10^6$	36.3±8.2	38.3±5.5	16.2±3.1*^{o}	39.6±8.9*	10.5±2.2	12.9±3.5$^+$
Absolute count of $CD8^+$, $\times10^6$	2.5±0.6	3.4±0.7	1.2±0.3	4.7±0.9	0.6±0.1	1.1±0.4

Note. p<0.05 compared to *PI+NS, 10.00; $^+$PI+MT, 10.00; ^{o}PI+NS, 15.00.

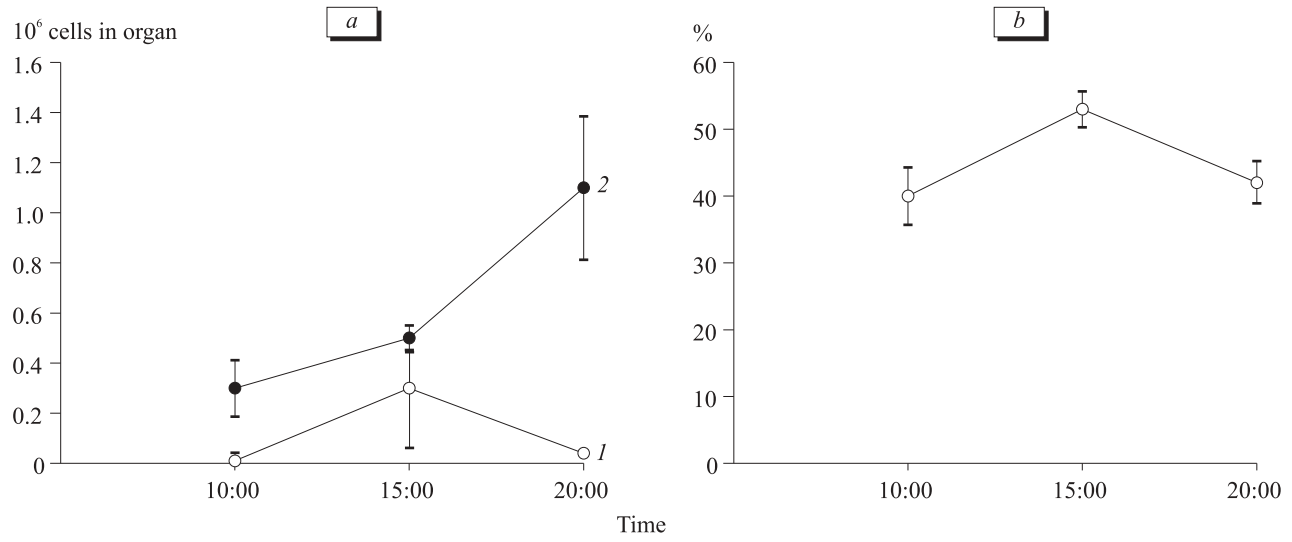

Fig. 1. Circadian fluctuations in cell composition of inguinal lymph nodes of mice under conditions of normal light/darkness regimen. *a*) absolute count of cells with $CD4^+8^+$ phenotype (*1*) and of $CD8^+$ cells (*2*); *b*) percentage of $CD4^+$ lymphocytes.

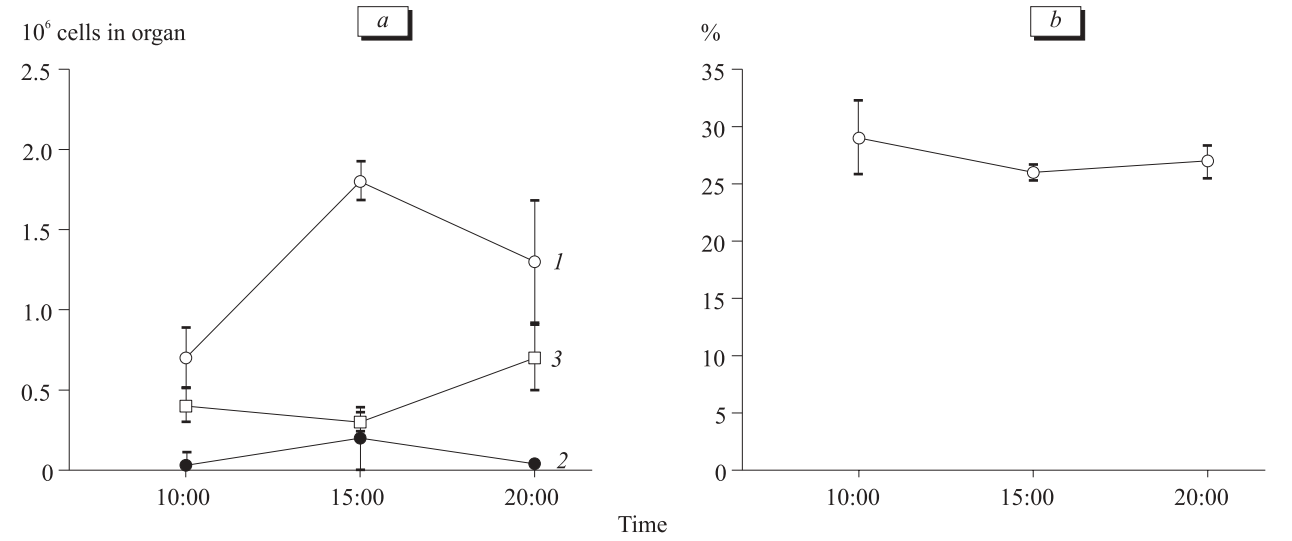

Fig. 2. Circadian changes in cell composition of inguinal lymph nodes under conditions of permanent illumination. *a*) absolute count of cells with $CD4^+$ phenotype (*1*), $CD4^+8^+$ (*2*), and $CD8^+$ cells (*3*); *b*) percentage of $CD8^+$ lymphocytes.

After injection of NS to group 3 animals the count of cells in the thymus and absolute count of lymphocytes with $CD4^+8^+$ phenotype decreased from 10.00 to 20.00 and from 15.00 to 20.00, the absolute count of $CD8^+$ decreased from 15.00 to 20.00 (Table 2). Hence, injections of MT solvent did not restore circadian dynamics of cell composition of the thymus disturbed by PI.

In mice exposed to PI melatonin treatment decreased the total number of cells in the thymus, absolute counts of $CD4^+8^+$, $CD8^+$, and $CD4^+$ thymocytes from 10.00 to 15.00 and increased this parameter at 20.00 (Table 2). Hence, the direction of circadian changes from 15.00 to 20.00 becomes similar to that in intact mice.

In inguinal lymph nodes from group 1 animals the absolute counts of $CD4^+8^+$ and $CD8^+$ lymphocytes increased from 10.00 to 20.00 and the percentage of lymphocytes with $CD4^+$ phenotype increased from 10.00 to 15.00 and decreased by 20.00 (Fig. 1).

The absolute counts of $CD4^+8^+$ and $CD8^+$ lymphocytes changed significantly in animals exposed to PI during the entire period of observation, similarly as in controls, but these parameters changed not from 10.00 to 20.00, but only from 10.00 to 15.00. The absolute count of $CD4^+8^+$ cells increased from 10.00 to 15.00, while the absolute count of $CD8^+$ lymphocytes decreased. The percentage of $CD8^+$ lymphocytes increased from 15.00 to 20.00 and the absolute count of $CD4^+$ cells from 10.00 to 20.00 (Fig. 2). In contrast

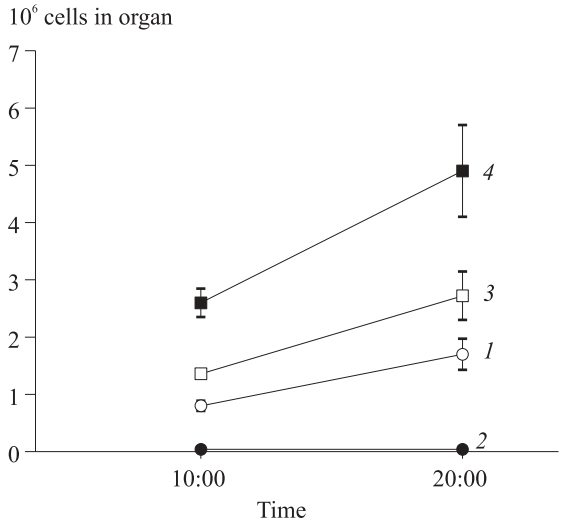

Fig. 3. Circadian variations in cellular composition of inguinal lymph nodes during exposure to permanent illumination after injection of normal saline. *1*) $CD8^+$; *2*) $CD4^+8^+$; *3*) $CD4^+$; *4*) total cell count.

to control animals in mice exposed to PI, the percentage of $CD4^+$ lymphocytes in inguinal lymph nodes did not exhibit circadian fluctuations. Significant changes in the subpopulation composition of lymphocytes in these organs were recorded only at 20.00, when the percentage of $CD4^+$ cells was higher in mice exposed to PI (47.5±6.58%) than in intact animals (41.7±2.97%; $p<0.05$).

The pattern of circadian variations in the parameters of mice exposed to PI changed after injection of NS. The absolute counts of $CD8^+$ and $CD4^+8^+$ lymphocytes changed significantly from 10.00 to 20.00 (similarly as in intact mice). The absolute count of $CD8^+$ cells increased from 10.00 to 20.00, but the absolute count of $CD4^+8^+$ lymphocytes decreased during this period. The absolute count of $CD4^+$ lymphocytes increased from 10.00 to 20.00, similarly as in mice exposed to PI without NS injection. After NS injection the total count of cells in lymph nodes started to change over the 24-h period, increasing significantly from 10.00 to 20.00 (Fig. 3). After injection of NS, which can be regarded as injection stress, partial recovery of circadian dynamics of the subpopulation composition of lymph nodes was observed (normalization of the absolute count of $CD8^+$ lymphocytes).

In group 4 only the level of lymphocytes with the $CD4^+8^+$ phenotype changed over 24 h: their percentage decreased from 10.00 (1.15±0.27%) and 15.00 (2.4±0.7%) by 20.00 (0.54±0.12%; $p<0.05$). The evening differences in the percentage of $CD4^+$ lymphocytes in the groups of mice with different illumination regimen (41.5±2.97% at normal illumination and 47.5±6.58% at permanent illumination, $p<0.05$) were retained after injection of MT (51.1±1.70%; $p<0.05$), their content increasing after MT injection in comparison with the control group. Circadian changes in this lymphocyte subpopulation disappearing after exposure to PI were not restored after injection of MT.

Hence, exposure to PI disturbs circadian variations in the subpopulation composition of lymphocytes in the central and peripheral lymphoid organs of experimental animals, which indicates a desynchronizing effect of this exposure on circadian organization of proliferation, differentiation, and migration in the immune system. Regular injection stress seems to "trigger" circadian biorhythms of the immune system, its synchronizing effect being more pronounced at the level of the peripheral lymphoid organs (inguinal lymph nodes). The synchronizing effect of MT is more pronounced in the central immune organ (thymus) and is virtually absent at the level of the lymph nodes. This is in line with a previous report [9] indicating that the main immunomodulating effects of MT are linked with modulation of the central component of T-lymphocyte differentiation.

The authors are grateful to Professor V. S. Shirinskii, Head of Immunopharmacology Laboratory, Institute of Clinical Immunology, Siberian Division of Russian Academy of Medical Sciences, for MT.

REFERENCES

1. E. B. Arushanyan, *Eksp. Klin. Farmakol.*, No. 5, 72-77 (1992).
2. E. B. Arushanyan and E. V. Beyer, *Ibid.*, No. 5, 73-80 (2002).
3. V. A. Baturin and E. B. Arushanyan, *Zh. Vyssh. Nervn. Deyat.*, No. 4, 681-687 (1990).
4. Yu. Ashoff, Ed., *Biological Rhythms* [in Russian], Moscow (1984), Vol. 1-2.
5. Yu. I. Borodin, V. A. Trufakin, A. Yu. Letyagin, and A. V. Shurlygina, *Circadian Biorhythms of Immune System* [in Russian], Novosibirsk (1992).
6. D. P. Cardinali, L. I. Brusco, R. A. Cultera, *et al.*, *Biol. Signals Recept.*, **8**, No. 1-2, 41-48 (1999).
7. V. M. Cassone, *Trends Neurosci.*, No. 13, 457-464 (1990).
8. G. J. M. Maestroni, *Melatonin and the Pineal Gland*, Paris (1992), P. 59.
9. G. J. M. Maestroni and A. Conti, *Neuroimmunomodulation*, No. 3, 325-332 (1996).
10. Ljubica Matijevic-Masik, *Rad. Hw. Akaol. Znan. i Umjeth. Med. Znan.*, **27**, 181-190 (1994).
11. M. Rafii-el-Idrissi, J. R. Calvo, D. Pozo, *et al.*, *J. Neuroimmunol.*, **57**, No. 1-2, 171-178 (1995).
12. S. Suzuki, S. Toyabe, M. Moroda, *et al.*, *Clin. Exp. Immunol.*, **110**, No. 3, 500-508 (1997).
13. Xu Feng, Li Jimg Cai, and Yang Ying-Bao, *Chin. Pharmacol. Bull.*, **10**, No. 6, 448-452 (1994).