Total Sleep Deprivation Induces an Acute and Transient Increase in NK Cell Activity in Healthy Young Volunteers

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Study Objectives: To investigate the effects of one night's total sleep deprivation (TSD) on NK cell activity, with rigorous control of circadian phase of sampling points as well as physical exercise level in association with sleep deprivation.

Design: The mean sleep onset time of each subject before starting the study was defined as his 0000 h. This study was composed of a Sleep-Sleep session (sleep times, 00:00 h - 08:00 h and 24:00 h - 32:00 h) and a Sleep-Wake session (sleep time, 00:00 h - 08:00 h) with TSD (24:00 h - 32:00 h) placed in a cross-over design with 2-week interval between each session. In each session, the subjects were rested in the supine position under dim light from 06:00 h to 36:00 h (for 42 hours).

Setting: University-based sleep and chronobiology laboratory

Participants: 10 healthy adult men (mean age, 20.9 y; age range, 19—23 y)

Interventions: NA

Measurements and Results: NK cell activity was measured every 4 hours from 12:00 h. NK cell activity during TSD (at 28:00 h) has been revealed to significantly increase (p=0.01) compared with the corresponding value in the Sleep-Sleep session. This effect was weaker at their usual waking time 32:00 h (p=0.07), and disappeared until 36:00 h (4 hours after awakening). The circadian rhythm phases (dim light melatonin onset time) were coincident between the 2 sessions.

Conclusions: The present findings suggest that one night TSD induces an acute and transient increase in NK cell activity that is not influenced by the effects of circadian rhythm or the amount of physical exercise undertaken during TSD.

Key words: NK cell activity; sleep deprivation; circadian rhythm; exercise; host defense

INTRODUCTION

EVIDENCE IS MOUNTING THAT SLEEP IS NOT MERELY A PASSIVE STATE OF RELAXATION TO COMPENSATE FOR FATIGUE ACCUMULATED DURING WAKING HOURS. For example, reports indicate that it plays a role in active modification of host defense functions. To evaluate the effects of natural sleep on neuroendocrine immune function, numerous research groups have investigated changes in certain immune functions when healthy subjects were subjected to total or partial sleep deprivation. Several of these groups have focused their attention on the effect of sleep deprivation on natural killer (NK) cell activity. NK cells are lymphocytes that lack antigenic specificity and are found in normal individuals. They are believed to play a role in mediating natural resistance against tumors and infections. Research to date has provided divergent findings on the effect of total or partial sleep deprivation on NK cell number and/or activity. Dinges et al. have investigated the effect of 64-hour sustained awake on NK cell activity in 20 young adults, and reported that NK cell activity increased during total sleep deprivation which effect were eliminated by recovery sleep. Born et al. have also found that NK cell numbers transiently increased during total sleep deprivation in 10 young male volunteers although they decreased until the following afternoon. Contrary to these findings, Moldofsky and his colleagues have indicated that total sleep deprivation could reduce NK cell activity in healthy male volunteers, as was also suggested by studies by Irwin et al. in which late-night partial sleep depriv-
METHODS

Experimental Subjects

The subjects were 10 healthy adult non-smoking men (mean age, 20.9 y; age range, 19—23 y). Before enrollment in the study, three physicians conducted rigorous physical and psychological evaluations of each subject. Subjects were surveyed for the following problems: a history of psychiatric disease which met Mini-International Neuropsychiatric Interview (M.I.N.I.),12 a structured diagnostic psychiatric interview for DSM-IV,13 infection within the preceding 12 months and history of allergy or other physical disease that could potentially affect immune function. Hematology tests including total numbers of WBC, erythrocytes, and platelets, leukocyte differential counts, hemoglobin, hematocrit as well as urinalysis were performed for all subjects; all results were within the normal range. Before the study began, the objectives of the study were thoroughly explained to potential subjects, and informed consent was obtained.

Procedures

Beginning seven days before the start of the study, excessive exercise, alcohol, and all medications were forbidden, and eight-hour sleep was enforced at each subject’s usual sleep onset time (base-line observation period). In the baseline observation period, regularity of sleep-wake rhythm was assessed using an actigraph fitted to the non-dominant wrist of each subject, after which the mean sleep onset time was computer-calculated according to the algorithm of Cole et al.14 and defined as his 00:00 hour. The study period was composed of a Sleep-Sleep session (SS session) and a Sleep-Wake session (SW session; subjects underwent total sleep deprivation), in a cross-over design with two-week interval between each session. In both the SS and SW sessions, each subject entered the sleep laboratory at eight hours before 00:00 hour (-08:00 hour). In the period before 06:00 hour, experimental clothing (identical cotton pajamas) was donned, the actigraph was fitted, the rectal temperature probe was inserted, and an indwelling catheter (heparin lock) for painless blood collection was inserted in a peripheral vein in the left forearm. At 06:00 hour, a 750-Cal meal and as much water as desired was given to each subject. Next, for 42 hours until the end of the study at 36:00 hour, subjects rested in the supine position desired was given to each subject. Next, for 42 hours until the end of the study at 36:00 hour, subjects rested in the supine position. Standing and walking were prohibited. A portable toilet in the adjoining room was used for defecation and urination. Movement to the toilet was assisted, and sitting was permitted during use of the toilet. Subjects were allowed to recline and sleep on a bed in the laboratory at 00:00-08:00 hour and 24:00-32:00 hour in the SS session, and at 00:00-08:00 hour only in the SW session. Sleeping was forbidden outside these time periods, and two or more laboratory staff members were constantly present to observe the subjects and provide conversation or assistance. Laboratory illumination was maintained at less than 10 lux during sleep, and at 100 lux near the subjects’ eyes at other times. The ambient temperature was maintained at 23±1°C throughout the study period. Every two hours beginning at 10:00 hour (except during 26:00-30:00 hour in the SS session), a 150-Cal snack, free of caffeine or other stimulants, and 100 cc of water were taken.

Evaluation of Sleep Quality Calculated by Actigraph Data

Throughout the study period, wrist activity was monitored with an actigraph (AMI Inc., Ardsley NY) around the non-dominant wrist of each subject. Actigraph data was analyzed for computer-calculated sleep-wake determinations.14 Nighttime sleep parameters for each subject were defined as follows: Sleep efficiency (SE), the total time asleep as a percentage of the total time in bed; awake time (AT), the total number of waking episodes that continued for at least 10 min during the sleep period; wake time after sleep onset (WASO), the accumulated time awake after the sleep onset; and sleep latency (SL), the time between bedtime and the sleep onset.

Evaluation of Circadian Rhythm Phase

Every hour from 12:00 h to 36:00 hour and every 30 minutes from 20:00-5:00 hour, blood samples were withdrawn painlessly. Samples were centrifuged for six minutes at 3000 rpm, and the serum produced was stored frozen at 20°C until analysis by later radioimmunoassay. Assays for melatonin were performed by laboratory technicians blind to the conditions of the experimental subjects. Parameters for melatonin rhythm were defined as follows: dim light melatonin onset time (DLMOn), the evening time at which serum melatonin concentrations reached 8.1 pg/ml, which was three-fold the detection limit of the radioimmunooassay kit used in this study; dim light melatonin offset time (DLMOff), the morning time at which serum melatonin concentrations decreased below 8.1 pg/ml; and the midtime of nocturnal rise (Midtime), the midtime between DLMOn and Off time.

Evaluation of Serum Cortisol Secretion

Every hour from 12:00 hour to 36:00 hour, blood samples were withdrawn painlessly. Samples were centrifuged for six minutes at 3000 rpm, and the serum produced was stored frozen at -20°C until analysis by later radioimmunoassay by laboratory technicians blind to the conditions of the experimental subjects. The area under the serum cortisol secretion curve from 24:00-36:00 hour was calculated.

Evaluation of Core Body Temperature

Every one minute from 12:00-36:00 hour, core body temperature (core BT) was monitored with the use of an ambulatory core BT monitoring system (Kohden Medical Inc., Tokyo) with the polyethylene-covered thermoprobe (0.01°C in accuracy) being inserted 10 cm into the rectum. Data at each measurement point were smoothed by means of a moving average procedure that included time points 15 minutes before and after each measurement point.

Measurement of NK Cell Activity

Samples of blood used for evaluation of NK cell activity were painlessly collected every four hours from 12:00-36:00 hour. At 24:00 hour and 32:00 hour in the SS session, blood samples were withdrawn immediately before subjects went to bed and immedi-
added to adjust the concentration to 1 x 10^6/ml. The target cells were washed three times with PBS, and RPMI 1640 medium was

**RESULTS**

The sleep parameters on the second night in the SS session are shown in Table 1. There were no significant differences in corresponding data between the second night and the baseline observation period for any sleep parameter (data not shown). All subjects were asleep during the blood collection procedure at 28:00 hour in the SS session, and analysis of the actigraph findings confirmed that any continuous arousal produced by the collection of blood lasted for less than five minutes.

Two-way analysis of variance showed a significant difference in the time course of changes in NK cell activity between the SW session and SS session (F=3.1, df=4, p=0.02, Figure 1a). The NK cell activity at 28:00 h in the SW session was significantly higher than the corresponding activity in the SS session (p= 0.01). This effect was weaker at 32:00 h (the end of the sleep session, p=0.07), and at 36:00 h (about four hours after awakening), there was no significant difference in NK cell activity between the two sessions.

Data for the three circadian phase parameters of melatonin secretion rhythm in the SS session and SW session are shown in Table 1. Obvious nocturnal melatonin secretion was seen in both sessions (Figure 1b). No significant differences between the SS session and SW session were seen for DLMOn, DLMOff, or Midtime.

The serum cortisol secretion patterns in the SS session and SW session are shown in Figure 1c. The area under the serum cortisol concentration curve at 24:00-32:00 hour, which corresponds to the time of sleep deprivation, was 42.0 ± 5.82 pg/ml*hr and 51.4 ± 6.73 pg/ml*hr in the SS session and SW session, respectively; the difference between the two sessions was not significant.

Changes in core BT in the period 21:00-36:00 hour in the SS session and SW session are shown in Figure 1d. Two-way analysis of variance showed a significant difference in the time course of changes in core BT between the SW and SS sessions, with core BT in the SW session significantly higher than that of the SS session in the period 28:30-31:30 hour (F=1.5, df=30, p=0.04).

**DISCUSSION**

Our research demonstrated that a single night’s sleep deprivation induces a significant acute and transient increase in NK cell activity in healthy young subjects. However, the effect continued for only eight hours or less (i.e., until their usual wake up time) and disappeared over 12:00 hour.

In the present experiment, the subjects were made to rest in the supine position during sleep deprivation, in order to rigorously control the effect of physical exercise on NK cell activity,11 that is inevitably increased during sleep deprivation as compared with when the individual is asleep.

In this study, we also focused on the effect of circadian variations in cellular immune function. In previous studies, NK cell counts7,8 and NK cell activity7,8 have been revealed to show obvious circadian variations. The present findings suggest that finely tuned control of the points in the circadian rhythm phase at which blood samples are collected for measurement of NK cell activity may be critical. In fact, as evidenced by the circadian variations in NK cell activity elucidated in the present study (Figure 1a),

<p>| Table 1—Sleep quality and rhythm phase of melatonin secretion in 10 subjects studied |
|----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Parameters</th>
<th>SS Session</th>
<th>SW Session</th>
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<tbody>
<tr>
<td><strong>Sleep Quality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Sleep Time (min)</td>
<td>443.3 ± 4.05</td>
<td>423.0 ± 4.12</td>
</tr>
<tr>
<td>Sleep Efficacy (%)</td>
<td>92.36 ± 0.84</td>
<td>88.7 ± 0.79</td>
</tr>
<tr>
<td>Awake Time (times)</td>
<td>3.1 ± 0.34</td>
<td>2.9 ± 0.31</td>
</tr>
<tr>
<td>WASO (min)</td>
<td>18.4 ± 1.57</td>
<td>20.1 ± 1.68</td>
</tr>
<tr>
<td>Sleep Latency (min)</td>
<td>14.9 ± 3.71</td>
<td>12.9 ± 3.64</td>
</tr>
<tr>
<td><strong>Rhythm Phase</strong></td>
<td></td>
<td></td>
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<tr>
<td>DLMOn (h : min)</td>
<td>24:32 ± 38.7</td>
<td>24:05 ± 42.6</td>
</tr>
<tr>
<td>DLMOff (h : min)</td>
<td>31:01 ± 33.4</td>
<td>30:57 ± 20.5</td>
</tr>
<tr>
<td>Midtime (h : min)</td>
<td>27:46 ± 29.1</td>
<td>27:31 ± 26.0</td>
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<tr>
<td><strong>Statistical Analysis</strong></td>
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<td>Two-way analysis of variance was used to compare cellular immune function, core BT level, and serum cortisol secretion level in the SS session and SW session. In the event of a significant difference between sessions, appropriate corresponding points were tested with the Mann-Whitney U-test. Data were expressed as mean values (plus or minus the standard error of the mean). Differences with p-values below 5% were regarded as significant.</td>
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substantial changes of 10% or more (with mean values ranging from 24.5% to 36.8%) occur in the four-hour period following waking (8-12 hours after going to sleep) during which blood samples have been taken in numerous previous studies. If blood samples are collected in this time period, the magnitude of circa-dian variations in NK cell activity produced by the disparities in blood sample collection times may overwhelm the effect of sleep deprivation itself, or diminish its effect. In addition, environmental (room) light condition during total sleep deprivation could also affect NK cell activity level even at the same clock time on the next day. Timed exposure to bright light ranging from 2500 lux to 13000 lux has been revealed to induce strong circadian phase-shifting up to several hours in human.15-17 Although the magnitude of circadian phase-shift by dimmer room light of 1000 lux or less has been shown to decrease in an intensity-dependent manner,18,19 long-lasting (all night) light exposure in experimental room, usually ranging between 400 and 1000 lux, could induce substantial phase-advance (or delay) according to subjects’ phase-response curve properties which also overwhelm the effect of sleep deprivation itself.

Previous studies that evaluated the effects of sleep deprivation on NK cell activity failed to precisely control the influence of circadian phase as well as experimental light condition.2,4-6,20 Some of these studies did not adopt cross-over design between sleep deprivation and natural sleep conditions,4-6,20 and also allowed a possible inconsistency in circadian timing of blood sampling up to two hours between 07:00 and 09:00 (clock time) during which substantial changes in NK cell activity might occur.5,6 To exclude as far as possible the effects of circadian variations of NK cell activity and to achieve uniformity of the circadian rhythm phase in which blood samples were collected, we strictly controlled light intensity in the experimental room under dim light (100 lux) as well as the sleep phase during seven days prior to entry in the study as the reference time of the circadian rhythm phase. By doing so, we achieved statistical agreement between the SS and SW session for all markers of the circadian rhythm phase, including the melatonin secretion on time, off time, and midtime. This ensured that the evaluation of cellular immune function in the SS and SW session was done at the identical circadian rhythm phase in the present study. It is reasonable to assume that there is only a small possibility that the increase in NK cell activity caused by total sleep deprivation was modified by effects attributable to variations in the circadian rhythm.

Given the technique for measuring NK cell activity employed in the present study, it is unclear whether the increase in NK cell activity during sleep deprivation was caused by a rise in the activity of NK cells themselves or reflected an increase in the number of NK cells as a proportion of all mononuclear cells. However, regarding the mechanism by which total sleep deprivation increases NK cell activity, we have postulated that the NK cell count in peripheral blood may have risen, via stimulation of adrenergic receptors on the membrane surface of NK cells.9 Previous research has shown that even though the NK cell activity in peripheral blood increases after adrenergic receptor stimulation by means such as catecholamine administration, exercise loading, or head-up tilt,21,22 there is no change in the cytotoxicity per NK cell when corrected with the NK cell count.22,23 It has been suggested that increases in the NK cell count in peripheral blood due to adrenergic receptor stimulation may be mediated by mechanisms such as the promotion of NK cell release from

Figure 1—Circadian variations in, and the effect of total sleep deprivation on (a) NK cell activity, (b) serum melatonin secretion, (c) serum cortisol secretion, and (d) core body temperature. The horizontal axis represents time, relative to 0000 h, defined as the mean sleep onset time during 7 days before entering the study. Shaded horizontal bars in the figure indicate the sleeping period (SS session) or the sleep deprivation period (SW session) on the second study day. Data are expressed as mean values (SS session: open circles; SW session: filled circles) plus or minus the standard error of the mean. See the main text for an explanation of the figure.
endothelia,24,25 or promotion of the liberation of NK cells into peripheral blood by constriction of splenic smooth muscle.22 Because such adrenalin-induced increases in the NK cell count are inhibited by propranolol, an adrenergic B1/B2 blocker,23,26 but not by metoprolol, a selective B1 antagonist,27 it can be conjectured that they are mediated by B2 receptors. These findings support the conjecture that the increase in NK cell activity during sleep deprivation observed in the present study was induced by an increase in the number of NK cells as a proportion of mononuclear cells. Unfortunately, since we did not directly evaluate variations in the blood concentration of catecholamines or autonomic function during sleep or sleep deprivation in our study, we do not know whether total sleep deprivation actually modified NK cell function via a sympathetic mechanism. However, the analysis of core BT data demonstrated that sleep deprivation produced a rise in core BT, as compared with the findings obtained during sleep. The rise in core BT was not mediated by an increase in exercise attributable to sleep deprivation. This finding indirectly suggests that the sympathetic system may dominate during sleep deprivation as compared with the situation during sleep.

In the present study, NK cell activity in the sleep deprivation session recovered rapidly during the daytime on the following sleep session. This suggests that the effect of sleep deprivation observed in the present study was induced by a rise in core BT, as compared with the findings obtained during sleep. The increase in core BT was not mediated by an increase in exercise attributable to sleep deprivation. This finding indirectly suggests that the sympathetic system may dominate during sleep deprivation as compared with the situation during sleep.

In the present study, NK cell activity in the sleep deprivation session recovered rapidly during the daytime on the following sleep session. This suggests that the effect of sleep deprivation for one night on the NK cell system may not be carried over, at least in young subjects. There have been several reports on the effects of the duration of stress on NK cell activity. In all stress models, including mental stress,26,28,29 physical stress,27 and emotional stress,30 it was reported that NK cell activity increased when the stress was acute. However, numerous reports contradict these findings, noting that NK cell activity is decreased in cases where it is presumed that chronic stress persists.31-34 Apart from short-duration shift work and jet lag, many forms of sleep disorder persist chronically. In the present study, we modeled the effects of a short-term sleep disorder on the biological defense mechanism, and it will now be necessary to investigate the effects of consecutive nights of chronic sleep disturbances on cellular immune function. If the type or duration of sleep disorder is found to affect immune function in some way, research interest should then turn toward whether this is manifested by physical effects, as demonstrated by an increase in the incidence of infection.

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REFERENCES

26. Benschop RJ, Nieuwenhuis EE, Tromp EA, Gadaert GL, Ballieux RE, van Doornen L. Effects of beta-adrenergic blockade on immunolog-