Chronic Insomnia and Immune Functioning

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Objective: The goal of this study was to investigate whether clinical insomnia is associated with immune alterations by comparing immune functioning between patients with chronic insomnia and good sleepers. **Methods:** The good sleepers group was composed of 19 adults with a regular sleep schedule and no complaint of sleep disturbances. The insomnia group was composed of 17 adults meeting criteria for a chronic primary insomnia disorder. Peripheral blood samples were taken at the interview (time 1) and before the second night of polysomnographic assessment (time 2) for immune measures, including enumeration of blood cell counts (ie, white blood cells, monocytes, lymphocytes, and CD3+, CD4+, CD8+, and CD16+/CD56+ cells), natural killer cell activity, and cytokine production (ie, interleukin-1β, interleukin-2, and interferon gamma). **Results:** Significant between-group differences were observed for CD3+, CD4+, and CD8+ cells, with higher levels found in good sleepers. In addition, a significant group-by-time interaction was found on monocyte counts. Although this was the only significant interaction effect observed, between-group differences were greater at time 2. **Conclusions:** This study suggests that chronic insomnia is associated with some immune alterations. More research is needed to determine the clinical significance of these findings. **Key words:** sleep, insomnia, immunity, lymphocytes, cytokines, psychoneuroimmunology.

ANCOVA = analysis of covariance; ANOVA = analysis of variance; EEG = electroencephalography; EMG = electromyography; EOG = electro-oculography; GS = good sleepers; HDRS = Hamilton Depression Rating Scale; IL = interleukin; IFN-γ = interferon gamma; INS = insomnia patients; ISI = Insomnia Severity Index; MANOVA = multivariate analysis of variance; NK = natural killer; PSG = polysomnography; REM = rapid eye movement; SCID = Structured Clinical Interview for DSM-IV; SE = sleep efficiency; TNF-α = tumor necrosis factor alpha; TWT = total wake time; WBC = white blood cells.

INTRODUCTION

Insomnia is a prevalent condition affecting between 9% and 12% of the adult population on a chronic basis (1–4). Insomnia may involve trouble falling asleep, frequent or prolonged nocturnal awakenings, early morning awakenings with an inability to return to sleep, or a combination of these complaints. It is commonly believed that sleep loss increases an individual's vulnerability to diseases and, conversely, that sleep has a determinant role in illness recovery. Although direct empirical evidence of the preventive and recuperative value of sleep on health is still needed, this hypothesis is partly supported by studies indicating that insomnia is associated with more frequent health problems, medical consultations, and hospitalizations relative to good sleep (3, 5–8). Furthermore, an association between mortality and sleep duration has been observed in epidemiological studies (9, 10).

The psychoneuroimmunological model proposes that psychological factors can have an impact on health through immune downregulation (11–14). Accordingly, it has been suggested that immune functioning acts as a mediator in the relationship between sleep and health. Specifically sleep would be essential for optimal host defense functioning, whereas sleep loss would be associated with immune changes increasing the vulnerability to pathogens (15–18). Research in animals provides some support for the hypothesis that sleep loss increases the risk of infections through alterations in immune functioning. Prolonged total sleep deprivation in rats produces septicemia and death, an effect that has been commonly attributed to a failure of host defense mechanisms against pathogens (19). Also, mice immunized to influenza virus and then inoculated with the virus fail to clear the virus from their lungs when they are sleep deprived at the time of inoculation (20).

The impact of sleep disturbance on immune functioning in humans has been mostly investigated by examining the effect of total sleep deprivation (ie, 48–72 hours without sleep). Despite some inconsistencies across studies, the findings generally suggest that total sleep deprivation is associated with immune activation, namely increased white blood cell (WBC), granulocyte, monocyte, lymphocyte, and NK cell counts, as well as increased NK cell activity (16, 17, 21). It has been suggested that monocytes, which can activate proinflammatory cytokines and consequently

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NK cell activity, play a central role in the immune response to sleep deprivation (21). A recent study showed increased plasma levels of TNF-α receptors and IL-6 after total sleep deprivation, which is consistent with this hypothesis (22). The pattern of results has been different in the few partial sleep deprivation experiments conducted. In one of these studies, early-night partial sleep deprivation was associated with a significant reduction in NK cell activity, number of NK cells, IL-2, and induced lymphokine-activated killer cell activity (23). Late-night partial sleep deprivation was also found to reduce NK cell activity (24), but it had no effect on serum concentrations of IL-2 (25).

Although these data are fairly consistent in demonstrating an effect of sleep deprivation on immunity, the direction of this relationship is variable depending on the duration of sleep deprivation. Moreover, experimental sleep deprivation may not generalize to clinical insomnia. Even partial sleep deprivation, a condition procedure that resembles most clinical insomnia (because individuals with sleep difficulties usually sleep a portion of the night), remains a short-term laboratory stressor that may not be comparable to clinical insomnia, which often develops a chronic course. Furthermore, there is a fundamental distinction between sleep deprivation, a procedure shortening sleep because of reduced opportunity to sleep, and insomnia, a complaint of reduced sleep despite the opportunity to sleep.

The sparse data available on the relationship between clinical insomnia and immunity have been obtained exclusively in the context of depression studies. In a study comparing depressed and nondepressed individuals on EEG measures, higher total sleep time, sleep efficiency, and duration of non-REM sleep were significantly associated with higher NK cell activity, independent of the presence of depression (26). In another study, increased severity of initial insomnia, as measured using the Hamilton Depression Rating Scale (HDRS), was significantly associated with decreased NK cell activity in patients with major depression, but there was no significant association between the HDRS total score and NK cell activity (27). We obtained similar results in women at risk for cervical cancer. More precisely, higher sleep satisfaction was associated with a higher concentration of CD4+ cells in circulating blood, even after controlling for depression (28).

Although these studies suggest that insomnia is associated with immunity, there are several methodological limitations that preclude clear conclusions about this relationship. One is the lack of an operational definition of insomnia. Indeed, insomnia was typically defined more as a sleep complaint, evaluated with instruments tapping general symptoms, rather than as an insomnia clinical syndrome, assessed by taking into account the frequency, severity, and duration of sleep disturbances. Moreover, all previous studies have used single measurements of immune functioning, which may vary considerably across time, and none have attempted to control for demographic and health behavior variables that may confound insomnia-immunity relationships (eg, age and exercise).

The goal of this study was to compare healthy individuals with chronic primary insomnia to good sleepers on a variety of immune parameters measured twice and after controlling for potentially confounding factors. It was hypothesized that patients with insomnia would display immune alterations (eg, decreased cellular immunity) compared with good sleepers.

METHODS

Participants

All participants were between 18 and 45 years of age. To be included in the study, insomnia participants (INS) had to present with a primary chronic insomnia disorder according to the combined criteria of the *International Classification of Sleep Disorders* (29), the *Diagnostic and Statistical Manual of Mental Disorders* (30), and those typically used in clinical research (31). These criteria, as assessed using the information from the clinical interview and sleep diaries, were as follows: 1) sleep-onset latency or time awake after sleep onset greater than 30 minutes per night, with a corresponding sleep efficiency (ratio of total sleep time to time spent in bed) lower than 85%; 2) these difficulties occurred for a minimum of 3 nights per week; 3) insomnia duration of at least 6 months; and 4) the sleep disturbance causes significant impairment of daytime functioning (eg, fatigue) or marked distress. Good sleepers (GS) had to meet the following criteria (at the clinical interview and in sleep diaries): 1) no subjective complaint of insomnia; and 2) a regular sleep schedule, defined as a sleep duration of 7 to 9 hours each night.

Individuals in both groups were excluded for the following reasons: 1) presence of another sleep disorder (eg, sleep apnea, periodic limb movements) as assessed by polysomnography (PSG); 2) presence of a current severe psychiatric disorder (eg, major depression, anxiety disorder, psychoactive substance dependence) as assessed using the Structured Clinical Interview for DSM-IV (SCID; Ref. 32); 3) self-reported acute or chronic medical disorder that might influence sleep and/or immunity (eg, cancer, cardiovascular disease, autoimmune disorders); and 4) recent (ie, past 2 weeks), self-reported use of medications known to affect sleep structure and/or immune functioning (eg, psychotropic, antihypertensive, anti-inflammatory medications). Pregnant women were also excluded.

Participants were recruited through media advertisements. A total of 143 persons requested information about the study. Each individual was initially screened using a phone interview to evaluate the main study inclusion and exclusion criteria. At that point, 92 (64%) individuals refused to participate because of a lack of interest or because they met at least one of the initial exclusion criteria. Among the remaining 51 potential participants who were invited for a clinical interview, 45 of them completed it. Six additional individuals were excluded after the interview because they did not meet criteria for an insomnia disorder ($N = 3$) or good sleep ($N = 1$) or because of evidence of periodic limb movements ($N = 1$) or current

mood disorder ($N = 1$). Of the 39 participants who met all study inclusion criteria, 36 completed all study procedures. One participant was excluded because an acute disease developed during the course of the study, and two quit just before the PSG assessments.

The INS group was composed of 17 participants (8 men, 9 women) with a mean age of 35.5 years (range = 18–44). Among the INS group, 35% reported difficulties initiating sleep, 6% reported trouble staying asleep during the night, and 59% reported both types of difficulty. The average duration of insomnia was 12 years (SD = 11.6, range = 1–35). The GS group was composed of 19 participants (10 men, 9 women) with a mean age of 32.4 years (range = 22–44). Participants were mainly white French-Canadian (92% of the total sample). Sixty percent of the total sample was single, 32% was married, and 8% was separated or divorced. Only two INS and five GS reported a past history of depressive disorder or adjustment disorder with depressed mood. Table 1 displays additional demographic characteristics for each group.

Procedures

Participants selected after phone screening were invited for a clinical interview to further assess their eligibility (see Fig. 1 for the time course of study procedures). In the meantime, two sleep diary forms with instructions were mailed to them along with a sociodemographic and medical questionnaire and the patient version of the Insomnia Severity Index (ISI-P; Ref. 31). Participants were asked to bring the completed questionnaires to the interview. At this evaluation session, written informed consent was first obtained, and two semistructured interviews, the Insomnia Interview Schedule (IIS; Ref. 31) and the SCID, were administered by two independent, trained clinicians. The clinician version of the ISI (ISI-C) was also completed by the clinician who administered the IIS after the interview. For participants still meeting inclusion criteria for the study, venous blood was drawn (time 1).

TABLE 1. Demographic Characteristics of Participants With Insomnia (INS) and Good Sleepers (GS)

Variable	INS ($N = 17$)	GS ($N = 19$)
Age, y, mean (SD)	35.5 (8.0)	32.4 (6.8)
Gender, % (*N*)		
Female	53.0 (9)	47.4 (9)
Male	47.1 (8)	53.0 (10)
Marital status, % (*N*)		
Married/living with partner	35.2 (6)	31.6 (6)
Single	52.9 (9)	63.2 (12)
Separated/divorced	11.8 (2)	5.3 (1)
Education level, % (*N*)		
Primary	5.9 (1)	0 (0)
Secondary/high school	17.6 (3)	10.5 (2)
College	23.5 (4)	15.8 (3)
University	52.9 (9)	73.7 (14)
Current occupation, % (*N*)		
Working (full and part time)	88.2 (15)	52.6 (10)
Unemployed	0 (0)	31.6 (6)
Student	11.8 (2)	15.8 (3)
Annual family income, % (*N*)		
≤$10,000	11.8 (2)	57.9 (11)
$10,001–$30,000	52.9 (9)	36.8 (7)
$30,001–$50,000	23.5 (4)	0 (0)
>$50,000	11.8 (2)	5.3 (1)

Participants were asked to continue completing the sleep diaries until the 2 consecutive nights in the laboratory. Participants usually slept in the laboratory during the following week, yielding a total of approximately 3 weeks of daily data for each participant. A second blood sample was taken before the second night in the laboratory (time 2). At both time assessments, blood was collected between 20:30 hours and 21:30 hours to control for diurnal variations. The health behaviors questionnaire was completed before both blood draws. INS participants who completed the study were offered a cognitive-behavioral treatment for insomnia as compensation for their participation; whereas GS participants received $50 (Canadian).

Measures

Sleep diary. Participants were instructed to complete their sleep diary every morning on arising. The sleep diaries monitored the following parameters: 1) daytime napping; 2) bedtime; 3) sleep-onset latency; 4) frequency of nocturnal awakenings; 5) duration of awakenings; 6) wake-up time; and 7) arising time. For the purpose of this study, two variables were derived from this information, total wake time (TWT), which is the sum of the time to fall asleep initially at bedtime plus all awakenings during the night, and sleep efficiency (SE), representing the ratio of total sleep time divided by the time in bed and multiplied by 100. Sleep diary data provide a reliable index of insomnia even though they do not always correspond exactly to PSG data (33).

Insomnia Severity Index. The ISI is a seven-item questionnaire that provides an index of insomnia severity (31). The seven items assess, on a 5-point scale, the perceived severity of sleep onset, sleep maintenance, early morning awakening problems, dissatisfaction with the current sleep pattern, degree of interference with daily functioning, noticeability of impairment due to sleep disturbance, and degree of worry or concern caused by the sleep problem. The total ISI score, obtained by summing the seven ratings, ranges from 0 to 28. The ISI-P has been found to have an excellent internal consistency and good convergent validity with the ISI-C and with corresponding variables of a sleep diary (34). In the present study, the French-Canadian version of the ISI was used; this version has been found to be equivalent to the English version (35), and both the self-administered (ISI-P) and clinician-rated (ISI-C) versions were used.

Polysomnography. All participants underwent 2 consecutive nights of sleep laboratory evaluation. A standard PSG montage that included EEG, EMG, and EOG monitoring was used. Sleep stages, respiratory disturbances, and limb movements were scored according to standardized criteria (36) by experienced technicians who were blind to the clinical status of participants (INS vs. GS). Respiration (ie, air flow, tidal volume, and oxygen saturation) and anterior tibialis EMG readings were recorded during the first night to detect sleep apnea or periodic limb movements. Sleep variables derived from PSG were the same as those derived from the sleep diaries.

Health Behaviors Questionnaire. Health behaviors that may potentially confound the relationship between insomnia and immune functioning were assessed using a questionnaire developed by our research team. This questionnaire assesses 1) smoking habits (ie, past and current smoking status and number of cigarettes in the past week), 2) caffeine consumption (ie, number of beverages in the past 48 hours), 3) alcohol use (ie, number of drinks in the past week and 48 hours), 4) drug use, 5) phase of menstrual cycle (menstruation, proliferative, or luteal phase), 6) quality of nutrition, and 7) exercise habits.

Quality of nutrition was evaluated using the two following indexes: 1) the typical number of days the individual eats three meals

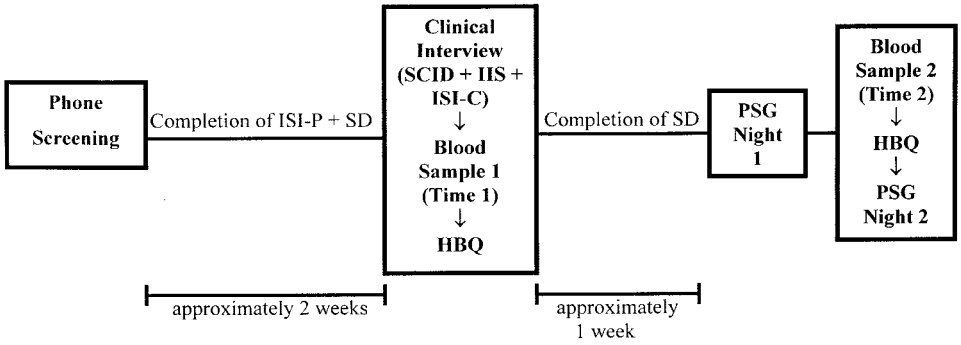

Fig. 1. Time course of study procedures. HBQ = Health Behaviors Questionnaire; IIS = Insomnia Interview Schedule; ISI-C = Insomnia Severity Index, clinician version; ISI-P = Insomnia Severity Index, patient version; PSG = polysomnography; SCID = Structured Clinical Interview for DSM-IV; SD = sleep diary.

(one item), and 2) typical compliance with "Canada's Food Guide to Healthy Eating" recommendations (eg, eating between 5 and 10 servings of fruits and vegetables per day; Ref. 37) using four 5-point scales (1 = never, 5 = 7 d/week), for a total score ranging from 4 to 20. Drug use was assessed by asking the respondent to specify which of 16 drugs (eg, marijuana, cocaine) they had used in the past week and in the past 48 hours. Because drug use was fairly uncommon in this sample, two dichotomous composite variables were created to indicate whether the respondent had used drugs during these two time periods. Finally, the time spent in various types of physical activity (ie, light, moderate, hard, and very hard) was assessed using the 7-day recall method (38) and transformed to yield an index of total energy expenditure. Metabolic equivalent (MET) values, representing exercise intensity, were assigned to each activity reported by the participants (ie, 1 for sleep, 1.5 for light activity, 4 for moderate activity, 6 for hard activity, and 10 for very hard activity); these values were then multiplied by the time spent in each type of activity and summed to yield a global measure of physical activity (39).

Immunologic assays. At each assessment point, 24 ml of venous blood was collected in three heparinized tubes. The tubes were centrifuged at 1800 rpm for 5 minutes at room temperature, and the buffy coat was collected (3 ml by tube). Analyses were performed by laboratory personnel blinded to the clinical status of participants (INS vs. GS).

WBC subsets were determined in whole blood by three-color direct immunofluorescence using a Coulter flow cytometer (Coulter EPICS Elite ESP, Beckman Coulter, Miami, FL). A minimum of 10,000 cells per sample was analyzed. To analyze lymphocyte surface antigens, monoclonal antibodies directly conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), or peridinin chlorophyll protein (PerCP) were used. Briefly, for each subset analysis, 10 μl of antibodies (TriTEST) were added to 50 μl of buffy coat and incubated for 20 minutes. Erythrocytes were then disintegrated using OPTILYSE-C reagent (Immunotech, Miami, FL). Enumeration by flow cytometry included the following cells: T cells (CD3+; CD3 FITC), T-helper cells (CD3+CD4+; CD3 FITC/CD4 PE/CD45 PerCP), T-suppressor/cytotoxic cells (CD3+CD8+; CD3 FITC/CD8 PE/CD45 PerCP), and NK cells (CD3−/CD16+CD56+; CD3 FITC/CD16+CD56 PE/CD45 PerCP). All antibodies and immunofluorescence reagents were purchased from Becton Dickinson (San Jose, CA). The absolute number per unit volume bearing each lymphocyte marker was determined by multiplying data obtained by flow cytometry with the absolute lymphocyte count derived from the complete blood count and differential. The complete blood count was performed within 2 hours after the blood was drawn; flow cytometry was conducted the next morning (within 12 to 15 hours after the blood draw).

Peripheral blood lymphocytes were separated by density gradient centrifugation on a Ficoll-Paque gradient (Amersham Pharmacia Biotech, Piscataway, NJ) the morning after the blood draw (within 12 to 15 hours) and were stored (20% dimethyl sulfoxide, 80% fetal bovine serum) at −80°C until the assay was performed (within 10 months). The NK cytotoxicity was determined by flow cytometry (40). This method is fast, reliable, and correlates well with the standard ^{51}CR-release assay, and there is no need to use radioactive material (41–43). Peripheral blood lymphocytes were thawed rapidly in a 37°C water bath, and a Ficoll-Paque was performed to remove dead cells. They were then washed with RPMI 1640 + 10% fetal bovine serum and kept for 30 minutes in a humidified 5% CO_2 atmosphere at 37°C. Effector cells were washed, counted, and adjusted to 5×10^6/ml. Target cells, K562, a human erythroleukemic cell line (American Type Culture Collection, Rockville, MD; accession number CCL243) in log phase, were washed, counted, and adjusted to 1×10^5/ml. Effector cells and target cells were then mixed at two ratios (E:T ratios: 12.5:1 and 6.25:1)[1] and incubated at 37°C in a humidified 5% CO_2 incubator for 10 minutes to promote conjugate formation. Then, 10 μl of propidium iodide (PI) working solution (100 μl/ml) was added to each tube and incubated for 90 minutes at 37°C in a 5% CO_2 atmosphere. Finally, cells were stored in a dark ice bath, and flow cytometric data acquisition was performed with a Coulter flow cytometer (Coulter EPICS Elite ESP, Beckman Coulter). To control spontaneous death of K562 cells in culture, target cells at 10^5/ml were incubated and counted in the flow cytometer on three occasions (ie, beginning, middle, and end) during the analysis, and NK cell activity data were adjusted accordingly. Moreover, to assess the influence of cryopreservation, blood was taken from three laboratory controls at three occasions at monthly intervals. Half of the samples were analyzed immediately on fresh cells and the other half were cryopreserved for one month. Results indicated that cryopreservation of blood samples had no influence on NK cell activity.

For determination of IL-1β, IL-2, and IFN-γ production, a whole-blood assay was performed (44). For stimulation of IL-1β, aliquots of 50 μl of buffy coat were resuspended in 445 μl of RPMI 1640 medium (Cellgro, Winset Canadian Laboratories, Saint-Bruno, Canada), and 5 μl of lipopolysaccharide (LPS) from *Escherichia coli* (0.1 mg/ml). For stimulation of IL-2 and IFN-γ, aliquots of 50 μl of buffy coat were resuspended in 440 μl of RPMI 1640 medium, and 10 μl of phytohemagglutinin (2%) was added. Every sample was stimu-

[1] Insufficient cells were available to perform the assay at ratios of 50:1 and 25:1.

lated in duplicate the morning after the blood draw (within 12 to 15 hours). The samples were then incubated for a minimum of 72 hours at 37°C with 5% CO_2. The supernatant were stored at −80°C until the assay was performed (within 10 months after the blood was drawn). A minimum incubation time of 72 hours was chosen on the basis of previous kinetic studies indicating that it provides a good estimate for the production of cytokines assessed in this study (45). All cytokine levels were measured by enzyme-linked immunosorben assay kits (Biosource International, Camarillo, CA). The sensitivities of the assays were 1 pg/ml for IL-1β, 5 pg/ml for IL-2, and 4 pg/ml for IFN-γ.

Statistical Analyses

Demographic, sleep, and immunological data were carefully screened for outliers and missing values using standardized procedures (46). Specifically, outlier detection criteria was set at $\alpha = 0.005$ within each experimental condition, and outliers were removed and treated as missing data. Because traditional methods of dealing with missing data are severely biased (47), missing data were estimated from the mean of 10 plausible data by using Expectation-Maximization (EM), a robust maximum-likelihood algorithm (47, 48). Missing data management was completed using the SAS MI procedure (49). However, some missing data could not be estimated, which explains why the total number of participants is not 17 INS and 19 GS for these analyses. Reasons for missing data are as follows: 1) two blood samples for complete blood count analysis coagulated, one in each group at time 1; 2) one INS participant had no blood drawn at time 2; 3) there were missing data on some covariates; and 4) some IFN-γ data exceeded the standard curve, which invalidated them.

Because of the relatively small sample size, univariate *t* tests were conducted to compare groups on demographic, health behavior, and sleep variables. The principal hypothesis was tested using repeated-measures (group by time) analyses of covariance. Following the suggestions of Frigon and Laurencelle (50), selection of covariates among all demographic and health behavior variables was based not on the presence of significant group differences on these variables but on covariate capacity to reduce error variance. All statistical analyses were conducted using SAS 8.2 statistical software (51). Bilateral tests were performed for all analyses, and the α level was set at 5%. Effect sizes are reported, as recommended by Wilkinson (52) and Cohen (53), to assess the magnitude of the effects tested and to help interpret non–statistically significant findings. Effect size calculations were conducted according to Cohen's criteria (54) with PowPal (55), an interactive program that computes effect sizes from summary statistics.

RESULTS

Preliminary Analyses

First-night effect. A repeated-measures (group by time) MANOVA was conducted on PSG variables to assess whether there was a significant first-night effect. This analysis revealed a significant time effect ($F(2,33) = 11.89$, $p < .0001$), indicating that sleep was significantly better on the second night of recording in both groups. Univariate analysis of variance time effects were significant on both sleep indices included in the analysis: TWT ($p < .0001$) and SE ($p < .0001$). Consequently only PSG data from the second night are hereafter reported.

Manipulation check. To assess the validity of the group assignment, the two groups were compared on ISI-P and ISI-C total scores, sleep diary (a daily average of all available data), and PSG indexes (see Table 2). Compared with the GS group, the INS group obtained significantly higher scores on both the ISI-P and the ISI-C (p values $< .001$). In addition, INS participants reported significantly poorer sleep than GS participants on TWT and SE variables when assessed by the sleep diary (p values $< .001$). However, no difference was found between the INS and GS groups on the same variables when assessed by PSG despite the presence of medium effect sizes (TWT: $d = 0.43$; SE: $d = 0.44$). To assess whether this lack of consistency between sleep diary and PSG data were attributable to patients' difficulties to reliably assess their sleep, correlations were calculated between PSG indices and sleep diary data completed by participants on nights spent in the laboratory. Highly significant correlations were found for both sleep variables on night 1 (TWT: $r = 0.41$, $p < .05$; SE: $r = 0.45$, $p < .01$) and night 2 (TWT: $r = 0.44$,

TABLE 2. Comparison of Participants With Insomnia (INS) and Good Sleepers (GS) on Sleep Variables

Variable	INS ($N = 17$)		GS ($N = 19$)		$t(34)$	p	d^a
	Mean	SD	Mean	SD			
Insomnia Severity Index							
Patient version	17.5	4.5	2.1	2.6	12.4	.001	>2.00
Clinician version	13.0	4.5	0.2	0.4	11.7	.001	>2.00
Sleep diary (average of all available data)							
Total wake time (min)	148.6	38.3	27.7	14.4	12.3	.001	>2.00
Sleep efficiency (%)	70.2	9.6	94.6	2.6	−10.1	.001	>2.00
Polysomnography (second night)							
Total wake time (min)	58.2	15.3	49.5	25.0	1.2	NS	0.43
Sleep efficiency (%)	86.9	3.5	88.9	5.5	−1.3	NS	0.44

[a] Cohen's *d*: small (0.20), moderate (0.50), and large (0.80) effect sizes.

$p < .01$; SE: $r = 0.48$, $p < .01$). Hence, participants were fairly good at assessing their sleep, and the lack of significant differences observed between the INS and GS groups on PSG assessments must be attributed to other reasons than a failure to compose "real" INS and GS groups because it was based only on subjective information.

Control variables. Correlational analyses were conducted on demographic (eg, age, education level, annual income, and history of depressive disorder) and health behavior (eg, smoking, nutrition, and alcohol and caffeine use) variables to identify variables that may confound the relationship between insomnia and immune functioning and should be used as covariates in subsequent analyses. Covariates for each immune parameter analyzed were as follows: compliance with Canada's healthy eating recommendations (WBC, lymphocytes, CD3+, and monocytes cells), alcohol consumption in the past 48 hours (WBC, CD8+, NK, and monocyte cells; NK cell activity at both ratios), height (CD4+ cells), weight (CD4+ cells), and physical activity (IFN-γ).

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Group comparisons. Adjusted means (and SDs) obtained from ANCOVAs by participants of each group on each immune variable are presented in Table 3, and results of repeated-measures ANCOVAs are presented in Table 4. Significant group effects were obtained on

TABLE 3. Comparison of Participants With Insomnia (INS) and Good Sleepers (GS) on Each Immune Parameter at Two Time Assessments[a]

Variable	INS			GS		
	N	Mean	SD	*N*	Mean	SD
WBC ($\times 10^6$/liter)						
Time 1	16	6624.6	1089.3	18	7167.5	1088.5
Time 2	16	6955.1	1548.0	18	6906.6	1547.0
Monocytes ($\times 10^6$/liter)						
Time 1	16	531.4	251.3	17	445.4	251.2
Time 2	16	418.6	203.6	17	573.6	203.5
Lymphocytes ($\times 10^6$/liter)						
Time 1	16	2370.4	731.7	18	2672.5	731.7
Time 2	16	2367.9	616.9	18	2809.2	616.9
T (CD3+) cells ($\times 10^6$/liter)						
Time 1	16	1711.2	546.4	18	1998.2	546.4
Time 2	16	1679.5	503.1	18	2124.1	503.1
T-helper (CD4+) cells ($\times 10^6$/liter)						
Time 1	16	1077.3	416.4	18	1276.4	414.8
Time 2	16	1023.0	302.9	18	1318.4	301.7
T-cytotoxic/suppressor (CD8+) cells ($\times 10^6$/liter)						
Time 1	16	559.1	192.0	17	688.6	191.9
Time 2	16	531.8	196.1	17	742.7	196.0
NK (CD16+/CD56+) cells ($\times 10^6$/liter)						
Time 1	16	177.8	88.2	18	175.2	88.2
Time 2	16	173.5	81.4	18	149.3	81.4
NK cell activity, 12.5:1 (%)						
Time 1	16	12.1	5.7	18	11.7	5.7
Time 2	16	9.8	3.9	18	8.2	3.8
NK cell activity, 6.25:1 (%)						
Time 1	16	7.6	4.4	18	8.0	4.4
Time 2	16	5.7	3.4	18	4.9	3.4
IL-1β (pg/ml)						
Time 1	16	1537.7	953.1	18	1586.3	953.0
Time 2	16	2153.9	715.0	18	1696.8	714.9
IL-2 (pg/ml)						
Time 1	16	865.5	1132.6	18	1088.0	1132.6
Time 2	16	198.2	166.6	18	150.2	166.6
IFN-γ (pg/ml)						
Time 1	10	1666.0	1189.3	9	1485.5	1195.1
Time 2	10	2330.9	1504.3	9	1841.4	1511.6

[a] Adjusted mean and SD obtained from ANCOVAs.

TABLE 4. Summary Statistics and Effect Size Calculations for Repeated-Measures ANCOVAs on Immune Parameters

Variable	*df* Error[a]	Group Effect		Time Effect		Interaction	
		F	*d*[b]	*F*	*d*	*F*	*d*
WBC	30	0.35	0.22	0.19	0.16	2.34	0.56
Monocytes	29	0.40	0.24	0.49	0.26	4.14**	0.76
Lymphocytes	31	3.20*	0.64	1.71	0.47	0.44	0.24
T (CD3+) cells	31	5.28**	0.83	1.02	0.36	0.86	0.33
T-helper (CD4+) cells	30	4.44**	0.77	0.42	0.24	0.80	0.33
T-cytotoxic/suppressor (CD8+) cells	29	10.2***	1.19	0.00	0.00	0.95	0.36
NK (CD16+/CD56+) cells	31	0.26	0.18	1.14	0.38	0.75	0.31
NK cell activity 12.5:1	31	0.59	0.28	6.38**	0.91	0.26	0.18
NK cell activity 6.25:1	31	0.03	0.06	4.60**	0.77	0.47	0.25
IL-1β	31	0.82	0.33	0.87	0.34	1.93	0.50
IL-2	31	0.20	0.16	0.09	0.11	0.47	0.25
IFN-γ	16	0.41	0.32	0.00	0.00	0.16	0.20

[a] Degrees of freedom error for between- and within-subject effects are identical under a 2 × 2 repeated-measures design.
[b] Cohen's *d*: small (0.20), moderate (0.50), and large (0.80) effect sizes.
* $p < .10$; ** $p < .05$; *** $p < .01$.

CD3+ ($p < .05$), CD4+ ($p < .05$), and CD8+ ($p < .01$) cell counts, and a marginally significant group effect was found on total lymphocyte count ($p = .08$). As shown in Figure 2, the INS group had lower counts of CD3+, CD4+, CD8+, and total lymphocyte cells compared with the GS group, a difference that was slightly more pronounced at the second blood draw. Moreover, a significant group-by-time interaction was obtained on monocyte counts ($p < .05$), and simple main effects revealed a significant group effect only at time 2 ($F(1,29) = 4.76$, $p < .05$). In fact, Figure 2 reveals a decrease of these cells in INS participants and an increase in GS participants across time. Finally, significant time effects were obtained only on NK cell activity at both ratios, indicating a reduction in both groups from time 1 to time 2 (data not illustrated).

Relationship between sleep and immune functioning. Pearson correlations were calculated between each immune variable and each sleep diary and PSG data assessed before blood draws. Analyses performed

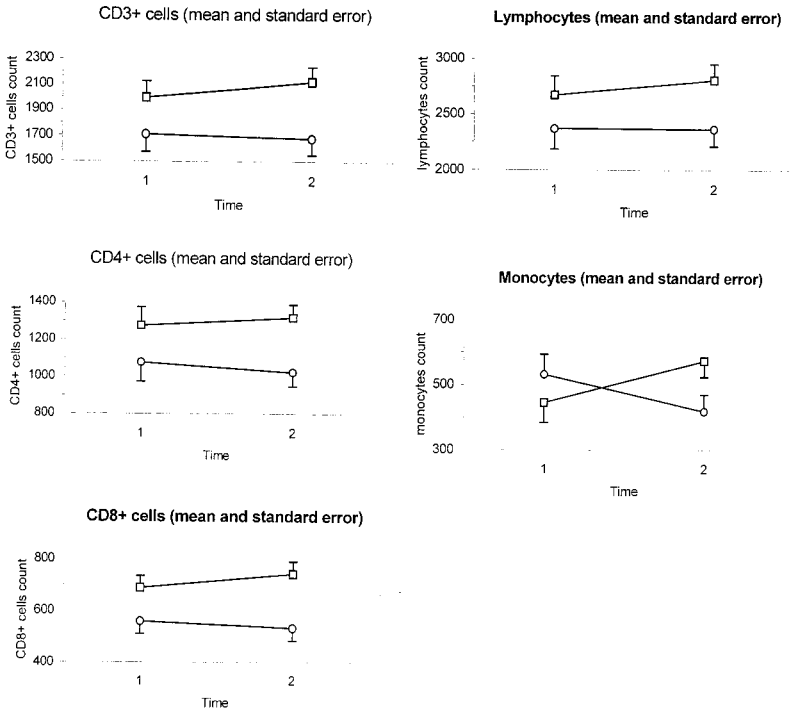

Fig. 2. Lymphocyte and monocyte counts in insomnia patients (INS, —○—) and good sleepers (GS, –□–) across time.

between sleep diary indices collected during the first 2 weeks of monitoring (between the phone screening and the clinical interview) and immune data from the first blood draw (time 1) performed subsequently did not reveal any significant correlations. Analyses conducted between sleep diary parameters obtained during the third week of monitoring (between the clinical interview and PSG recordings) and immune data from the second blood draw (time 2) revealed more significant correlations. Greater TWT (r (34) = −0.33, p = .06) and lower sleep efficiency (r(34) = 0.33, p = .06) were associated with a reduced number of CD3+ cells. In addition, higher levels of TWT (r(34) = −0.44, $p <$ .01) and lower levels of SE (r(34) = 0.45, $p <$ .01) were significantly associated with reduced number of CD8+ cells.

Pearson correlations were also performed between sleep parameters derived from the first PSG evaluation and immune variables collected the next day (second blood draw, time 2) to assess the influence of spending a night in the laboratory on sleep quality. Only one correlation was significant, and it was in the opposite direction of our hypothesis. In fact, higher SE was associated with lower monocytes level (r(35) = −0.39, $p <$ .05). Finally, correlations were conducted between ISI (patient and clinician versions) and immune data derived from the first blood draw. None of these correlations was significant.

DISCUSSION

The goal of this study was to evaluate the relationship between primary chronic insomnia and immune function by comparing healthy individuals with insomnia to good sleepers on a variety of immune parameters. Results of this study partially support the hypothesis that chronic insomnia is associated with immune alterations. Specifically, chronic insomnia was significantly associated with lower counts of lymphocyte subpopulations (ie, CD3+, CD4+, and CD8+ cells) and was marginally associated with lower total lymphocyte count. However, the INS and GS groups did not significantly differ on WBC count, NK cell activity, or cytokine production. A significant interaction was obtained on monocyte levels, and further analysis showed lower counts in the INS group relative to the GS group at time 2.

These findings are consistent with the existing literature suggesting a downregulation effect of experimental total sleep deprivation on cellular immune functioning (16, 17, 21, 22). Naturalistic poor sleep quality has also been found to be associated with lower lymphocyte counts (ie, CD4+ cells) (28). However, the absence of effect of chronic insomnia on NK cell activity and cytokine production is unexpected and difficult to explain considering previous investigations demonstrating an effect of experimental sleep deprivation and clinical insomnia symptoms on these variables (23–27) and considering that immune variables are generally highly correlated. However, calculations of effect sizes suggest that it may be at least partially attributable to a lack of statistical power. Indeed, small to moderate effect sizes for group effects were obtained on NK cell activity at the 12.5:1 ratio (d = 0.28) and on the production of IL-1β (d = 0.33) and IFN-γ (d = 0.32). Hence, a study with a larger sample size could have yielded a more consistent pattern of results.

Although a significant group-by-time interaction effect was observed only on monocyte counts, interaction effects on other immune variables, with an average effect size of 0.32, deserve some comments. As shown in Figure 2, between-group differences on lymphocyte counts were greater at time 2. In fact, the number of each lymphocyte subset increased in the GS group, whereas it slightly decreased in the INS group across time (effect sizes for interaction effects: d = 0.33–0.36). This pattern is even more obvious for monocytes. These findings are consistent with correlational analyses showing stronger relationships between immune data at time 2, specifically CD3+ and CD8+ cells, and continuous sleep diary variables collected the previous week. Thus, this study suggests that chronic insomnia is associated with some changes in immune response, but this association was stronger at the second time assessment despite a general pattern of temporal stability of immune measures (only NK activity significantly varied across time).

The first time assessment occurred at the clinical interview and was scheduled at that time to give a measure of "usual" immune functioning, not contaminated by the study procedures, whereas the second time assessment occurred the day after the first PSG night. Thus, it seems that the first PSG night potentiated the relationship between insomnia and immunity. However, because no consistent relationships were obtained between sleep variables assessed by the first PSG night and all immune measures assessed the next day (time 2), it does not seem that immune functioning assessed at time 2 was influenced by the quality of the sleep the patients had in the laboratory the night before.

However, the similarity of these results with findings of previous studies that showed an influence of chronic stress on immune responsiveness to acute stress is striking (56–58). In one of these studies, augmentation or no change in lymphocyte subsets (ie, CD3+, CD8+, CD57+) was observed in individuals with low chronic stress, whereas a reduction in the

same immune variables was found in individuals with high chronic stress after exposure to acute stress (56). Likewise, immunosuppression (ie, monocytes) or no change (ie, CD3+, CD4+, CD8+ cells) was observed in patients with chronic insomnia, whereas some immunoenhancement was observed in good sleepers after their first PSG assessment in this study, a laboratory procedure that may be interpreted as a stressful experience. Hence, patients with chronic insomnia and good sleepers would react differently to the acute stress caused by the sleep laboratory procedure, the same way patients with or without chronic stress show differential immunological reactivity to acute experimental stressors.

It is important to discuss the discrepancy obtained between subjective and objective sleep measures, because one may argue that groups were not composed of "real" insomniacs and "real" good sleepers. Insomnia is a condition that is defined primarily by a subjective complaint of poor sleep and by a subjective dissatisfaction with sleep. Several previous studies comparing subjectively defined groups of insomnia patients and good sleepers also found no or few differences on PSG measures (eg, Refs. 59 and 60; for a review, see Ref. 61), a puzzling finding that has been interpreted in a variety of ways (62). First, it has been suggested that insomnia patients might exaggerate their sleep difficulties. However, correlations performed in this study between PSG indices and sleep diary data assessing the same nights suggest that patients' subjective estimation of their sleep is fairly reliable. Alternatively, this lack of consistency can be explained by the weak ecological validity of PSG assessments. Because insomnia is a conditioned response in which the stimuli that are typically associated with sleep (eg, bedtime rituals and bedroom environment) become associated with the fear of not sleeping and performance anxiety, sleep may not be the same in the laboratory as it usually is at home (63, 64). Consequently it is often necessary to conduct assessments for several consecutive nights (a recent study even suggests that an entire week of PSG recordings is necessary; Ref. 65). Because of budget constraints, we performed only 2 nights of recording in the present study, which left usable data for only the second night after data from the first night had to be discarded because of a significant first-night effect. Thus, it is our opinion that the discrepancy observed between subjective and objective measures do not invalidate the present comparison between insomnia patients and good sleepers, because both groups were carefully selected on the basis of interview and sleep diary data and seemed to reliably assess their sleep. It is also plausible that immunity is linked more to the subjective experience of insomnia, and its associated distress, than to sleep disturbances that are detectable only by PSG assessment.

The present study is characterized by some weaknesses, including the use of a cross-sectional design, which precludes conclusions about causal relationships. Moreover, although the study had enough power to detect large differences, the small sample size may have prevented the detection of more modest, but perhaps clinically meaningful, differences between groups. The study is further limited by the lack of measurement of potential mediators in the relationship between insomnia and immunity, such as corticosteroids (eg, cortisol and catecholamines) and growth hormone. Finally, similar to most previous psychoneuroimmunological studies, this study did not investigate the clinical relevance of observed immune alterations in terms of impact on health status. Hence, areas for future research include the use of longitudinal or experimental designs (eg, intervention study), the use of larger samples, the assessment of potential mechanisms linking insomnia and immunity, the evaluation of differential reactivity to acute stress, and the verification of the clinical impact of immune alterations observed on health status (eg, on the occurrence of infections).

Despite these limitations, the present study offers some methodological improvements compared with previous studies. First, we evaluated various immune parameters simultaneously to obtain a more general measure of immune functioning. In addition, compared with previous studies that used a single assessment of immunity, we evaluated these parameters twice, with a week interval, which allowed measurement of temporal stability. Finally, several demographic and health behavior variables that could confound the relationship between insomnia and immunity were evaluated and statistically controlled when necessary.

It has been believed since ancient times that sleep loss can lead to illness. Although the present study was not designed to verify that broad hypothesis, it suggests that chronic insomnia affects host defenses. Further research is needed to investigate whether these alterations have health implications and to identify potential mediating factors.

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