

Shift of Monocyte Function Toward Cellular Immunity During Sleep

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Background: Sleep is considered to strengthen immune defense. We hypothesized that sleep achieves this effect by shifting the balance between types 1 and 2 cytokine activity toward increased type 1 activity, thereby supporting adaptive cellular immune responses.

Methods: We analyzed monocyte-derived type 1 (interleukin 12 [IL-12]) and type 2 (IL-10) cytokines by means of multiparametric flow cytometry in healthy human subjects (n=11) during a regular sleep-wake cycle and 24 hours of wakefulness.

Results: Sleep increased the number of IL-12-producing monocytes and concurrently decreased the number of IL-10-producing monocytes, thereby inducing clear rhythms in these cells, with maximum numbers at 2:20 and 11:30 AM, respectively. The rhythms were completely absent during continuous wakefulness. Correlation analyses and supplementary in vitro studies sug-

gest that high prolactin and low cortisol levels are factors contributing to the shift in the IL-12/IL-10 ratio toward increased IL-12 activity during sleep.

Conclusions: Monocyte-derived IL-12 and IL-10 play a critical role for tuning the synapse between antigen-presenting cells and lymphocytes. By preferentially supporting type 1 IL-12 activity, sleep induces a 24-hour oscillation between predominant types 1 and 2 cytokines and, in this way, acts to globally increase the efficacy of adaptive immune responses. Improving sleep could represent a therapeutic option to enhance the success of vaccinations and success in the treatment of diseases (eg, atopic dermatitis and human immunodeficiency virus infection) that are characterized by type 2 cytokine overactivity.

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THERE ARE HINTS THAT THE immune system is organized in time with a predominance of type 1-mediated immune defense at night.^{1,2} The balance of proinflammatory type 1 cytokines (interleukin [IL] 2, interferon- γ , and IL-12) and anti-inflammatory type 2 cytokines (IL-4 and IL-10) crucially controls immune function, with type 1 cytokines overall supporting cellular aspects of immune responses and type 2 cytokines moderating the type 1 response. An excessive production of either cytokine type leads to inflammation and tissue damage on the one hand and to susceptibility to infection and allergy on the other hand.³ To prevent overactivity, the type 1-type 2 cytokine balance is tightly regulated by cross-inhibition and via a supraordinate neuroendocrine control.⁴

Emerging evidence indicates a vital role of sleep for efficient immune defense.⁵ Specifically, sleep deprivation in healthy sub-

jects⁶ and chronic sleep deficits in insomnia,⁷ alcoholism,⁸ stress,⁹ and the course of aging⁹ are associated with a shift of the type 1-type 2 cytokine balance toward type 2 activity. The clinical relevance of this function of sleep is indicated, eg, by findings of an increased type 2-mediated allergic skin reaction after sleep deprivation in patients with atopic dermatitis.¹⁰ Also, sleep deprivation after inoculation in healthy humans suppresses antibody responses to hepatitis A virus and influenza vaccinations.^{11,12} The IgG subclass profiles in response to both vaccinations is highly restricted to type 1 cytokine-dependent IgG1 and IgG3.^{13,14} Previous studies have demonstrated a sleep-associated shift toward type 1 cytokine activity in T cells.⁶ However, the specific effect of sleep on the cells of the innate immune system, which serve as antigen-presenting cells (APC) and which can induce such a shift, has not been assessed so far, despite the apparent key role these cells play in the regulation of the type

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1–type 2 cytokine balance.¹⁵ Herein, we show that nocturnal sleep (compared with wakefulness) shifts monocyte cytokine production to type 1 cytokines, as a prerequisite for a sleep-associated predominance of helper T-cell 1 (T_H1)–mediated adaptive immune defense. We measured cytokine activity repeatedly in human monocytes representing direct precursors of APC that, unlike APC, can easily be assessed in blood samples.¹⁶ To dissect circadian influences from those of sleep on the type 1–type 2 cytokine balance, we analyzed cell counts of monocytes producing IL-12 (IL-12+) and IL-10 (IL-10+) by means of multiparametric flow cytometry during a regular sleep-wake cycle and 24 hours of continuous wakefulness in healthy human subjects.

METHODS

SUBJECTS AND PROCEDURE

Participants included 11 physically and mentally healthy men with a mean \pm SD age of 25 \pm 1 years. They were nonsmokers, did not experience sleep disturbances, and were not taking any medication. Acute illness was excluded by results of a physical examination and an extended routine laboratory investigation. (To have a homogeneous sample with regard to sleep and endocrine variables, women were not included in this study. There is no evidence of sex differences in the relationship between sleep and immunity.) Subjects were adjusted to the experimental setting by spending 1 adaptation night in the laboratory. The study protocol was approved by the local ethics committee. All subjects gave written informed consent.

Experiments were performed according to a within-subject crossover design. Each man participated in 2 experimental conditions, each starting at 8 PM and ending 24 hours later at 8 PM the next day. One condition (sleep) included a regular sleep-wake cycle, whereas in the other condition (wake) subjects remained awake throughout the 24-hour experimental period. Both experimental sessions for a subject were separated by at least 4 weeks, and the order of conditions was balanced across subjects. In the sleep condition, sleep was allowed from 11 PM (lights off) to 7 AM. During the wake condition, subjects stayed awake in bed in a half-supine position from 11 PM to 7 AM. During this time, they watched television, listened to music, and talked to the experimenter at normal room light (about 300 lux).

During both conditions, blood was sampled first at 8 PM, then every 1.5 hours from 11 PM to 8 AM, and every 3 hours from 8 AM to 8 PM the next day. Blood samples were drawn via an intravenous forearm catheter that was connected to a long, thin tube and enabled blood collection from an adjacent room without disturbing the subject's sleep. Sleep stages were determined off-line from polysomnographic recordings following standard criteria.¹⁷

DETERMINATION OF IL-12+ AND IL-10+ MONOCYTE NUMBERS AND HORMONE LEVELS

Cytokine numbers were evaluated at the single-cell level by means of multiparametric flow cytometry. Immediately after drawing, heparinized venous blood samples were diluted 1:1 with RPMI media and incubated with lipopolysaccharide (catalogue L-4391; Sigma-Aldrich Corp, St Louis, Mo) at a final concentration of 10 ng/mL at 37°C in a 5% carbon dioxide atmosphere for 6 and 24 hours to stimulate production of IL-12 and IL-10, respectively, from monocytes. For detection of the intracellular cytokines, antibodies (IL-12/APC [allophycocya-

tin], p40/p70, clone C11.5, and IL-10/PE [phycoerythrin], clone JES3-19F1) and reagents from BD Biosciences, San Jose, Calif, were used, and standard procedures were performed according to the manufacturer's instructions as described previously.¹⁸ At least 10 000 CD14⁺ HLA-DR–positive cells were gated and subsequently analyzed for the expression of IL-12 and IL-10 on a flow cytometer (FACSCalibur; BD Biosciences) using commercially available software (CellQuest; BD Biosciences). Double IL-12+/IL-10+ monocytes were difficult to detect with the assay. In pilot experiments with a modified assay, these cells represented only about 15% of the IL-10+ monocytes and varied in parallel with this population. Hence, they were not included in this analysis.

Growth hormone (GH), prolactin, and cortisol levels were measured in serum using commercial assays (Immulite; DPC-Biermann GmbH, Bad Nauheim, Germany). Norepinephrine level was measured in plasma by means of standard high-performance liquid chromatography.

IN VITRO TESTING OF HORMONAL MEDIATORS

To test the influence of changes in hormone concentrations during nocturnal sleep on the type 1–type 2 cytokine balance in vitro, we performed supplementary experiments in 12 men with blood drawn at 1 AM during regular sleep. Subject characteristics and procedures were the same as in the main experiments. Numbers of IL-12+ and IL-10+ monocytes were determined after preincubation for 10 minutes at room temperature of the samples with GH antibody (1- μ g/mL antihuman GH antibody; R&D Systems, Minneapolis, Minn), prolactin antibody (1- μ g/mL antihuman prolactin antibody; R&D Systems), cortisol (4- and 20- μ g/dL [110- and 552-nmol/L]; Sigma-Aldrich Corp), and norepinephrine (170- and 510-pg/mL [1.0- and 3.0-nmol/L]; Sigma-Aldrich Corp). (Pilot experiments showed that GH antibody together with prolactin antibody, each at a concentration of 1 μ g/mL, completely blocked the proliferation of a lactogen-dependent Nb2 cell line in the presence of human serum containing GH and prolactin at concentrations normally observed during sleep.) Further stimulation and staining protocols were identical to those in the main experiments.

STATISTICAL ANALYSIS

Data are presented as mean \pm SEM. Statistical analysis was based on repeated-measures analysis of variance with subsequent post-hoc contrasts. Analysis of variance factors for the main experiment included condition (sleep or wake), period (nighttime or daytime), and time (single time points). To identify circadian (24-hour) rhythms, cosinor analysis was performed with Chronolab software (Vigo, Spain). Pearson product moment correlation coefficients were calculated to assess the relationships between the percentages of IL-10+ and IL-12+ monocytes and hormonal levels. Missing single values were replaced by linear interpolation, which occurred in less than 2% of the data.

RESULTS

SLEEP AND PERCENTAGES OF IL-12+ AND IL-10+ MONOCYTES

Polysomnographic recordings ensured that subjects slept normally in the sleep condition. **Table 1** summarizes sleep variables together with subjects' characteristics. Slow-wave sleep predominated in the early half of the night, whereas rapid eye movement sleep dominated during the late half of the night.

Table 1. Subjects' Characteristics and Sleep Variables

Subject Characteristic (n = 11)	Mean ± SEM Value
Age, y	25.0 ± 1.0
BMI	23.5 ± 1.0
Sleep variables, min	
Total sleep time	443.3 ± 17.2
Stage 1	32.7 ± 7.9
Stage 2	236.8 ± 17.4
Slow wave sleep	76.9 ± 6.7
REM sleep	73.7 ± 6.7
Sleep-onset latency	21.4 ± 5.0
REM sleep latency	125.7 ± 18.6

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); REM, rapid eye movement.

During the regular sleep-wake cycle, the percentage (with reference to total number of monocytes) of IL-12+ monocytes showed a distinct increase during sleep, which peaked at about 2 AM and was completely blocked when subjects remained awake at night ($F_{1,10}=12.25$; $P=.006$ for condition \times period interaction). **Figure 1A** shows significant differences of nighttime means and at single time points. This increasing effect of sleep on IL-12+ monocytes was also confirmed ($P=.01$) in analyses on values correcting for the slight but non-significant difference between sleep and wake conditions during the presleep period. Cosinor analysis confirmed a significant rhythm only during the regular sleep-wake cycle ($P=.02$), with a peak at 2:20 AM (± 38 minutes) and an amplitude of $9.4\% \pm 2.5\%$. In contrast to the dynamics of IL-12+ monocytes, the percentage of IL-10+ monocytes was lowered during sleep compared with continuous wakefulness ($F_{1,10}=7.42$; $P=.02$ for condition \times period interaction [Figure 1B]). Suppression of IL-10+ monocytes during nocturnal sleep resulted in a consistent 24-hour oscillation of these cells peaking at 11:53 AM (± 71 minutes) with an amplitude of $1.3\% \pm 0.5\%$ as revealed by cosinor analysis ($P=.03$). During continuous wakefulness, the percentage of IL-10+ monocytes distinctly increased during the night. However, this increase was too variable to express a significant circadian rhythm ($P=.06$). As a consequence of the opposite effects of sleep on percentages of IL-12+ and IL-10+ monocytes, the nighttime ratio of IL-12+/IL-10+ monocytes was about 4-fold higher when subjects slept than when they remained awake during this time ($F_{1,10}=8.43$; $P=.02$ condition \times period interaction [Figure 1C]). Again, cosinor analysis indicated a significant 24-hour rhythm of this ratio only during the regular sleep-wake cycle (peak time, 1:31 AM ± 53 minutes; amplitude, $4.4\% \pm 1.3\%$; $P=.03$), and did not do so during continuous wakefulness ($P=.37$).

HORMONES AND THEIR ASSOCIATION WITH IL-10+ AND IL-12+ MONOCYTES

Plasma levels of GH, prolactin, cortisol, and norepinephrine showed their well-known temporal patterns during the regular sleep-wake cycle (**Figure 2**). Compared with

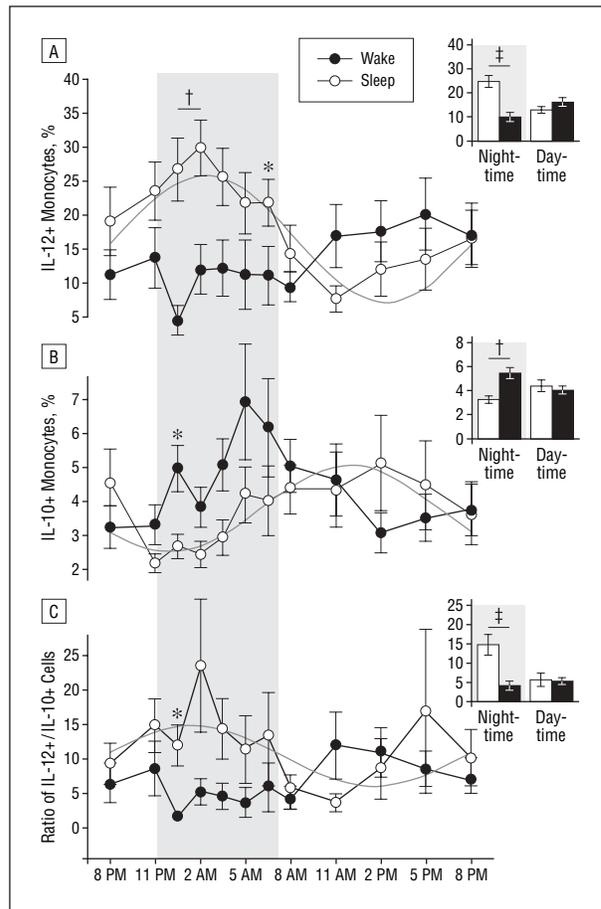


Figure 1. Mean \pm SEM numbers of cytokine-producing monocytes during the regular sleep-wake cycle (sleep) and continuous wakefulness (wake). Percentages of interleukin (IL) 12-producing (IL-12+) monocytes (A) and IL-10+ monocytes (B) and the ratio of IL-12+/IL-10+ monocytes (C) are shown. Grey lines illustrate adapted cosine curves for confirmed circadian rhythm in the sleep condition; shaded area depicts sleep time. Bar graphs (right) indicate mean \pm SEM values for nighttime and daytime. * $P<.05$. † $P<.01$. ‡ $P<.001$.

the levels during continuous wakefulness, nocturnal sleep distinctly increased concentrations of GH, particularly during the early night, and concentrations of prolactin ($F_{1,10}=18.9$ and $F_{1,10}=27.2$, respectively; $P<.001$ for condition \times period interaction). In contrast, norepinephrine plasma levels dropped during sleep ($F_{1,10}=5.4$; $P=.04$). Nighttime plasma cortisol concentrations were lower, as expected, than during daytime levels ($P<.001$, for 24-hour rhythm identified by cosinor analysis), but remained uninfluenced by sleep.

To elucidate whether the clear dependence of changes in IL-12+ and IL-10+ monocytes on sleep is linked to sleep-associated release of hormones, we calculated correlations between hormonal levels and percentages of IL-12+ and IL-10+ monocytes for the sleep condition (**Table 2**). Numbers of IL-12+ monocytes correlated positively with prolactin levels and negatively with cortisol levels and IL-10+ monocyte numbers, whereas IL-10+ monocyte numbers correlated positively with norepinephrine levels. Allowing a time lag of 3 hours for cortisol to act on IL-12+ monocytes increased the respective correlation to $r=0.38$ ($P<.001$).

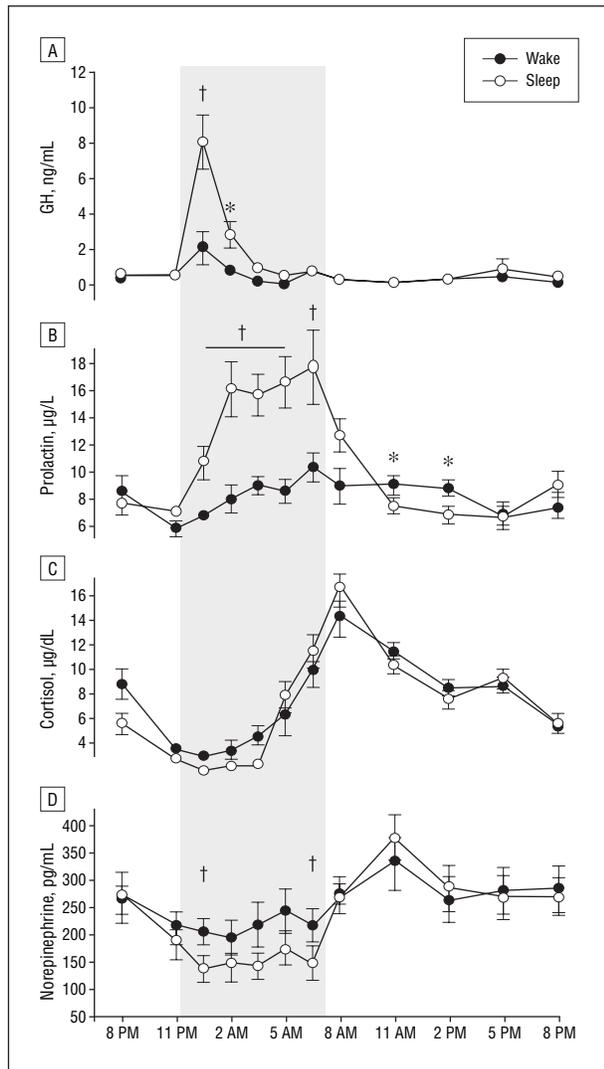


Figure 2. Mean \pm SEM hormone concentrations of growth hormone (GH) (A), prolactin (B), cortisol (C), and norepinephrine (D) during the regular sleep-wake cycle (sleep) and continuous wakefulness (wake) in 11 men. Shaded area indicates sleep time. To convert GH to picomoles per liter, multiply by 44; prolactin to picomoles per liter, multiply by 43.48; cortisol to nanomoles per liter, multiply by 27.59; and norepinephrine to nanomoles per liter, multiply by 0.00591. Significance is indicated for pairwise comparison between both conditions. * $P < .05$. † $P < .01$.

EFFECTS OF HORMONES ON IL-10+ AND IL-12+ MONOCYTES IN VITRO

To explore the contribution of the hormonal changes that are a hallmark of nocturnal sleep to the regulation of monocyte cytokine production, we performed supplementary experiments in which blood samples were drawn from sleeping subjects at 1 AM (when the sleep-associated hormonal changes are most pronounced) and determined numbers of IL-12+ and IL-10+ monocytes after addition of GH and prolactin antibodies, cortisol, and norepinephrine to block peak levels of GH and prolactin and to increase endogenous nadir levels of stress hormones. Prolactin antibody ($P < .05$) and cortisol at concentrations of 4 μ g/dL (110 nmol/L) ($P < .01$) and 20 μ g/dL (552 nmol/L) ($P < .001$), respectively, reduced the number of IL-12+ monocytes, whereas the number of IL-10+ monocytes

Table 2. Correlation Analyses*

	IL-12+ Monocytes	IL-10+ Monocytes
GH	+0.16	-0.12
Prolactin	+0.19†	-0.12
Cortisol	-0.25‡	+0.08
Cortisol at -3 h§	-0.38	+0.10
Norepinephrine	-0.16	+0.23†
IL-12+ monocytes		-0.47

Abbreviations: GH, growth hormone; IL-10+, interleukin (IL) 10-producing; IL-12+, IL-12-producing.

*Data are expressed as Pearson product moment correlation coefficients across subjects between percentages of cytokine-positive monocytes and blood concentrations of hormones during the regular sleep-wake cycle.

† $P < .05$.

‡ $P < .01$.

§Correlations with cortisol levels were additionally calculated for a time lag of minus 3 hours.

|| $P < .001$.

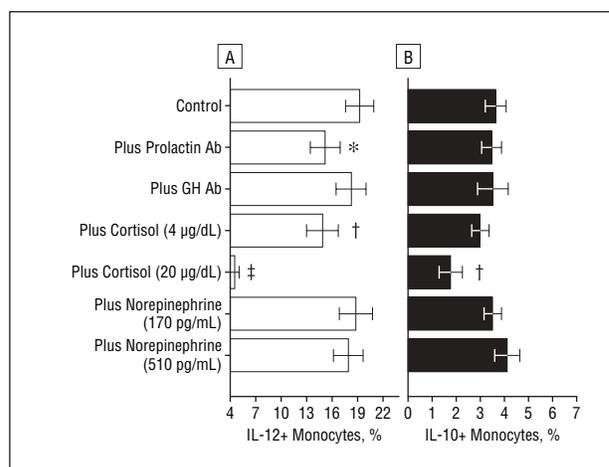


Figure 3. In vitro assessment (mean \pm SEM) of hormonal effects on cytokine activity. We evaluated interleukin (IL) 12-producing (IL-12+) (A) and IL-10+ (B) monocytes after substance administration in blood sampled at 1 AM from 12 sleeping men. Control values refer to untreated samples. Conversion factors for SI units are given in the legend for Figure 2. Ab indicates antibody; GH, growth hormone. Significance is indicated for pairwise comparisons with control values. * $P < .05$. † $P < .01$. ‡ $P < .001$.

was decreased at cortisol concentrations of 20 μ g/dL (552 nmol/L) ($P < .01$; **Figure 3**).

COMMENT

Our study shows that nocturnal sleep in humans increases the percentage of IL-12+ monocytes and concurrently decreases the percentage of IL-10+ monocytes. Thereby, sleep organizes in time the pattern of types 1 and 2 cytokine activity leading to rhythms that are synchronized to the regular sleep-wake cycle and absent during continuous wakefulness. Whereas the maximum number of IL-12+ monocytes occurs during nocturnal sleep (at 2:20 AM), the maximum number of IL-10+ monocytes occurs during daytime wakefulness (at 11:53 AM). Nocturnal sleep thus represents a condition where the type 1-type 2 cytokine balance is shifted maximally toward type 1 (ie, IL-12) activity.

Our results of a sleep-dependent nighttime increase in IL-12+ monocyte numbers agree with those of previous studies indicating a nocturnal peak of cytokine production in blood cultures during the regular sleep-wake cycle for IL-12¹⁹ and other type 1 cytokines (IL-2 and interferon- γ).^{2,20} Also consistent with our results, the ratio of interferon- γ and IL-10 production in lipopolysaccharide-stimulated whole blood cell culture was found to peak at approximately 4 AM during regular sleep-wake conditions,¹⁹ although IL-10 production in that study showed a diverging maximum at 9 PM. Such differences most likely reflect our assessment of cytokine activity linked to specific cells directly by means of flow cytometry at the single-cell level, whereas previous studies concentrated on a less specific assessment of cytokine activity in cell cultures¹⁹ or serum samples.²¹

The most striking and novel result of our experiments is the clear dependence of the cytokines' rhythm on sleep and its complete absence during continuous wakefulness. Obviously, 24-hour rhythms in monocyte-derived IL-12 and IL-10 and in the respective type 1–type 2 cytokine balance are induced primarily by sleep. Although, to our knowledge, the effects of sleep and wakefulness on IL-12 and IL-10 activity have not yet been thoroughly investigated, investigation of other cytokines has likewise revealed alterations in support for a sleep-dependent augmentation of type 1 responses.²² Thus, sleep increases production of the type 1 T-cell cytokine IL-2 in whole blood cell culture,²⁰ whereas numbers of T_H2 cells producing IL-4 is reduced during early sleep.⁶ Also, our findings are in line with those of studies^{6–10} that examined the effects of experimental sleep deprivation and disordered sleep in patients on the type 1–type 2 balance. The overall less consistent picture resulting from those investigations likely reflects the less specific assays used grasping type 1 cytokine activity mainly of T-cell origin.^{6,20} Our focus on monocytes as precursors of APC provides a more straightforward reflection of the critical mechanisms regulating the type 1–type 2 cytokine balance, because the interaction between APC and naive T cells represents the first and most important step that essentially determines the strength and type of the succeeding immune response.¹⁵

In search of potential neuroendocrine mechanisms underlying the sleep-induced shift in the type 1–type 2 cytokine balance, we measured plasma concentrations of several immunoactive hormones with characteristic secretory patterns during nocturnal sleep. Our data confirmed the well-known sleep-dependent rise in prolactin and GH concentrations, the latter focusing during the slow-wave sleep–rich early part of sleep.^{6,11} Plasma cortisol and norepinephrine concentrations were decreased during nighttime and enhanced during daytime, with only a slight amplification of the drop in norepinephrine concentration by sleep. Growth hormone and prolactin are known to be able to shift the type 1–type 2 balance toward type 1, whereas cortisol and norepinephrine can shift it toward type 2.^{23,24} Indeed, IL-12+ monocytes, prolactin, and GH on the one side and IL-10+ monocytes, cortisol, and norepinephrine on the other side exhibit strikingly parallel temporal dynamics across the sleep-wake cycle (Figures 1 and 2),

whereby the inverse relationship between these rhythms possibly reflects that they mutually inhibit each other. In line with this view, our analyses confirmed positive correlations between prolactin level and IL-12+ monocyte numbers and between norepinephrine level and IL-10+ monocyte numbers and, consistent with previous experiments,¹⁹ a negative correlation between cortisol level and IL-12+ monocyte numbers (Table 2). Suppressive actions of cortisol on mitogen-stimulated cytokine production are known to develop with some delay (1–4 hours),^{19,25} which explains why this negative correlation between cortisol level and IL-12+ monocyte numbers is distinctly increased when cortisol activity precedes that of IL-12+ monocytes by 3 hours. In addition, we observed a robust negative correlation between IL-12+ and IL-10+ monocyte numbers, which strongly speaks for a direct contribution of the well-known antagonism between these cytokines to their inverse time course across the sleep-wake cycle.²⁶

A contribution of prolactin (alone or in combination with other factors) to the sleep-induced shift toward type 1 cytokine activity is supported by our supplementary *in vitro* experiments. However, results from *in vitro* studies may not reflect *in vivo* conditions. In these experiments, the blockade of endogenous levels of prolactin with prolactin antibody led to a distinct reduction of IL-12+ monocyte numbers in sleeping subjects' blood. The same effect was achieved by adding cortisol to these sleep-associated samples containing minimum amounts of endogenous cortisol. Comparable effects of prolactin and cortisol on lipopolysaccharide-stimulated IL-12 production in cell culture have been previously reported.^{2,19,24,27–29} Cortisol *in vitro* not only led to the expected decrease in IL-12+ monocyte numbers²⁹ but, at concentrations of 20 $\mu\text{g/dL}$ (552 nmol/L), also reduced IL-10+ monocyte numbers. However, this effect was less pronounced, so that overall cortisol administration induced a bias toward type 2 cytokine activity that has been likewise reported by others.^{24,28–30} The failure of GH and norepinephrine to affect IL-12+ and IL-10+ monocytes in our *in vitro* study argues against an essential contribution of these hormones to the regulation of the type 1–type 2 balance, although in light of other studies such an effect cannot be entirely ruled out.²⁴ Other hormones not examined herein may also contribute to the sleep-associated shift toward type 1 cytokine activity. Melatonin is known to stimulate type 1 activity² and could have been suppressed by light exposure during nocturnal wakefulness. In a previous study¹⁸ using identical lighting conditions, there were no significant differences in melatonin levels between sleep and wakefulness. Therefore, it is unlikely that differences in melatonin levels could account for the observed effects of the sleep-associated shift of cytokine activity.

Nocturnal wakefulness suppressed IL-12+ monocyte numbers on average to approximately 40% of sleep levels and enhanced IL-10+ monocyte numbers to approximately 170% of sleep levels. The size of these effects is comparable to alterations seen in various clinical conditions.^{31,32} Patients with rheumatoid arthritis show alterations reminiscent of an exaggerated sleeplike pattern of immune and endocrine changes with high

IL-12/IL-10 ratios, high levels of prolactin, a hyporesponsive hypothalamic-pituitary-adrenal axis, and a maximum of type 1-dependent disease symptoms occurring during nocturnal sleep.^{19,24,27,33,34} A reversed pattern is evident in patients infected with human immunodeficiency virus, who typically exhibit low IL-12/IL-10 ratios that deteriorate prognosis, in combination with signs of weak cellular immunity, hypoprolactinemia, high levels of cortisol, and disturbed sleep.^{3,5,35,36} On this clinical background, our data implicate a critical function of sleep for organizing a balanced oscillation in type 1 and type 2 cytokine activity and corroborate the view that sleep supports adaptive immunity.^{5,11,37}

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