Sleep Deprivation and Activation of Morning Levels of Cellular and Genomic Markers of Inflammation

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Background: Inflammation is associated with increased risk of cardiovascular disorders, arthritis, diabetes mellitus, and mortality. The effects of sleep loss on the cellular and genomic mechanisms that contribute to inflammatory cytokine activity are not known.

Methods: In 30 healthy adults, monocyte intracellular proinflammatory cytokine production was repeatedly assessed during the day across 3 baseline periods and after partial sleep deprivation (awake from 11 PM to 3 AM). We analyzed the impact of sleep loss on transcription of proinflammatory cytokine genes and used DNA microarray analyses to characterize candidate transcription-control pathways that might mediate the effects of sleep loss on leukocyte gene expression.

Results: In the morning after a night of sleep loss, monocyte production of interleukin 6 and tumor necrosis factor α was significantly greater compared with morning levels following uninterrupted sleep. In addition, sleep loss induced a more than 3-fold increase in transcription of interleukin 6 messenger RNA and a 2-fold increase in tumor necrosis factor α messenger RNA. Bioinformatics analyses suggested that the inflammatory response was mediated by the nuclear factor κB inflammatory signaling system as well as through classic hormone and growth factor response pathways.

Conclusions: Sleep loss induces a functional alteration of the monocyte proinflammatory cytokine response. A modest amount of sleep loss also alters molecular processes that drive cellular immune activation and induce inflammatory cytokines; mapping the dynamics of sleep loss on molecular signaling pathways has implications for understanding the role of sleep in altering immune cell physiologic characteristics. Interventions that target sleep might constitute new strategies to constrain inflammation with effects on inflammatory disease risk.

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ABOUT ONE THIRD OF THE population of the United States experiences frequent difficulties with sleep initiation,[1] with an even higher prevalence of such sleep complaints among patients with chronic inflammatory disorders such as cardiovascular disease.[2,3] Given that epidemiological data implicate poor sleep as a predictor of chronic disease risk and mortality in some[3-7] but not all studies,[8] it is increasingly important to consider the consequences of sleep loss on inflammatory mechanisms. The risk of a wide spectrum of medical conditions, including cardiovascular disease, arthritis, diabetes mellitus, certain cancers, and functional decline, is associated with activation of cellular signals that initiate expression of inflammatory cytokines such as interleukin (IL) 6.[9,10]

Experimental sleep deprivation has been found to alter immune responses[11,12] and is reported to increase circulating levels of inflammatory markers such as IL-6, tumor necrosis factor (TNF) α, and C-reactive protein,[13-15] with significant elevations after only 1 night of sleep loss.[15] The cellular sources of proinflammatory cytokine activity are not known, although monocytes, which make up about 5% of circulating leukocytes, are a major contributor to proinflammatory cytokine production in peripheral blood.

In this study, we sought to clarify the functional basis for altered inflammatory response after sleep loss by measuring the production of proinflammatory cytokines by monocytes following ligation of the Toll-like receptor 4 with lipopolysaccharide. Toll-like receptors mediate innate immune responses to common pathogens,[16] and aberrant increases of Toll-like receptor activity have been linked to inflammatory diseases such as rheumatoid arthritis,[17] Crohn disease,[17] and heart failure.[18] We also examined the upstream sources of cellular inflammatory cytokine expression by testing the impact of sleep loss on gene expression; transcription of IL-6 messenger RNA (mRNA) and TNF-α mRNA was quantified before (ie,

at baseline) and after sleep deprivation in peripheral blood mononuclear cell populations. Finally, we sought to determine whether increased inflammatory gene expression might constitute 1 element of a more general genomic response to sleep deprivation. We used DNA microarray analyses to identify broad categories of genes that showed altered expression following sleep loss, and bioinformatics analyses characterized specific transcription-control pathways that might mediate those effects (eg, activity of the hormone-responsive transcription factor CREB [cyclic adenosine monophosphate response element binding protein] or the proinflammatory mediator, nuclear factor κB). An experimental model of partial sleep deprivation (PSD) was used because it is hypothesized that loss of sleep during part of the night, as opposed to total sleep deprivation, replicates the kind of sleep loss that is ubiquitous in the general population and also resembles the reduction of sleep duration that is often found in persons with chronic medical disorders.

METHODS

SUBJECTS

The subjects included 30 healthy volunteers (17 men and 13 women) (mean [SD] age, 37.6 [9.8] years; range, 25-59 years), who gave informed consent; the institutional review board of the University of California, Los Angeles (UCLA), approved the protocol. Inclusion criteria required that subjects be healthy as assessed by medical interview and physical examination, with reference range results from screening laboratory tests; none had a history of an inflammatory disorder, cancer, or chronic or active infections. Subjects had body mass indices (calculated as weight in kilograms divided by the square of height in meters) less than 30, were nonsmokers, fulfilled the criteria from *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Revised*, for never mentally ill,[19] and regularly slept between 10:30 PM and 7:30 AM as confirmed by 2-week sleep diaries.

PROTOCOL

Subjects spent 4 days (24-hour periods) in the National Institutes of Health General Clinical Research Center. After adaptation to the sleep laboratory with screening for sleep apnea and nocturnal myoclonus, subjects underwent 3 days of baseline testing (ie, prior to PSD) and 1 day of testing after PSD. During each night, subjects underwent testing with polysomnography, with ambient light dimmer than 50 lux. During baseline testing, uninterrupted sleep occurred between 11 PM and 7 AM, whereas during PSD, sleep was restricted between 3 AM and 7 AM. Subjects were prohibited from exercise and were behaviorally monitored to ensure that they were awake during the day and during the PSD period.

Blood samples were obtained via an indwelling venous forearm catheter at 8 AM, 12 PM, 4 PM, 8 PM, and 11 PM during baseline 1, baseline 2, baseline 3, and after PSD, with samples analyzed for expression of intracellular proinflammatory cytokines in monocyte populations. Hence, a total of 15 measures was obtained prior to sleep deprivation (ie, baseline) with 5 measures obtained after PSD.

ASSAYS

Monocyte intracellular production of IL-6 and TNF-α was assessed by flow cytometry.[20] Briefly, heparin-treated blood was mixed with 100 pg/mL lipopolysaccharide (Sigma, St Louis, Mo) and 10 µg/mL brefeldin A (Sigma) and incubated for 4 hours at 37°C. After cells were permeabilized in fluorescence-activated cell sorting permeabilizing buffer (BD Biosciences, San Jose, Calif) and fluorescence-conjugated antibodies were added, about 12 000 $CD14^+$ events were counted to determine the net stimulated percentage of cytokine-secreting monocytes, with quadrant coordinates set based on unstimulated cells.

In 10 subjects (5 men and 5 women), real-time quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) was used to quantify gene expression for IL-6 and TNF-α relative to cellular housekeeping genes at baseline and after PSD. After isolation of 10^6 peripheral blood mononuclear cells by Ficoll density gradient centrifugation, total RNA was extracted (Rneasy; Qiagen, Valencia, Calif) with mRNA concentrations for IL-6 and TNF-α quantified by real-time RT-PCR using a 1-step thermal cycling protocol (iScript One-Step RT-PCR kit with SYBR Green, a fluorescent, double-stranded, DNA-binding reporter molecule; Bio-Rad, Hercules, Calif). About 100 ng of the total RNA sample was assayed with the following primers: IL-6 sense 5′-CAGCTATGAACTCCTTCTCCACAAGC-3′ and antisense 5′-CTGAGATGCCGTCGAGGATGTACCG-3′; TNF-α (Biosource, Camarillo, Calif); glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sense 5′-GAAGGTGAAGGTCGGAGTC-3′ and antisense 5′-GAAGATGGTGATGGGATTTC-3′. Reverse transcription was performed for 30 minutes at 50°C, followed by 15 minutes of reverse transcriptase denaturation at 95°C and 40 cycles of DNA amplification (95°C for 15 seconds, 60°C for 1 minute). Expression of mRNA was normalized to human GAPDH by subtraction of threshold cycles (Ct) (normalized target Ct = target Ct – GAPDH Ct) and quantified as a fold change after PSD relative to baseline (fold change = 2 [stimulated normalized target Ct – baseline normalized target Ct]).

In 5 subjects (3 women and 2 men), genome-wide mRNA expression profiles were assayed in 10 µg of leukocyte total RNA using a high-density oligonucleotide array (U133a; Affymetrix, Santa Clara, Calif) at baseline and after PSD. All assays were performed by the UCLA DNA Microarray Core (Affymetrix) following the manufacturer's standard protocol, with sample quality assured for RNA concentration and purity using a bioanalyzer (Agilent, Palo Alto, Calif) prior to probe synthesis. Following hybridization of fluorescent complementary RNA probes, microarrays were imaged using a scanner (Affymetrix), and low-level gene expression values were derived using GeneChip Operating Software (GCOS) (Affymetrix) with default parameter settings (as described in the next subsection).

STATISTICAL ANALYSES

Data were analyzed using SAS statistical software (version 9.13; SAS Inc, Cary, NC). Missing values were handled using cell-wise deletion. To determine the effects of PSD on monocyte intracellular proinflammatory cytokine expression, repeated measures of mixed-model analysis of variance were performed using a 2 (condition: baseline, PSD) × 5 (times: 8 AM, 12 PM, 4 PM, 8 PM, and 11 PM) design. The baseline was determined by obtaining an average value for each time point across baseline 1, baseline 2, and baseline 3 after confirming that there was no overall baseline day effect. The statistical significance of the difference between baseline and PSD mRNA concentrations was assessed using a paired *t* test conducted on log-transformed values of GAPDH-normalized mRNA expression values from RT-PCR analyses.

To identify specific genes showing differential expression in microarray assays of leukocyte samples between baseline and

PSD, we performed standard paired comparison analyses using GCOS, with default analysis parameters, which is known to be conservative in declaring differential gene expression.[21] Differences between an individual baseline sample and a PSD sample were declared significant at a P value of less than .01; a set of significantly increased genes was defined as transcripts showing statistically significant increases for 3 or more of the 5 paired samples analyzed (which would be extremely rare by chance alone; binomial $P<.001$). Functional commonalities among those differentially expressed genes were identified using GOstat (Gene Oncology Statistics) bioinformatics software[22] (http://gostat.wehi.edu.au/) to define specific functional Gene Ontology database categories overrepresented among induced genes. To identify upstream signal transduction pathways that might mediate effects of PSD on gene expression dynamics, we used the Transcription Element Listening System[23] (TELiS) (http://www.telis.ucla.edu), searchable database software to determine transcription factor binding motifs that were overrepresented in promoters of genes that are significantly up-regulated following PSD vs baseline sleep. The GOstat and TELiS analyses used default settings (GOstat: false discovery rate, $P<.01$; TELiS: significance threshold, $P<.05$ in an analysis of 600 base pairs upstream of transcription start sites with 0.90 stringency match to vertebrate motifs in the TRANSFAC database [http://www.gene-regulation.com/pub/databases.html#transfac]).

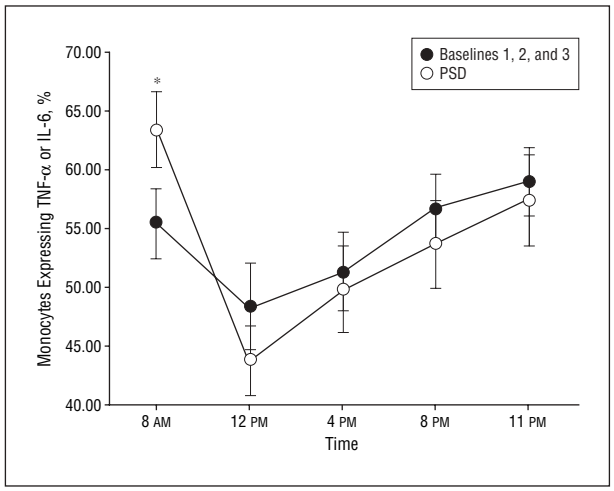

Figure 1. Differences in expression of interleukin (IL) 6 and tumor necrosis factor (TNF) α in lipopolysaccharide-stimulated CD14+ cells between baseline and partial sleep deprivation (PSD) conditions. Data are represented as mean±SEM. *$P<.05$.

RESULTS

Partial sleep deprivation induced an increase in the capacity of monocytes to express IL-6 and TNF-α immediately following a night of PSD with a condition × time interaction ($F_{4,82}=2.3$; $P=.07$ [**Figure 1**]). Compared with baseline levels, the percentage of monocytes expressing IL-6 and TNF-α was significantly increased at 8 AM after PSD ($t_{107}=-2.3$; $P<.05$); this increase normalized at subsequent time points. The fixed effect for time was significant ($F_{4,95}=14.5$; $P<.001$), but there was no condition effect ($F_{1,25}=0.4$; $P=.52$). Similar results were found for the percentage of monocytes expressing IL-6 alone or of TNF-α alone (condition × time interaction) ($F_{4,82}=10.7$, $P<.001$; $F_{4,82}=2.2$, $P=.07$). **Figure 2** displays representative flow cytometric results at 8 AM for 1 person at baseline and after PSD.

Exploratory analyses examined whether there were sex differences in the capacity of monocytes to produce proinflammatory cytokines following PSD; no main effect for sex was found ($F_{1,217}=0.4$; $P>.40$), and there was no condition × time × sex interaction ($F_{8,217}=0.3$; $P>.92$), which indicates similar responses in men and women. In addition, we examined whether PSD-induced increases in delta sleep[24-26] were associated with changes

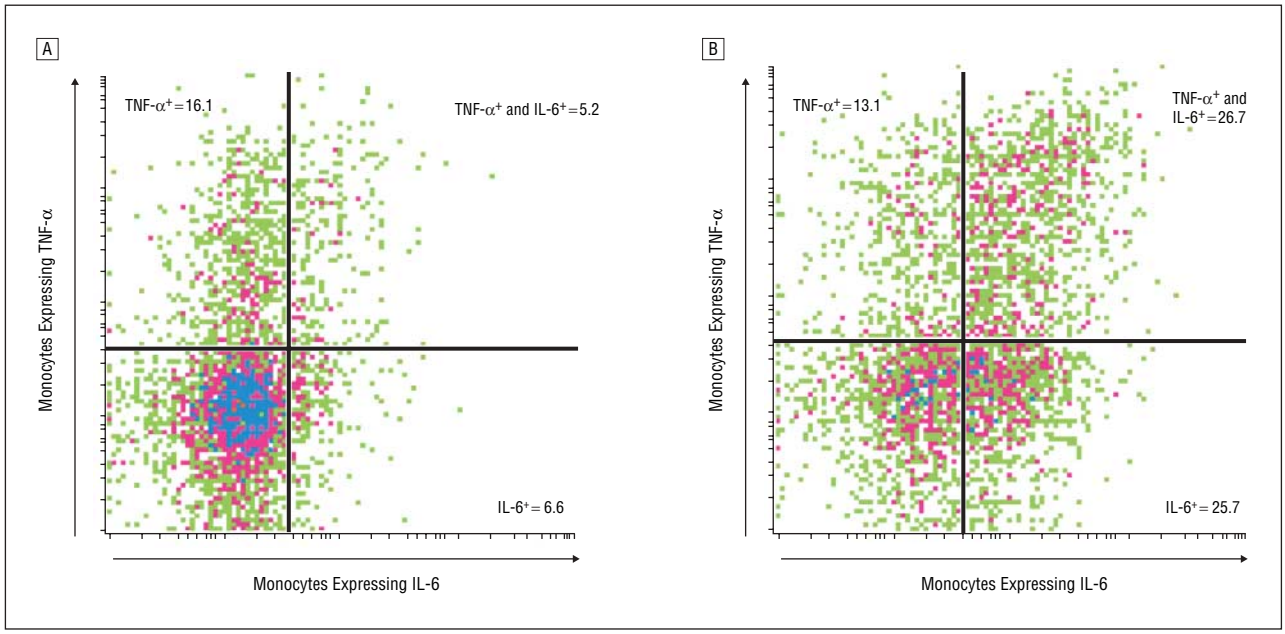

Figure 2. Representative expression of interleukin (IL) 6 and tumor necrosis factor (TNF) α in lipopolysaccharide-stimulated CD14+ cells from a participant (A) at baseline and (B) at partial sleep deprivation (PSD). Numbers indicate the percentages of the fraction of CD14+ cells that are positive for TNF-α alone (upper left), TNF-α and IL-6 (upper right), and IL-6 alone (lower right). In the baseline condition, 72.1% of the CD14+ cells are negative for both IL-6 and TNF-α whereas only 34.5% of the CD14+ cells are negative for both IL-6 and TNF-α in the PSD condition.

in monocyte expression of proinflammatory cytokines. Partial sleep deprivation induced increases in the percentage of delta sleep compared with normal sleep ($t_{1,29}$=−2.1; P=.05) (data not shown), but neither percentage of delta sleep during PSD nor increases of delta sleep from baseline to PSD correlated with monocyte proinflammatory cytokine expression (all P values >.30). Finally, additional analyses examined the effect of PSD on the circulating number of monocytes; compared with baseline, the number of monocytes was not different at the 8 AM time point after PSD ($t_{1,29}$=−0.3; P>.85). Differences in monocyte numbers do not account for the varying levels of intracellular proinflammatory cytokine expression.

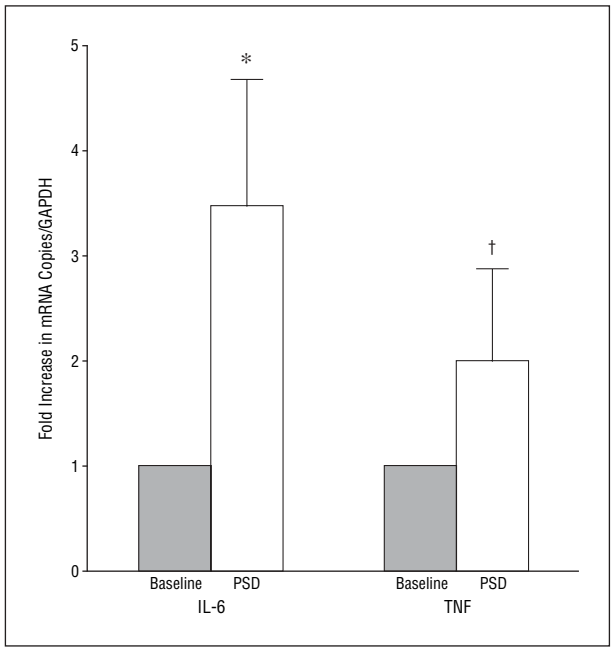

Figure 3. Differences in reverse transcriptase–polymerase chain reaction gene expression of interleukin (IL) 6 and tumor necrosis factor (TNF) α between baseline and partial sleep deprivation (PSD). Data are represented as mean±SEM fold change in target messenger RNA (mRNA) concentration in 3 replicates after normalization to cellular glyceraldehyde-3-phosphate dehydrogenase (GAPDH). *P<.05. †P=.05.

To determine whether PSD altered proinflammatory cytokines at the level of gene expression, mRNAs for IL-6 and TNF-α were quantified at the 8 AM time point at baseline and after PSD. Both proinflammatory cytokine genes were activated in the morning following PSD with an average 3.5-fold increase over baseline levels in concentrations of IL-6 mRNA (t=2.9; P<.05) and an average 2-fold increase over baseline levels in concentrations of TNF-α mRNA (t=2.3; P=.05 [**Figure 3**]).

To determine whether observed changes in proinflammatory cytokine gene expression might be part of a more general genomic response to sleep deprivation, we performed global gene expression profiling using microarray assays. Among the 22 283 transcripts surveyed, high-stringency data analysis identified a set of 22 genes that was significantly up-regulated (P<.01) after PSD relative to baseline (listed in the **Table**). One transcript was significantly down-regulated by PSD using the same highly stringent criteria (*GOLGIN-67*). **Figure 4** graphically portrays that genomic fingerprint of PSD, with red intensity reflecting the degree of up-regulation and green intensity denoting down-regulation in these comparisons. Elements of the up-regulated gene ensemble included the master circadian regulator *PER1*, several immediate early

Table. Gene Transcripts Induced by Sleep Deprivation

Probe Name	Gene Symbol*	Common Gene Name
202644_s_at	*TNFAIP3*	TNF-α–induced protein 3†
205067_at	*IL1B*	IL-1β†
202014_at	*PPP1R15A*	Protein phosphatase 1, regulatory (inhibitor) subunit 15A‡
210042_s_at	*CTSZ*	Cathepsin Z‡
204373_s_at	*CAP350*	Centrosome-associated protein 350‡
217022_s_at	*IGHA2 /// MGC27165*	Immunoglobulin heavy constant α 2 (A2m marker) /// hypothetical protein MGC27165‡
37028_at	*PPP1R15A*	Protein phosphatase 1, regulatory (inhibitor) subunit 15A‡
204440_at	*CD83*	CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)‡
204285_s_at	*PMAIP1*	Phorbol-12-myristate-13-acetate-induced protein 1‡
201110_s_at	*THBS1*	Thrombospondin 1‡
205114_s_at	*CCL3 /// CCL3L1 /// MGC12815*	Chemokine (C-C motif) ligand 3 /// chemokine (C-C motif) ligand 3-like 1 /// chemokine (C-C motif) ligand 3-like, centromeric‡
202393_s_at	*KLF10*	Kruppel-like factor 10‡
201631_s_at	*IER3*	Immediate early response 3‡
202859_x_at	*IL8*	IL-8‡
202887_s_at	*DDIT4*	DNA damage–inducible transcript 4‡
212099_at	*RHOB*	*ras* Homologue gene family, member B‡
202768_at	*FOSB*	FBJ murine osteosarcoma viral oncogene homologue B‡
212830_at	*EGFL5*	EGF-like domain, multiple 5‡
38037_at	*HBEGF*	Heparin-binding EGF-like growth factor‡
211506_s_at	*IL8*	IL-8‡
201044_x_at	*DUSP1*	Dual-specificity phosphatase 1‡
202861_at	*PER1*	Period homologue 1 (*Drosophila*)‡

Abbreviations: EGF, epidermal growth factor; FBJ, Finkel-Biskis-Jinkins; IL, interleukin; TNF-α, tumor necrosis factor α.
*Backslashes indicate that the same gene has 2 gene symbol names as well as 2 common names.
†Significant increase for 4 participants (80%).
‡Significant increase for 3 participants (60%).

genes that mark cellular signal transduction (*FOSB, IER3,* and *PMAIP1*), the *ras* family guanine nucleotide exchange factor *RHOB*, the cell adhesion factor thrombospondin 1 (*THBS1*), the lysosomal cysteine proteinase cathepsin Z (*CTSZ*), several epidermal growth factor–related genes (*EGFL5* and *HBEGF*), multiple protein phosphatases (*DUSP1* and *PPP1R15A*), and multiple inflammatory response genes including IL-1β (*IL1B, IL8, CD83, CCL3,* and *TNFAIP3*).

The GOstat bioinformatic analysis identified several functional commonalities among PSD-induced genes, including predominant roles in immune response and inflammation (Gene Ontology terms 0006952, 0006452, 0008009, 0006954, and 0050900), wound healing (0009613 and 0009611), cell cycle regulation (0000074, 0007049, 0051726, and 0042127), angiogenesis (0001525, 0001568, 0045765, and 0048514), apoptosis (0008219, 0012501, 0043066, 0043069, and 0006916), growth factor activity (0005154 and 0008083), negative regulation of cellular physiologic characteristics (0051243), and stress response and behavior (0006950 and 0007610) (all Gene Ontology terms overrepresented at a false discovery rate of $P<.05$).

To identify signal transduction pathways that might potentially mediate leukocyte transcriptional responses to PSD, we used TELiS bioinformatics software to define transcription factor–binding motifs that were overrepresented in the promoters of genes that were specifically up-regulated by PSD compared with baseline. Results indicated regulation by cyclic adenosine monophosphate/protein kinase A–induced transcription factors of the CREB/activating transcription factor family ($P<.001$), the protein kinase C–induced AP-1 family ($P<.001$), the proinflammatory nuclear factor κB/Rel family ($P=.02$), and the mitogen-activated protein kinase–inducible E-26 transformation–specific transcription factor family typified by E-26–like protein 1 ($P=.001$). Thus, results for signal transduction analyses are consistent with Gene Ontology bioinformatics and suggest that PSD modulates cellular function through multiple physiologic signaling pathways that include hormones, proinflammatory cytokines, and cellular growth factors.

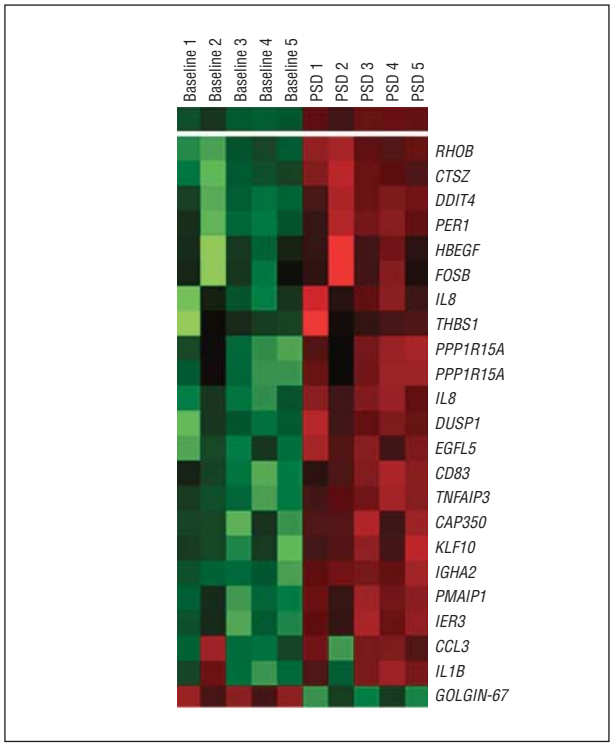

Figure 4. Gene expression fingerprint of partial sleep deprivation (PSD) compared with baseline in 5 subjects. Genome-wide transcription profiling was performed using high-density oligonucleotide microarray assays with differential expression of 23 genes ($P<.01$) as shown in a standardized heat plot (red indicates increased expression; green, decreased expression); 22 genes were up-regulated ($P<.01$); 1 transcript was down-regulated after PSD compared with baseline or normal sleep.

COMMENT

This study provides the first evidence of an alteration in a functional cellular innate immune response following sleep loss. In the morning after a night of sleep loss, lipopolysaccharide ligation of TLR4 triggered significantly greater production of IL-6 and TNF-α in peripheral blood monocyte populations relative to morning levels following uninterrupted sleep. In addition, sleep loss has an impact on the expression of proinflammatory cytokine genes, inducing a more than 3-fold increase in transcription of IL-6 mRNA and a 2-fold increase in TNF-α mRNA.

Altered expression of proinflammatory cytokine genes emerged as 1 element of a more complex ensemble of functional genomic alterations induced by sleep loss, which also included enhanced expression of the circadian clock gene *PER1* and multiple immediate early response genes, signal transduction mediators, and growth factor–related genes. Structure-function bioinformatics analyses further suggested that leukocyte transcriptional response to sleep deprivation involves multiple signal transduction pathways that include the nuclear factor κB inflammatory signaling system and classic hormone and growth factor response pathways such as the CREB/activating transcription factor, activator protein 1, and E-26 transformation specific families of transcription factors. The present genomic analyses should be considered preliminary owing to the small number of cases analyzed. However, the statistically significant effects that do emerge, even with these limited data, provide new targets for future analyses that seek to map the molecular processes mediating the effects of sleep loss on cellular physiologic characteristics in the immune system and on physiologic and metabolic functions more generally. In addition, remodeling of the leukocyte gene expression profile by sleep physiologic characteristics opens up new vistas regarding the possible dynamic role of sleep in general homeostasis.

It is known that aberrant increases of TLR activity are found in association with rheumatoid arthritis,[17] Crohn disease,[17] and heart failure,[18] whereas transient changes in monocyte production of proinflammatory cytokines might not translate into an increased risk for disease. Furthermore, increases in monocyte responses to lipopolysaccharide stimulation correlate with increases in circulating inflammatory markers,[20] which in turn induce

systemic inflammation and metabolic changes. Small elevations in circulating inflammatory mediators, for example, have been associated with the syndrome of insulin resistance and type 2 diabetes mellitus, independent of adiposity.[27] Moreover, a shorter sleep duration,[28] as well as sleep deprivation[29] and cumulative partial sleep loss,[30] are reported to correlate with impaired glucose tolerance, insulin resistance, and diabetes mellitus.

Inflammation also plays a fundamental role in the development and progression of atherosclerosis.[31] Difficulty falling asleep or maintaining sleep is significantly associated with risk for nonfatal myocardial infarction or cardiovascular death, even after adjustment for multiple risk factors of coronary heart disease.[32,33] Furthermore, rates of sudden cardiac death, myocardial infarction, and ischemic stroke are lowest during nocturnal sleep, then increase after awakening.[34-36] We have previously found that PSD induces marked increases in cardiovascular responses[24] as well as in sympathoadrenal activity on awakening.[24,37] In turn, adrenergic output is known to facilitate in vivo release of inflammatory mediators into circulating blood,[38,39] although catecholamines in vitro are reported to suppress proinflammatory cytokine production.[40,41] Consistent with in vivo observations, the bioinformatic analyses described herein suggest that CREB mediates genomic effects of sleep loss; the cyclic adenosine monophosphate/protein kinase A/CREB signaling pathway constitutes a primary mediator of catecholamine signaling through leukocyte β-adrenoreceptors. If studies using adrenergic antagonists identify sympathoadrenal activity as a mediator of genomic responses to sleep deprivation, such data would suggest new pharmacologic approaches for protecting patients against the detrimental biological effects of sleep loss.

Herein, we used a functional assay of immune response to stimulation to identify the specific cellular and molecular mechanisms that might contribute to previously reported variations in static cytokine levels; increases in monocyte production of proinflammatory cytokine were found at the beginning of the wake phase following sleep loss. Similarly, modest sleep restriction induced increases of circulating levels of IL-6 immediately following awakening, with increases recurring during the early evening; in men, sleep restriction induced increases of TNF-α levels only immediately following awakening.[13] In contrast, total sleep deprivation is associated with increases in circulating IL-6 during the late afternoon and early evening.[42] Given that monocyte production of proinflammatory cytokine contributes to increases in circulating levels,[20] we speculate that morning elevations in cellular proinflammatory cytokine expression might have consequences for subsequent daytime elevations.[13]

Loss of sleep during only part of the night is one of the most common complaints of persons who experience environmental or psychological stress,[43,44] travel across time meridians, engage in shift work,[43] or have a psychiatric disorder.[45] Our results show that a modest amount of sleep loss activates cellular and genomic markers of inflammation, and these responses are associated with up-regulation of molecular signaling pathways that mediate increases in the transcription of the *IL-6* and *TNF* genes. Confirmatory studies that use signal antagonists will be required to fully validate these potential mechanisms, but the results presented herein support the general principle that sleep loss alters molecular processes that drive cellular immune activation and induce inflammatory cytokines. These data should also motivate further investigations to define the effects of sleep loss on inflammatory mechanisms with implications for cardiovascular and chronic inflammatory disorders in humans. Testing of interventions that target sleep and/or its biological consequences might identify new strategies to constrain inflammation and to promote health as people age.

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Author Contributions: The principal investigator, Dr Irwin, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors have reviewed and approved the final version of this manuscript.
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