Sleep and Immune Regulation

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Introduction

While it has long been recognized that inadequate sleep at night is followed by daytime sleepiness, only in the last few decades has sleep deprivation begun to be viewed as a significant health problem with negative consequences including obesity, diabetes, cardiovascular disease, and hypertension. These specific pathologies are all associated with inflammation and emphasize the crucial relationship between sleep and immune function. Given the high prevalence of insufficient sleep that has been reported in both civilian and veteran populations in recent years [1], understanding this relationship has become increasingly important.

Although the research on inflammatory pathologies related to sleep has largely been conducted in the last 20 years, our understanding of this sleep–immune relationship stems from research conducted in the early twentieth century. At that time, scientists in both Japan and France discovered that injecting cerebrospinal fluid from sleep-deprived dogs into well-rested dogs resulted in deep slumber in these otherwise healthy animals [2, 3]. This seminal research was largely unrecognized until the early 1960s, when several independent research groups began to focus their attention on the humoral regulatory mechanisms for sleep, using similar methods to those used by Ishimori and Pieron [4–6]. Following that work, Pappenheimer and colleagues demonstrated a similar transfer of cerebrospinal fluid from sleep-deprived goats to well-rested rats (resulting in sleep induction) and further characterized the sleep-inducing agent as "Factor S," a muramyl peptide [7]. These breakthroughs led directly to our current body of clinical and preclinical studies of sleep and immune function.

Accumulating and novel data from the experimental literature as well as field-based research have provided us with a more comprehensive understanding of this complex relationship. Experimental studies of sleep loss have now examined the sleep–immune relationship across multiple domains, including white blood cell counts, inflammatory mediators, and transcription factors (e.g., [8–10]). This review will present an overview of the current state of the field with respect to both innate and acquired immunity. Additionally, emerging research from clinical trials or intervention studies, which have the potential to further illuminate the bidirectional nature of the relationship between sleep and immunity, will be presented when available.

In order to best understand the sleep–immune relationship, it is also necessary to consider the relationship of hormones with respect to both sleep and immunomodulation. Many hormones demonstrate a circadian pattern, and levels of these hormones can also be affected by sleep loss. Please refer to Fig. 12.1 for a schematic of the diurnal rhythms of immune and endocrine markers and Fig. 12.2 for the impact of sleep deprivation on relevant endocrine markers. While a full description of what is known about the relationship between sleep and endocrine function is beyond the scope of this chapter, we will briefly review sleep-related endocrine effects that facilitate a balanced and well-functioning host defense system, with an emphasis on cortisol and hormones associated with energy regulation and metabolism. For a more comprehensive review of sleep and endocrine relationships, please refer to [11].

Cortisol Cortisol has long been recognized as a powerful immunomodulating hormone with a known diurnal rhythm [12]. The relationships between the diurnal rhythm of cortisol and a range of white blood cells as well as cytokines and their receptors have been well documented [13–15]. The fact that it has endogenous immunomodulating potential has

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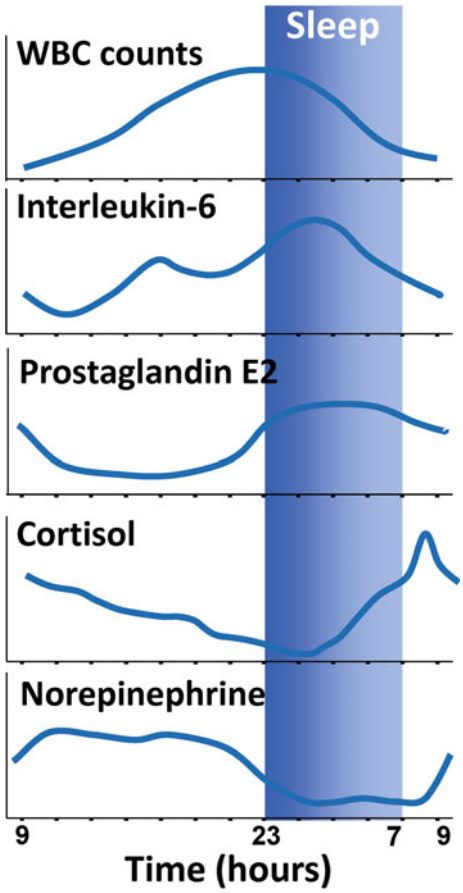

Fig. 12.1 Diurnal rhythms of immune and endocrine markers (schematic). Without sufficient and good quality sleep, rhythms are upregulated or displaced (i.e., shifting of nadirs and peaks)

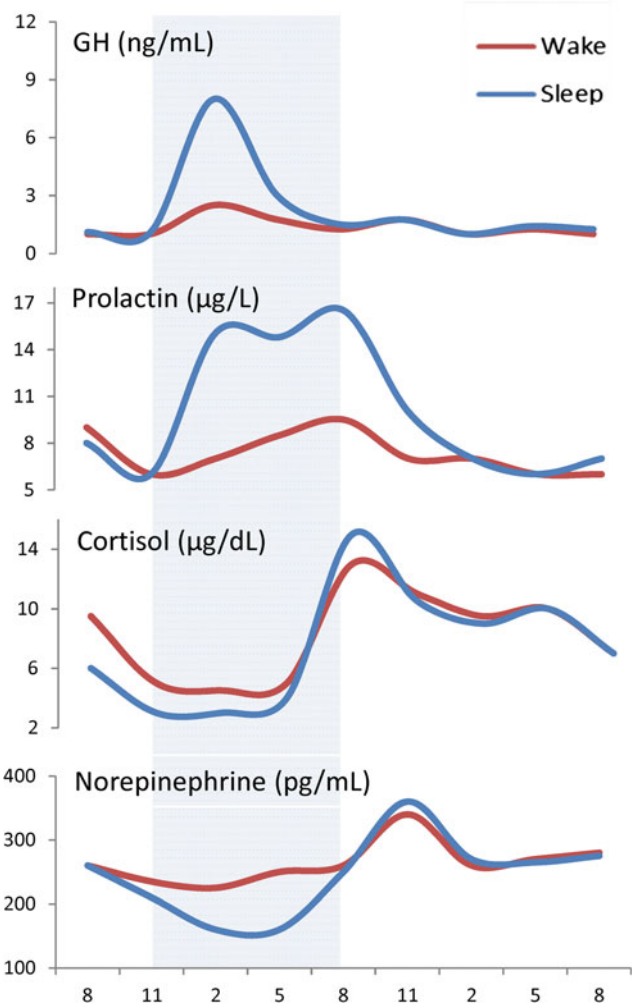

Fig. 12.2 Immunoregulatory hormones are affected by total sleep deprivation (schematic)

also been recognized at least since the 1990s when Pollmächer and colleagues showed that an injection of lipopolysaccharide LPS given in the morning when cortisol is at its peak had a greatly diminished effect on the febrile response, compared to when the challenge was administered in the evening, when cortisol was close to its nadir [16]. This effect was seen despite similar IL-6 and TNF-alpha production in response to challenge, suggesting that the endogenous corticoids have a suppressive effect on the pro-inflammatory effects of these cytokines [16]. In addition, sleep has been shown to be associated with activation of the mineralocorticoid receptor, which has been suggested as a potential mechanism through which sleep supports adaptive immunity [17].

Energy metabolism and adipose tissue hormones It is also known that sleep deprivation alters basal metabolic function, which can contribute to changes in immunological homeostasis [18]. Both total and partial sleep loss have been shown to slow glucose metabolism [19, 20]. Thus, insufficient sleep may be an important contributing mechanism in the clinical development of insulin resistance. Additionally, acute total and sustained partial sleep deprivation have been shown to reduce the diurnal rhythm amplitude [21] and levels [22] of peripheral circulation levels of leptin, an adipocyte hormone that signals satiety to the brain. Levels of ghrelin, a hormone that signals hunger to the brain, and subjective appetite are also increased during partial sleep deprivation [23]. Both changes in insulin resistance and glucose metabolism, and in appetitive hormones may lead to weight gain and changes in adiposity. As adiposity, particularly visceral adiposity, contributes to the production of interleukin 6 (IL-6) and C-reactive protein (CRP) [24, 25], these changes in endocrine system function have a direct impact on the sleep–immune relationship. These hormone changes are particularly relevant when considering the increasing prevalence of obesity [26]

and the development of the metabolic syndrome [27] along with the frequency of chronic insufficient sleep [1], in modern society.

Experimental

Largely conducted in laboratory settings, experimental sleep deprivation (or sleep restriction) studies provide a highly controlled environment in which the effects of sleep loss on immune parameters can be assessed. To date, the vast majority of these studies have been conducted in healthy young adults that have undergone thorough medical screenings in order to provide the clearest understanding of the mechanisms underlying the relationship between sleep and immunity. The field is currently lacking controlled studies that examine how sleep loss affects immune function in potentially more vulnerable populations (e.g., older adults, chronic illness).

Historically, the majority of experimental studies of sleep loss involved acute (total) sleep deprivation—the length of which ranged from 24 to 126 h. Experimental studies of sleep loss have examined the sleep–immune relationship primarily in whole cell counts and in inflammatory mediators of the immune system. There are multiple studies examining how white blood cell counts and differential cells respond under conditions of total sleep deprivation. One of the earliest studies, which was conducted in the 1960s, healthy volunteers were sleep-deprived for up to five nights and days, after which increases in white blood cells, particularly neutrophils, were observed [28]. Subsequent studies of total sleep deprivation report consistent increases in leukocyte number and function [8, 15, 29–31], as well as in some white blood cell count (WBC) subsets (neutrophils [8, 28, 30, 31] and monocytes [8, 15, 30]). Together, these findings collectively suggest that sleep loss activates the innate immune system, as well as the adaptive immune system (discussed below). This conclusion is supported by more recent studies demonstrating that total sleep loss is also associated with upregulation of a range of inflammatory markers, including IL-1 beta, IL-6, CRP TNF-alpha, and prostaglandin E2 in some studies, although results are not as uniformly consistent across studies [32–37].

More recent studies have utilized shortened sleep protocols, ranging from a single night to more chronic sleep restriction paradigms, in which participants' sleep is restricted to a particular shortened length of time each night. It is thought that this model of sleep restriction, rather than the model of total sleep loss, may more closely model the real-world phenomena of chronic insufficient sleep. Similar to participants enrolled in studies of total sleep deprivation, sleep restriction studies typically examine the effects of sleep restriction on healthy adults who typically sleep about 8 h a night. This allows for a controlled dose of sleep loss of similar relative magnitude (compared to habitual duration) across participants. In one of the earliest partial sleep loss studies, participants were restricted to four total hours of sleep per 24 h day over four nights, with sleep opportunities divided into two 2-h naps—one of which occurred in the afternoon and one at night [35]. In this study, no increases in IL-6, TNF-alpha, or TNF-alpha receptors were observed; however, in a parallel total sleep loss condition (see above), increases in TNF-alpha receptors and IL-6 were found [35]. Indeed, effects of partial sleep loss on TNF-alpha and its receptor have been mixed, with some studies finding significant increases (men only [37]) while others do not [35, 38]. IL-6 findings have been more consistent, with deprivation paradigms of sleep restricted to 6 h for 7 nights [37], 4 h/night for 10 nights [33], and 4 nights of sleep restricted to 4 h of split sleep [35] reporting increases in IL-6. Increases in other inflammatory markers have also been observed, including CRP [33, 36, 38] and IL-1 beta [36], although, again, not all studies show this consistent increase [9].

While collectively these experimental, laboratory-based studies of sleep loss demonstrate a significant upregulation of the acute host defense and inflammatory response, there are several additional factors that should be taken into consideration when interpreting these results. Many of these studies are small and thus have the potential to be underpowered, particularly when multiple immune markers are measured. Additionally, basal levels of these immune markers demonstrate considerable variability resulting from a range of factors, including individual factors (stress, temporal proximity to physical activity or meal intake, body mass index, and other metabolic factors), circadian factors (IL-6 in particular has been shown to have circadian variation [13, 39]), and measurement effects (e.g., effects of catheterization [40] and potential sample contamination [41]). All of these factors can increase the variability of the data and have the capability to mask potentially small, yet important changes in immune markers following sleep loss. Collectively, these findings reinforce the need for careful methodological consideration when comparing outcomes across studies.

More recent work has begun to explore the physiological mechanisms underlying the relationships between insufficient sleep, circadian rhythm disruption and negative health outcomes, focusing on the level of gene expression. Moller-Levet and colleagues compared transcriptome analyses after either one week of sufficient sleep (8.5 h/night) or one week of insufficient sleep (5.7 h/night) and found that expression of genes affected by insufficient sleep was associated with not only circadian rhythms and sleep homeostasis (including IL-6), but also oxidative stress and metabolism [10]. A follow-up study by the same research

team found that mis-timed sleep alone also leads to a reduction in rhythmic transcripts [42]. Together, these findings suggest that the immune system is affected by sleep alterations even at the molecular level. We expect that the study of gene expression in relation to sleep loss and immune parameters will be an increasing area of research focus in the future.

In addition to these direct relationships between sleep loss and increases in immune and inflammatory markers, there is a wealth of evidence that sleep supports components of the adaptive immune response. The effects of insufficient sleep on adaptive immune system components have been mostly tested using vaccination models, and exploring the magnitude and time line of the primary antibody response. In one of the first laboratory studies in this area, two groups of adults received a hepatitis A vaccination; one group was deprived of sleep for one night prior to the vaccination, and the other was allowed a full night of sleep. The well-rested group was observed to have an immune response of nearly twice the magnitude of their sleep-deprived comparison subjects [43]. In a follow-up study, vaccination-induced T helper (Th) cell and antibody (AB) responses were monitored over a one-year period following a series of three hepatitis A vaccines, with participants randomized to either sleep or wake the night *following* each vaccine administration. Again, the individuals in the sleep condition had twice the frequency of antigen-specific Th cells and an increased fraction of Th1 cytokine-producing cells; these differences remained significant over the one-year follow-up period [44]. There is also direct evidence that sleep duration can impact the immune systems response to an actual viral challenge, as demonstrated by Cohen and colleagues [45]. In this study that used both field and experimental techniques, habitual sleep duration was assessed via self-report prior to entering the laboratory, where they were then inoculated with a dose of the common cold virus. Participants who slept seven or fewer hours/night were at three times greater risk of developing a cold [45].

Two more recent studies provide additional support for this relationship between sleep and the adaptive immune response. Benedict and colleagues investigated the effects of acute sleep deprivation on the antibody titer response to a novel influenza A H1N1 virus (swine flu) [46]. Twenty-four healthy students received the vaccine after a single night of sleep deprivation or regular sleep. Of note, the antibody response in the sleep-deprived group was 60% lower in the early observation phase of the immune response (day five following the vaccine) although this difference was observed only in males [46]. The authors suggest that acute sleep deprivation may delay the induction of adaptive immune responses in males, but that it does not have lasting effects on the antibody titer response to influenza vaccination, with equivalent responses in both sexes on follow-up days 10, 17, and 52. These results were consistent with those of an earlier pilot study that reported lower antibody titer against a seasonal influenza virus only in the early period (10 days) but not later periods (after 28 days) following a brief exposure to acute sleep deprivation [47]. These studies suggest that insufficient sleep may delay, or at least reduce, the adaptive immune response.

Collectively, these experimental sleep loss studies demonstrate that sleep loss can affect a range of established markers of the inflammatory system, including IL-6, CRP, IL-1 beta, TNF-alpha, and prostaglandin E2. Many of these inflammatory markers are considered to be pro-inflammatory in nature; however, it is important to be mindful that the regulation of inflammatory parameters is complex and is often bidirectional, such that increases in one inflammatory parameter may eventually function to down-regulate another aspect of the system. Additionally, as described above, there is significant variation in the experimental parameters (e.g., the duration and timing of sleep loss, diet, body positional, and activity control of study participant); this, in combination with the study population itself, may help explain patterns of immune responses in a given study compared to another. Replication of specific protocols and expansion of study participants to include more vulnerable populations will provide valuable information to help us better understand the differential pattern of results.

Field and Population Studies

Field studies, which include both epidemiological studies and large-scale non-laboratory-based research, complement experimental or laboratory-based protocols. This area of research helps provide a better understanding of the relationship between sleep and immune function in a real-world setting, allowing for better estimations of the effects of, for example, the chronicity of sleep loss. This real-world validity, however, is countered to some extent by the loss of experimental control when research moves beyond the laboratory. While well-conducted studies are able to control or covary for an appropriate range of factors, they cannot execute the degree of control seen in laboratory studies given their much larger scale. Considered together, experimental and field-based studies provide a more comprehensive picture of the sleep–immune interaction.

There are numerous of epidemiological studies linking short sleep duration with negative health outcomes (e.g., body mass index, risk of hypertension [48, 49]), as well as risk of all-cause mortality. Several meta-analyses have found increased risk of all-cause mortality in both habitual short and long sleep durations, with elevated risks with short sleep of 10–12 and 23–30% with long sleep [50, 51]. While not directly linked with immune function, the overall

relationship between sleep duration and *all*-cause mortality suggests that there may be an underlying shared mechanistic link, and immune function and inflammation is one viable candidate. This hypothesis is supported by other population-based studies that have examined the relationship between sleep duration and inflammatory mediators associated with body mass index (BMI) or adiposity, which we discuss further below.

There is a large base of research demonstrating that insufficient sleep is associated with obesity, both cross-sectionally and prospectively, even when sleep duration is measured objectively [52, 53]. Given that adiposity, particularly visceral adiposity, is associated with higher circulating levels of inflammation [24, 25], the association between sleep and adiposity can also be considered from a perspective of an independent relationship between inflammatory mediators and sleep duration. To date, there are a limited number of studies that can allow for such comparisons. The available data suggest that there is weak evidence for an independent relationship between short sleep and inflammation. In one study of 907 adults (Wisconsin Sleep Cohort study), no relationship between levels of CRP and sleep duration was reported [54], while in another study of over 4600 individuals, sleep disturbances were associated with higher CRP levels in women, but not men [55]. Notably, increases in inflammatory markers associated with long sleep durations have also been reported [56, 57]. While these studies control for a range of potential covariates and comorbid factors, it is still an open question as to whether there is an independent relationship between long sleep durations and inflammation, or rather there is a yet-unidentified/unaddressed factor that is driving this relationship.

These differential results emphasize that very large study samples are required for this area of research, in order to both insure adequate representation across a range of sleep duration and also to allow for statistical control of the contributions of a range of potentially confounding factors. As described in the experimental studies section, methodological variation such as time of day of sample collection and the collection procedures themselves may have an impact on the results, independent from the actual population studied. Interpretation of these findings is also complicated given that epidemiological studies have largely relied on self-reports of habitual sleep duration. Research suggests that healthy population may overestimate by self-report their actigraphically measured sleep duration, while populations with sleep disorders (e.g., insomnia) may over-report time awake/under-report sleep duration [58–60].

Self-assessment of sleep duration via a sleep diary and objective sleep duration, as measured by a night of polysomnography, may lead to differing outcomes, however. Objectively measured shorter sleep duration has been found to be correlated with fasting levels of TNF-alpha, after controlling for covariates; however, in the same study, self-reported longer sleep durations were significantly associated with increased high-sensitivity (hs) CRP and IL-6 [61]. The authors suggest that self-reported sleep duration and single-night polysomnography may be measuring different constructs of sleep [61], which appears supported by the literature on differential findings between objectively and subjectively measured sleep durations described above. Nonetheless, these studies provide more evidence that the inflammatory system is sensitive to actual sleep duration and potentially also by longer-term subjective estimates of sleep patterns. These methodological challenges add an extra layer of complexity in our understanding of study findings, particularly relative to the experimental sleep loss literature. In addition to the investigation of basal changes in inflammatory markers in relationship to sleep duration, a few studies have looked at the inflammatory response or reactivity to challenge, such as to stress. For example, Heffner and colleagues conducted a study in which IL-6 levels were assessed at baseline and 60 min after a stressful testing period in a sample of older adults (greater than 50 years old). Individuals categorized as poor sleepers had a significantly larger inflammatory response to this acute stressor, which was not mediated by negative affect or perceived stress [44].

Field studies have also provided important insights into the relationship between sleep and adaptive immune function. Using the vaccine challenge model, Prather and colleagues investigated whether naturally occurring sleep influenced the specific immune response to a hepatitis B vaccination [62]. This larger study examined relationship between self-reported sleep duration and the primary and secondary antibody response, as well as clinical protection status after the 3rd vaccination in this series. The authors found that shorter sleep duration was associated with lower secondary antibody levels, translating into a 56% increase in secondary antibody levels with each additional hour of sleep [62]. Additionally, shorter sleep durations in this study were associated with a decreased likelihood of being clinically protected after the third vaccine in the series. Specifically, sleeping fewer than 6 h a night was associated with a significant risk of remaining unprotected against hepatitis B infection compared to those sleeping more than seven hours [62]. This relationship between sleep duration and immune response to system challenge is further supported by findings from a large (N = 56,953) study of women, which found that sleeping less than 5 h a night increased the risk of pneumonia by 70% over the four-year observation period, compared to women sleeping 8 h per night [63].

Qualitative aspects of sleep, rather than duration per se, have also been shown to modulate immune function. In one early study, 11 young adults with insomnia, who were otherwise healthy, were compared to 11 age- and sex-matched healthy control subjects. These subjects were studied in the laboratory for four nights; while there were no

differences in mean 24-h levels of IL-6 or tumor necrosis factor, a shift in the secretion timing (from nighttime to daytime) of these cytokines was observed [64]. In a similar study comparing individuals with chronic insomnia to healthy controls, increases in nocturnal IL-6 were observed in the insomnia sample [65]. Total IL-6 levels were inversely correlated with subjective sleep quality and the amount of slow wave sleep and were positively correlated with the amount of wake time at night [65], suggesting that there may be specific relationships between sleep symptoms and immune markers. However, not all studies have reported a relationship between insomnia symptoms and increased markers of inflammation. One large study of 8547 Norwegians with insomnia found no consistent associations with specific symptoms of insomnia and hsCRP levels [66]. Inconsistencies between studies may have largely to do with the wide range of insomnia phenotypes and definitions. For example, more recent findings suggest that inflammatory and other systems abnormalities are present particularly in insomniacs with objectively verified sleep abnormalities (such as short sleep duration, low sleep efficiency) [67].

A growing number of studies have also examined relationships between sleep quality and inflammation, with mixed results. While one large study found that inflammation was not related to an overall index of sleep quality [56], another smaller study found a significant relationship between sleep quality and production of IL-1 beta [68]. Two additional studies report relationships between sleep quality and inflammation in women: one in a female-only study sample (CRP, [69]) and the other in a prospectively assessed, mixed gender sample (multiple inflammatory markers, [70]). Together, this body suggests that there may be some relationship between impaired sleep quality, particularly at the disorder level (e.g., insomnia disorder), and inflammation; however, there are likely multiple factors contributing to these relationships (e.g., sex, medication use, chronicity of sleep disturbance, body mass index) that are yet to be fully understood.

Clinical Trials/Intervention Studies

The studies presented thus far in this chapter have demonstrated the relationship between acute and chronic sleep durations and immune function. There is far more that can still be learned from both experimental and field studies in this area; an exciting new area of research lies in clinical trials and intervention research. Although it is well documented that sleep can successfully be improved (e.g., through treatment of insomnia) [71, 72], few studies to date have incorporated a physiological measurement arm. In the most recent and largest of these studies, 123 older adults were randomized to either cognitive behavioral therapy for insomnia (CBT), Tai Chi Chi (TCC), or a sleep seminar education control (SS) [73]. Treatment was conducted in weekly 2-h group sessions over four months. In addition to CBT outperforming the other two conditions with respect to insomnia remission rates and improvements in sleep parameters, CBT was also associated with a reduced risk of CRP levels at the 16-month follow-up point compared to the SS control condition. Further, remission of insomnia was also associated with lower levels of CRP [73]. The authors suggest that observed inflammatory effects have implications for the cardiovascular morbidity observed with sleep disturbances in epidemiological studies [73].

Two additional studies in medical populations (breast cancer and peritoneal dialysis patients) with insomnia have also demonstrated significant changes in immune markers. In one pilot study, dialysis patients completed either individual CBT-I or a sleep education control condition, and effects on five inflammatory markers were assessed. While sleep quality improved in the intervention group, the authors observed decreases in only one inflammatory marker, IL-1 beta [74]. In another small study, 57 women with insomnia secondary to breast cancer were assigned to CBT or a control condition. The authors report that patients treated with CBT had higher levels of IFN-gamma and a smaller increase in lymphocytes at post-treatment compared to control patients. Additional changes in multiple markers were observed from pre- to post-treatment, including WBCs, lymphocytes, IFN-gamma, and IL-1 beta [75]. Interpretation of specific immune changes following sleep improvement is complex, particularly in these samples of medical populations; however, it does appear that modifying sleep has the potential to effect direct changes in immune function. While more research is needed in this area, these important findings suggest an avenue by which improving sleep can affect underlying medical conditions and also help shed light on our understanding of the mechanisms underlying the sleep duration and health risk epidemiological literature.

Another new area examining the reversibility of inflammatory and autonomic changes related to insufficient sleep is sleep extension. One pilot study in this area assessed 22 adults with hypertension or pre-hypertension and a habitual sleep duration of less than 7 h a night; participants were then randomized to a control condition or an intervention designed to facilitate extension of bedtimes by 60 min/night over a six-week period [76]. Beat-to-beat blood pressure, and stress and inflammatory markers were both assessed pre- and post-treatment in a controlled laboratory setting. Sleep duration increased significantly in the extension group by an average of 35 min/night, and this was accompanied by a significant decrease in systolic blood pressure and

nonsignificant decreases in WBC, IL-6, CRP, and norepinephrine [76]. While small in scale, this study suggests that reversing chronic insufficient sleep even for a period of six weeks can result in significant improvement in autonomic markers related to inflammation.

There is also a small amount of intriguing research that has assessed the relationship between sleep and inflammatory activity in an intervention model by testing the effects of an anti-inflammatory medication (tocilizumab, an antibody against the IL-6 receptor) for six months on sleep disturbances in a small sample of women with rheumatoid arthritis [77]. Researchers found that this IL-6 receptor antibody treatment led to a slight, but significant improvement in sleep quality that was independent of decreased disease activity [77]. This study will require replication but provides more evidence to support the responsivity aspect of the relationship between sleep and immune function, where poor sleep can result in a stronger inflammatory response to stress (see [78]) and reducing the inflammatory response (with use of medication) can improve sleep quality [77].

Conclusion

Accumulating experimental data from laboratory-based studies of sleep loss suggest that insufficient sleep affects basal levels of immune and inflammatory mediators, as well as the inflammatory reactivity to stressors. In field settings, short sleep duration has been associated with increased inflammatory markers, reduced antibody responses, and lower clinical protection induced by vaccinations. Epidemiological and intervention data also suggest that infection rates are higher when sleep durations are lower. This raises the possibility that interventions designed to improve sleep may reduce disease risk to infections through either direct protection by the early and adaptive immune systems or indirect antibody support mechanisms.

While the mechanisms underlying the sleep–immune relationship are yet to be fully understood, preliminary evidence suggests that inflammatory mediators may not only be affected by changes in basal levels of various immune regulatory hormones, but also by changes in receptor density or sensitivity of immune cells to hormonal signals. Future research in this area will benefit from greater understanding of individual differences, and genetic and epigenetic factors so that interventions can be optimized to better address biological system-specific risk.

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