The relationship between sleep and innate immunity

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Sleep regulates inflammatory responses, and the innate immune system affects sleep. Interleukin-1 beta, tumor necrosis factor alpha, growth hormone-releasing hormone, prolactin, and nitric oxide are somnogenic substances. Sleep deprivation, such as chronic insomnia or obstructive sleep apnea, affects cytokine production, glial function, natural killer cell activity, and inflammasome function. This review will discuss the relationship between sleep and innate immunity.

Keywords: Sleep, Innate immunity, Cytokines, Microglia, Inflammation

Introduction

Numerous studies have focused on the relationship between sleep and immunity. Conditions such as chronic insomnia or obstructive sleep apnea, which result in sleep deprivation, are reciprocally associated with innate immunity [1,2]. Sleep regulatory substances, which control sleeping patterns, exhibit oscillations in the brain according to sleep/wake cycles. Interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), growth hormone-releasing hormone, prolactin, and nitric oxide (NO) are known proinflammatory, somnogenic (sleep regulatory) substances [1,2]. When injected into the central nervous system (CNS), sleep regulatory substances systemically induce either an increase or decrease in sleep. Inhibiting or removing sleep regulatory substances leads to changes in sleep patterns, and such substances are altered in response to pathogens [1,3].

Both sleep and innate immunity are influenced by circadian rhythms (Figure 1). In humans, leukocyte trafficking increases with peak circulating immune cell numbers at night. Leukocyte oscillation does not occur without stimuli like the light-dark cycle. Macrophage function and inflammatory responses are also regulated by circadian rhythms. Key proteins including BMAL1 in the mammalian molecular circadian clock affect circadian-immune interactions, and CLOCK protein increases nuclear factor kappa B (NF-κB) pathway activity and proinflammatory cytokine production [1,4-7].

Various autoimmune disorders have been linked to sleep disturbances. Patients with autoimmune diseases often complain of sleep disorders. Autoimmune encephalitis (AE) patients commonly complain of sleep disturbances such as hypersomnia, insomnia, and parasomnia [8,9]. It has been demonstrated that sleep and circadian disturbances interact with autoimmune diseases such as systemic lupus erythematosus (SLE) and multiple sclerosis. There are suggestions that sleep deprivation plays a role in the etiology of autoimmune diseases in animal models of SLE and reports linking short sleep duration to progression to SLE [10]. In patients with rheumatoid arthritis, dysregulation of the sleep-wake cycle occurs due to changes in the inflammatory profile, leading to increased pain severity via excessive inflammatory activity.
This review aims to explore the relationships and mechanisms between sleep and the innate immune system.

**Main text**

**Sleep regulates innate immunity**

Lack of sleep influences immunity and affects both innate immunity and the secretion of inflammatory cytokines. Consequently, lack of sleep is associated with conditions related to immune mechanisms, such as cardiovascular disease, depression, and type 2 diabetes mellitus. In animal experiments, continuous sleep deprivation in mice induces an IL-2Rγ-dependent cytokine storm-like inflammatory syndrome that results in death within four days. Sleep deprivation increases efflux of prostaglandin D2 (PGD2) across the blood–brain barrier via adenosine triphosphate (ATP)-binding cassette transporter C4 (ABCC4) [12,13]. Sleep deprivation diminishes natural killer (NK) cell function, leading to an increased risk of viral infections and heightened cancer risk [14,15]. Decreased antibody production also renders individuals more vulnerable to common infections. Additionally, sleep disturbances are associated with the hypothalamic-pituitary-adrenal axis and inflammatory cytokines such as IL-6 and TNF-α [16]. Inflammasomes, in particular, the nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, are related to short sleep duration [17].

**Microglia**

Microglia, innate immune cells of the CNS, respond to sleep deprivation by secreting inflammatory signals and exhibit a sleep-bound response. Microglia are responsible for homeostatic neuromodulatory function during sleep. Inhibiting microglial cytokine production results in a decrease in non-rapid eye movement (NREM) sleep and a reduction in delta power, following sleep deprivation. Although the exact mechanisms remain unclear, microglia exhibit a reactive disposition after sleep deprivation, which is evidenced by changes in morphology, motility, phagocytic behavior, and secretion of inflammatory cytokines. Furthermore, there is an association between the inflammatory activity displayed by microglia and the drive for sleep [18].

**Natural killer cells**

NK cells play a crucial role as an early defense mechanism against pathogens and in recognizing virus-infected cells and tumor cells. As sleep deprivation affects immune surveillance, a study targeting individuals with short sleep duration showed that NK cell activity was decreased in those who self-reported short sleep duration [14]. Additionally, sleep deprivation is associated with poor prognosis in cancer patients. In animal experiments, chronic sleep deprivation in mouse models led to a decrease in NK cells and increased tumor progression due to immunosuppression in the tumor microenvironment [19].

**Inflammasomes**

Inflammasomes are complexes of receptors and sensors of the innate immune response. NLRP3 is one of the well-recognized inflammasomes that is activated by pathogen-associated molecular patterns and danger-associated molecular patterns, initiates inflammatory responses by enhancing proinflammatory cytokines, and activates NF-κB signaling pathways [17]. In a study with chronic insomnia patients with objectively determined short sleep duration, the NLRP3 inflammasome was upregulated compared to healthy controls. NLRP3 inflammasome activation was associated with short sleep duration, reduced slow wave sleep, and sleep fragmentation. This was evidenced by experimental assessment of NLRP3 inflammasome protein levels, including NLRP3, apoptosis-associated speck-like protein containing a caspase recruitment domain, caspase 1, IL-1β, and IL-18 cytokine levels [20].

**Sleep alters cytokine levels**

Sleep deprivation modifies molecular mechanisms that stimulate cellular immune activation and trigger the production of inflammatory cytokines. Sleep rebound is marked by aug-
mented sleep duration and heightened sleep intensity, delineated by electroencephalogram (EEG) delta wave amplitude. Sleep homeostasis stands as a defining attribute of sleep, with its mechanisms likely entailing the generation and release of sleep regulatory substances, including IL-1 and TNF [7,21]. TNF-α protein levels increase in the brain under both arousal and sleep deprivation conditions [1,7].

When morning blood samples were collected from 30 healthy adults who had insufficient nocturnal sleep over a period of three days, gene transcription analyses were conducted based on DNA microarray technology to assess monocyte intracellular proinflammatory cytokines. The transcription of IL-6 messenger RNA (mRNA) increased three-fold, while TNF-α mRNA increased two-fold [12,22].

A study compared control and cognitive behavioral therapy (CBT) in 72 hemodialysis patients with sleep disturbances. The CBT group showed improvement in indicators like the Pittsburgh Sleep Quality Index, the Fatigue Severity Scale, the Beck Depression Inventory, and the Beck Anxiety Inventory. Additionally, high-sensitivity C-reactive protein (CRP), IL-18, and oxidized low-density lipoprotein levels were decreased in the CBT group [23]. In a randomized control trial with insomnia patients aged 55 or older, the CBT treatment group showed reduced levels of inflammatory markers like CRP and decreased monocyte production of proinflammatory cytokines [24].

**Innate immunity affects sleep**

**Glia**

Glia comprise approximately 90% of brain cells and interact with neurons to modulate inflammation and participate in the secretion and clearance of neurotransmitters through autocrine and paracrine mechanisms. Because of their electrical actions, glia are likely a key component in the genesis of sleep [1,25]. Furthermore, microglia, one of the prominent innate immune cells in the CNS, are associated with the homeostatic neuromodulatory process during sleep. It is well-documented that the administration of substances such as minocycline, which inhibits microglial cytokine production, results in a reduction in NREM sleep. This phenomenon resembles an increase in brain proinflammatory signals observed during the sleep rebound response, which occurs after prolonged wakefulness [18].

**Cytokines**

Sleep regulates cytokine expression, while cytokine release alters sleep. In the 1980s, it was observed that administering cerebrospinal fluid from sleep-deprived dogs to control dogs resulted in increased sleep. This finding led to the hypothesis that substances regulating sleep are produced in response to waking, and later studies revealed their association with inflammation [1,3,26]. IL-1, TNF, and IL-6 have been found at increased levels during sleep or in the early morning [26]. In addition, IL-1β and TNF-α alter the expression of circadian genes, including altering E-box regulatory element activation through CLOCK-BMAL [27,28].

1) **IL-1β**

The central or systemic injection of IL-1 or TNF has been observed to prolong the duration of NREM sleep and increase EEG delta power in various animal models and humans. Administration of inhibitors such as soluble receptors or IL-1RA suppresses spontaneous sleep. The cytokine IL-1 activates sleep-active neurons in the hypothalamus but inhibits wake-active neurons [7].

2) **TNF-α**

The somnogenic function of TNF-α as an endotoxin-induced cytokine and endogenous pyrogen associated with tumor necrosis was elucidated in 1987. The TNF-α receptor TNF receptor 1 is produced in various CNS cells, including neurons and glia, while TNF receptor 2 is found in immunocytes. TNF-α activates multiple inflammatory cellular pathways, including the NF-κB signaling pathway [1,7]. Systemic or central injection of TNF-α increases the duration of NREM sleep and enhances EEG delta power during NREM sleep. Administration of high concentrations of TNF-α reduce the duration of REM sleep [1,7]. In rabbits treated with muramyl dipeptide, a somnogenic substance from bacterial cell wall peptidoglycan that prolongs NREM sleep duration, the administration of TNF-α inhibitor suppressed the response to muramyl dipeptide. Additionally, increased TNF-α production has been observed in pathogen-induced NREM sleep, such as bacterial or influenza infections [1,29].

3) **IL-6**

IL-6 has pleiotropic effects and somnogenic influence in that it stimulates the hypothalamic-pituitary-adrenal axis, promoting the secretion of adrenocorticotropic hormone, cortisol, and growth hormone. Administration of IL-6 induces reductions in delta sleep during the initial half of the night. In inflammatory conditions, elevated levels of IL-6 cause sleep-related symptoms such as somnolence and fatigue and trigger activation of the hypothalamic-pituitary-adrenal axis [30]. When IL-6 is administered to rats, it brings about
dose-dependent changes in NREM sleep. Reports have shown changes in fatigue and sleep architecture, including decreased slow wave sleep in healthy adults, following injections of recombinant human IL-6 [5]. A study conducted on rabbits administered IL-6 via intraventricular injection in varying doses showed fever but no changes in sleepwake activity, suggesting a low likelihood of somnogenic function [31]. In a study targeting chronic insomnia patients, a four-day consecutive sleep laboratory recording was conducted, and 24-hour IL-6 plasma level measurement was performed. Increased daytime secretion of IL-6 adversely affected quality of life and sleep quality. The peak time of IL-6 secretion also shifted from the morning to the evening hours, from 4 AM to 7 PM, in the insomnia group compared to the control [32]. IL-6 is also related to sleep quality. Sleep loss results in excessive IL-6 secretion during the day, and daytime IL-6 plasma concentration inversely correlates with the quantity of slow wave sleep [33,34]. In addition, subcutaneous administration of IL-6 prior to sleep initiation in healthy subjects led to delayed REM sleep latency and decreased REM sleep duration, with no alteration in the total amount of NREM sleep [35].

4) Prostaglandin
The enzyme cyclooxygenase (COX) converts arachidonic acid to prostaglandin H2 [36]. COX-2, found in most mammalian cells including macrophages and microglia, modulates inflammation and sleep. Inflammatory sleep regulatory substances, such as IL-1β and TNF-α, induce the expression of COX-2. Inhibiting COX-2 activity attenuates spontaneous NREM sleep and sleep induced by TNF-α [7,36]. Additionally, sleep is inhibited by the COX-1 and COX-2 inhibitor acetaminophen and PGD2 and prostaglandin E2, which are downstream products of COX activity and are implicated in the regulation of sleep and wakefulness [37,38].

Sleep deprivation increases the efflux of PGD2 across the blood-brain barrier via ABCC4. Increased blood concentration of PGD2 also stimulates increased levels of circulating neutrophils, an effect that is dependent on the PGD2 receptor 1 (DP1). Blocking PGD2 efflux by inhibition of ABCC4 or DP1 or in Ptgds−/− or Abcc4−− mice reduces neutrophilia and increases the survival of sleep-deprived mice [13].

Cellular inflammatory pathways
Multiple inflammatory pathways relate to sleep regulation and modulate both sleep regulatory substances and sleep itself. Among these pathways, IL-1β and TNF-α are particularly noteworthy as they not only upregulate themselves, but also influence the expression of multiple other cytokines. NF-κB, NO, and COX inflammatory cellular pathways are implicated in the regulation of sleep, while other cellular pathways also play a role in sleep patterns.

NF-κB, a transcription factor, responds to cytokines, pathogens, neurotransmitters, and ATP [39]. Present in all nucleated cells, including neurons and glia, NF-κB transcriptionally activates proinflammatory sleep regulatory substances, such as IL-1β and TNF-α, following its activation and nuclear localization. In contrast, anti-inflammatory and anti-somnogenic sleep regulatory substances, like IL-4 and IL-10, inhibit NF-κB activation. Inhibition of NF-κB activation has been shown to reduce spontaneous NREM sleep [40]. Interestingly, mice lacking the NF-κB p50 subunit exhibit enhanced spontaneous NREM and REM sleep, with heightened responsiveness to lipopolysaccharide resulting in increased NREM and attenuated REM sleep [41]. The NF-κB activation process is regulated by numerous pro- and anti-inflammatory molecules; thus, NF-κB appears to serve a pivotal regulatory role in the control of sleep, although the full extent of its inflammatory-related interactions with sleep remains to be elucidated.

NO synthase (NOS) catalyzes the conversion of arginine to NO in the presence of nicotinamide adenine dinucleotide phosphate and dioxygen. Inducible NOS (iNOS) and neuronal NOS (nNOS) are produced by various brain cells, including macrophages and microglia. The sleep regulatory effect of NO is complex and influenced by various sleep regulatory substances. Brain iNOS exhibits circadian variation and increases with sleep propensity [42], as evidenced by elevated levels during sleep deprivation in rats. Administration of NO precursors or donors enhances both NREM and REM sleep, while inhibition of iNOS suppresses sleep [43]. Mice lacking iNOS or nNOS exhibit reduced NREM sleep and enhanced REM sleep in response to influenza challenge compared to their counterparts possessing these genes.

Infection and autoimmune disease
After infection, alterations in sleep occur due to microbial production mediated by toll-like receptors or NOD-like receptors. In animals, the total duration of slow wave sleep decreases after infection, while the duration of REM sleep decreases in severe inflammatory states [29]. The epidermal antimicrobial peptide gene acts as a somnogen in Caenorhabditis elegans, promoting sleep via epidermal growth factor receptor signaling [44]. Infection is accompanied by excessive slow wave sleep and fever, which are believed to be regulated by the hypothalamus despite being governed by different areas. During normal NREM sleep, brain temperature decreases
es, leading to a reduction in brain metabolic rate and the execution of restorative functions [29]. Pattern recognition receptor activation induces an inflammatory response with the production of sleep regulatory substances, such as IL-1 and TNF, which reach the brain and promote NREM sleep. In higher doses, these sleep regulatory substances may also suppress REM sleep [35].

Narcolepsy, considered to have an autoimmune pathogenesis, involves the loss of hypocretin neurons in the lateral hypothalamus. The incidence of narcolepsy increased during the 2009 H1N1 influenza pandemic, and streptococcal infection is also considered a possible environmental risk factor [45].

AE is a CNS autoimmune disorder, and sleep disturbance may co-occur. A review published by Koo [8] in 2023 suggested sleep disturbances in AE and specific AE linked to sleep disorders. Insomnia is mostly reported to be associated with autoantibodies. Sleep breathing disorders such as hypoventilation are observed in N-methyl-D-aspartate receptor encephalitis and sleep apnea in immunoglobulin-like cell adhesion molecule 5 (IgLON5) disease; hypersomnolence in leucine-rich glioma-inactivated 1 (LGII) and Ma antibodies; narcolepsy in Ma and Hu antibodies; REM sleep behavior disorder in contactin-associated protein-like 2 (Caspr2), LGI1, IgLON5, and Ma antibodies; and disorders of arousal including NREM parasomnia in Caspr2 and IgLON5 [9,46]. Diurnal variations in rest-wake activity and sleep are regulated by the suprachiasmatic nucleus, the master circadian clock, located in the hypothalamus. In a case of AE associated with circadian disruption, with antibodies against Caspr2 characterized by nocturnal insomnia and daytime hypsomnolence, circadian fluctuations in melatonin level were absent, and the overall melatonin concentration was reduced. In addition, hypothalamic and endocrine disruptions in AE were reported in Ma1/Ma2 antibody encephalitis cases [46].

**Summary**

Sleep is pivotal in regulating innate immunity by influencing the secretion of inflammatory cytokines. Continuous sleep deprivation induces an inflammatory state, reduces NK cell function, and alters microglial activity and dysregulation of the NLRP3 inflammasome, which impact sleep patterns. Glial cells, especially microglia, contribute to the homeostatic neuromodulatory process during sleep, influencing sleep patterns. Cytokines such as IL-1β, TNF-α, and IL-6 play essential roles in the bidirectional relationship between sleep and inflammation, modulating sleep patterns and being influenced by sleep deprivation. Crucial cellular inflammatory pathways, including NF-κB and NOS, regulate both sleep and immune responses to pathogens. Infections induce changes in sleep patterns through toll-like receptors, demonstrating the intricate interplay between sleep, immunity, and infection. Examining the potential consequences of sleep deprivation on innate immunity and exploring the mechanisms by which innate immunity modulates sleep could yield valuable insights. Understanding sleep physiology related to innate immunity has the potential to advance our clinical approaches to disorders related to both sleep and the immune system.

**Conflicts of Interest**

Kyung-Il Park has been Editor-in-Chief of *Encephalitis*, and he was not involved in the review process of this article. No other potential conflict of interest relevant to this article was reported.

**Author Contributions**

Conceptualization, Supervision: Lee Y, Park KI; Data curation, Formal analysis, Methodology, Visualization, Investigation, Resources, Software, Validation: Lee Y; Project administration: Park KI; Writing–original draft: Lee Y; Writing–review & editing: Lee Y, Park KI.

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