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Circadian rhythm of leucocytes and lymphocyte subsets and its possible correlation with the function of the autonomic nervous system

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SUMMARY

There are physiological variations in the levels of leucocytes. Among these, the circadian rhythm is very important in terms of the magnitude. Since newly identified lymphocyte subsets (i.e. extrathymic T cells) have recently been detected, a comprehensive study of the circadian rhythm was conducted. All leucocytes were found to vary in number or proportion with a circadian rhythm and were classified into two groups. One group—granulocytes, macrophages, natural killer (NK) cells, extrathymic T cells, $\gamma\delta$ T cells, and $CD8^+$ subset—showed an increase in the daytime (i.e. daytime rhythm). The other group—T cells, B cells, $\alpha\beta$ T cells, and $CD4^+$ subset—showed an increase at night. Humans are active and show sympathetic nerve dominance in the daytime. Interestingly, granulocytes and lymphocyte subsets with the daytime rhythm were found to carry a high density of adrenergic receptors. On the other hand, lymphocyte subsets with the night rhythm carried a high proportion of cholinergic receptors. Reflecting this situation, exercise prominently increased the number of cells with the daytime rhythm. These results suggest that the levels of leucocytes may be under the regulation of the autonomic nervous system.

Keywords circadian rhythm leucocytes lymphocyte subset granulocytes autonomic nervous system

INTRODUCTION

It is well known that various immunoparameters vary in number and function, depending on physical conditions in the host [1,2]. One such phenomenon is circadian variation in the number of leucocytes and lymphocyte subsets in humans and animals [3–5]. In earlier studies, such rhythms of lymphocytes were estimated by morphological and other criteria. In a subsequent study, lymphocyte subsets were estimated by MoAbs, and a unique pattern of the circadian rhythm was reported [6–8]. In this study, experiments were further conducted for the following purposes: (i) since recent studies have revealed the existence of new lymphocyte subsets in humans, i.e. $CD56^+$ T cells and $CD57^+$ T cells of extrathymic origin [9,10], it is desirable that an overall picture of circadian variation of leucocytes and lymphocyte subsets should be obtained; (ii) it should also be elucidated what is the major factor causing the circadian rhythm of leucocytes and lymphocyte subsets.

The results obtained in the present study in humans demonstrate that there are two groups of leucocytes with different peak times of the circadian rhythm. One group consists of cells with a daytime rhythm, namely, cells which increase in number or proportion in the daytime. This group includes monocytes, granulocytes, natural killer (NK) cells, extrathymic T cells, $CD8^+$ cells, and $\gamma\delta$ T cells. The other group comprises cells with a night rhythm, namely, cells which increase in number or proportion at night. This group includes T cells (including $\alpha\beta$ T cells), B cells, and $CD4^+$ cells.

Many, if not all, of the cells with the daytime rhythm tend to express a higher level of adrenergic receptors on the surface. In contrast, cells with the night rhythm were found to carry a large proportion of cholinergic receptors on the surface. This raises the possibility that physical activity, which accompanies the high production of catecholamines or inversely the high production of acetylcholine (ACh), determines the rhythm of leucocytes. Supporting this speculation, it was found that all cells with the daytime rhythm increased in number after exercise. In conjunction with accumulating evidence for adrenergic and cholinergic receptors on leucocytes [11,12], it is concluded that physical activity, a function of the autonomic nervous system, causes the circadian rhythm of leucocytes and lymphocyte subsets.

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SUBJECTS AND METHODS

Subjects

The subjects in this study were 10 healthy men, their ages ranging from 26 to 48 years. Two of them, subject 1 being 48 years old and subject 2 being 28 years old, were also tested after exercise. The samples for a circadian rhythm were taken every 4 h from the beginning at 8.00 am. All donors worked and slept in bed, in a regular way.

Immunoparameters examined

Peripheral blood (5 ml) was aspirated into a heparinized syringe. Two millilitres of the blood were sent to the Central Laboratory Unit of Niigata University to examine the numbers and proportions of leucocytes, including granulocytes and lymphocytes, and to measure the serum levels of adrenaline and noradrenaline. The enumeration of leucocyte count was usually done within several hours after sampling. During this time, we confirmed that the result was not affected.

The remaining 3 ml of the blood was used to determine the levels of various lymphocyte subsets. Mononuclear cells (MNC) were isolated by Ficoll–Isopaque gradient (1·077 g/ml) centrifugation [5].

Two-colour immunofluorescence tests were applied to identify lymphocytes subsets, NK cells, extrathymic T cells, and conventional T and B cells [10]. Two-colour staining for CD3 and CD16 (or CD56 or CD57) identified $CD3^-CD16^+$ NK cells (or $CD3^-CD56^+$ or $CD3^-CD57^+$ NK cells), $CD3^+CD16^+$ extrathymic T cells (or $CD3^+CD56^+$ or $CD3^+CD57^+$ extrathymic T cells), and $CD3^+CD16^-$ conventional T cells (or $CD3^+CD56^-$ or $CD3^+CD57^-$ conventional T cells). Two-colour staining for CD3 and T cell receptor (TCR) $\alpha\beta$ (or TCR $\gamma\delta$) or for CD3 and CD4 (or CD8) was also performed to identify $\alpha\beta$ T cells (or $\gamma\delta$ T cells) and $CD4^+$ T cells (or $CD8^+$ T cells). $CD20^+CD5^-$ B cells were identified by two-colour staining for CD20 and CD5. All MoAbs used were obtained from Becton Dickinson (Mountain View, CA). The fluorescence-positive cells were analysed by FACScan (Becton Dickinson).

Cell purification

To determine the presence of adrenergic or cholinergic receptors on various cell fractions, cell purification was performed. Monocytes were purified from whole MNC by adherence to a plastic surface [13]. Granulocytes were isolated from the buffy coat of the blood (sedimented by 6% dextran sulphate) by Ficoll–Isopaque gradient centrifugation. All lymphocyte subsets were isolated by the cell sorter after two-colour staining in various combinations of MoAbs as shown above. Purity was > 98%.

Serum level of catecholamines

Plasma at the indicated time was used to measure the concentration of adrenaline and noradrenaline. Its concentrations were analysed by the high performance liquid chromatography (HPLC) system, as described elsewhere [14]. Preliminary experiments of separated aliquots in one sample showed us that experiment-to-experiment variation was < 2 pg/ml for adrenaline and < 10 pg/ml for noradrenaline.

Measurement of β-adrenergic receptors

Pre-incubation of 2×10^5 cells with 1 μM propranolol was first performed in Eagle's MEM medium supplemented with 6 mM HEPES (Nissui Pharmaceutical Co., Tokyo, Japan) and 2% heat-inactivated newborn calf serum for 90 min at 30°C. Cells were then incubated with different concentrations (10–200 pM) of ^{125}I-cyanopindolol (^{125}I-CYP; Amersham Corp., Arlington Heights, IL) in a total volume of 600 μl medium [11,12]. After being washed three times with medium, cell pellets were obtained to measure isotope-binding levels in a gamma counter. Specific ^{125}I-CYP binding was determined by subtracting the value in the presence of 1 μM propranolol from the total counts in its absence.

Identification of cholinergic receptors on the cell surface

Purified cell fractions (2×10^5 cells) were first incubated with a medium containing ACh esterase (AChE) (1·0 U/ml). This incubation at 37°C for 5 h is critical to eliminate saturated ACh on the cell surface of leucocytes [15]. Cells were harvested and washed twice with medium. FITC-conjugated α-bungarotoxin (α-BT; Sigma Chemical Co., St Louis, MO) was then added at various concentrations to the cell pellet for 30 min at 4°C. After washing, fluorescence-positive cells were analysed by FACScan. This method identified the proportion of cells carrying cholinergic receptors (i.e. nicotinic acetylcholine receptors; nAChR).

Exercise

Subjects 1 and 2 ran for 1 h, starting at 2.00 pm. Immunoparameters were examined at three points of time, i.e. before, just after, and 4 h after the exercise.

Statistical analysis

Rogers' method was applied to estimate the rhythmicity [16]. Student's *t*-test was also used to estimate the difference of some parameters.

RESULTS

Circadian rhythm of granulocytes, lymphocytes and monocytes in the blood

In this study, we first confirmed the pattern of variation in the number of leucocytes in the peripheral blood over the 24-h period of a day. Data from five healthy donors are represented (Fig. 1). Although the number of total leucocytes was seen to be relatively constant, the number (as well as the proportion) of granulocytes, lymphocytes and monocytes varied, showing a circadian rhythm. The number and proportion of granulocytes and monocytes increased in the daytime, whereas the number and proportion of lymphocytes increased at night (statistical analysis of these parameters is included at the top of Table 2). Since granulocytes and monocytes comprise > 60% of the total leucocytes, the variation in the number of total leucocytes slightly resembled this variation. This tendency was previously reported by us as well as by other investigators [3–8]. Since we have already reported the day-to-day variation and annual variation in some of these factors [16], we did not repeat it.

Stability of the value in experiments

In the present study, we enumerated many immunoparameters, namely the proportion of various lymphocyte subsets. To determine the variation of such values between experiments, one sample was separated into three aliquots and the values were enumerated independently (Table 1). It was confirmed that the variaton was very small.

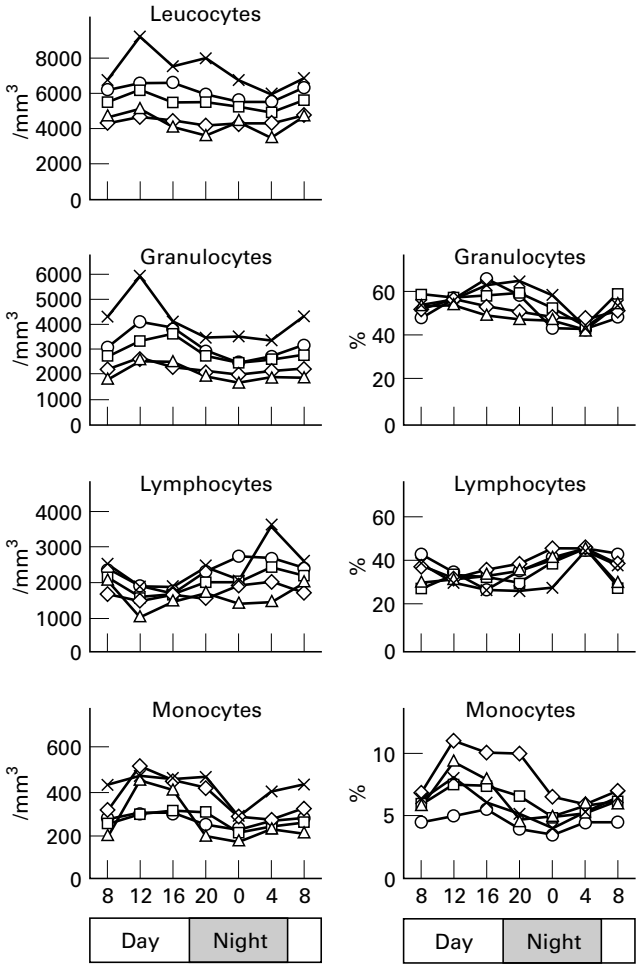

Fig. 1. Circadian rhythm in the number and proportion of total leucocytes, granulocytes, lymphocytes, and monocytes. Five healthy donors were examined on various immunoparameters every 4 h from the beginning at 8.00 am during the 24-h period of a day. It was confirmed that the number and proportion of granulocytes and monocytes had daytime rhythm, while those of lymphocytes had night rhythm. The same symbols in Figs 1–3 indicate data from the same individual.

Circadian rhythm of lymphocyte subsets was classified into two groups

Two-colour staining in various combinations was conducted to identify lymphocyte subsets in the peripheral blood, e.g. NK cells, extrathymic T cells, and conventional T and B cells. Data from five healthy subjects are represented (Fig. 2). The significance of the rhythms is statistically analysed in Table 2. By two-colour staining for CD3 and CD16 (or CD56 or CD57), $CD3^-CD16^+$ (or $CD56^+$ or $CD57^+$) NK cells, $CD3^+CD16^+$ (or $CD56^+$ or $CD57^+$) extrathymic T cells, and $CD3^+CD16^-$ (or $CD56^-$ or $CD57^-$) conventional T cells were determined. NK cells and extrathymic T cells (except $CD3^+CD16^+$ cells) were estimated to vary with a circadian rhythm, showing an increase in the proportion in the daytime. In contrast, conventional T cells varied with a circadian rhythm, showing an increase in the proportion at night. $\gamma\delta$ T cells increased in the daytime, whereas $\alpha\beta$ T cells increased at night. $CD4^+$ T cells increased at night, whereas $CD8^+$ T cells increased in the daytime. B cells as well as T cells were also identified by two-colour staining for CD20 and CD5. Both $CD20^+CD5^-$ B cells and $CD20^-CD5^+$ T cells were evaluated to vary with a circadian rhythm, showing an increase in proportion at night.

Table 1. Stability of the value in experiments

Subpopulation		Mean ± s.d. (%)
$CD3^-CD16^+$	NK	19·4 ± 0·4
$CD3^-CD56^+$	NK	19·0 ± 0·6
$CD3^-CD57^+$	NK	8·2 ± 0·8
$CD3^+CD16^+$	extrathymic T	0·4 ± 0·2
$CD3^+CD56^+$	extrathymic T	4·6 ± 0·5
$CD3^+CD57^+$	extrathymic T	5·8 ± 0·8
$CD3^+CD16^-$	T	61·5 ± 0·9
$CD3^+CD56^-$	T	58·3 ± 2·5
$CD3^+CD57^-$	T	56·4 ± 2·7
$\alpha\beta$ T	(% of $CD3^+$T)	86·7 ± 0·9
$\gamma\delta$ T	(% of $CD3^+$T)	13·3 ± 0·9
$CD4^+$ Th		33·0 ± 0·5
$CD8^+$ Tc		32·0 ± 1·2
$CD20^+$ B		9·7 ± 0·9

Mean and s.d. were obtained from three independent experiments.

Circadian rhythm in the serum level of catecholamines

One of the greatest circadian variables is physical activity. It may influence the production level of catecholamines. In these experiments, the variation in serum level of catecholamines was first confirmed in five healthy donors (Fig. 3). Sera were obtained at the same times as their circadian rhythms had been examined previously. The levels of both adrenaline and noradrenaline varied with a circadian rhythm, showing an increase in the daytime ($P < 0{\cdot}001$ in Roger's method).

Cells with daytime rhythm expressed a higher level of adrenergic receptors

Leucocytes are known to express adrenergic receptors on their surface. In this regard, it was examined whether the expression level of adrenergic receptors on various types of leucocytes was related to the pattern of their circadian variation (Fig. 4a). ^{125}I-CYP (β-adrenergic agonist) was used to identify the specific binding for β-adrenergic receptors with or without pretreatment with propranolol (1 μM). It was found to be greater on granulocytes than on lymphocytes.

Similarly, specific binding for adrenergic receptors was compared among various cell fractions purified by the cell sorter (Fig. 4b). Monocytes, granulocytes, NK cells and $CD56^+$ T cells (of extrathymic origin) showed a greater density of adrenergic receptors, whereas lymphocyte subsets, i.e. $CD4^+$, $CD8^+$ and B cells, showed a lower density. Cells which had a clear daytime rhythm (i.e. phagocytic cells and NK cells) tended to express a higher level of adrenergic receptors.

Cells with night rhythm expressed a higher level of cholinergic receptors

We recently established a method of directly identifying nAChR on lymphocytes [15]. Since nAChR on lymphocytes is usually saturated by ACh itself, pretreatment of lymphocytes with AChE for 5 h is required to induce the specific binding of FITC–α-BT.

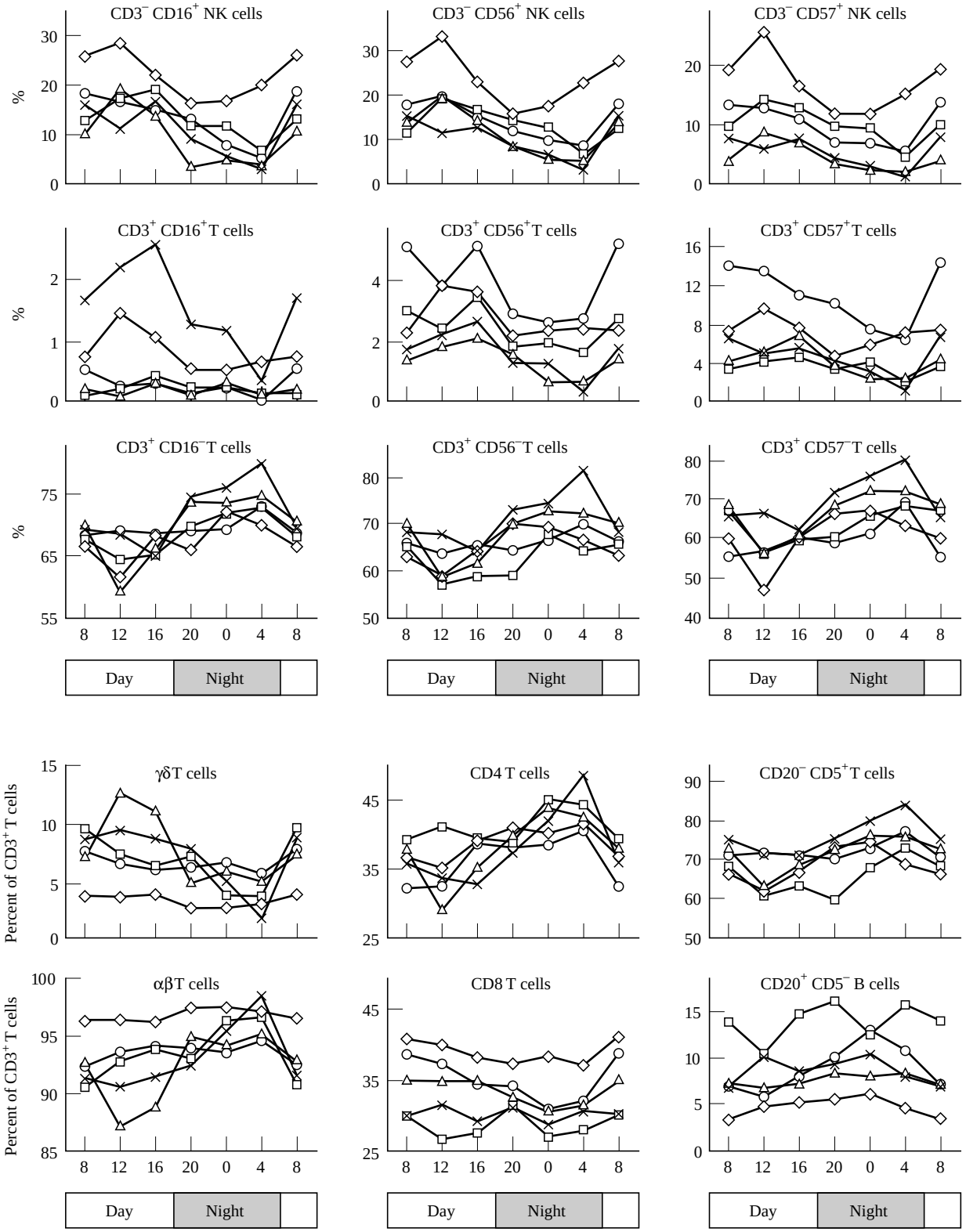

Fig. 2. Circadian rhythm of lymphocyte subsets. Five healthy donors were examined. Natural killer (NK) cells, extrathymic T cells, and conventional T cells, as well as other lymphocyte subsets, were identified by two-colour staining in various combinations of MoAbs. Lymphocyte subsets were classified into two groups, namely, a daytime rhythm group (e.g. NK cells and extrathymic T cells) and a night rhythm group (e.g. conventional T and B cells).

Table 2. Statistical analysis of immunoparameters

Subpopulation		Increase	*r*	*P*
Leucocytes		Daytime	27903	<0·001*
Granulocytes		Daytime	281·4	<0·001*
Lymphocytes		Night	163·5	<0·001*
Monocytes		Daytime	34·3	<0·001*
$CD3^-CD16^+$	NK	Daytime	77·0	<0·001*
$CD3^-CD56^+$	NK	Daytime	79·5	<0·001*
$CD3^-CD57^+$	NK	Daytime	51·8	<0·001*
$CD3^+CD16^+$	extrathymic T		3·8	=0·15
$CD3^+CD56^+$	extrathymic T	Daytime	12·8	=0·002*
$CD3^+CD57^+$	extrathymic T	Dayime	30·4	<0·001*
$CD3^+CD16^-$	T	Night	336·7	<0·001*
$CD3^+CD56^-$	T	Night	325·9	<0·001*
$CD3^+CD57^-$	T	Night	314·4	<0·001*
$\alpha\beta$ T		Night	466·4	<0·001*
$\gamma\delta$ T		Daytime	33·8	<0·001*
$CD4^+$ Th		Night	190·2	<0·001*
$CD8^+$ Tc		Daytime	163·8	<0·001*
$CD20^+$ B		Night	45·8	<0·001*

Data are results from 10 donors, including five donors indicated in Figs 1–3 and an additional five donors.

*Significant.

Using this method, the level of cholinergic receptors on purified fractions of lymphocytes was examined (Fig. 5). $CD3^+$ T cells, $CD20^+$ B cells and $CD56^+$ NK cells were found to contain a greater proportion of $nAChR^+$ cells than the proportion contained by granulocytes. In this study, we showed only a relative difference in the expression of cholinergic receptors among various subsets.

Exercise induced an increase in the number and proportion of cells with daytime rhythm

There was a tendency for the group of cells with the daytime rhythm to have a higher level of adrenergic receptors. If this finding is valid, exercise (i.e. high physical activity accompanied by a high production of catecholamines) may induce some specific variation patterns. This possibility was examined in two healthy subjects who ran for 1 h. The number and proportion of leucocytes and lymphocyte subsets were enumerated at three points of time

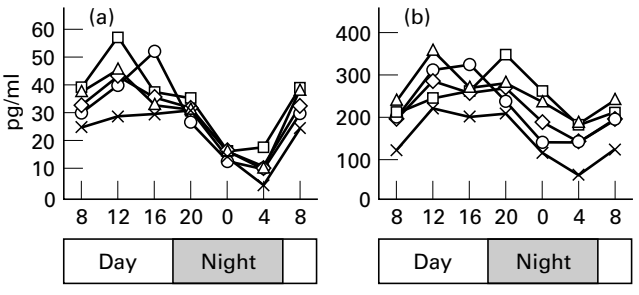

Fig. 3. Circadian variation in the serum levels of adrenaline (a) and noradrenaline (b). To confirm whether physical activity changes the function of the sympathetic nervous system, catecholamine levels in the blood were examined in five healthy donors.

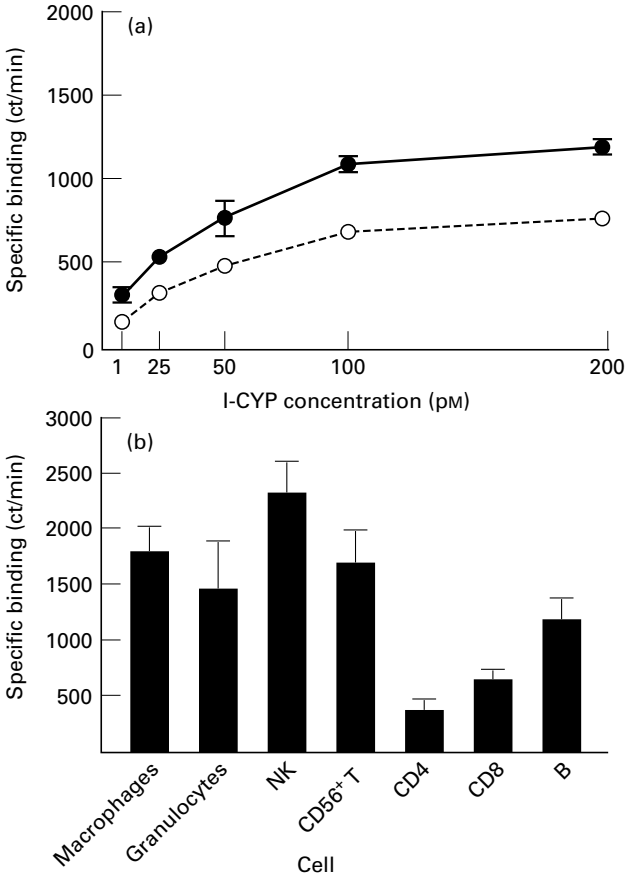

Fig. 4. Expression of adrenerigc receptors on leucocytes. (a) Specific binding of ^{125}I-cyanopindolol (^{125}I-CYP) on granulocytes (●) and lymphocytes (○). (b) A comparison of the expression of adrenergic receptors among leucocytes and various lymphocyte subsets. The sampling time was 10.00 am for the experiments of Figs 4 and 5. Monocytes, granulocytes, NK cells, and $CD56^+$ T cells (of extrathymic origin) with the daytime rhythm expressed a higher level of adrenergic receptors on the surface. The mean and 1 s.d. of the data from three donors are represented.

before and after exercise (Fig. 6). In both subjects, the number of total leucocytes and granulocytes increased substantially at the post-exercise time (i.e. just and 4 h after exercise). Although the proportion of granulocytes decreased just after exercise, their absolute number increased slightly. In the case of lymphocytes, the absolute number and proportion transiently increased (just after exercise), but these retured to normal or even lower levels 4 h after exercise. As shown later, this increase in the number and proportion of lymphocytes was due to the increase in the number and proportion of NK cells and extrathymic T cells.

All proportional increases of lymphocyte subsets seen after exercise were for cells with daytime rhythm

The variation in the proportion of lymphocyte subsets was then examined after exercise (Fig. 7). The proportion of NK cells and extrathymic T cells increased just after exercise, without exception, in both subjects. In sharp contrast, the proportion of conventional T cells decreased prominently. Similarly with regard to the circadian rhythm, the proportion of $\gamma\delta$ T cells increased, whereas that of $\alpha\beta$ T cells and $CD4^+$ T cells decreased. Thus, exercise induced an increase in the proportion of the subsets

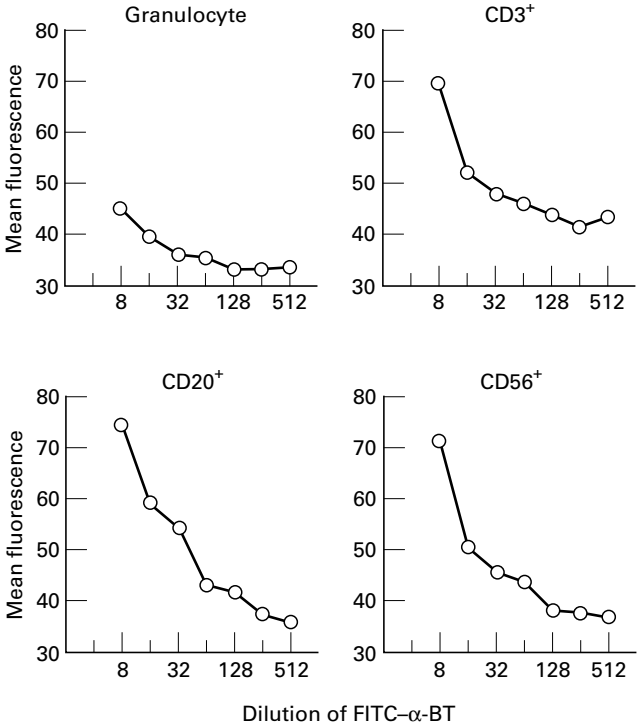

Fig. 5. A comparison of the expression of cholinergic receptors among leucocytes and lymphocyte subsets. All cells were pre-incubated with 1·0 U/ml acetylcholine esterase (AChE) for 5 h and then stained with FITC-conjugated α-bungarotoxin (α-BT). There was an order in the expression level of nicotinic acetylcholine receptor (nAChR), i.e. $CD20^+$ B cells > $CD3^+$ T cells > $CD56^+$ NK cells > granulocytes. Representative results from three experiments are depicted.

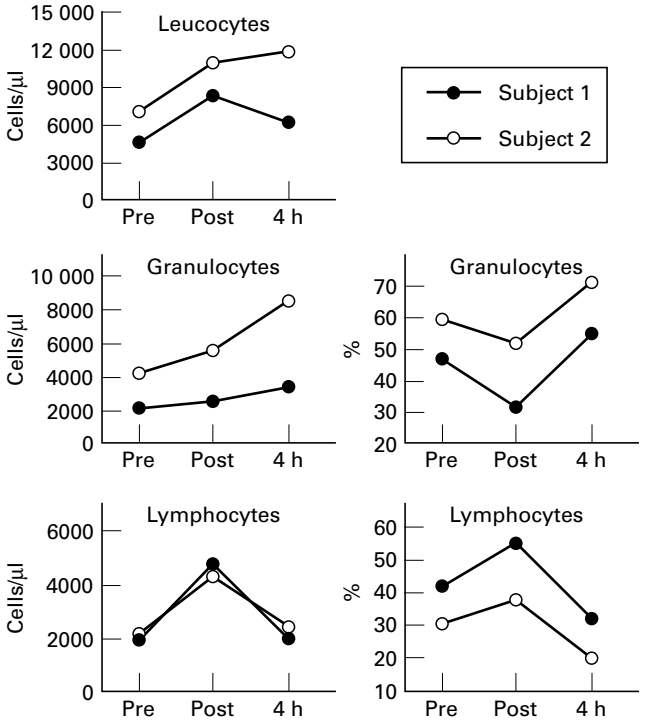

Fig. 6. Variation in the number or proportion of granulocytes and lymphocytes after exercise. Two healthy subjects ran for 1 h and various immunoparameters in the peripheral blood were examined at the indicated points of time. Just after exercise, the number of lymphocytes increased and that of granulocytes gradually increased thereafter. Subsequent experiments (see Fig. 7) revealed that the increase of lymphocytes was due to that of NK cells. The sampling for the experiments of Fig. 6 was started at 2.00 pm.

with the daytime rhythm. The levels of T cells estimated by CD8 and $CD20^+CD5^-$ B cells were relatively stationary after exercise.

DISCUSSION

In this study, we first confirmed that granulocytes and monocytes varied in number and proportion, showing a circadian rhythm with a peak in the daytime (i.e. daytime rhythm), whereas total lymphocytes showed a peak at night (i.e. night rhythm). Based on this earlier evidence [3–8], experiments were then conducted to characterize the rhythm of various lymphocyte subsets, including possible extrathymic T cells (i.e. $CD56^+$ T cells and $CD57^+$ T cells) [9,10]. It was found that cell populations could be classified into two groups: one group with daytime rhythm includes granulocytes, monocytes, NK cells, extrathymic T cells, $\gamma\delta$ T cells, and $CD8^+$ cells. The other, with night rhythm, includes conventional T and B cells, $\alpha\beta$ T cells, and $CD4^+$ cells. It is well known that NK cells, extrathymic T cells and $\gamma\delta$ T cells are involved in natural immunity and are more primitive than conventional T (and $\alpha\beta$ T) and B cells in phylogenetic development. Therefore, cell populations with daytime rhythm might belong to a primitive lineage of leucocytes.

$CD8^+$ cells consist of both conventional T cells and extrathymic T cells. In usually healthy pearsons, $CD8^+$ extrathymic T cells are much more dominant than $CD8^+$ conventional T cells. In this situation, total $CD8^+$ cells may behave as the cells with daytime rhythm.

The next objective of this study was to determine what causes the circadian rhythm of leucocytes in the body. Previously, we found that mice (which are nocturnal) have a circadian rhythm of lymphocytes in all tested organs, which is opposite to that of humans [17]. A secretion burst of glucocorticoids is seen in both humans and mice just before the start of physical activity (e.g. an early morning secretion of glucocorticoids in humans) [5]. In other words, the early morning secretion of glucocorticoids stimulates and awakes us, and results in the subsequent daily rhythm of our activity, accompanying the serum elevation of catecholamines. Therefore, we used adrenalectomized mice in an attempt to identify possible influences on the circadian rhythm [17]. These mice lost such circadian rhythms, implying some hormonal regulation. However, because of impaired mobility, they also lost the variation of their physical activity round the clock. Changes in the activity of the autonomic nervous system might affect leucocyte subsets, some of them carrying adrenergic or cholinergic receptors [11,12]. There are many reports on changes in blood leucocytes after exercise [2,12,18–20].

We therefore compared adrenergic or cholinergic receptors among leucocytes and various lymphocyte subsets in this study. There was a tendency for cells with daytime rhythm to express a higher density of adrenergic receptors on the surface, while cells with night rhythm were found to express a

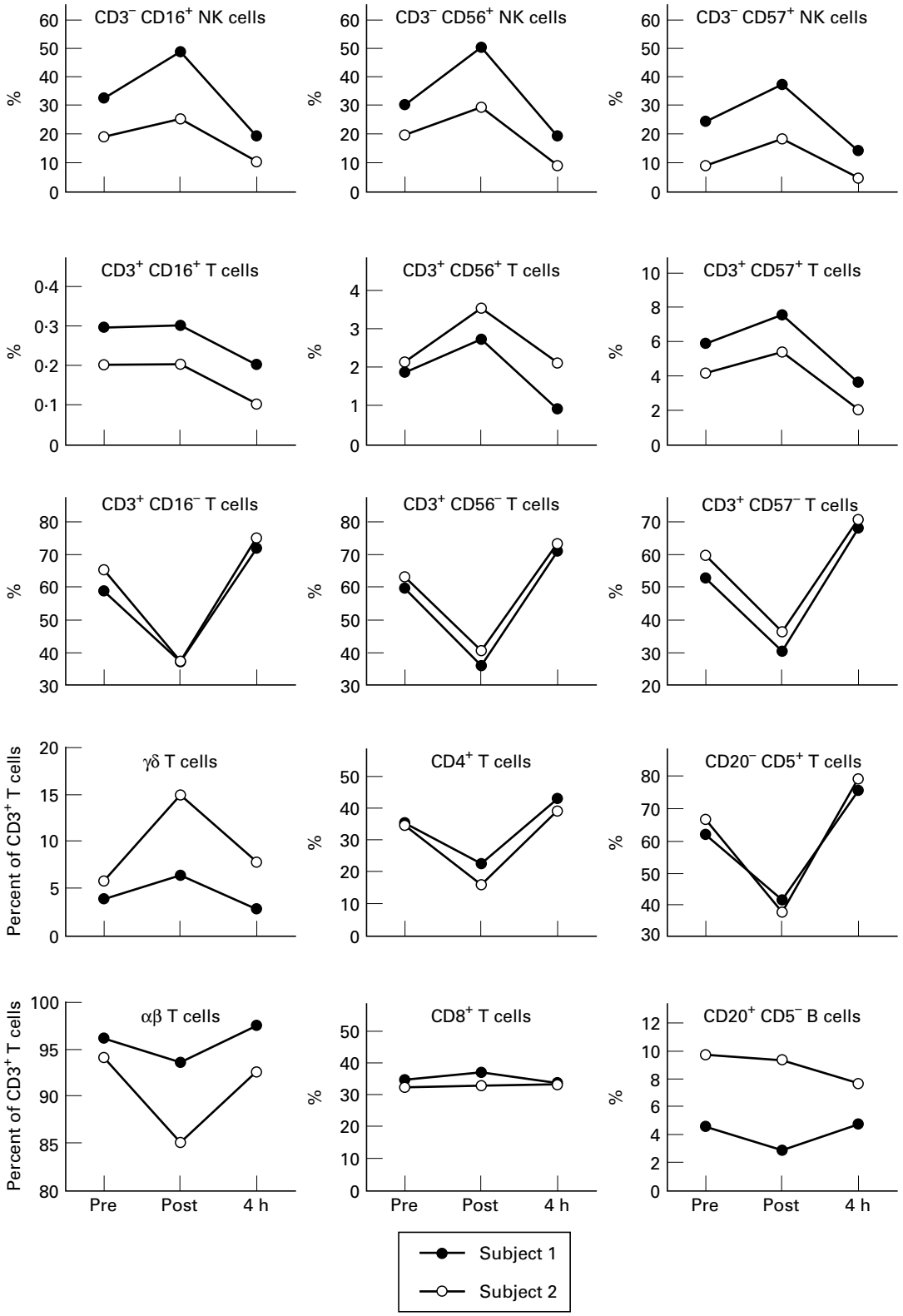

Fig. 7. Variation in the proportion of lymphocyte subsets after exercise. Two healthy subjects ran for 1 h and various immunoparameters in the peripheral blood were exmained at the indicated points of time. Exercies induced an increase in the proportion of cells with the daytim rhythm (i.e. cells carrying a higher level of adrenergic receptors), although there was a time lag (e.g. granulocytes).

high proportion of cholinergic receptors. This raises the possibility that the rhythm is determined by a combination of the expression of both receptors. For example, B cells which had night rhythm exceptionally expressed a high density of adrenergic receptors, but also expressed a high proportion of cholinergic receptors.

If physical activity and the resultant changes in the activity of the autonomic nervous system do indeed produce a circadian rhythm of leucocytes, exercise should induce an increase in the number of those subsets with daytime rhythm. This predicton was confirmed by our present finding of a prominent increase in number or proportion of cells with daytime rhythm, including granulocytes, NK cells, extrathymic T cells, etc. In conjunction with the data on the production of catecholamines in the blood, in which the production increased in the daytime or after exercise, the physical activity and resultant sympathetic nerve strain might be a direct factor causing the variation of leucocytes. Consequently, most subsets with night rhythm tended to decrease after exercise. Some effects on the leucocyte circulation (e.g. from the blood to the lymph nodes) might also be associated with this phenomenon [16].

The most rapid changes were seen in granulocytes as well as NK cells. A similar phenomenon was observed by other investigators, namely, the administration of catecholamine was found to increase NK cells in the peripheral blood [21]. Given a quick response of NK cells or granulocytes, many investigators postulate the existence of an accessible reservoir of these populations somewhere. In the above cited study, the authors revealed that the response of NK cells to an administration of catecholamine did not change in patients who underwent splenectomy. In this regard, the spleen may not be the site of a reservoir (or marginal pool) of these cells. In a preliminary study using mice, we demonstrated that the bone marrow itself might be the site of such a reservoir, especially of granulocytes [22]. As is well known, the life span of granulocytes is very short (i.e. 2 or 3 days). In this respect, the turnover itself of granulocytes is very quick. Therefore, the differentiation of granulocytes in the bone marrow and their export to the periphery might be accelerated by sympathetic nerve stimulation. The bone marrow possibly has such a great potential.

It is obvious that granulocytes, NK cells, and extrathymic T cells, as well as monocytes, are more primitive (i.e. developed phylogenetically earlier) than conventional T and B cells [23]. The primitive lineage of leucocytes is considered to carry dominantly such adrenergic receptors, rather than cholinergic receptors on the surface. The present results, therefore, lead us to speculate that primitive leucocytes are more efficiently activated by sympathetic nerve stimulation, while both $CD4^+$ $\alpha\beta$ T cells and B cells are activated by parasympathetic nerve stimulation. In other words, there is a shift towards the innate immune system during exercise in the daytime. Conversely, specific responses to small fragments of microbial and foreign antigens, which are too small to be phagocytosed by leucocytes, might be favoured at night.

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REFERENCES

1 Abo T, Kumagai K. Studies of surface immunoglobulins on human B lymphocytes. III. Physiological variations of SIg^+ cells in peripheral blood. Clin Exp Immunol 1978; **33**:441–52.

2 Edwards AJ, Bacon TH, Elms CA, Verardi R, Felder M, Knight SC. Changes in the populations of lymphoid cells in human peripheral blood following physical exercise. Clin Exp Immunol 1984; **58**:420–7.

3 Elmadjian F, Pincus G. A study of the diurnal variations in circulating lymphocytes in normal and psychotic subjects. J Clin Endocrinol 1946; **6**:287–4.

4 Carter JB, Barr GD, Levin AS, Byers VS, Ponce B, Fudenberg HH, German DF. Standardization of tissue culture conditions for spontaneous thymidine-2-^{14}C incorporation by unstimulated normal human peripheral lymphocytes: circadian rhythm of DNA synthesis. J Allergy Cin Immunol 1975; **56**:191–205.

5 Abo T, Kawate T, Itoh K, Kumagai K. Studies on the bioperiodicity of the immune response. I. Circadian rhythms of human T, B, and K cell traffic in the peripheral blood. J Immunol 1981; **126**:1360–3.

6 Ritchie AWS, Oswald I, Micklem HS, Boyd JE, Elton RA, Jazwinska E, James K. Circadian variation of lymphocyte subpopulations: a study with monoclonal antibodies. Brit Med J 1983; **286**:1773–5.

7 Bertouch JV, Roberts-Thomson PJ, Bradley J. Diurnal variation of lymphocyte subsets identified by monoclonal antibodies. Brit Med J 1983; **286**:1171–2.

8 Levi FA, Canon C, Blum J-P, Mechkouri M, Reinberg A, Mathe G. Circadian and/or circahemidian rhythms in nine lymphocyte-related variables from peripheral blood of healthy subjects. J Immunol 1985; **134**:217–22.

9 Takii Y, Hashimoto S, Iiai T, Watanabe H, Hatakeyama K, Abo T. Increase in the proportion of granulated $CD56^+$ T cells in patients with malignancy. Clin Exp Immunol 1994; **97**:522–7.

10 Okada T, Iiai T, Kawachi Y, Moroda T, Takii Y, Hatakeyama K, Abo T. Origin of $CD57^+$ T cells which increase at tumor sites in patients with colorectal cancer. Clin Exp Immunol 1995; **102**:159–66.

11 Khan MM, Sansoni P, Silverman ED, Engleman EG, Melmon KL. Beta-adrenergic receptors on human suppressor, helper, and cytolytic lymphocytes. Biochem Pharmacol 1986; **35**:1137–42.

12 Ratge D, Wiedemann A, Kohse KP, Wisser H. Alterations of β-adrenoceptors on human leukocyte subsets induced by dynamic exercise: effect of prednisone. Clin Exp Pharm Phys 1988; **15**:43–53.

13 Abo T, Sugawara S, Amenomori A, Itoh H, Rikiishi H, Moro, I, Kumagai K. Selective phagocytosis of Gram-positive bacteria and interleukin 1-like factor production by a subpopulation of large granular lymphocytes. J Immunol 1986; **136**:3189–97.

14 Yazawa K, Wang CH, Maruoka Y, Nakajima T, Saito H, Nishiyama N. Determination of catecholamines and their metabolites in adrenals of stress-loaded and wild suncrus (*Suncus murinus*). Jap J Pharmacol 1989; **51**:443–5.

15 Toyabe S, Iiai T, Fukuda M, Kawamura T, Suzuki S, Uchiyama M, Abo T. Identification of nicotinic acetylocholine receptors of lymphocytes in the periphery as well as thymus in mice. Immunology, in press.

16 Kawate T, Abo T, Hinuma S, Kumagai K. Studies on the bioperiodicity of the immune response. II. Co-variations of murine T and B cells and a role of corticosteroid. J Immunol 1981; **126**:1364–7.

17 Maisel AS, Harris T, Rearden CA, Michel MC. β-adrenergic receptors in lymphocyte subsets after exercise. Alterations in normal individuals and patients with congestive heart failure. Circulation 1990; **82**:2003–10.

18 Maisel AS, Knowlton KU, Fowler P, Rearden A, Ziegler MG, Motulsky HJ, Insel PA, Michel MC. Adrenergic control of circulating lymphocyte subpopulations. Effects of congestive heart failure, dynamic exercise, and terbutaline treatment. J Clin Invest 1990; **85**:462–7.

19 Murray DR, Irwin M, Rearden CA, Ziegler M, Motulsky H, Maisel AS. Sympathetic and immune interactions during dynamic exercise. Mediation via a β_2-adrenergic-dependent mechanism. Circulation 1992; **86**:203–13.

20 Iversen PO, Arvesen BL, Benestad HB. No mandatory role for the spleen in the exercise-induced leucocytosis in man. Clin Sci 1994; **86**:505–10.
21 Schedlowski M, Hosch W, Oberbeck R, Benschop RJ, Jacobs R, Raab H-R, Schmidt RE. Catecholamines modulate human NK cell circulation and function via spleen-independent β_2-adrenergic mechanisms. J Immunol 1996; **156**:93–99.
22 Tsukahara A, Tada T, Suzuki S *et al*. Adrenergic stimulation simultaneously induces the expansion of granulocytes and extrathymic T cells in mice. Biomed Res 1997; **18**:237–46.
23 Abo T, Watanabe H, Iiai T *et al*. Extrathymic pathways of T-cell differentiation in the liver and other organs. Int Rev Immunol 1994; **11**:61–102.