Seasonal Modulation of the Circadian Time Structure of Circulating T and Natural Killer Lymphocyte Subsets from Healthy Subjects

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Abstract

A seasonal modulation of the circadian time structure of circulating T and natural killer (NK) lymphocyte subtypes was documented in five healthy men aged 24–36 yr. Venous blood was obtained every 4 h for 24 h from each subject in January, March, June, August, and November 1984. Three subjects were also studied in April and/or August and/or November 1983 for the T subsets only. Mononuclear cells were isolated on Ficoll-Paque gradient and aliquots were incubated with OKT3, OKT4, OKT8, or HNK-1 monoclonal antibodies for characterizing all, T, T helper, T suppressor-cytotoxic, and NK lymphocytes, respectively, under an epifluorescence microscope. An effect of both sampling time and study month was statistically validated ($P < 0.01$) with both two-way analysis of variance and cosinor for the peripheral counts in total, pan-T, T helper, and NK lymphocytes (cells per cubic millimeter). Seasonal changes affected both the circadian patterns and the 24-h mean values. Thus the double amplitude (total extent of variation) of the circadian rhythm in circulating total, T, and T helper lymphocytes varied between 0 in March ($P > 0.30$; no rhythm) and up to 46–68% of the 24-h-mean (M) in November, with acrophases (times of maximum, Ø) localized in the first half of the night ($P < 0.001$). Maximal values were found at 8:30 h for both T suppressor-cytotoxic and NK lymphocytes; a smaller second peak was also found at 20:30 h, and a 12-h rhythm was validated by cosinor ($P < 0.0001$), with no patient change in waveform along the year scale. A circannual rhythm was statistically validated by cosinor for total (Ø in November), pan-T (Ø in March), T suppressor-cytotoxic (Ø in December), and NK lymphocytes (Ø in October). A rhythm with a period equal to 6 mo was found for circulating T helper cells with Ø occurring both in April and October. Seasonal variations in the incidence of several immunologically related diseases may correspond to an endogenous circannual time structure.

Introduction

Large seasonal variations in several immune functions have been reported in various animal species including mice (1), guinea pigs (2), dogs (3), and human beings (4–10). Seasonal variations are commonly explained by environmental changes. Nonetheless, endogenous circannual rhythms in numerous variables have been demonstrated in animals kept in a standardized environment for several years (11, 12, and many others). With regard to immune functions, the lymphocyte blastogenic response of mice exhibited a reproducible circannual rhythm for 2 consecutive yr despite the fact that these animals had been kept in the same photoperiodic and nutritive environment (1). Similar results have been observed in the ground squirrel (13). Reproducible circannual rhythms have also been found for 3–5 consecutive yr for the in vivo migration of polymorphonuclear cells (14) and for the splenic natural killer (NK)[1] cell activity (15) in mice kept in a standard photoperiodic environment. Immune functions are also organized along a circadian time structure, as documented in preceding articles by us (16, 17) and by others (18–22).

Discrepant results had been reported in the literature, however, with regard to circannual variations in circulating T and/or B lymphocyte counts in human beings (5, 7, 10, 19). Other discrepant reports pertained to the circadian rhythmicity in the T helper/T suppressor-cytotoxic ratio (20, 21, 23) and to that in phytohemagglutinin-induced lymphoblastic transformation in humans (19, 22, 24). This function test is known to primarily involve T lymphocytes. In an initial study on the circadian organization of circulating T and B lymphocyte subsets, we noticed changes that might have been related to circannual rhythms (16, 25). Such rhythms are known to be capable of modulating any circadian parameter of a given variable, including the circadian mean, amplitude, acrophase (peak time), or even the 24-h periodicity itself. For instance, the circadian acrophase of plasma testosterone was found to occur 6–8 h later in June as compared with January in young men (26). A circadian rhythm in plasma luteinizing hormone was only found in the second half of the year in young men in two independent studies (27, 28). We hypothesized that the circadian time structure of the immune system would be subjected to a profound circannual modulation which would account for apparently discrepant results (16, 23). The end points studied were the counts of circulating T and NK (29, 30) lymphocyte subsets in healthy young men. Both cell types are involved respectively in the mounting of delayed immune response and in immediate cytotoxicity. The circulating counts of such cells constitute usual clinical gauges for evaluating the immune status of a patient.

It was believed that knowledge of the rhythmicity of such immune cells would lead to a better understanding of the sea-

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1. *Abbreviations used in this paper:* ANOVA, analysis of variance; 2A, double amplitude (total extent of variation accounted for by the fitted cosine function); M, mesor (rhythm-adjusted mean); τ, period (length of a cycle); Ø, acrophase (localization of maximum in fitted cosine function).

sonal variations in the incidence of several immunologically related diseases such as viral infections (31) and Hodgkin's lymphoma (32).

Methods

Subjects

Five apparently healthy male subjects volunteered for this study (median age 33 yr, range 24–36 yr). They were synchronized by daily activities and nocturnal rest. On each study day and during the 2 d preceeding it, they were recumbent from ≅ 23:00 h to ≅ 07:00 h; breakfast was taken at ≅ 07:30 h, lunch at ≅ 12:45 h, and dinner at ≅ 20:30 h. With the use of both clinical and routine biological examinations, neither acute nor chronic infection was diagnosed, at least during the month preceding and that following the study. One subject had a past history of childhood asthma, and two were heavy smokers (15 and 25 cigarettes per day) but with no or minimal smoking (less than five cigarettes/24 h) during the study.

Protocol

Blood (40 ml) was drawn every 4 h for 24 h starting at 08:30 h, in January, March, June, August, and November 1984 from all subjects. Thus, on each of these 5 mo, seven samples were obtained in each subject. For each sample, 13 ml of blood was collected in standard EDTA-containing tubes and was processed for immediate hemogram determination and subsequent hormonal analysis; 27 ml was collected in syringes containing 3 ml of heparin and was processed for the determination of lymphocyte surface markers. In addition, several subjects participated in a similar protocol for the determination of lymphocyte subsets only: subject 1 in April, August, and November 1983, subject 3 in August 1983, and subject 4 in November 1983.

Hemogram

The total leukocyte count (cells per cubic millimeter of blood) was determined by automatic hemocytometer (model ELT8, Ortho Diagnostic Systems, Inc., Raritan, NJ) within 30 min of each blood collection. Slides were made and stained with May-Grunwald-Giemsa. The proportions of lymphocytes and monocytes were determined microscopically, with 300 cells counted per slide. The total number of circulating lymphocytes per cubic millimeter was calculated.

Lymphocyte surface marker determinations

Within 30 min of collection, each blood sample was processed for isolation of mononuclear cells. Peripheral blood was collected on heparin (Liquemine, Roche, Neuilly, France), and mononuclear cells were separated on a Ficoll-paque gradient at 1,000 *g* for 20 min. The cells were washed twice at 4°C in Hanks' balanced saline solution containing 5% fetal calf or human AB serum. Cell viability was performed by means of trypan blue exclusion. One million cells in 0.1 ml of Hanks' with 5% fetal calf serum were incubated and with 5 μl of OKT3, OKT4, OKT8 monoclonal antibodies (Orthoclone, Aubervilliers, France) and HNK-1-fluorescein isothiocyanate (FITC) (Leu7, Becton Dickinson, 94 Rungis, France). Incubation was performed at 4°C for 20 min and assayed as described by Ritz et al. (33). Cells were than washed twice and again incubated at 4°C for 20 min with 150 μl of goat anti-mouse antibody conjugated with fluorescein for indirect immunofluorescence (Nordic, Le-Perray-en-Yvelines, France). After two additional washes, cells were examined under a Zeiss epifluorescence microscope. Fc receptors were blocked in the presence of 5% fetal calf serum or 5% human AB serum. A control was performed by incubating cells with the fluoresceinated goat anti-mouse antibody only. 200 cells were counted. All counts were performed by the same investigator, the coefficient of variation of this technique being 5% when performed by her. The quality control for activity had been performed on each lot of monoclonal antibodies in addition to the fact that they were produced by the same hybridoma cell lines and checked by the manufacturer. Lots 305 and 306 were used for OKT3 respectively in January–March 1984 and in June–August–November 1984. Lots 405, 406, and 407 were used for OKT4 in January (405), March (406), and June, August, and November 1984 (407). Lot 806 was used for OKT8 in all months but November 1984 (lot 809). Finally, the same Leu7 lot was used for all study months. For each lot change, comparison between both the old and the new was performed on peripheral lymphocytes obtained from two healthy donors and from two cancer patients in remission after chemotherapy. Highly reproducible results (within limits < 10%) have been observed among different lots since 1980 when their routine use was started in our clinical department. The total number in each circulating lymphocyte subtype was determined by multiplying the proportion of fluorescent cells by the number of circulating lymphocytes.

Statistical analyses

Data were expressed both in their conventional units and as percentages of the individual 24-h or yearly mean for each variable and time point. This latter method was used in order to minimize interindividual differences occurring in mean values of lymphocyte subsets, which has been previously documented (16).

Means and 1 SEM were computed for each time point, each variable, and on each study month. Time series were analyzed both by three-way analysis of variance (ANOVA) and by the cosinor method (34). The three-way ANOVA considered three factors as potential sources of variance, e.g., subject, sampling time of day, and sampling month, and a two-way interaction between time of day and month of sampling. The cosinor method characterized a rhythm by the parameters of the fitted cosine function approximating all data. Several periods (τ) were a priori considered, e.g., the circannual fundamental with $\tau \cong 1$ yr and its first harmonic with $\tau \cong 6$ mo, and the circadian fundamental with $\tau \cong 24$ h and its first harmonic with $\tau \cong 12$ h. A rhythm was detected if the null amplitude hypothesis was rejected with $P < 0.05$ (from an F test); however, amplitude and acrophase could be approximated if $0.05 \leqslant P \leqslant 0.10$. The rhythm characteristics estimated by this linear least squares method include the mesor (M, rhythm-adjusted mean), the double amplitude (2A, the difference between the minimum and maximum of fitted cosine function), and the acrophase (ϕ, time of maximum in fitted cosine function, with December 31st at midnight as ϕ reference). They are given with their 95% confidence limits if $P < 0.05$. The cosinor method was applied to individual and pooled time series.

Results

Seasonal variations

The data from the five subjects obtained on all 5 mo of study in 1984 were first analyzed by three-way ANOVA for effects of subject, circadian time, and circannual stage. The effects of both subject and circadian time were statistically validated for all variables. The role of the month of study was also found with statistical significance for total, T helper, and HNK-1$^+$ lymphocytes. Moreover, an interaction between the circadian and the circannual stages of sampling was documented ($P < 0.05$) for total, all T, and T helper lymphocytes.

Seasonal modulation of circadian rhythms

Cosinor analyses were performed on the time series from each month of study for each variable. Circadian rhythms in total, OKT3$^+$, and OKT4+ lymphocytes were statistically validated in January, August, and November, but neither in March nor in June. The circadian acrophases of all three variables were localized in the first half of the night span. The circadian amplitudes were smaller in summer than in winter or fall. The OKT4$^+$/OKT8$^+$ ratio exhibited a circadian rhythm only in June, August, and November, with similar acrophases (at

~ 2:00 h) and similar double amplitudes (~ 60% of the mesor).

An ultradian rhythm with τ = 12 h characterized the HNK-1+ subset on all months except June, when a 24-h rhythm was detected. A similar finding was observed for the OKT8$^+$ subset on all study months, except August when a 24-h rhythm was suggested. A 12-h rhythm was also found for the OKT4+ subset in March, and a 12-h harmonic was statistically validated for total, OKT3$^+$ and OKT4$^+$ lymphocytes both in November and in January but neither in June nor in August.

Thus a seasonal modulation of circadian rhythmicity was documented by both ANOVA and cosinor for total, pan-T, and T helper lymphocytes. Such an effect relied on cosinor analysis alone for HNK-1$^+$ cells, T suppressor-cytotoxic lymphocytes, and the T helper/T suppressor-cytotoxic ratio.

Because no circadian rhythm was documented either in March or in June for total, T, or T helper lymphocytes, data from both study months were pooled. No circadian rhythm was detected ($P \geq 0.10$) by cosinor for any of these variables. Conversely, data from January, August, and November were pooled and significant circadian rhythms were found for all three variables by cosinor ($P < 0.001$). Such a seasonal modulation of the circadian time structure is depicted in Figs. 1–3.

No circadian rhythm was detected for the OKT4$^+$/OKT8$^+$ ratio in January or in March, when taken separately. After pooling the time series from both of these months, however a circadian rhythm was validated (P = 0.04) with 2A = 30% of

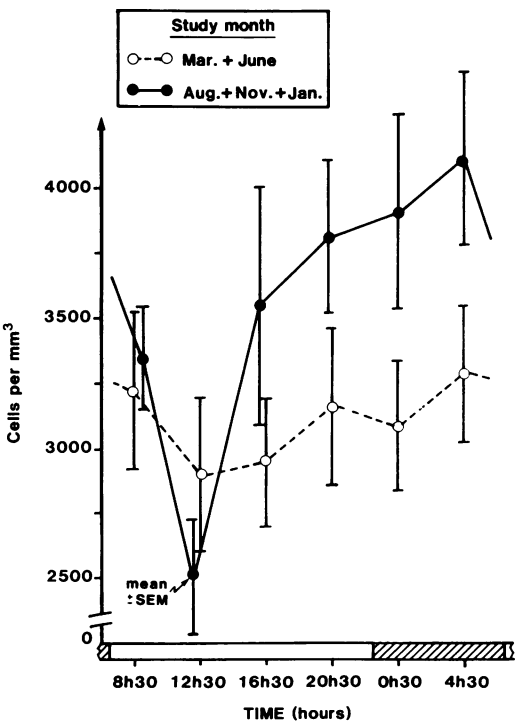

Figure 1. Circadian variation in the count of circulating total lymphocytes from human peripheral blood in January, August, and November, as opposed to March and June. Cosinor analysis yielded the respective following parameters: (*a*) for March and June, P (from an F test of the rejection of the null amplitude test) = 0.07, M = 3,121±126 cells/mm^3, 2A = 14% of M, ϕ = 2.20 h; (*b*) for August, November, and January, $P < 0.001$, M = 3,550±130 cells/mm^3, 2A = 35±16% of M, ϕ = 1.00±1.40 h.

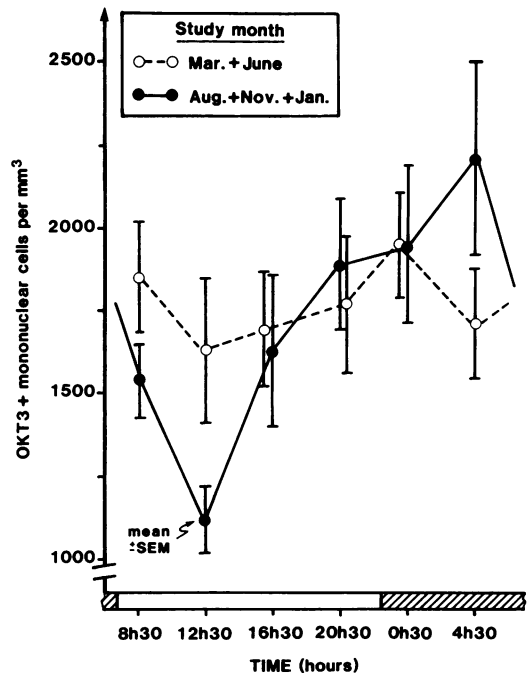

Figure 2. Circadian variation in the count of circulating OKT3$^+$ lymphocytes from human peripheral blood in January, August, and November, as opposed to March and June. Data are expressed as percentages of each individual's 24-h mean for each study month. Cosinor analysis yielded the respective following parameters: (*a*) for March and June, P = 0.07, M = 1,780±80 cells/mm^3, 2A = 17% of M, ϕ = 2.00 h; (*b*) for August, November, and January, $P < 0.001$, M = 1,720±80 cells/mm^3, 2A = 48±20% of M, ϕ = 1.00±1.30 h.

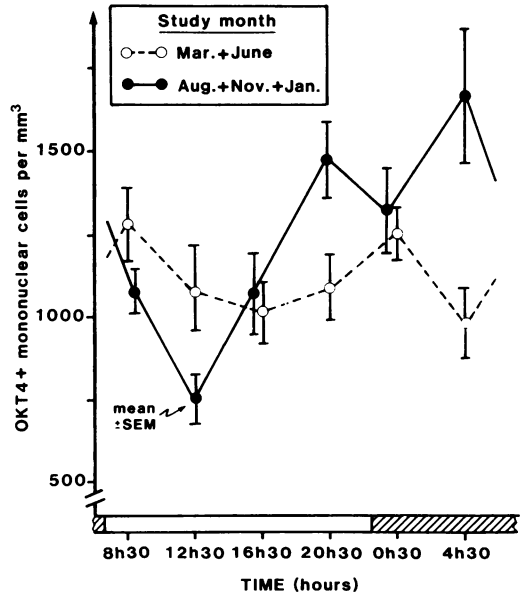

Figure 3. Circadian variation in the count of circulating OKT4$^+$ lymphocytes from human peripheral blood in January, August, and November, as opposed to March and June. Data are expressed as percentages of each individual's 24-h mean for each study month. Cosinor analysis yielded the respective following parameters: (*a*) for March and June, P = 0.42, M = 1,130±50 cells/mm^3; (*b*) for August, November, and January, $P < 0.0001$, M = 1,230±50 cells/mm^3, 2A = 52±20% of M, ϕ = 1.00±1.20 h.

the M and Ø = 23:10 h. A statistically significant circadian rhythm was found on the other 3 mo of study, considered separately or pooled ($P < 0.0001$). In the latter case, 2A was 65% of the M and Ø was localized at 1:30 h (Fig. 4). Such differences in mean 2As were statistically significant ($t = 2.6$; $P < 0.05$).

Circannual rhythms

Circannual rhythms were validated by cosinor for total lymphocytes, $OKT3^+$, and $OKT8^+$ cells. Their double amplitudes were close to 20% of the mesor, e.g., they were smaller than the corresponding circadian ones. A circannual rhythm also characterized HNK-1^+ cells, with a large, double amplitude (60% of the mesor) exceeding that of both the circadian rhythm and the 12-h rhythm. Acrophases of total lymphocytes, $OKT3^+$, $OKT8^+$ and HNK-1^+ cells were respectively localized in November, March, December, and October. A rhythm with $\tau \cong 6$ mo was detected only for $OKT4^+$ lymphocytes with acrophases occurring both in April and in October (Figs. 5 and 6). No circannual rhythmicity was found for the $OKT4^+/OKT8^+$ ratio at a group level.

Reproducibility of circadian time-qualified values 1 yr apart

This aspect was documented on four circadian profiles from three subjects for total and T lymphocytes. The median difference (Δ) in individual 24-h mean (M) 1 yr apart was < 22%, and that in individual peak time (P) was < 2 h for each variable. The poorest reproducibility was achieved for the count of

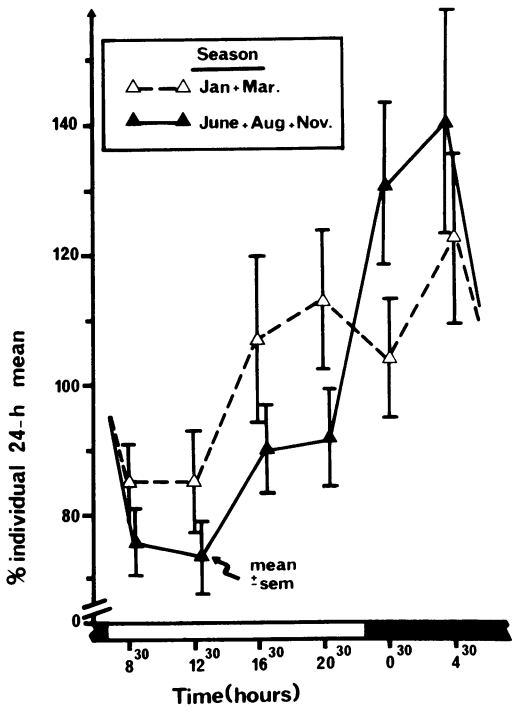

Figure 4. Circadian variation in the $OKT4^+/OKT8^+$ ratio in peripheral human blood in January and March, as opposed to June, August, and November. Data are expressed as percentages of each individual's 24-h mean for each study month. The following circadian parameters were obtained by cosinor analysis: (*a*) for January and March, $P = 0.04$, M = 2.7±0.2, 2A = 30%±28% of M, Ø = 23.10± 5.40 h; (*b*) for June, August, and November, $P < 0.0001$, M = 3.0±0.2, 2A = 65±30% of M, Ø = 1.30±1.50 h.

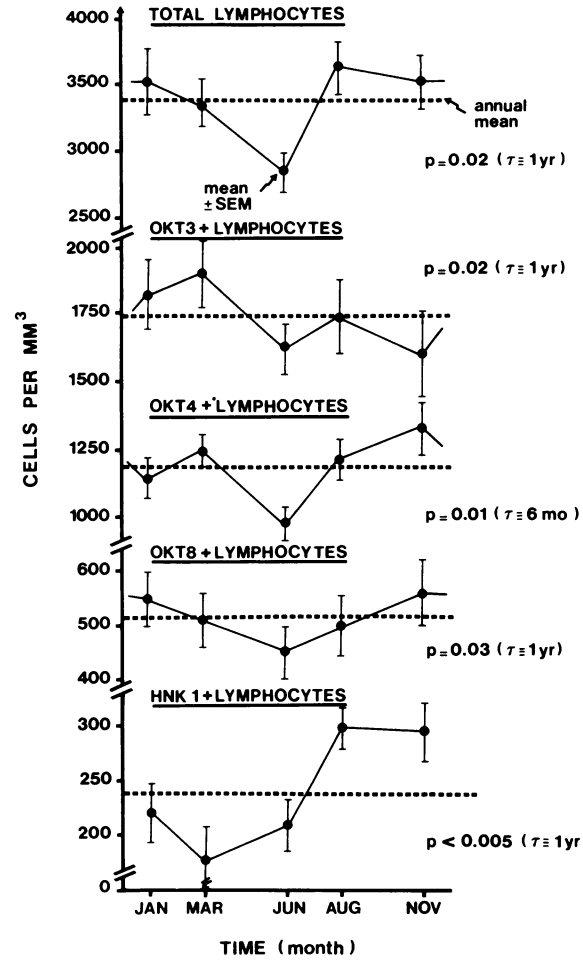

Figure 5. Seasonal variations in the 24-h mean count of circulating total, $OKT3^+$, $OKT4^+$, $OKT8^+$, and HNK-1^+ lymphocytes from five 24–35-year-old, healthy men. The *P* value from cosinor analysis of an *F* test of the rejection of the null-amplitude hypothesis (for a rhythm with a chosen period τ) is indicated on the right of each graph.

$OKT3^+$ lymphocytes (ΔM = −22%; range = −31, +10%; ΔP = +2 h; range = −8, +4 h) and the best one for $OKT4^+$ lymphocytes (ΔM = 0%; range = −9, +30%; ΔP = 0 h; range = 0, +4 h). Thus a rather good intra-individual seasonal reproducibility was achieved especially for $OKT4^+$ and $OKT8^+$ lym-

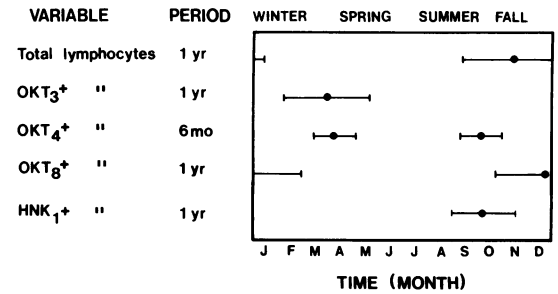

Figure 6. Seasonal time structure of circulating total lymphocytes and T and $HNK1^+$ subtypes in five healthy young men, as represented by an acrophase chart. The maximum (acrophase, Ø) is shown with its 95% confidence limits, as yielded by cosinor analysis, for a period τ = 1 yr or 6 mo.

phocytes and their ratio with regard to the 24-h mesor and for all variables with regard to the timing of their circadian peak. Moreover, a circannual rhythm in the 24-h mesor of the $OKT4^+/OKT8^+$ ratio was statistically validated by cosinor in subject 1 for whom a total of eight circadian time series were available over a 2-yr span.

Discussion

The present study demonstrates that the characteristics of the circadian rhythms in the five immunologic variables investigated were seasonally modulated. The circadian mesor exhibited a circannual rhythm for total, T, T suppressor-cytotoxic, and HNK-1 lymphocytes with a maximum estimated respectively in November, March, December, and October. A 6-mo rhythm was documented for T helper lymphocytes, with estimated maxima occurring in both April and October. The double amplitudes of such circannual or circahemiannual rhythms ranged between 15% and 25% of the circannual mesor and were smaller than the corresponding circadian or circahemidian ones except for the $HNK\text{-}1^+$ cells. $HNK\text{-}1^+$ mononuclear cells include two T cell subsets which may or may not express T3 receptor. NK cell activity is primarily exerted by $HNK\text{-}1^+$ $OKT3^-$ cells (30). Furthermore, $HNK\text{-}1^+$ cells may also express the T suppressor-cytoxic phenotype ($Leu\ 2a^+$, equivalent to $OKT8^+$) (35, 36). Irrespective of study month, $HNK\text{-}1^+$ cells exhibited a circadian rhythm as opposed to $OKT8^+$ cells which only displayed one of 12 h. This suggests that the temporal variations in the circulating counts of $HNK\text{-}1^+$ cells to some degree reflect those of NK cells. The intra-individual reproducibility of such circannual rhythms was shown in the three subjects studied once to three times during the previous year. The results further support the view that pan-T, T helper, T suppressor-cytotoxic, and NK lymphocytes vary rhythmically along the 1-yr scale. Such a circannual rhythmicity was also found for the T helper/T suppressor-cytotoxic ratio in a single individual followed for 2 yr. Although no such rhythm was statistically validated at a group level, this suggests that interindividual differences may be found with regard to the circannual rhythmicity in this ratio.

A circannual rhythm in the 24-h mesor of circulating leukocytes was demonstrated by Reinberg et al. (4) with highest values occurring in early December, e.g., very close to the maximum found for total lymphocytes in the present study. Nonetheless, no circannual rhythm in morning values of total leukocytes or lymphocytes has been found by others (7, 19). In a recent study, large seasonal variations in morning total leukocyte count were found in young men, but not in men older than 40 yr (37). A reproducible circannual rhythm in the count of circulating B lymphocytes was documented in a healthy young Japanese subject sampled monthly between 9:00 and 11:00 h for 2 consecutive yr, with a peak in August (summer) and a nadir in January (winter) (5). Such results were confirmed at a group level by the same investigators (5). Others have been reported the reverse in U. S. hospital employees, e.g., that the count of circulating B cells was highest in January–February (winter) and lowest in August–September (summer) in healthy subjects (10). However, no indication was given about the sampling time of day or about the subject's characteristics or their synchronization along the 24-h scale or whether they were shift workers. Such results cannot thus be interpreted.

The seasonal time structure herein described for T and NK lymphocyte subsets may be related to that in lymphocyte enzymatic activity (6) with a peak in summer, or that in lymphocyte mitotic index with a peak in winter (38).

The circadian double amplitude of total, T, and T helper lymphocytes varied between 0% (no rhythm) in March and 46–60% of the mesor in November or January. With regard to the T helper/T suppressor-cytotoxic ratio, a circadian rhythm was not detected either in January or in March, but it was validated in the other seasons with 2As equal to 67% of the 24-h mesor both in June and in August. Thus, apparent discrepancies in the literature with regard to the presence or absence of a physiologic circadian rhythm in T lymphocyte–related variables (16–25, 39, 40) may be accounted for by a circannual modulation of circadian rhythmicity, as hypothesized. In addition, the acrophases of the statistically validated circadian or circahemidian rhythms remained similar throughout the year.

A lack of detection of group circadian rhythms in circulating total pan T and T helper lymphocytes both in March and in June was intriguing to us. A technical failure seemed unlikely since this phenomenon was observed on 2 mo of study for each of these variables. Since the group of five subjects had been split into two subgroups, the risk of additive rather than random errors in lymphocyte count was further minimized. Moreover, a 12-h rhythm in both $OKT8^+$ and $HNK1^+$ lymphocytes was observed on 4 and 3 mo of study, respectively, including March, with similar rhythm characteristics. Of 15 reports in which circadian changes in the peripheral lymphocyte count are described, the season of study is only given in five (including three by us) (16–25, 39–43). Of those, none was performed in March or in June. Moreover, in a study performed in May the mean peak-trough difference in lymphocyte count was only 200 cells/mm^3 (40).

A latent infectious process that would recur periodically and alter the circadian time structure of the immune system at a subclinical level cannot be ruled out since viral dormancy has been documented to last for several months in humans (44). Such an explanation was considered as possibly accounting for the circannual rhythm in the plasma concentration of antirubella antibodies observed in a young woman for the 7 consecutive yr after infection (45). Nonetheless, an alteration of circadian rhythmicity of several variables has been documented in specific seasons in laboratory rodents kept in a standardized environment. This was notably the case for the number of brain receptors for several neurotransmitters (46, 47), the number of committed bone marrow stem cells (48), and host tolerance for the anticancer agent *cis*-dichlorodiammineplatinum (49). In healthy men, the circadian rhythmicity in plasma luteinizing hormone was lacking in the first half of the year in two independent studies (27, 28) and the circadian acrophase in plasma testosterone occurred ~ 6 h later in summer as compared with winter (26). Similar results were observed in the present study with regard to this hormone.[2] Such facts, however result from the analysis of data pooled from several individuals. Thus, the lack of detection of circadian rhythmicity at a group level may result from an interindividual desynchronization, e.g., individual circadian rhythms may ex-

2. Data and statistical analysis are available from the authors upon request.

hibit period lengths differing from precisely 24 h. This phenomenon might be more likely to occur at specific times of the year.

As a support to this assumption, the endogenous, so-called free-running circadian period of body temperature was shorter in March–April than in July–August in healthy human beings kept under temporal isolation (50). In longitudinal studies carried out in Paris in June, oral temperature exhibited a period that differed from 24 h with statistical significance in two of five apparently healthy men, despite the fact that these subjects followed their usual social routine (51). Similar results had been obtained earlier both in men and women (52). In the present study, despite no circadian rhythm in $OKT4^+$ lymphocytes detected at a group level in March or June, maximal individual values were almost twice as high as trough values; their respective timing differed, however, among subjects. Since all subjects followed a similar rest–activity schedule throughout the study span, this does not account for the apparent desynchronization. Rather, an endogenous circannual time structure might allow for circadian desynchronization to occur in winter and/or spring. Such a phenomenon may contribute to the marked spring peak in the incidence of several viral diseases such as rubella, mumps, etc. (31) and to that of Hodgkin's disease in young men (32). A lack of circadian organization of the immune system may represent a favorable condition in which these and other diseases may develop.

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References

1. Brock, M. A. 1983. Seasonal rhythmicity in lymphocyte blastogenic response of mice persists in a constant environment. *J. Immunol.* 130:2686–2688.

2. Godfrey, H. P. 1975. Seasonal variation of induction of contact sensitivity and of lymph node T lymphocytes in guinea pigs. *Int. Arch. Allergy Appl. Immunol.* 49:411–414.

3. Shifrine, M., L. Rosenblatt, N. Taylor, N. Hetherington, V. Matthews, and F. Wilson. 1980. Seasonal variations in lectin-induced lymphocyte transformation in beagle dogs. *J. Interdiscip. Cycle Res.* 11:219–231.

4. Reinberg, A., E. Schuller, N. Delasserie, J. Clench, and M. Helary. 1977. Rythmes circadiens et circannuels des leucocytes, protéines totales, immunoglobulines A, G et M. Etude chez 9 adultes jeunes et sains. *Nouv. Presse Med.* 6:3819–3823.

5. Abo, T., and K. Kumagai. 1978. Studies of surface immunoglobulin on human B lymphocytes. *Clin. Exp. Immunol.* 33:441–452.

6. Paigen, B., E. Ward, A. Reilly, L. Houten, H. L. Gurtoo, J. Minowada, K. Steeland, M. B. Havens, and P. Sartori. 1981. Seasonal variation of aryl hydrocarbon hydroxylase activity in human lymphocytes. *Cancer Res.* 41:2757–2761.

7. Bratescu, A., and T. Teodorescu. 1981. Circannual variation in the B cell/T ratio in normal human peripheral blood. *J. Allergy Clin. Immunol.* 68:273–280.

8. Shifrine M., A. Garsd, and L. S. Rosenblatt. 1982. Seasonal variation in immunity of humans. *J. Interdiscip. Cycle Res.* 13:157–165.

9. Munch-Petersen, B., K. Wallevik, and M. Faber. 1985. Seasonal variations in UVR-induced DNA synthesis and in UVR inhibition of phytohemagglutinin-stimulated proliferation of human lymphocytes. *Scand. J. Clin. Lab. Invest.* 45:37–44

10. MacMurray, J. P., J. P. Barker, J. D. Armstrong, L. P. Bozzetti, and I. N. Kuhn. 1983. Circannual changes in immune function. *Life Sci.* 32:2363–2370.

11. Pengelley, E. T., and S. M. Asmundson. 1969. Free-running periods of endogenous circannian rhythm in the golden-mantled ground squirrel, *Citellus lateralis. Comp. Biochem. Physiol.* 30:177–186.

12. Schwab, R. G. 1971. Circannian testicular periodicity in the European starling in the absence of photoperiodic change. *In* Biochronometry. Proceedings of a Symposium at the National Academy of Sciences USA. M. Menaker, editor. 428–447.

13. Sidky, Y. A., J. S. Hayward, and R. F. Reith. 1972. Seasonal variations of the immune response of ground squirrel kept at 22–24°C. *Can. J. Physiol. Pharmacol* 50:203–206.

14. Bureau, J. P., M. Coupé, L. Garelly, and G. Labrecque. 1984. Circannual rhythms of PMN migration induced by BCG in intact mice. *Annu. Rev. Chronopharmacol.* 1:337–340.

15. Pati, A. K., J. Florentin, V. Chung, M. De Sousa, F. Lévi, and G. Mathé. 1987. Circannual rhythm in natural killer cell activity of murine splenic lymphocytes. *Cell. Immunol.* 108:227–234.

16. Lévi F., C. Canon, J. P. Blum, M. Mechkouri, A. Reinberg, and G. Mathé. 1985. Circadian and/or circahemidian rhythms in nine lymphocyte-related variables from peripheral blood of healthy subjects. *J. Immunol.* 134:217–222.

17. Lévi, F., C. Canon, Y. Touitou, J. Sulon, M. Mechkouri, E. Demey-Ponsart, J. P. Touboul, J. M. Vannetzel, I. Mowzowicz, A. Reinberg, and G. Mathé. 1988. Circadian and circahemidian rhythms in circulating T lymphocyte subtypes and plasma concentrations of testosterone, total and free cortisol in five healthy men. *Clin. Exp. Immunol.* In press.

18. Abo, T., T. Kawate, K. Itoh, and K. Kumagai. 1981. 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.* 126:1360–1363.

19. Haus, E., D. Lakatua, J. Swoyer, and L. Sackett-Lundeen. 1983. Chronobiology in hematology and immunology. *Am. J. Anat.* 168:467–517.

20. Ritchie, W. S., I. Oswald, H. S. Micklem, J. E. Boyd, R. A. Elton, E. Jazwinska, and K. James. 1983. Circadian variation of lymphocyte subpopulations: a study with monoclonal antibodies. *Br. Med. J.* 286:1773–1775.

21. Bertouch, J. V., P. Roberts-Thompson, and J. Bradley. 1983. Diurnal variation of lymphocyte subsets identified by monoclonal antibodies. *Br. Med. J.* 286:1171–1172.

22. Tavadia, H. B., K. Fleming, P. D. Hume, and H. W. Simpson. 1975. Circadian rhythmicity of human plasma cortisol and PHA-induced lymphocyte transformation. *Clin. Exp. Immunol.* 22:190–193.

23. Lévi, F., C. Canon, J. P. Blum, A. Reinberg, and G. Mathé. 1983. Large-amplitude circadian rhythm in helper: suppressor ratio of peripheral blood lymphocytes. *Lancet.* ii:462–463.

24. Eskola, J., H. Frey, G. Molnar, and E. Soppi. 1976. Biological rhythm of cell-mediated immunity in man. *Clin. Exp. Immunol.* 26:253–257.

25. Canon, C., F. Lévi, Y. Touitou, J. Sulon, E. Demey-Ponsart, A. Reinberg, and G. Mathé. 1986. Variations circadienne et saisonnière du rapport inducteur ($OKT4^+$)/suppresseur ($OKT8^+$) dans le sang veineux de l'homme adulte sain. *C.R. Acad. Sci. Ser. III Sci. Vie.* 302:519–524.

26. Reinberg, A., M. Lagoguey, J. M. Chauffournier, and F. Cesselin. 1975. Circannual and circadian rhythms in plasma testosterone in five healthy young Parisian males. *Acta Endocrinol.* 80:732–741.

27. Reinberg, A., M. Lagoguey, F. Cesselin, Y. Touitou, J. C.

Legrand, A. Delasalle, J. Antreassian, and A. Lagoguey. 1978. Circadian and circannual rhythms in plasma hormones and other variables of five healthy young human males. *Acta Endocrinol.* 88:417–425.

28. Touitou, Y., M. Lagoguey, A. Bogdan, A. Reinberg, and H. Back. 1983. Seasonal rhythms of plasma gonadotrophins: their persistence in elderly men and women. *J. Endocrinol.* 96:15–22.

29. Abo, T., and C. M. Balch. 1981. A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J. Immunol.* 127:1024–1029.

30. Abo, T., M. D. Cooper, and C. M. Balch. Characterization of HNK-1$^+$ (Leu 7) human lymphocytes. I. Two district phenotypes of human NK cells with different cytotoxic capability. *J. Immunol.* 129:1752–1757.

31. Reinberg, A., and M. Smolensky. 1983. Biological Rhythms and Medicine. Cellular, Metabolic, Physiopathologic and Pharmacologic Aspects. Springer-Verlag, Inc., New York. 305–320.

32. Newell, G. R., H. K. Lynch, J. M. Gibeau, and M. R. Spitz. 1985. Seasonal diagnosis of Hodgkin's disease among young adult. *J. Natl. Cancer Inst.* 74:53–56.

33. Ritz, J., L. M. Nadler, A. K. Bahn, J. N. McConarty, J. M. Pesando, and S. F. Schlossmann. 1981. Expression of common acute lymphoblastic leukemia antigen (CALLA) by lymphomas of B-cell and T-cell lineage. *Blood.* 58:648–657.

34. Nelson W., Y. L. Tong, and F. Halberg. 1979. Methods for cosinor rhythmometry. *Chronobiologia.* 6:305–323.

35. Porwit-Ksiazek, A., P. Amon, T. Skiazek, and P. Biberfeld. 1983. Leu 7$^+$ (HNK-1$^+$) cells. II. Characterization of blood Leu 7$^+$ cells with respect to immunophenotype and cell density. *Scand. J. Immunol.* 18:495–499.

36. Lanier, L. L., E. G. Engleman, P. Gatenby, G. F. Badcock, N. L. Werner, and L. A. Herzenberg. 1983. Correlation of functional properties of human lymphoid cell subsets in surface marker phenotypes using multiparameter analysis and flow cytometry. *Immunol. Rev.* 74:143–160.

37. Gidlow, D. A., J. F. Church, and B. E. Clayton. 1983. Haematological and biochemical parameters in an industrial workforce. *Ann. Clin. Biochem.* 20:341–348.

38. Sinha, A. K., A. Linscombe, B. Gollapudi, G. Jersey, and R. Flake. 1986. Cytogenetic variability of lymphocytes from phenotypically normal men. Influence of age, smoking, season and sample storage. *J. Toxicol. Environ. Health.* 17:325–345.

39. Knapp, M. S., and R. Pownall. 1984. Lymphocytes are rhythmic: is this important? *Br. Med J.* 289:1328–1330.

40. Miyawaki, T., K. Taga, T. Nagaoka, H. Seiki, Y. Susuki, and N. Taginuchi. 1984. Circadian changes of T lymphocyte subsets in human peripheral blood. *Clin. Exp. Immunol.* 55:618–622.

41. Carter, J. B., G. D. Barr, A. S. Levin, V. S. Byers, B. Ponce, and H. D. Fudenberg. 1975. 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 Clin. Immunol.* 56:191–205.

42. Heitbrock, H. W., R. Mertelsman, and M. Garbercht. 1976. Circadian rhythm of RNA polymerase B activity in human peripheral blood lymphocytes. *Int. J. Chronobiol.* 3:255–261.

43. Ramot, B., F. Brok-Simoni, E. Chiveidman, and Y. E. Askenazi. 1976. Blood leucocyte enzymes. III. Diurnal rhythm of activity in isolated lymphocytic leukemia patients. *Br. J. Haematol.* 34:79–87.

44. Muchmore, H. G., A. J. Parkinson, J. E. Humphries, E. N. Scott, D. A. McIntosh, L. V. Scott, M. K. Cooney, and J. A. R. Miles. 1981. Persistent parainfluenza virus shedding during isolation at the south pole. *Nature (Lond.).* 289:187–189.

45. Rosenblatt, M. S., M. Shifrine, N. Hetherington, T. Paglieroni, and R. MacKenzie. 1982. A circannual rhythm in rubella antibody titers. *J. Interdiscip. Cycle Res.* 13:81–88.

46. Kafka, M. S., A. Wirz-Justice, and D. Naber. 1981. Circadian and seasonal rhythms in α- and β-adrenergic receptors in the rat brain. *Brain Res.* 207:409–419.

47. Naber, D., A. Wirz-Justice, M. S. Kafka, I. Tobler, and A. Borbely. 1981. Seasonal variations in the endogenous rhythm of dopamine-receptor binding in rat striatum. *Biol. Psychol* 16:831–835.

48. Aardal, N. P. 1984. Circannual variations of circadian periodicity in murine colony-forming cells. *Exp. Hematol.* 12:61–67.

49. Lévi, F. 1982. Chronopharmacologie de trois agents doués d'activité anticancéreuse chez le rat et la souris. Thèse de Doctorat d'Etat ès-Sciences, Université Paris VI.

50. Wirz-Justice, A., R. A. Wever, and J. Aschoff. 1984. Seasonnality in free-running circadian rhythms in man. *Naturwissenschaften.* 71:316–322.

51. Reinberg, A., F. Lévi, A. Bicakova-Rocher, J. P. Blum, M. M. Ouechni, and N. Nicolai. 1984. Biologic time-related changes in antihistamine and other effects of chronic administration of mequitazine in healthy adults. *Annu. Rev. Chronopharmacol.* 1:61–64.

52. Bicakova-Rocher, A., A. Gorceix, and A. Nicolai. 1984. Circadian and ultradian rhythm period alterations in apparently healthy subjects with and without placebo. *Annu. Rev. Chronopharmacol.* 1:169–171.