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Cellular adhesion molecule expression, nocturnal sleep, and partial night sleep deprivation

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Abstract

Sleep is hypothesized to have a role in the regulation of the immune system. This study evaluated the nocturnal expression of cellular adhesion molecules, Mac-1 and L-selectin on monocytes and lymphocytes during a full nights sleep and following a partial night of sleep deprivation (PSD). Healthy male subjects ($n = 16$) had an increase in the percentage of Mac-1 positive lymphocytes across the baseline night. Whereas, the percentage of Mac-1 positive lymphocytes was reduced and L-selectin positive lymphocytes and monocytes were greater during the PSD night as compared to the baseline night. These data indicate that acute sleep disruption is associated with alterations in cellular adhesion molecule expression, with implications for the regulation of immune cell trafficking.

Keywords: Cellular adhesion molecules; L-selectin; Mac-1; Monocytes; Lymphocytes; Sleep deprivation

1. Introduction

Sleep is thought to have a role in the homeostatic regulation of the immune system. Across a night of normal sleep, there is evidence that natural killer (NK) cell activity increases (Irwin et al., 1996; Redwine et al., 2003a,b), stimulated ex vivo production of interleukin-2 (IL-2) is enhanced (Born et al., 1997), there is a rise in the nocturnal response of lymphocytes to pokeweed mitogen stimulation (Moldofsky et al., 1989), Th1 versus Th2 cytokine expression shifts toward a Th1 predominance (Petrovsky and Harrison, 1995; Redwine et al., 2003a), and circulating levels of IL-6 increase (Bauer et al., 1994; Redwine et al., 2000; Vgontzas et al., 1999). Although data are limited, nocturnal increases of NK activity, IL-2 production, lymphocyte proliferation to pokeweed mitogen, and IL-6 expression appear to be related to sleep-dependent, rather than circadian, mechanisms (Born et al., 1997; Moldofsky et al., 1989; Redwine et al., 2000, 2003a; Vgontzas et al., 1999); as these nocturnal immune changes are attenuated during a period of wakefulness or sleep deprivation. In addition, a broader body of research has examined the effects of sleep loss on *daytime* measures of immune system functioning (as reviewed by Benca and Quintas, 1997) and found that sleep deprivation leads to daytime increases of IL-6 expression (Shearer et al., 2001; Vgontzas et al., 1999) and either decreases or increases of daytime NK activity with different results possibly due, in part to the duration of the sleep loss (Born et al., 1997; Dinges et al., 1994; Moldofsky et al., 1989). Given that cellular adhesion molecule expression is critical in the recruitment and migration of immune cells to sites of inflammation and infectious challenge (Ortaga et al., 1997, 1999), we examined nocturnal and daytime levels of cellular adhesion molecule expression in relation to normal sleep. We also evaluated the effects of partial night sleep deprivation (PSD) on cellular adhesion molecule expression immediately after the period of sleep loss and in the subsequent morning. Partial night sleep loss is arguably similar to the kinds of sleep loss found in people who work extended hours or shiftwork

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(late shift) or students who often study and/or work late, but are still required to rise early.

Expression of the adhesion molecule, L-selectin, tethers immune cells to the endothelium, which enhances binding to locally expressed ligands. With subsequent immune activation, L-selectin is cleaved (Marschner et al., 1999) and integrins such as macrophage associated antigen (Mac-1) and lymphocyte functional antigen (LFA-1) transition into the active state (Tohya and Kimura, 1998), which lead to firm adhesion and transendothelial migration via interaction with endothelial counter-ligands such as intercellular adhesion molecule-1 (ICAM-1). Release of proinflammatory cytokines is thought to initiate cleavage of L-selectin and expression of Mac-1 (Black and Garbutt, 2002). Moreover, recent observations suggest that cellular adhesion molecules play a role in atherogenesis and infectious disease by mediating adherence and migration of leukocytes across the endothelium and to inflammatory sites (Black and Garbutt, 2002).

The role of nocturnal sleep and sleep loss on cellular adhesion molecule expression is not known. In sleep apneic patients, daytime circulating levels of soluble adhesion molecules such as ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), and L-selectin (Chen et al., 2000; Ohga et al., 1999) were found to be elevated along with increased expression of cellular adhesion molecule integrin, CD11c (Dyugovskaya et al., 2002). However, in these studies, measures were limited to a single morning time point and changes in sleep apneic patients may be due to oxidative stress rather than disordered sleep.

The present study measured cellular adhesion molecule expression in healthy individuals who regularly slept between 23:00 and 07:00 h, without either sleep apnea or nocturnal myoclonus. Cellular adhesion molecule expression was measured during a full nights sleep as well as a night of PSD to evaluate the effects of nocturnal sleep and sleep loss on the immunocyte expression of Mac-1 and L-selectin. Early night PSD was used as the experimental sleep paradigm, which has been employed previously in our laboratory (Irwin et al., 1996; Redwine et al., 2000). Since sleep loss is known to induce a rebound increase of delta sleep (Irwin et al., 2003) early night PSD provides an opportunity to examine whether changes of immune function are due to the effects of sleep loss or to the increased depth of recovery sleep in the later half of the deprivation night.

Given the known effects of sleep on immune cell function, with increases in markers of immune activation (e.g., IL-6) (Bauer et al., 1994; Redwine et al., 2000; Vgontzas et al., 1999), we hypothesized that Mac-1 would increase from 03:00 to 06:30 h, whereas this increase would be attenuated during a night of PSD. For L-selectin, immune activation is associated with reductions in the expression of this cellular adhesion molecule. Consequently, in contrast with Mac-1, we hypothesized that normal nocturnal sleep would be associated with decreases of L-selectin from 03:00 to 06:30 h, whereas nighttime PSD would induce an increased expression of this adhesion molecule during the same time interval.

2. Subjects and methods

2.1. Subjects

Subjects were identified through a standardized search strategy of the San Diego area involving the placement of flyers and advertisements in local newspapers. Male volunteers ($n = 16$) between the ages of 26 and 64 (mean age = 48.8, $SD = 10.6$) were identified for the study of which nine were African American and seven were European American. All subjects were free of physical illness as determined by examination; none had histories of recent (<2 weeks) viral infections or diseases (e.g., autoimmunity or cancer) that would influence immune function or sleep. Prior to entry into the study, medication histories were obtained and subjects who were taking medications known to alter the immune system or sleep were excluded. Because depression may affect sleep and immune activity (Irwin et al., 2003), and because psychological stress is associated with changes in cellular adhesion molecule expression (Mills et al., 1999), severity of depressive symptoms was evaluated using the 17-item Hamilton Depression Rating Scale (HDRS; Hamilton, 1967) by a trained interviewer with a test–retest reliability of 0.94 on total scores (Endicott et al., 1981; Irwin et al., 1990). The HDRS was administered between 10:00 and 11:00 h one to two days prior to beginning the study. All subjects scored low for symptoms of depression ($M = 0.56 \pm 1.2$) and showed no evidence of a clinical depression. Two weeks prior to entry into the study, sleep–wake activity diaries were obtained; all volunteers slept regularly between the hours of 22:00 and 07:00 with average total sleep of 7.0 ± 0.7. None had evidence of sleep phase disorder, recent shift work, sleep loss of <6 h per night, or insomnia.

2.2. Procedures

The sleep protocol involved four nights. The first night was used as an adaptation night where subjects underwent recordings of oxygen desaturation and tibial myoclonus to exclude those with either sleep apnea or nocturnal myoclonus. On the second night, EEG sleep was monitored using polysomnography (PSG) based on methods from Irwin et al. (2000). On the third night, EEG sleep was again measured by PSG along with nocturnal blood sampling prior to sleep and throughout the night as described further below. On the fourth

night, subjects underwent early night partial sleep deprivation (PSD-E). The data in the present study represent sleep on nights 3 and 4, baseline, and PSD-E. EEG sleep data from a cohort of this sample have been previously reported (Redwine et al., 2003a).

For each of the experimental nights, subjects arrived at the laboratory between 20:00 and 21:00 h. On nights 3 and 4, between 20:30 and 21:30 h, an intravenous catheter was inserted into a forearm vein and electrodes were placed for EEG, electrooculography, and submental electromyography recordings; sleep EEG measures were obtained during continuous PSG recordings between 22:00 and 06:30 h. On night 3, lights were turned off at 23:00 h. On night 4, subjects were kept awake until 03:00 h. Lights were dimmed during the PSD night period (23:00 to 03:00 h) from approximately 150 lux to around 10 lux. We have found that this very low intensity of light does not suppress the secretion of melatonin but is important towards helping maintain the subject's awake state (Redwine et al., 2000). Awake status was monitored by a sleep technician who observed subject behavior and EEG. All subjects included in the present analyses remained supine throughout the entire nocturnal period; a bedside urinal was used if subjects needed to urinate during the night. Subjects that were not already awake were awakened after the last blood draw at 06:30 h. In the morning following completion of the nocturnal protocol, participants left the sleep laboratory and returned to their daily routine with exposure to their usual amounts of light; napping behavior or use of substances such as alcohol or caffeine was not allowed.

For blood sampling, the intravenous catheter was connected to a long thin plastic tube that enabled blood collection from an adjacent room. We have previously demonstrated that placement of an intravenous catheter and nocturnal sampling of blood can be achieved without altering sleep amount or sleep depth (Irwin et al., 2003). For this study, two blood samples, 03:00 and 06:30 h, were analyzed for cellular adhesion molecule expression. The 03:00 h sample provided a comparison of nocturnal sleep versus acute sleep loss for about 4 h, whereas the 06:30 sample provided a comparison of a full night of nocturnal sleep versus partial night sleep loss. In addition, assessment of the EEG sleep during the later half of the night allowed for testing the relation between recovery sleep depth and morning levels of Mac-1 and L-selectin expression. In contrast, a protocol with PSD in the later part of the night (subjects are awakened at 03:00 h after lights out at 23:00 h) would not allow evaluation of the effects of sleep intensity changes. Between intravenous catheter insertion and blood samplings, continuous heparinized isotonic saline was infused, totaling about 1000 mL across the nocturnal period. Immediately after blood was obtained, the sample was put into tubes containing EDTA and kept at room temperature until assay between 1 and 5 h later. Pilot studies performed in our laboratory revealed that keeping tubes at room temperature did not change cellular adhesion molecule expression over a 5-h period. Samples that were held for 8 h or longer show declines of adhesion molecule expression.

Sleep records were visually scored according to the criteria of Rechtschaffen and Kales (1968). The derived EEG sleep variables were divided into three groups: sleep continuity indices, sleep architecture indices, and REM sleep indices. Sleep continuity indices included total sleep time, sleep efficiency (time spent asleep/total recording period $\times$ 100), and sleep latency [time from beginning of the recording period "lights out" to the first minute of stage 2 or rapid eye movement (REM) sleep followed by at least 8 min of sleep in the next 9 min]. While some studies define sleep onset by three contiguous epochs of stage 1 sleep (1.5 min), stage 1 may be unstable and subject to arousals; the onset of stage 1 sleep may not represent the onset of a continuous sleep period. Sleep architecture indices included amount (minutes) and percentage of time spent in different stages of sleep (i.e., stage 1, stage 2, stage 3, stage 4, and delta or the sum of stages 3 and 4 sleep, and REM sleep). A REM period was defined by not less than three consecutive minutes of REM sleep as described by Rechtschaffen and Kales (1968). Indices for REM sleep included REM activity (a visually scored estimate of the frequency of rapid eye movements with each minute of REM sleep scored 0–8), REM density (REM activity divided by REM time), REM duration first period (amount of time for first REM period), REM latency (time from sleep onset to first REM period), and REM latency corrected (REM latency minus intervening time spent awake or in movement). The three sleep research technicians who scored EEG sleep records showed high scoring inter-reliability (10% of the records were double scored): sleep latency ($r = .96$), REM latency ($r = .99$), REM density ($r = .91$), amounts of stages 3 and 4 ($r = .85$), and total sleep time ($r = .99$).

2.3. Cellular adhesion molecule measures

Flow cytometry (FACScan, Becton–Dickinson, San Jose, CA) using SimulSET software was used with CD45 gating to quantify leukocytes and cellular adhesion molecules. As previously described (de Rossi et al., 2003), aliquots of whole blood (490 μL) from each time point were stimulated for 30 min at room temperature with 10 μL of 10^{-5} of a bacterial peptide, *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) diluted in serum-free Roswell Park Media Institute (RPMI) media, thus looking at the ability of the cells to become activated by a pathogenic stimulus. The reaction was stopped with 10 mL of cold phosphate-buffered saline (PBS) and the blood was centrifuged for 10 min at 800*g*. Most of the supernatant was removed, leaving 800 μL of PBS/

pellet that was separated into four tubes of 200 μL each. The samples were incubated at room temperature for 15 min with phycoerythrin (PE) conjugated monoclonal antibodies for L-selectin (CD62L) and Mac-1 (CD11b), and fluorescein (FITC) for CD45 (Becton and Dickenson). An isotypic control (IgG-PE) was used for each assay to determine nonspecific staining. After incubation, the red blood cells were lysed with FACSLyse solution (Becton and Dickson), followed by washing with 2 mL of PBS, centrifugation for 10 min 800*g*, and aspiration of the supernatant. Red blood cell lyses was performed rather than density gradient centrifugation, since a significant loss of monocyte L-selectin expression has been observed with density gradient centrifugation methods (Stibenz and Buhrer, 1994). Labeled cells were fixed with 2% formaldehyde in PBS and analyzed by flow cytometry within 24 h. Lymphocytes, monocytes, and granulocytes were identified by forward and side scatter using flow cytometry. Cell density was determined by mean fluorescence per cell.

3. Statistical analyses

All data were entered and analyzed using SPSS version 11.5 for Windows. To avoid reductions in statistical power due to missing data points, a small number of absent values were replaced by single-point multiple imputation using NORM version 2.03 as this technique has been shown to provide accurate statistical estimates based on the available data (Schafer and Graham, 2002). A within-subjects repeated measures design was used for the present study because of the recognized inter-individual differences in both EEG sleep measures (e.g., Hohagen et al., 1993; Irwin et al., 2003; Uthgenannt et al., 1995) and cellular adhesion molecule expression (e.g., Goebel and Mills, 2000; Mills and Dimsdale, 1996). To evaluate the changes in cellular adhesion molecules over time, a two-factor (time and night) repeated measures analysis of variance (ANOVA) was conducted. It is important to note that other statistical techniques can accommodate within-subjects experimental designs involving multiple time points (i.e., mixed model analysis); however, a repeated measures ANOVA was chosen for its robustness to violations of normality and ability to control for subject variability (Keppel, 1991) as well as its simplicity, interpretability, and pervasive use within the literature. Pairwise comparisons based on a priori hypotheses were also performed to evaluate the changes in CAM expression from 03:00 to 06:30 on the baseline and PSD-E nights. Extreme values were identified using boxplots and analyses were run with and without outliers to confirm that the results were consistent.

Descriptive statistics were examined for sleep continuity, sleep architecture, and REM measures for the baseline and PSD-E nights. Paired-samples *t* tests were performed to evaluate the differences between the nights. Correlations between the cellular adhesion molecule measures with delta percent for two halves of the baseline night (11:00 to 3:00 and 03:00 to 06:30) and the later part of the PSD-E night (03:00 to 06:30) were explored.

4. Results

The subjects slept an average of 314.1 ± 60.5 min, which was reduced to 171.7 ± 20.2 min on the PSD-E night. Table 1 shows the means for the various EEG sleep measures obtained during the baseline and PSD-E nights. There were no significant differences in total numbers of lymphocytes and monocytes between the two nights. Portions of these findings have been previously reported in our evaluation of disordered sleep, nocturnal cytokines, and immunity in alcoholics (Redwine et al., 2003a,b).

4.1. Mac-1 positive lymphocytes and monocytes

The percentage of Mac-1 positive lymphocytes differentially changed across the two nights: night × time interaction ($F(1,14) = 13.22$; $p = .01$) with increased expression during the baseline night and no change during the PSD-E night (Fig. 1). In contrast, the percentage of Mac-1 positive monocytes did not change across either the baseline or PSD-E nights.

4.2. L-selectin positive lymphocytes and monocytes

The percentage of L-selectin positive lymphocytes also differentially changed across the two nights: night × time interaction ($F(1,14) = 4.59$, $p < .05$). However, unlike the pattern of change for Mac-1 expression, nocturnal increases in the expression of L-selectin were greater during the PSD-E night as compared to the baseline night (Fig. 2). A similar result was found for the percentage of L-selectin positive monocytes: night × time interaction ($F(1,14) = 9.34$, $p < .01$) with greater increases during the PSD-E night compared to the baseline night (Fig. 3). Furthermore, there was a night × time interaction for density (the intensity of fluorescence per cell, indicating the amount of L-selectin expression on each cell) of L-selectin on monocytes: ($F(1,14) = 5.94$, $p < .05$) in which density increased during the PSD-E night but not during the baseline night (Fig. 4).

4.3. Sleep stages and cellular adhesion molecule expression

Healthy adults show increases in sleep depth as measured by relative amount of delta sleep following

Table 1
EEG sleep measures during baseline and partial sleep deprivation

	Baseline		Partial sleep deprivation			
	Mean	*SD*	Mean	*SD*	*t*	*p*
Sleep continuity						
Total sleep time (min)*	314.06	60.53	171.69	20.18	10.103	.002
Sleep efficiency (%)*	71.86	14.63	83.64	8.10	−3.74	.002
Sleep latency (min)*	25.41	29.33	9.28	4.97	2.32	.035
Sleep architecture						
Stage 1						
(min)*	22.13	9.68	6.75	4.16	5.805	.000
(%)*	7.32	3.21	4.08	2.55	3.125	.007
Stage 2						
(min)*	207.69	48.67	105.69	21.29	9.792	.000
(%)*	66.19	8.63	61.86	11.63	2.235	.041
Stage 3						
(min)	15.94	20.89	12.13	16.26	1.378	.188
(%)	5.20	7.34	6.90	9.48	−1.693	.111
Stage 4						
(min)	4.41	8.50	4.31	8.44	.066	.948
(%)	1.47	2.91	2.56	4.98	−1.305	.212
Delta						
(min)	20.63	27.98	16.44	22.11	1.244	.233
(%)	6.67	9.86	9.46	12.87	−2.016	.062
REM						
(min)*	63.91	29.41	42.81	16.20	3.275	.005
(%)	20.91	7.94	24.60	8.10	−1.947	.070
REM measures						
Latency (corrected)*	66.34	33.96	44.07	25.75	2.59	.020
Density*	1.60	1.15	1.27	0.71	2.15	.049
Duration (first per.)*	15.79	7.86	25.53	11.22	−3.31	.005

Baseline and partial sleep deprivation nights were compared for differences in sleep continuity, architecture, and REM.
* $p < .05$ between baseline and partial sleep deprivation nights.

recovery from sleep deprivation (Irwin et al., 2003). To address whether changes of monocyte and lymphocyte adhesion molecule expression were due to changes of delta sleep during PSD-E baseline nights, the relationships between relative amounts of delta sleep and levels of Mac-1 and L-selectin expression monocyte were tested

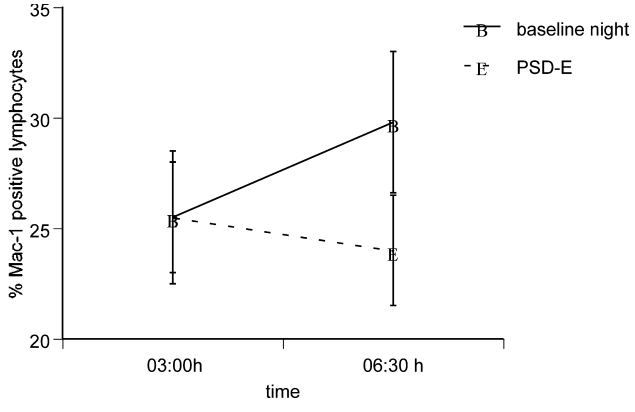

Fig. 1. The percentage of Mac-1 positive lymphocytes, during the nocturnal period of a full nights sleep (baseline) and following a period where subjects were kept awake until 03:00 h (PSD-E). Error bars reflect standard errors of the mean.

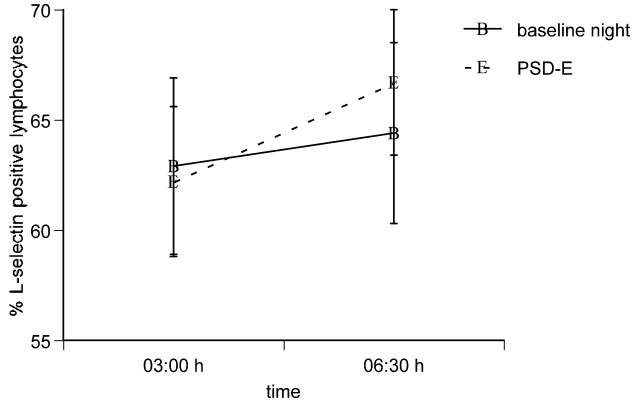

Fig. 2. The percentage of L-selectin positive lymphocytes, during the nocturnal period of a full nights sleep (baseline) and following a period where subjects were kept awake until 03:00 h (PSD-E). Error bars reflect standard errors of the mean.

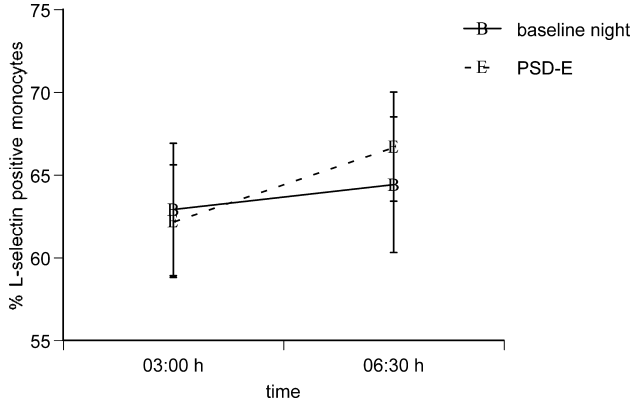

Fig. 3. The percentage of L-selectin positive monocytes, during the nocturnal period of a full nights sleep (baseline) and following a period where subjects were kept awake until 03:00 h (PSD-E). Error bars reflect standard errors of the mean.

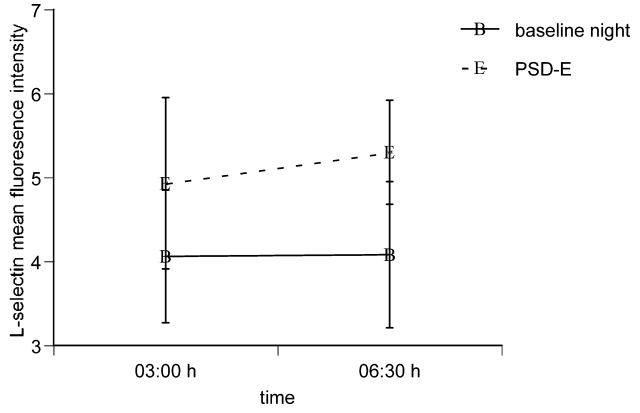

Fig. 4. The density of L-selectin on monocytes (L-selectin mean fluorescence intensity), during the nocturnal period of a full nights sleep (baseline) and following a period where subjects were kept awake until 03:00 h (PSD-E). Error bars reflect standard errors of the mean.

with Spearman correlations. Relative amounts of delta sleep (% delta sleep) during the first or second half of the baseline night, or the second half of the PSD-E night (the first half of the night was defined as 23:00 to 03:00 h and the second half of the night was defined as 03:00 to 06:30 h), were not correlated with monocyte Mac-1 and L-selectin or lymphocyte Mac-1 or L-selectin at either 03:00 or 06:30 h during the baseline or PSD-E nights. However, delta sleep was missing at baseline and PSD for three people, of which one person was missing delta sleep on both nights. In order to determine if age was a factor in the differences in immune response to sleep deprivation we examined the relationships between age and both EEG and cellular adhesion molecule expression. Within this sample, age was not correlated with either sleep EEG or cellular adhesion molecule variables.

5. Discussion

The present study examined the expression of cellular adhesion molecules, Mac-1 and L-selectin, during a night of normal sleep and during a night of partial sleep deprivation (PSD). Cellular adhesion molecules are important in the migration of immune cells to sites of inflammation and infectious challenge. During a night of normal sleep, percentage of lymphocytes expressing macrophage associated antigen (Mac-1) increased from 03:00 to 06:30 h, whereas staying awake until 03:00 h interfered with nocturnal increases of Mac-1. For L-selectin, a differential pattern of change was also found between the two nights; increases of L-selectin occurred during partial night sleep deprivation, but not during a night of normal sleep. Levels of Mac-1 or L-selectin were not correlated with relative amounts of delta sleep during the night of sleep deprivation, indicating that the differential responses of Mac-1 and L-selectin were likely due to the effects of sleep restriction rather than the effects of a more intense sleep during the second half of the partial sleep deprivation night.

The data from this research are in agreement with previous studies that have shown that nocturnal sleep is associated with changes of immune cell and cytokine activity. The increase in the percentages of Mac-1 positive lymphocyte and monocyte expression found during the latter half of the nocturnal period corresponds to the increase observed in some aspects of cellular immunity, such as NK activity (Irwin et al., 1996) and cytokine secretion patterns across the night (Bauer et al., 1994; Irwin et al., 1999; Vgontzas et al., 1999; Redwine et al., 2003a). Furthermore, consistent with the hypothesis that these nocturnal changes are dependent on sleep, sleep deprivation in a laboratory setting is associated with decreases in nocturnal and daytime measures of NK activity (Irwin et al., 1996; Redwine et al., 2003a), as well as, nocturnal decreases of IL-2 and IL-6 secretion (Born et al., 1997; Redwine et al., 2000; Vgontzas et al., 1999). Similarly, in the present study Mac-1 positive lymphocyte expression was blunted the morning after nighttime PSD, suggesting a decrease in the cell's abilities to adhere and migrate to sites of infection.

Lower levels of L-selectin expression during a night of normal sleep, as compared to nighttime PSD, are also consistent with the notion that sleep is associated with measures of immune activation. In the transition of immune cells into the active state (Tohya and Kimura, 1998), L-selectin is cleaved (Marschner et al., 1999) and released into the circulation (Redwine et al., 2003b), which may be reflected by a reduction of L-selectin expression on immune cells in circulation. Subsequently, there is an upregulation of integrins such as Mac-1 and LFA-1, which allows for cellular adhesion and migration to sites of inflammation. Therefore, lower levels of L-selectin expression during normal sleep may reflect

increased cleavage of the molecule and consequently the shifting of immune cells into an active state.

Importantly, the changes of cellular adhesion molecule expression in relation to sleep and sleep loss are not likely due to the effects of psychological stress. Redwine et al. (2000) have previously shown that the profile of cortisol secretion is not altered following nighttime PSD. Moreover, the effects of stress on adhesion molecule expression contrast with the findings related to sleep loss. Nighttime PSD led to an increase of L-selectin, whereas the administration of a psychological stressor induced decreases of L-selectin (Redwine et al., 2003b).

In contrast to a previous study where there were negative correlations between the two adhesion molecules following a stress task (Redwine et al., 2003b), the present study did not find significant correlations between Mac-1 and L-selectin expression at either time point or on either night. This further demonstrates that stress responses and sleep loss responses are mediated via different mechanisms and initiate different pathways and responses. One explanation is that cell activation occurs in response to stress and L-selectin cleavage directly results in the expression of Mac-1. The time course of the stressor is limited to 15 min and there is a negative relationship between the expression of L-selectin and Mac-1. In contrast, during sleep deprivation that lasts several hours, cells may have a reduction in cell activation reflected by reduced Mac-1 expression and increased L-selectin expression, but the temporal duration of these changes obscures detection of a direct relationship. To accurately determine whether changes in cellular adhesion molecule expression during sleep translate into altered functional ability, future studies are needed with more frequent sampling to test whether alterations in cellular adhesion molecule expression in relation to sleep deprivation are associated with changes in cellular adhesion and migration.

The study results are limited in that only males were included in the sample. However, previous research has suggested that there were not differences between males and females in basal L-selectin or integrin, LFA-1 expression (Mills and Dimsdale, 1996).

Since differences between the two nights were found only at 06:30 h and not at 03:00 h, it was possible that changes did not reflect acute sleep restriction, but that alterations at 06:30 h were a sign of responses to a period of more intense sleep and rebound on the sleep deprivation night. Given that sleep loss is known to induce a rebound increase of delta sleep (Irwin et al., 2003), correlations between delta sleep and CAMs were measured. Analyses revealed no associations between delta sleep and Mac-1 or L-selectin expression at 06:30 h. Thus, alterations of Mac-1 and L-selectin more likely reflect delayed affects of partial night sleep deprivation. However, they may also represent shifts in circadian expression of these molecules, which was not evaluated in this study since subjects were awakened after the 06:30 blood draw and no additional blood samples were obtained. Although we have previously found that PSD does not alter the profile of nocturnal melatonin (Redwine et al., 2000), this issue should be evaluated in future studies since it has been reported that all leukocyte classes exhibit significant circadian-like variation in L-selectin expression (Niehaus et al., 2002). Nevertheless, in our previous studies, PSD showed no effect on the profile of secretion of the circadian hormone, melatonin (Redwine et al., 2000).

In conclusion, increasing evidence suggests that acute sleep deprivation is associated with alterations in immune activity, which supports the role of sleep in regulating immune system function. These data show that nocturnal sleep is also associated with alterations in the expression of cellular adhesion molecules with implication for adherence and migration of immune cells to sites of inflammation, although, the clinical significance of these findings in not yet known. Whether chronic sleep disturbances result in alteration of cellular adhesion molecule expression has not been examined, although chronic insomnia has been found to increase nocturnal levels of circulating norepineprine (Irwin et al., 2003) which have been found to regulate immune cell traffic by inducing changes in L-selectin and Mac-1 (Goebel and Mills, 2000; Redwine et al., 2003b). Given epidemiologic data linking insomnia and the risk of cardiovascular disease and mortality (Foley et al., 1995; Kripke et al., 2002; Mallon et al., 2002), we speculate that sleep disturbance may contribute to changes in adhesion molecule expression, which may be involved in atherogenesis and increased disease risk.

Acknowledgments

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