

Molecular mechanisms in allergy and clinical immunology

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Links between the innate immune system and sleep

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Sleep is a fundamental physiologic process with unknown functions. It is divided into 2 distinct states: non-rapid-eye-movement sleep and rapid-eye-movement sleep. After acute infection with nonneurotropic agents, there are stereotypic changes in non-rapid-eye-movement sleep, particularly increased time spent in slow-wave sleep, and often a reduction of time spent in rapid-eye-movement sleep. It is now recognized that both infection-associated sleep and spontaneous sleep are regulated, in part, by immune mediators called cytokines. This review provides brief tutorials on the elements of the innate immune system that detect infection, how sleep is characterized in the laboratory, issues regarding the interpretation of sleep effects on immune function, the interaction of sleep with circadian rhythms and stress, and some of the microbial products, cytokines, and neuropeptides associated with sleep regulation. We also summarize our current understanding of the role of sleep in host defense and asthma exacerbation. (*J Allergy Clin Immunol* 2005;116:1188-98.)

Key words: Sleep, infection, innate immunity, virus, bacteria, toll-like receptors, cytokines, circadian rhythms, stress, asthma

The innate immune system of mammals comprises numerous antimicrobial mechanisms,¹ some of which can be traced back to the first multicellular organisms. Until recently, study of the innate immune system has taken a back seat to the more evolutionarily advanced acquired immune system. In the last decade, however, the emphasis in immunology has shifted to innate immune mechanisms with the discovery of certain key concepts and molecular classes described below.² The innate immune system appears to have 2 primary functions: rapid isolation and destruction of invading pathogens (or foreign cells, such

Abbreviations used

APR: Acute-phase response
dsRNA: Double-stranded RNA
EEG: Electroencephalographic
GHRH: Growth hormone-releasing hormone
NK cells: Natural killer cells
NLR: NACHT-leucine-rich repeat bearing proteins
NOD: Nucleotide-binding oligomerization domain
NREMS: Non-rapid-eye-movement sleep
PAMP: Pathogen-associated molecular pattern
PGD₂: Prostaglandin D₂
REMS: Rapid-eye-movement sleep
SWS: Slow-wave sleep
TLR: Toll-like receptor

as tumors or transplants) through inflammatory processes and antigen recognition and processing for the acquired immune system. The acquired immune system, in turn, uses antibodies and cytotoxic cellular mechanisms that help clear residual microorganisms and, through immunologic memory, speed up their detection and removal in future reinfections.

In the context of infection, both of these innate immune functions require distinguishing pathogenic microorganisms from the self. In recent years, microbiologists have characterized an evolutionarily conserved receptor system that appears to be the major cell membrane-bound system for pathogen recognition. This receptor system has been named the Toll-like receptor (TLR) system after the Toll system of fruit flies,³ where this class of pathogen-associated molecular pattern (PAMP) recognition receptors was first identified. Currently, TLRs comprise 13 receptors (some found in mice, some found in human subjects, and most shared by both species⁴) that recognize a range of PAMPs unique to microorganisms. These PAMPs include the LPS on the surface of gram-negative bacteria (TLR4), lipopeptides on gram-positive bacteria and mycoplasma (TLR2, TLR1, and TLR6),^{5,6} fungal polysaccharides (TLR2), bacterial flagellae (TLR5 and TLR11),⁶ unmethylated bacterial and viral DNA (TLR9),⁷ guanosine-uridine-rich viral RNA (TLR7 and TLR8),⁷

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or the double-stranded RNA (dsRNA) induced during viral replication (TLR3).³ TLRs all possess amino-terminal leucine-rich repeats that are responsible for PAMP recognition, as well as a carboxy-terminal TLR1 receptor domain required for intracellular signaling.⁶ Most TLRs are expressed on the cell surface, but those that recognize nucleic acids are all expressed in endosomal compartments.⁶ Soluble factors are also involved in pathogen recognition and can work in association with TLRs and with more generic scavenger, complement, and lectin receptors on phagocytes.¹

Selected bacterial PAMPs, the peptidoglycans, are also sensed by a family of cytoplasmic proteins, termed the nucleotide-binding site/leucine-rich repeat proteins.⁸ This family of pathogen recognition receptors has recently been dubbed NLRs (NACHT-leucine-rich repeat bearing proteins) for convenience.⁹ The best studied members of this cytoplasmic system are the nucleotide-binding oligomerization domain (NOD) proteins NOD1 and NOD2.⁶ Peptidoglycans are a major structural component of all bacterial cell walls, and NODs can distinguish gram-negative and gram-positive peptidoglycans.¹⁰ There is controversy as to whether peptidoglycans are recognized by TLR2 on the cell surface, as well as intracellular NLRs.¹¹ Elucidation of the PAMPs for TLRs and related proteins have provided valuable insights into the cause of such human diseases as Crohn's bowel disease and recurrent bladder infections.⁶

Major effector cells in the innate immune system are the circulating phagocytic leukocytes (neutrophils and monocytes), natural killer cells (NK cells), natural killer T lymphocytes (NK T cells), and $\gamma\delta$ T lymphocytes. Fixed macrophages and dendritic cells are widely distributed throughout tissues. All of these cells express TLRs that recognize pathogens. TLRs have only recently been found on NK cells¹² and $\gamma\delta$ T cells.¹³ A central function of phagocytes is to recognize, engulf, and (ideally) destroy pathogens through several mechanisms, often involving oxygen and nitrogen free radicals. NK cells and cytotoxic NK T cells also play a key role in eliminating infected or otherwise altered cells, such as tumor cells.^{2,14} Dendritic cells are key antigen-processing cells and form a central link between innate and acquired immunity.² Tissue mast cells (which also express TLRs¹⁵) and enterochromaffin cells¹⁶ also play a role in innate immunity through release of vasoactive factors, such as histamine and serotonin. All of these cells release cytokines when invaded by microbes or when stimulated by other cytokines or mediators released in the course of inflammation.

Cytokines are an enormously complex network of peptide-signaling molecules that are synthesized by immune cells activated by PAMP recognition.¹⁷ Cytokines are also made by infected epithelial cells, endothelial cells, and virtually any other cell when appropriately stimulated, although the specific types made¹⁸ and quantities produced per cell might differ from phagocytes. More than 100 cytokines have been identified, several of which are key regulators of allergy and asthma responses.¹⁹ The cytokines are generally classified as proinflammatory

(type I) or anti-inflammatory (type II) and include classical endocrine hormones, such as prolactin and growth hormone, as well as chemotactic chemokines and immunomodulatory type I IFNs as subsets.¹⁴

The release of cytokines from infected cells alerts neighboring cells through paracrine mechanisms that the host is under attack. This cytokine signaling induces protective cytokines (eg, IFNs) in those neighboring cells and stimulates chemotaxis of inflammatory cells, such as neutrophils, to supplement local defenses. In sufficient concentrations cytokines spill into the lymph and blood to act on the brain, liver, and bone marrow. Circulating cytokines act on brain capillary endothelium to induce pyrogenic prostaglandins and enter the brain parenchyma through specific transporters and at sites lacking a blood-brain barrier, such as the organum vasculosum of lamina terminalis and the median eminence of the hypothalamus.²⁰ Once in the brain, proinflammatory cytokines induce themselves, as well as prostaglandins and anti-inflammatory cytokines.²¹ Extensive evidence indicates that cytokines also signal the brain through the vagus nerve (although this has recently been questioned²²) and can thereby induce cytokine synthesis in selected regions of the brain.²⁰ The accumulated systemic and brain proinflammatory cytokines initiate a complex and protective physiologic response termed the acute-phase response (APR).²³ The most commonly studied physiologic APR is fever. Our laboratory has characterized one of the even more complex APRs, excess slow-wave sleep (SWS). Several recent reviews^{17,24-32} have discussed the humoral regulation of physiologic and pathologic sleep by microbial products, cytokines, and hormones. This article will summarize and update these reviews with respect to selected microbial products, infective organisms, cytokines, and neuropeptides. In addition, we will discuss our limited knowledge of the host defense role of sleep. Finally, we will discuss the relationship of sleep to asthma.

BASICS OF SLEEP

We spend a third of our lives asleep, but despite a century of study,³⁰ we have very little understanding of why we sleep. All mammals sleep, although in radically different amounts and patterns. For example, aquatic mammals sleep on one side of the brain at a time to avoid drowning.³³ In an effort to better understand physiologic sleep, our studies have focused on sleep alterations that occur after challenge with microbial products of the type detected by TLRs and NODs or after actual infection.

Whether physiologic or pathologic, sleep is divided into 2 states: non-rapid-eye-movement sleep (NREMS; quiet sleep) and rapid-eye-movement sleep (REMS; paradoxical sleep or dream sleep). These 2 states manifest very differently and appear to be regulated by different regions of the brain.³⁴ NREMS and REMS are defined by electroencephalographic (EEG) brain wave patterns, the amount of eye movement, and brain temperature by using EEG

electrodes, electromyographic electrodes, and thermistors applied in or near the brain, respectively.³⁵ The process of collecting and integrating all of these various data to quantify sleep states is termed polysomnography.

NREMS is divided into 4 stages in human subjects, the latter 2 of which are characterized by high-amplitude EEG slow-wave activity (also called delta waves: frequencies between 0.5 and 4 Hz). In human subjects sleep is usually consolidated into an 8-hour period during the night. In contrast, most mammals are polyphasic sleepers. For instance, a rat SWS episode seldom lasts more than a few minutes, and sleep episodes occur throughout the day and night, although as a nocturnal species, they do sleep more during the day. We have observed in animals that the total amount of SWS is increased during infection or after challenge with microbial products or certain cytokines.²⁵ Another characteristic of infection-associated SWS is increased amplitudes of the EEG delta waves, which are thought to be a measure of sleep intensity.³⁶ Time spent in NREMS and slow-wave amplitudes are the 2 measures that best characterize sleep changes after infection.

REMS comprises about 25% of the sleep time in human subjects but less than 10% of the sleep time in rodents.³³ Time in REMS is reduced in more severe inflammatory states. One of the intrinsic regulators of REMS appears to be endogenous type I IFNs because spontaneous REMS is substantially suppressed in the absence of the type I IFN receptor.³⁷

Frequently, excess SWS and fever (or hypothermia in small animals) coincide in infections, and because both are induced by the same stimuli, they are often assumed to be linked. Both SWS and body temperature are regulated by the hypothalamus, although by different regions. There are many circumstances in which these 2 APRs can be distinguished, and they appear to be independently regulated.³⁸ In fact, brain temperature characteristically decreases along with decreasing brain metabolic rate in normal NREMS, which is thought by some to reflect a restorative or restful function of NREMS.³⁹

Sleep regulation is intimately associated with circadian rhythm regulation. Obvious evidence of the association is that diurnal species sleep at night, whereas nocturnal species sleep primarily during the day.⁴⁰ Although sleep rhythms can be disrupted by eliminating external cues, such as light-dark cycles, when those cues are available, sleep rhythms are clearly controlled by a circadian pacemaker in the suprachiasmatic nucleus of the hypothalamus, as are temperature rhythms and numerous other biologic rhythms.⁴⁰ Several genes expressed both in the suprachiasmatic nucleus and in nonneural tissues are involved in circadian rhythm regulation,⁴¹ and the use of gene microarrays reveals that expression of several hundred genes in the mouse liver vary in a cyclical pattern over the course of a 24-hour day.⁴¹ Currently, it is believed that the timing of sleep and wakefulness is mediated by 2 interconnected processes: a homeostatic regulatory process that increases during waking and decreases during sleep, together with a circadian clock-dependent mechanism.⁴¹ Clock-dependent

mechanisms clearly influence sleep-wake cycles during jet lag and appear responsible for chronic sleep disorders associated with inappropriate sleep-wake cycles, such as those that occur in shift workers.⁴¹ The genes involved in circadian regulation of sleep are being vigorously investigated. Genes that appear important for sleep are the period genes and the cryptochrome genes that regulate them.⁴²

SLEEP DEPRIVATION

The importance of sleep for various host functions is generally studied by examining the effects of either acute or chronic sleep deprivation on the function of interest. Regrettably, sleep deprivation paradigms are not standardized, and it is difficult to compare results from different laboratories. More importantly, it is not possible to isolate sleep as an independent variable because during sleep, almost every physiologic variable changes relative to wakefulness. Furthermore, sleep regulation is intimately associated with the hypothalamic-pituitary axis,⁴³ as indicated by the inhibition of spontaneous sleep with antagonists of corticotrophin-releasing hormone, such as astressin.⁴⁴

Although prolonged sleep loss is not associated with classical stress markers,^{29,45} perhaps because of hypothalamic failure,⁴⁶ acute sleep loss is a potent stimulus of stress hormones in animals.^{45,47,48} The stress component might reflect the method used for overcoming the sleep urge because human volunteers do not demonstrate the increases in glucocorticoids seen in laboratory animals,⁴⁹ perhaps because they make a conscious decision to participate. (Alternatively, the sleep deprivation methods used in animals might be inherently more stressful than those used in human volunteers.) Experimental studies comparing REMS deprivation in rats with other classical laboratory stressors such as foot shock and restraint reveal that stress hormone responses, such as increased corticosterone and decreased testosterone levels, are greatest after acute REMS deprivation and are similar in rats to those seen after foot shock, a potent stressor.⁴⁷ Subacute sleep deprivation induces stress ulcers in rats.⁵⁰ Restraint stress, well known to be immunosuppressive,⁵¹ is a much milder inducer of stress hormones than is REMS deprivation,⁴⁷ but it can lead to an increase in REMS in rats.⁵² Regardless, acute stress is a powerful modulator of immune function,⁵¹ and the effects of sleep deprivation on immunity in animals must be interpreted with care. Furthermore, most studies of immune competence in human volunteers use stimulated blood leukocyte responses. Such *ex vivo* studies can be confounded by the catecholamine increases associated with sleep deprivation,⁵³ which in turn might result in alterations in white blood cell populations that can lead to sampling errors.⁵⁴ Functional studies, such as analysis of vaccine responses, might give a more accurate assessment of sleep deprivation effects on immunity in human subjects than can classical *ex vivo* analyses.

EXCESS SWS AS AN APR

Sleepiness is anecdotally a frequent early response to acute infections, especially during more debilitating infections with agents such as influenza virus. The need to lie down and sleep can be overwhelming in such infections. Sleepiness and excess sleep are reported subjectively after viral challenge infections in human volunteers⁵⁵ and natural infections associated with fatigue, such as infectious mononucleosis.⁵⁶ More objective polysomnographic measurements have been conducted in human subjects with infectious mononucleosis⁵⁷ or HIV-1⁵⁸ (chronic infections) or challenged with a rhinovirus causing the common cold (an acute infection).⁵⁹ Although fatigue and excessive sleep time are commonly seen in infectious mononucleosis, sleep architecture (ie, relative amounts and quality of NREMS and REMS) is not changed.⁵⁷ In HIV-1-infected asymptomatic patients,⁶⁰ striking increases in SWS are seen before the onset of clinical AIDS.⁵⁸ Increased SWS has also been induced with certain HIV viral proteins,⁶¹ although large doses are required, and rigorous removal of microbial contaminants⁶² (easily acquired during purification⁶³) has not been conducted in these studies. In the localized rhinovirus infection, total sleep is reduced, perhaps as a result of discomfort.⁵⁹ (These findings of reduced sleep contradict self-report studies conducted after rhinovirus challenge.⁵⁵) A fatal rabies virus (a classical neurotropic virus) infection of the mouse brain results in an almost total loss of NREMS over time, with substantial increases in awake time, which is consistent with the insomnia seen in human patients with rabies.⁶⁴ A more severe strain of rabies results in early development of a pathologic sleep state during which REMS and waking are no longer detectable in the EEG spectrum.⁶⁴

Because it is difficult to conduct polysomnographic studies in sick human subjects, animal models with non-neurotropic agents have been devised to further characterize infection-associated sleep changes.³⁵ As indicated earlier, quantification of sleep is a complex process requiring maintenance of electrode placement and continuous monitoring of EEG patterns, eye motion, and brain temperature,³⁵ and only in the last 25 years has prolonged polysomnography become routine in small experimental animals.

Because of the notable association of excess sleep with influenza infections in human subjects (although not documented with polysomnography), influenza has also been the infection model most extensively investigated in animals with respect to sleep characteristics.

Initially, studies of sleep in response to influenza were conducted in rabbits, in which the influenza virus undergoes only partial replication. Large doses of virus given intravenously result in a rapid and short-term (about 6-hour) increase in NREMS, as well as short-term fever.⁶⁵ Killed virus is not active, suggesting that at least partial replication is necessary.⁶⁵ Pretreatment of the rabbits with either virus⁶⁵ or synthetic dsRNA⁶⁶ blocks these and other APRs, suggesting that both virus and dsRNA induce mediators (perhaps anti-inflammatory cytokines)

capable of suppressing the virus-induced APR. These studies also suggest that the formation of dsRNA during partial viral replication⁶⁷ plays a role in the rabbit influenza viral APR.⁶⁶

Subsequently, influenza models were developed in mice. Certain human strains of influenza virus have been serially passed through the lungs of mice and are adapted to complete replication in the mouse respiratory tract. Large doses of these viral strains cause a lethal pneumonitis similar to human viral pneumonia when administered intranasally. With smaller viral doses, the mice can survive, depending on their genetic background. In both types of infection, the mice manifest a marked decrease in body temperature, loss of appetite, and a substantial loss of body weight.⁶⁸ The time of onset of these responses is dose dependent, but once initiated, the APR progresses with the same features, if of somewhat different magnitude.⁶⁸ Sleep changes in high-dose infections resemble those seen in the rabbit but are accompanied by a profound hypothermia rather than fever.^{69,70} This hypothermic response appears to be protective in mice,⁷¹ as is fever in larger species,⁷² although if the body temperature decreases to less than 32°C, the mice almost certainly die.⁷³ In addition to increased time spent in NREMS, time spent in REMS is suppressed.⁶⁹ Baseline sleep and sleep responses to influenza in mice are substantially affected by the mouse strain used.⁷⁴ A characteristic feature of the NREMS response to virus in both mice and rabbits is an increase in slow-wave (delta) amplitudes, suggesting an increased intensity of SWS.³⁶ In mice sleep changes induced by influenza infection are dependent on expression of the chemokine macrophage inflammatory protein 1 α .⁷⁵

A series of studies has been conducted of sleep changes occurring during systemic bacterial or fungal infections of rabbits.⁶¹ These studies include both human pathogens and a natural pathogen of rabbits, *Pasteurella multocida*.⁶¹ Sleep changes after these challenges are similar to those seen with influenza, although route, dose, and invasiveness of the bacterium affect the kinetics and magnitude of the sleep response. Increases in NREMS and delta amplitudes, as well as reduced REMS, are seen in all rabbit infection models examined.⁶¹

In summary, increased NREMS and decreased REMS are characteristic of every nonneurotropic acute infection in which sleep has been characterized, whether viral, bacterial, fungal, or parasitic.⁶¹ The degree to which these sleep states are altered during infection depends on the severity of the infection. The nature of the infective organism seems less important, although the time course of the sleep effect varies greatly with the microbe used, and the sleep architecture is substantially affected by animal strain.⁷⁴

MICROBIAL PRODUCTS, CYTOKINES, AND SLEEP

The links between the immune system and sleep were first identified in the 1970s, when a sleep-inducing factor

BRAIN CYTOKINE CASCADE INVOLVED IN SLEEP REGULATION

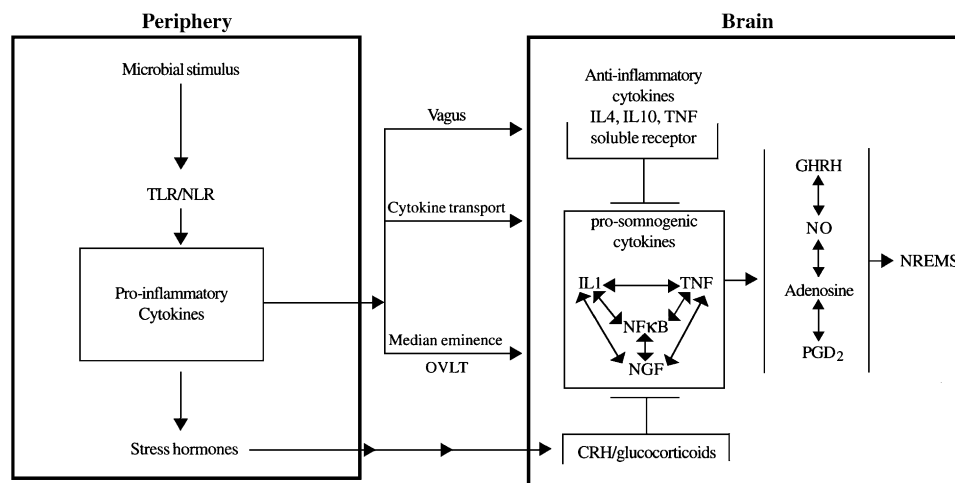


FIG 1. Cytokine networks are involved in sleep regulation. A variety of stimuli, including microbial stimuli, promote production of brain cytokines such as IL-1 β , TNF- α , and nerve growth factor (NGF). The initial microbe-induced cytokines are activated at the site of infection, usually in the periphery (*left box*) via pathogen-associated molecular pattern recognition receptors such as Toll-like receptors (TLR) and NACHT-leucine-rich repeat bearing proteins (NLR). Peripheral cytokines induce stress hormones and both cytokines and hormones make their way to the brain via a variety of mechanisms. Many cytokines promote non-rapid eye movement sleep (NREMS). These cytokines also promote production of each other, thereby forming positive feedback loops. There are several mechanisms used to dampen these positive feedback loops such as the inhibitors of cytokines (*lower right box*) and anti-somnogenic cytokines (*upper right box*). It is likely that all of these mechanisms are involved in physiological sleep regulation as well as the sleep responses to microbial challenge. Effector downstream mechanisms are shown in the right box; several of those molecules are also used by the innate immune system for anti-microbial actions. CRH, corticotrophin-releasing hormone; IGF-1, insulin-like growth factor 1; GHRH, growth hormone releasing hormone; NO, nitric oxide; PGD₂, prostaglandin D₂; OVLT, organum vasculosum of lamina terminalis; NFKB, nuclear factor κ B.

isolated from human urine, Factor S, was chemically characterized.³⁰ Factor S was shown to be a muramyl peptide derived from bacterial peptidoglycan,³⁰ a chemically unique cell-wall component of all bacteria. Macrophages have several enzymes capable of degrading peptidoglycan into lower-molecular-weight muramyl peptides.⁷⁶ Such peptidoglycan fragments are of the type recognized by NOD proteins, and their biologic activities are greatly affected by small changes in structure (eg, amidation-deamidation).⁷⁷ These early observations suggest that the breakdown of normal flora might be contributing peptidoglycan fragments that can act as physiologic regulators in the brain,⁷⁸ a theory for which there is supporting evidence⁷⁹ but little direct evidence to date.

Fortuitously, at the same time that Factor S was being characterized, other workers were investigating the immunologic properties of the peptidoglycan muramyl dipeptide, a potential vaccine adjuvant.³⁰ Subsequently, muramyl dipeptide—and Factor S—related peptidoglycans were all shown to induce the key immunoregulatory cytokine IL-1 β .³⁰ Then IL-1 β was shown to be a potent somnogen, as well as a potent pyrogen.²³ In fact, IL-1 β is one of the most neurologically active molecules known, being somnogenic and pyrogenic after central administration of femtomolar quantities.²³ Subsequent studies revealed that bacterial LPS, LPS components (eg, lipid A), and viral and

synthetic dsRNA, as well as killed and living bacteria, increase NREMS and reduce REMS in rabbits.⁸⁰ All of these microbial factors also induce IL-1, TNF- α , IL-6, and a host of other cytokines. It is highly probable that all microbial PAMPs will be shown to alter sleep, as well as temperature, responses in appropriate models through their induction of proinflammatory cytokines.

Infections represent just one form of systemic inflammation. Other disease states that involve systemic inflammation include cancer,^{14,81} trauma,⁸² and autoimmune diseases.²⁶ Sleep has been characterized in only a few studies of patients with cancer,⁸³⁻⁸⁵ and alterations are difficult to separate from the effects of cancer pain or anxiety. In autoimmune diseases, such as rheumatoid arthritis, sleep studies reveal a characteristic pattern of sleep fragmentation that can be dissociated from pain.²⁶ Recent studies in patients with arthritis given an antagonist of TNF- α to reduce joint inflammation show a reduction in the daytime sleepiness often reported by these patients.⁸⁶ Sleep disorders are also associated with chronic fatigue syndrome and fibromyalgia, probably as a consequence of the systemic inflammation associated with these disorders.²⁶ Low levels of systemic inflammation, as detected by the increased levels of the APR protein C-reactive protein, are associated with atherosclerosis and severe periodontal disease.⁸⁷ Whether these low levels of systemic

inflammation affect spontaneous sleep is unknown.⁸⁸ The specific cytokines initiating sleep changes seen in more severe inflammatory diseases have not been rigorously defined; all of the known proinflammatory cytokines are implicated as discussed later.

Several other cytokines, including anti-inflammatory cytokines, have been implicated in sleep regulation by infusing them centrally into animals.²⁴ In general, proinflammatory cytokines increase SWS, and anti-inflammatory cytokines, such as IL-4 and IL-10, inhibit sleep (Fig 1).²⁴

EFFECTS OF SLEEP DEPRIVATION ON IMMUNE PARAMETERS

Studies of leukocyte population changes and cytokine levels in various sleep deprivation models have been performed in human volunteers.⁴⁹ Different deprivation schedules result in different immune outcomes, particularly in terms of leukocyte numbers and cell types.⁴⁹ In subjects sampled at 15, 39, and 63 hours during total sleep deprivation and also after recovery sleep, plasma levels of IL-1 β tend to increase slightly during deprivation and decrease to less than baseline levels on recovery.⁴⁹ IFN- γ levels fluctuate across the sleep deprivation period and decrease substantially on recovery.⁴⁹ No systematic variation is seen in plasma IL-2, IL-6, IL-12, or TNF- α levels in this study.⁴⁹ Another study revealed increases in soluble TNF- α receptor 1 and IL-6 levels in human volunteers subjected to simulated spaceflight (with extensive partial sleep deprivation), whereas soluble TNF- α receptor 2, TNF- α protein, IL-2 receptor, and IL-10 levels do not change.⁸⁹ The amplitude of the diurnal rhythm of the metabolic regulator leptin is reduced during total sleep deprivation.⁹⁰ Another sleep deprivation paradigm examined plasma IL-6, growth hormone, cortisol, and melatonin in partially sleep-deprived subjects.⁹¹ IL-6 and growth hormone levels are altered in response to the sleep schedule and sleep stage, whereas the cortisol and melatonin levels do not change.⁹¹ The interpretation of the IL-6 results has been complicated by the observation that the tissue damage from an indwelling catheter changes IL-6 levels independently of experimental manipulations.⁹² Other factors that must be controlled in such studies are the sleep deprivation parameters used and the time of day the blood is drawn for leukocyte harvest and cytokine measurements.⁴⁹

Another approach to dissecting the role of cytokines in sleep is to examine sleep changes when cytokines or cytokine receptors of interest are mutated or blocked. Such studies demonstrate distinct changes in spontaneous sleep when IL-1, TNF, or the key transcription factor for these cytokines, nuclear factor κ B, are blocked.²⁴ IL-6 deficiency alters the NREMS response to LPS in mice⁹³ but does not appear to be involved in regulation of spontaneous sleep in mice.⁹⁴ The absence of the type I IFN receptor, which binds such IFNs as IFN- α , reduces spontaneous

REMS but does not substantially affect spontaneous NREMS.³⁷ Although cytokines are implicated in the sleep changes that occur during infection and mice with null mutations in cytokine genes permit one to determine whether the cytokine is important in sleep, these studies can be complicated by the increased susceptibility to infections that might result from the loss of the cytokines.⁹⁵ Our laboratory is currently investigating the effects on sleep and body temperature in cytokine-deficient mice given a very low dose of influenza virus that can be controlled by noncytokine innate mechanisms. We are also examining sleep and body temperature in a dsRNA viral model that obviates the infection issues in these mice.⁹⁶ These studies should help characterize the role of proinflammatory cytokines and type I IFNs in virus-induced sleep and hypothermia responses.

Studies of the neurologic effects of IL-1 and TNF have defined some of their specific actions on brain regions associated with sleep regulation.²⁷ IL-1 stimulates sleep-active neurons in the preoptic area of the anterior hypothalamus, inhibits serotonergic neurons in the raphe, enhances adenosine levels in the hippocampus, and stimulates prostaglandin D₂ (PGD₂) production by the hypothalamus, neocortex, and hippocampus.²⁷ (Both adenosine and PGD₂ are considered sleep regulatory substances.) TNF might also stimulate sleep-active neurons in the preoptic area of the anterior hypothalamus, inhibits noradrenergic neurons in the locus ceruleus, and also stimulates PGD₂ synthesis.²⁷ IL-1 has also been implicated in regulation of the somatotrophic axis, specifically through stimulation of growth hormone–releasing hormone (GHRH) synthesis in GHRHergic neurons and upregulation of GHRH receptors. GHRH is a potent stimulant of NREMS: central injection of 1 nmol/kg in rabbits induces 53 minutes of excess NREMS within the first 6 hours after injection.²⁷ GHRH-receptor deficient dwarf mice have suppressed NREMS and REMS; the latter is restored by restoring growth hormone, but NREMS is not restored with growth hormone.⁹⁷ These dwarf mice also fail to respond to influenza challenge with increased NREMS.⁹⁸ The GHRH-inhibitory peptide somatostatin suppresses NREMS.²⁷ Another member of the somatotrophic axis, insulin-like growth factor, inhibits or promotes NREMS depending on dose.²⁷

It is important to recognize that IL-1 β and TNF- α are also involved in physiologic regulation of sleep unrelated to inflammation.²⁴ The levels of cytokines both in the brain and in the cerebrospinal fluid correlate with sleep propensity (eg, during sleep deprivation).²⁴ In human subjects IL-1 levels in the plasma are highest at sleep onset, and plasma TNF- α levels correlates with SWS EEG activity.²⁴ Extensive evidence implicates IL-1 and TNF in physiologic body temperature and appetite regulation, as well as sleep regulation.⁹⁹ IL-1 homologs have been found in all vertebrates, as well as invertebrates.¹⁰⁰ The evolutionary significance of IL-1 is demonstrated by the fact that the TLR system is derived from the type 1 IL-1 receptor, homologs of which are seen in plants, as well as all animals examined.¹⁰⁰

OTHER SLEEP REGULATORY SUBSTANCES

In addition to cytokines and somatotrophic hormones, numerous other neuropeptides, classical endocrine hormones (eg, prolactin), growth factors (eg, nerve growth factor), prostaglandins other than PGD_2 , and adenosine also are involved in sleep regulation. Evidence for a sleep regulatory role for these factors has been reviewed in depth²⁷ and is beyond the scope of this article. Although many details remain to be worked out, a cascade of effectors ranging from cytokines to nitric oxide participate in sleep regulation.^{27,98} A highly simplified sketch of the cascade of known sleep regulatory substances induced by infection is provided as Fig 1. The sequence of events starts in the periphery with microbial invasion, phagocytosis, and release of PAMPs into the tissue; TLR/NLR detection of the PAMPs; and induction of proinflammatory cytokines by local epithelium and phagocytes. These cytokines then act on the brain and brain endothelium through endothelial receptors, specific transporters, penetration of the blood-brain barrier at circumventricular organs and the median eminence, or nerve transport. Within the brain, cytokines induce other proinflammatory cytokines in the hypothalamus and other brain regions. These cytokines in turn induce NREM sleep and regulators, such as nitric oxide and GHRH. The proinflammatory cytokines also induce anti-inflammatory cytokines, such as IL-10, soluble receptors, glucocorticoids, and receptor antagonists, to dampen the inflammatory events.

SLEEP AND IMMUNE COMPETENCE

The importance of adequate and appropriate sleep for health maintenance was anecdotally recognized in the Bible.¹⁰¹ However, scientifically demonstrating a role for sleep in immune competence has proved difficult because, as mentioned earlier, it is not possible to isolate sleep *per se* as an independent variable. Studies of T-cell subsets in association with sleep show that T_H1 cells (associated with cellular immunity) increase relative to T_H2 cells (associated with humoral immunity) early in the sleep cycle, but the ratios reverse during late sleep.¹⁰² Functional consequences of acute sleep deprivation on responses to vaccines in human volunteers have been demonstrated.^{103,104} One whole night of sleep deprivation before vaccination suppresses the antibody response to hepatitis A vaccine about 50% on day 28.¹⁰³ Six nights of partial sleep deprivation during which the subjects were inoculated with influenza virus vaccine also showed a 50% reduction in antibody levels 10 days after vaccination, but by 3 weeks, antibody levels were similar in both groups.¹⁰⁴ Chronic sleep loss in shift workers is associated with increased infections,¹⁰⁵ although, of course, many other factors in the lives of these workers other than sleep loss could account for this phenomenon.

One study of influenza vaccination in mice shows a marked immunosuppression in sleep-deprived Swiss mice versus normal-sleeping control animals; sleep-deprived

mice have lower antibody titers and viral clearance.¹⁰⁶ Another influenza study in the BALB/c strain of mouse suggests that sleep deprivation enhances existing immunity.¹⁰⁷ (There are substantial differences in SWS responses to influenza in different mouse strains.⁷⁴) Challenge of nonimmune mice with influenza after sleep deprivation reveals a protective effect of diminished sleep time: sleep-deprived mice have reduced dissemination of the virus and enhanced viral clearance.¹⁰⁷ In another influenza model in C57BL/6 or Swiss mice subjected to sleep deprivation, constant light, or restraint stress, no effect of sleep deprivation or constant light on viral antibody titers or viral clearance is seen.¹⁰⁸ In this model restraint stress reduces lung inflammation, but sleep deprivation or constant light do not.¹⁰⁸ Effects of sleep deprivation on an intravenous bacterial challenge in rabbits does not reveal sleep deprivation-induced clinical changes other than enhanced fever.¹⁰⁹

Chronic sleep loss in rats results in a lethal sepsis that probably results from a breakdown of intestinal defenses and systemic invasion by the intestinal flora.^{110,111} These animals also demonstrate hormonal secretion patterns compatible with hypothalamic failure.^{46,111} A similar hormonal pattern is seen in military trainees subjected to prolonged sleep loss, together with numerous other physical stressors.¹¹²

A careful analysis of sleep patterns in bacterially or *Candida* species-infected rabbits shows a correlation between favorable outcomes (eg, survival) and the quantity and quality of SWS.¹¹³ Overwhelming doses of bacteria result in reduced total NREMS and a poor prognosis.¹¹³ This study, although correlative, is the most direct demonstration to date of a relationship between increased NREMS and recovery from infection.

SLEEP AND ASTHMA

It is widely recognized by emergency department personnel and parents that many asthma attacks occur at night when the patient is trying to sleep.¹¹⁴ Up to 75% of asthmatic subjects are awakened by breathing difficulties at least once a week, and approximately 40% experience nocturnal symptoms on a nightly basis.¹¹⁵ Not all asthmatic patients experience a majority of their attacks at night, and allergists often distinguish patients with nocturnal asthma from those whose attacks do not consistently occur at night. However, up to 90% of asthmatic subjects experience nocturnal symptoms severe enough to awaken them from sleep.¹¹⁶

Circadian aspects of asthma¹¹⁶ have been examined together with the interaction of asthma attacks with sleep *per se*.¹¹⁷ Sleep posture and any associated sleep-disordered breathing¹¹⁷⁻¹¹⁹ certainly contribute to asthma exacerbation during sleep, and circadian changes in lung function also appear important.^{114,120} Effective treatment of sleep apnea can reduce nocturnal asthma symptoms in patients with this sleep disorder.¹²¹ Other physiologic changes associated with sleep, such as reduced lung volume,

intrapulmonary blood pooling, and upper airway narrowing, might also aggravate asthma attacks.¹¹⁷ Vagal tone, neurogenic inflammation, and airway hyperresponsiveness are increased at night and most likely also contribute to asthma severity.¹¹⁶ At the biochemical level, circadian rhythms in catecholamine and cholinergic receptors are probably relevant to nocturnal asthma.¹²²

Immune parameters associated with asthma, particularly IgE levels, show a circadian rhythm in asthmatic subjects, although unexpectedly, the serum concentration nadir occurs at night.¹²³ Cytokine secretion in general also displays a rhythm.^{123,124} Serum IL-4, IL-5, and IFN- γ levels are higher in asthmatic subjects than in healthy subjects at both 4 PM and 4 AM; only IFN- γ levels correlate with airway responsiveness to methacholine.¹²⁵ IL-1 β and IFN- γ induction *ex vivo* show a maximum around the time of sleep onset and a minimum level in the morning hours.¹²⁶

Several effector cells, particularly mast cells and eosinophils, secrete a range of cytokines and other inflammatory mediators on activation by IgE-allergen interactions, including IL-5, leukotrienes, and histamine.¹²⁷ IL-5 levels correlate with reduced forced expiratory volume,¹²⁸ whereas increased bronchoalveolar lavage IL-1 β levels are associated with nocturnal asthma.¹²⁹ The major mediators of asthmatic bronchospasm, the leukotrienes, are increased at night in patients with nocturnal asthma.¹³⁰ The complex interactions of leukocytes, cytokines, leukotrienes, and other inflammatory mediators in asthma have been recently reviewed and are beyond the scope of this article.¹²⁷

The inflammation accompanying certain acute viral infections exacerbates asthma and wheezing and appears to either stimulate or suppress future asthma attacks.¹³¹ Analyses of cytokine changes in asthmatic patients experiencing respiratory infections reveal diminished anti-inflammatory IL-10 production in asthmatic subjects compared with control patients.¹³² Epithelial cells from asthmatic individuals appear to have an intrinsic deficiency in antiviral cytokine production (such as IFN- β).¹³³ Whether virally induced somnolence (which includes “excess” SWS) has any special properties *vis-à-vis* asthma is not known.

ASTHMA AND SLEEP

Asthma, in turn, affects sleep. Obviously, sleep deprivation occurs when a patient is kept awake all night trying to breathe. Recent studies suggest that asthmatic subjects experience chronic sleep deprivation, even when clinically stable.¹³⁴ Experimental sleep deprivation appears to reduce the bronchoconstriction that occurs during sleep in asthmatic patients.¹³⁵ Selective REMS deprivation reduces plasma histamine levels and increases plasma epinephrine levels in rats, factors that could ameliorate asthma.¹³⁶ As discussed earlier, the effects of sleep deprivation cannot readily be dissociated from the effects of

stress, and therefore such studies provide little information regarding sleep effects *per se* on asthma.

CONCLUDING REMARKS

Unexpected links between the innate immune system and sleep regulation have been revealed in the last 25 years. Although the evidence is limited, it is likely that maintenance of immune function is one reason why we sleep. The importance of sleep to recovery from infection remains more intuitive than grounded in hard evidence. The evidence that sleep plays a role in nocturnal asthma is more extensive but is difficult to dissociate from intrinsic circadian changes in inflammation, as well as physiologic changes associated with sleep. The sheer complexity of sleep regulation combined with immune regulation will make it extremely difficult to dissect out key interactive mechanisms and thus therapeutic targets relevant to nocturnal asthma and other immune disorders. However, the burgeoning field of sleep medicine might well offer assistance to asthmatic patients. It would certainly seem to be appropriate to assess asthmatic patients for treatable sleep-disordered breathing.

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