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Differential acute effects of sleep on spontaneous and stimulated production of tumor necrosis factor in men

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ABSTRACT

Tumor necrosis factor (TNF) is considered a key molecule in the regulation of sleep in health and disease. Conversely, sleep compared to sleep deprivation can modulate TNF release, but overall results are conflicting. In this study we focused on the influence of sleep on spontaneous, i.e., unstimulated TNF production, which might be involved in sleep regulation under normal non-infectious conditions, and on lipopolysaccharide (LPS)-stimulated TNF production, which reflects the capacity of the immune system to respond to a pathogen. To this end, we monitored 10 healthy men during a regular sleep-wake cycle and during 24 h of wakefulness while blood was sampled repeatedly to analyze circulating TNF levels in serum as well as intracellular TNF production in monocytes spontaneously and after stimulation with LPS employing whole blood cell cultures. In addition we assessed numbers of monocyte subsets and levels of various hormones in blood. In comparison with nocturnal wakefulness, sleep acutely decreased serum TNF levels, with no parallel decrease in spontaneous monocytic TNF production, but was associated with a striking nighttime increase in the percentage of TNF producing monocytes after stimulation with LPS. The following day circulating TNF showed a reverse pattern with higher levels after regular sleep than after the nocturnal vigil. The mechanisms mediating the differential effects of sleep on circulating TNF (acutely decreased) vs. stimulated monocytic TNF production (acutely increased) remain unclear, although explorative correlational analyses pointed to a regulatory involvement of cortisol, norepinephrine and prolactin. The acute enhancing effect of sleep on LPS stimulated monocytic TNF production adds to the notion that nocturnal sleep favors immune defense to a microbial challenge.

1. Introduction

In the bidirectional crosstalk between sleep and the immune system, tumor necrosis factor (TNF) is a key signal. TNF, together with other cytokines such as interleukin (IL)-1 is a 'sleep regulatory substance' and has a role in the homeostatic regulation of sleep in normal physiological conditions (Bryant and Curtis, 2013; Krueger et al., 1999). Thus, neuronal activity during wakefulness triggers the release of TNF, which in turn promotes non-rapid eye movement (NREM) sleep and enhances slow wave activity (Churchill et al., 2008; Krueger et al., 2008). In accordance with its sleep regulating function, brain levels of endogenous TNF fluctuate with the sleep-wake cycle with a peak during the early resting time and a trough after elapsed sleep (Bredow et al., 1997; Cearley et al., 2003; Floyd and Krueger, 1997; Krueger et al., 1999). Moreover, during experimental sleep deprivation, spontaneous TNF production in the brain is increased (Taishi et al., 1999; Zielinski et al., 2014). However, this pattern characteristic for a sleep regulatory substance has not been obtained in human studies measuring circulating TNF levels in blood: 24 h observations revealed either no overt rhythm (Gudewill et al., 1992; Togo et al., 2009; Vgontzas et al., 2004) or a morning peak in levels of TNF (Vgontzas et al., 2002, 2003). In sleep deprivation experiments in healthy subjects circulating TNF remained unchanged (Irwin et al., 2004; Lekander et al., 2013; Ruiz et al., 2012; Shearer et al., 2001; Vgontzas et al., 2007) or increased only at certain times after prolonged periods of sleep loss, i.e., in the afternoon after 34 h of total sleep

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deprivation (Chennaoui et al., 2011) or in the morning after 7 days of sleep restriction (Chennaoui et al., 2011; Vgontzas et al., 2004). However, chronic sleep disturbances were indeed found to be associated with robust increases in circulating TNF levels, in particular during the daytime period, in patients with insomnia (Vgontzas et al., 2002) and sleep apnea (Minoguchi et al., 2004; Nadeem et al., 2013), as well as in habitual short sleepers (Patel et al., 2009) and in pregnant women (Okun and Coussons-Read, 2007). Along with increases in circulating levels of IL-1, IL-6 and C-reactive protein (CRP) these signs of low-grade systemic inflammation have been proposed to explain the observed connection between sleep debt and an increased risk for inflammatory diseases (Ananthakrishnan et al., 2013; Grandner et al., 2013; Irwin, 2014; Kinnucan et al., 2013; Mullington et al., 2009; Seay et al., 2013).

Apart from these subtle but persisting increases in TNF during low-grade systemic inflammation, high amounts of TNF are produced chiefly by activated macrophages and monocytes upon an infectious challenge. As such TNF serves the acute regulation of local and systemic inflammation and, thus, the efficient eradication of pathogens. TNF boosts innate and adaptive immune responses to bacterial and viral infections through multiple mechanisms: it enhances (i) antigen-presenting cell activation, migration and maturation (Banchereau et al., 2000; Kaisho et al., 2001), (ii) phagocytosis (Hess et al., 2009), (iii) prostaglandin production (Baud et al., 1992), (iv) the acute-phase response (Gabay and Kushner, 1999), (v) T cell proliferation (Cope et al., 1994), and (vi) its own production via positive autocrine feedback mechanism (Blasi et al., 1994; Imamura et al., 1987). Employing stimulation with the toll-like receptor (TLR)2/TLR4 ligand LPS several human studies revealed a circadian rhythm in the production of TNF that peaks during the night (Entzian et al., 1996; Hermann et al., 2006; Petrovsky et al., 1998; Zabel et al., 1993). The contribution of sleep to this rhythm, however, is presently obscure as respective studies provided rather mixed outcomes with enhancing (Weil et al., 2009), suppressing (Uthgenannt et al., 1995), and no (Ashley et al., 2013; Born et al., 1997; Haack et al., 2001) effects of sleep on LPS-stimulated TNF production.

Against this backdrop in the present experiments we aimed to assess in humans the impact of sleep on both spontaneous and stimulated production of TNF by monocytes as well as on levels of TNF circulating in blood. Based on previous reports in sleep apnea patients (Minoguchi et al., 2004), we expected parallel changes in circulating levels of TNF and spontaneous production of TNF by monocytes, although the latter are only one of the sources of TNF in the body. We hypothesized that in our healthy subjects, both measures display the temporal pattern typical for a sleep regulatory substance, acutely decreasing across nocturnal sleep and increasing during subsequent wakefulness. In contrast, given the supportive function of sleep for host defense (Lange et al., 2011), for LPS-stimulated production of TNF we anticipated an acute sleep-dependent increase specifically during nighttime. In order to cover the possibility that effects of sleep on cytokine production are restricted to certain time intervals (Chennaoui et al., 2011), or even are opposite in direction during night and daytime (Redwine et al., 2000; Vgontzas et al., 1999), we performed repeated blood draws covering an entire 24-h period. In addition, to control for potential confounding influences of cell composition (Born et al., 1997) we analyzed TNF production on the single cell level in monocytes by flow cytometry and measured also the proportion of two monocyte subsets which are known to differ both in their capacity to produce TNF and in their regulation by sleep and the circadian system (Dimitrov et al., 2007, 2013; Nguyen et al., 2013; Ziegler-Heitbrock et al., 2010).

Because the effect of sleep on the immune system is believed to be caused by sleep-associated changes in immunoregulatory hormones such as growth hormone (GH), prolactin, testosterone, cortisol, epinephrine, and norepinephrine, we also measured these hormones in blood and performed supplementary in vitro experiments to elucidate their role in mediating the influence of sleep on LPS stimulated TNF production (Besedovsky et al., 2012; Bouman et al., 2005; Cipollaro de et al., 1998; Petrovsky, 2001; Severn et al., 1992).

2. Materials and methods

2.1. Participants

Ten physically and mentally healthy men were included in the study (mean age 25.3, range 18–30 years, mean body mass index 23.5, range 20–26 kg/m^2) from a larger parent trial that investigated sleep, circadian rhythm, and immune parameters such as IL-6, IL-12, and blood cells counts (Dimitrov et al., 2007, 2009). They were non-smokers, did not suffer from sleep disturbances, and were not taking any medication at the time of the experiments. None had a medical history of any relevant chronic disease or mental disorder. Acute illness was excluded by physical examination and routine laboratory investigation, including chemistry panel, CRP concentration <6 mg/L, and a white blood cell (WBC) count <9000/μL. Women were not included in the study to keep the subject sample most homogenous with regard to sleep and endocrine parameters.

The men were synchronized by daily activities and nocturnal rest. They had a regular sleep-wake rhythm for at least 6 weeks before the experiments. During the week preceding the study, they were required to turn off lights for nocturnal sleep between 11 pm and 11:30 pm, to get up by 7 am the next morning, and not to take any naps during the day. Adherence to these instructions was exclusively confirmed by questionnaires. All subjects spent one adaptation night in the laboratory in order to become accustomed to the experimental setting. The presence of signs of sleep disturbances, including apnea and nocturnal myoclonus, was excluded by interview and by recordings during this night. The study was approved by the Ethics Committee of the University of Lübeck, and all participants gave written informed consent.

2.2. Procedure

Experiments were performed according to a within-subject cross-over design. Each man participated in two experimental conditions, each starting at 8 pm and ending 24 h later. One condition ('sleep') included a regular sleep-wake cycle whereas in the other condition ('wake') subjects remained awake throughout the 24-h experimental period. Both experimental sessions for a subject were separated by at least four weeks, and the order of conditions was balanced across subjects. In the 'sleep' condition, sleep was allowed between 11 pm (lights off) and 7 am in the morning. On the 'wake' condition, subjects stayed awake in bed in a half-supine position between 11 pm and 7 am. During this time they were watching TV, listening to music and talking to the experimenter at normal room light (about 300 lux).

On both conditions, blood was sampled first at 8 pm, then every 1.5 h between 11 pm and 8 am, and every 3 h between 8 am and 8 pm the next day. Blood was sampled via an intravenous forearm catheter which was connected to a long thin tube and enabled blood collection from an adjacent room without disturbing the subject's sleep. To prevent clotting, about 700 mL of saline solution were infused throughout the 24-h experimental period. The total volume of blood sampled during a session was 250 ml. Blood samples were always processed immediately after sampling.

Sleep and sleep stages were determined off-line from polysomnographic recordings following standard criteria

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