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Review

Circadian nature of immune function

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ABSTRACT

The primary physiological role of the circadian system is to synchronize and coordinate organ systems, particularly in response to dynamics in the environment. The immune system is under direct circadian control by systemic cues and molecular clocks within immune cells. The master circadian pacemaker called the suprachiasmatic nucleus (SCN) conveys timing information to the immune system through endocrine and autonomic pathways. These signals promote phase coherence of peripheral clocks in the immune system, and also govern daily variations in immune function. The coordination of immune response may compose an anticipatory state for optimal immune response. Interactions between circadian and immune systems are bidirectional, in that immune factors can modulate phasing of circadian clocks. Circadian disruption, such as environmental desynchronization and/or anomalous molecular clock functions, may lead to lack of system coordination, and particular vulnerabilities to infection and disease may develop.

Contents

1. Introduction

Circadian rhythms are ubiquitous in mammals governing many aspects of cellular and behavioral physiology. Circadian rhythms are endogenous oscillators with periods of approximately 24 h, which are generated by networks of central and peripheral clocks. The ability of the circadian system to adapt to changing external and internal states allows organisms to optimize their physiological tasks and maintain coordination among multiple organ systems. This synchrony seems to be critical for maintaining homeostatic regulation, as circadian disruptions are associated with negative health outcomes. Human studies have reported circadian desynchronization to the environment and discoordination among clocks is associated with a higher incidence of several types of cancer (Schernhammer et al., 2001, 2003). Additionally, evidence from animal models has begun to elucidate the potential mechanisms linking alterations in biological rhythms with disease.

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Perturbations of circadian rhythms by either external stressors (e.g., shift-work and jet-lag), or internal stressors (e.g., psychological distress), may negatively impact health by impairing immune function. The immune system is critical for defending an organism against bacterial and viral infections and other diseases, including cancer, and emerging evidence indicates immune function is under tight circadian regulation. Circadian information is transmitted to immune tissues by neural and endocrine signals, and most, if not all, immune cells contain molecular clock components, which have been shown to mediate immune responses, including natural-killer cell (NK) cytotoxicity, phagocytosis, and inflammation. Therefore, in this review, we evaluate the primary pathways by which timing information is conveyed to the immune system and the involvement of clock genes in regulating immune response. We also discuss how immune factors are able to modulate circadian timing by acting on central and peripheral clocks. Lastly, we highlight several studies explicitly exploring the impact of circadian disruption on immune function, particularly in relation to cancer and inflammation.

2. Autonomic and endocrine outputs of the circadian system communicate time-of-day information to immune tissues

In mammals, the circadian system is a complex hierarchically organized network of central and peripheral oscillators. The central circadian pacemaker in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus contains specialized neurons that receive photic input through the retinohypothalamic tract (RHT) and non-photic cues by disparate neural inputs (Rosenwasser, 2009). The convergence of these inputs in the SCN integrates both environmental and physiological signals in order to coordinate downstream brain areas and organ systems via neural and endocrine outputs (Guo et al., 2006). The SCN produces diffusible signals, including several neuropeptides, and direct axonal projections to communicate with specific neuronal populations in order to generate circadian patterns of behavior and physiology (Inouye and Kawamura, 1979; Meyer-Bernstein et al., 1999; Silver et al., 1996). Between the SCN and other hypothalamic nuclei, as well as other brain areas, extensive axonal connections are present, including two major areas for regulating stress and immune response – the paraventricular and arcuate nuclei (PVN; ARC, respectively) (Kalsbeek and Buijs, 2002; Saeb-Parsy et al., 2000).

2.1. SCN–PVN-sympathetic pathways

Although it is likely other pathways exist, the SCN conveys circadian information to the immune system by mediating activity of autonomic and endocrine neurons of the PVN (Fig. 1; Arjona and Sarkar, 2008; Boyadjieva et al., 2001). In the PVN, "preautonomic" neurons, both sympathetic and parasympathetic, are controlled by inhibitory and excitatory SCN efferents (Kalsbeek et al., 2008). Although the specific neuronal targets of SCN projections in PVN need to be determined, these most likely include oxytocin and corticotropin-releasing hormone (CRH) producing neurons (Stanley et al., 2010). Further, these specific PVN neurons govern autonomic inputs to peripheral tissues through modulation of sympathetic preganglion neurons and parasympathetic neurons of the dorsal nucleus of the vagus (Buijs et al., 2003; Dibner et al., 2010). Primary and secondary lymphoid organs, which include the thymus, spleen and lymph nodes, receive extensive autonomic inputs (Bellinger et al., 1993). For example, sympathetic noradrenergic input to the spleen originates from hypothalamic neurons, and upon stimulation, releases norepinephrine (NE) to mediate activity of NK cells, macrophages, among other lymphocytes (Bellinger et al., 1993; Cano et al., 2001; Dokur et al., 2004; Elenkov et al., 2000; Nance and Burns 1989). Recently, we demonstrated NE input to the spleen follows a robust circadian rhythm, which when abolished by sympathetic denervation, selectively disrupts the daily variations observed in cytokines and cytolytic factors in splenocytes and NK cells (Logan et al., 2011). In line with results in other tissues, these data suggest rhythmic sympathetic inputs modulate, but do not fully dictate, the phase of the rhythms in peripheral tissues (Vujovic et al., 2008). Similar to the spleen, the liver receives inputs from clock controlled sympathetic inputs and also contains

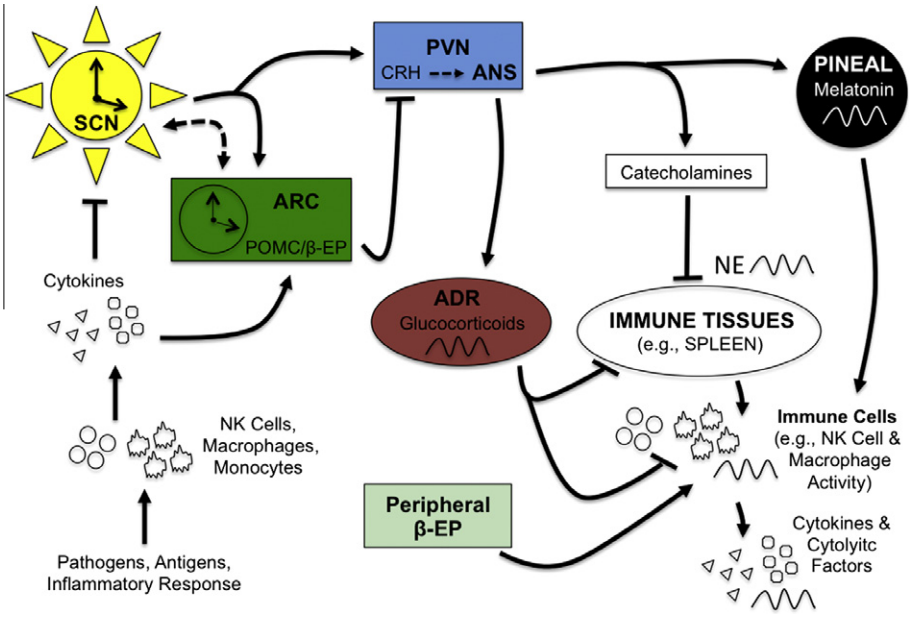

Fig. 1. Conceptual framework of biological clocks regulating immune function. Internal clocks within the suprachiasmatic nucleus (SCN, yellow), arcuate nucleus (ARC, green), and immune cells govern circadian rhythms of immune function. Both the SCN and ARC send projections to the paraventricular nucleus (PVN, blue) of the hypothalamus to modulate autonomic nervous system (ANS) endocrine and neural signaling to peripheral immune tissues. Under activated conditions, immune factors and cytokines also modulate clocks in the SCN and ARC. ADR, adrenals; β-EP, beta-endorphin; CRH, corticotropin releasing hormone; NK, natural-killer cells; POMC, proopiomelanocortin.

large numbers of lymphocytes (Cailotto et al., 2005; Gao et al., 2009; Terazono et al., 2003), suggesting hepatic NK cells are also entrained by rhythmic sympathetic input and may be critical for circadian regulation of immune response to cancer, as these cells have increased tumor cell cytotoxicity compared to those found in the blood and spleen (Vermijlen et al., 2002). Therefore, the neuroimmune axis comprised of the SCN, PVN, and sympathetic nervous system provides a temporal framework by which immune cells operate. The SCN appears to be a critical modulator of this system by balancing sympathetic and parasympathetic outflow to peripheral tissues (Buijs et al., 2003; 2008).

2.2. SCN–ARC pathways: involvement of opioids in circadian regulation of immune cells

Circadian rhythms of immune cells may also involve the endogenous opioid peptide beta-endorphin (β-EP). β-EP is derived from precursor proopiomelanocortin (POMC), which also give rise to adrenocorticotropic hormone (ACTH) after peptide cleavage (Nakanishi et al., 1979). In the brain, β-EP producing perikarya are primarily found in the ARC, where they send projections to many brain areas, particularly within the hypothalamus (O'Donohue and Dorsa, 1982; Wilcox et al., 1986). Bidirectional excitatory and inhibitory connectivity between the ARC and the SCN allows for control and feedback of circadian networks (Saeb-Parsy et al., 2000). Also, the ARC may be capable of generating rhythms in hormonal output and electrical activity independent of SCN modulation, including the production of POMC (Chen et al., 2004; Wyse and Coogan, 2010; Guilding and Piggins, 2007). Interestingly, we have shown cytokine expression and cytolytic activity of NK cells maintain a circadian rhythm that is quite similar to the peak and nadirs of POMC in the ARC (Chen et al., 2004), and when rhythms in ARC POMC are dampened or abolished, so are those in NK cells (Arjona et al., 2004; Arjona and Sarkar, 2006a,b; Chen et al., 2004). Thus, β-EP producing neurons in the ARC may intricately control circadian rhythms in NK cells and possibly other lymphocytes (Fig. 1). These actions may be mediated by CRH neurons in the PVN that regulate sympathetic signaling, as administration of either CRH antagonists in the PVN, or a ganglion blocker prevents hypothalamic β-EP's stimulatory effect on NK cell cytotoxicity (Boyadjieva et al., 2006), indicating β-EP's positive effects on NK cell function involves inhibiting CRH actions on sympathetic neural signals to peripheral tissues. In addition to their presence in the brain, POMC peptides are found in the intermediate and anterior lobes of the pituitary gland (Rossier et al., 1977), in lymphocytes (Labuz et al., 2010; Sitte et al., 2007; Smith and Blalock, 1981), and in macrophages (Lolait et al., 1984; Mousa et al., 2004). Indeed, β-EP regulates many immune functions, such as enhancing the production of interferon-gamma (IFN-γ) and granzyme B (GZMB) (Dokur et al., 2004; Boyadjieva et al., 2001), promoting splenic lymphocyte proliferation during an immune challenge (Boyadjieva et al., 2002), and increasing cytotoxicity of NK cells through action on δ- and μ-opioid receptors (Boyadjieva et al., 2004). Therefore, we propose the SCN, internal clocks in the POMC neurons of the ARC, and peripheral β-EP maintain circadian rhythms in immunocompetent cells, especially NK cells.

2.3. Endocrine regulators of immune function

Endocrine hormones also regulate the circadian rhythms of immune cells. The two major hormones involved are glucocorticoids and pineal melatonin. Circadian rhythms in peripheral glucocorticoid levels are controlled by SCN modulation of autonomic input to the adrenal glands and corticotropin-releasing hormone producing neurons in the PVN (Dibner et al., 2010). Local adrenal clocks also regulate glucocorticoid release and sensitivity of the gland to circulating ACTH (Dickmeis, 2009). Circulating glucocorticoids may be a major conveyor of circadian information to immunocompetent cells (Haus and Smolensky, 1999). Indeed, glucocorticoids are known to control cytokine production, leukocyte distribution, cell proliferation, and apoptosis (Fu and Lee, 2003). Melatonin is another regulator of the immune system and oscillates in antiphase with glucocorticoids. In mammals, circadian rhythms of pineal melatonin are generated by SCN-autonomic outputs. In bone marrow cells, pineal melatonin controls diurnal rhythms of leukocyte proliferation, cytokine production, and NK cell activity (del Gobbo et al., 1989; Drazen et al., 2001; Haldar et al., 1992; Matsumoto et al., 2001). Pro- and anti-inflammatory cytokines in the spleen are also regulated by rhythmic melatonin (Naidu et al., 2010). Thus, the opposing rhythms of glucocorticoids and melatonin, peaking during the day and night, respectively, are potential circadian regulators of the immune system. On the other hand, lack of plasma corticosterone and melatonin rhythms do not translate to a loss of rhythmicity in specific immune cells (Arjona and Sarkar, 2006a; Goto et al., 1989; Yang et al., 2009; Yellon and Tran, 2002), suggesting other mechanisms, such as sympathetic innervation and intracellular clocks are necessary for rhythm generation in immune tissues.

3. Components of the molecular clock play an integral role in circadian rhythms of the immune system

3.1. The molecular clock

In order to orchestrate coherency across multiple systems, outputs of the SCN coordinate semi-autonomous oscillators in peripheral tissues (Yamazaki et al., 2000; Yoo et al., 2004). In peripheral tissues, molecular clocks provide circadian modulation of tissue-specific physiology and metabolic programs (Hastings et al., 2003). Several interlocking positive and negative transcriptional–translational feedback loops form the molecular clock, which is driven by rhythmic near 24 h expression of clock genes and their protein products (Fig. 2; Reppert and Weaver, 2002). In the primary loop, CLOCK and BMAL1, thereby heterodimers bind to E-box promoter elements to promote the transcription of *Period (Per1,2,3)* and *Cryptochrome (Cry1,2)* genes. As protein levels increase, PER and CRY associate and translocate into the nucleus directly repressing CLOCK/BMAL1, thereby inhibiting their own transcription. Thus, the expression of positive factors CLOCK and BMAL1 and negative factors PER and CRY, are held in antiphase of one another providing circadian timing at the molecular level. The stability and robustness of the amplitude and period of the clock is provided by post-translational modifications, involving *Casein kinase 1 epsilon* and *delta* (*CK1ε,δ*) (Etchegaray et al., 2009; Gallego and Virshup, 2007; Meng et al., 2008). Auxiliary loops, involving retinoic acid nuclear hormone receptors, REV-ERBs and RORs also provide stability to the clock. REV-ERBs and RORs can repress or promote transcription of *Bmal1*, respectively, while PER abundance inhibits REV-ERB activity (Preitner et al., 2002; Emery and Reppert, 2004). Epigenetic mechanisms also regulate timing of molecular clocks, such as histone acetytransferase (HAT) and deacetyltransferase activity (HDAC), DNA methylation, and others. For example, transcription of *Per* and *Cry* is activated by phosphorylation in SCN and acetylation in liver of histone H3 (Crosio et al., 2000; Etchegaray et al., 2003; Nakahata et al., 2008), possibly involving HAT activity of CLOCK to H3 (Doi et al., 2006). Interactions between clock genes controlled by transcriptional, post-translational, and epigenetic states are necessary to generate circadian timing at the cellular level. In regards to the immune system, further research may uncover valuable evidence involving the role

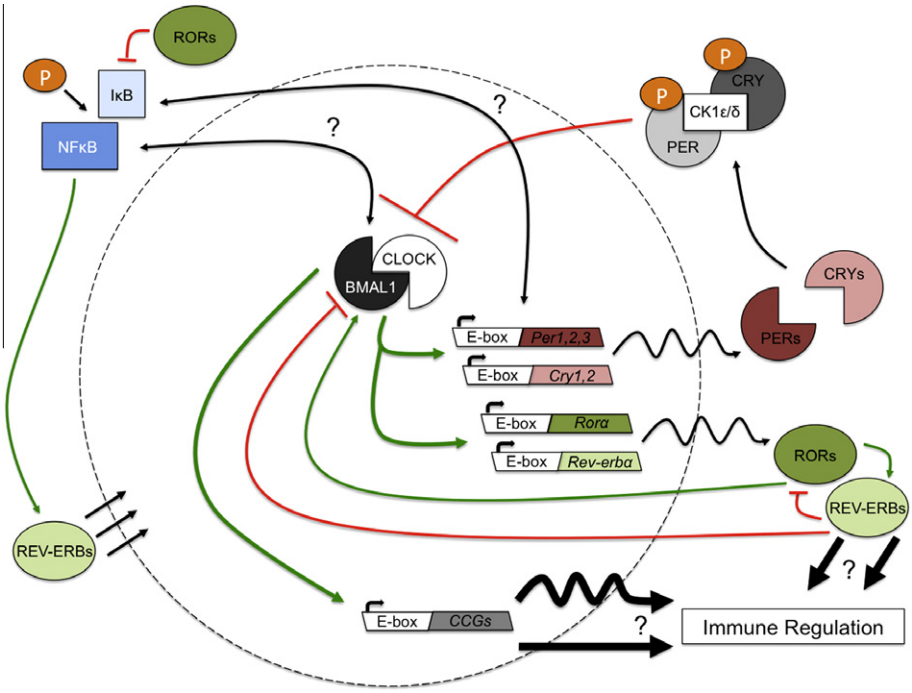

Fig. 2. Molecular connections between immune and biological clock pathways. The molecular clock consists of positive and negative autoregulatory feedback loops controlling circadian timing. The major components of the molecular clock are positive elements, CLOCK (white) and BMAL1 (black), and negative elements, PER and CRY (shades of red). CLOCK/BMAL1 heterodimers bind to E-box sequences to induce transcription of Per1–3 and Cry1,2, which translocate the cytoplasm to undergo translation. As PER and CRY proteins accumulate, they associate and translocate into the nucleus interacting directly with CLOCK/BMAL1 to inhibit their own transcription. BMAL1 and CLOCK also controls expression of CCGs (gray). Some CCGs are related to immune function. The nuclear receptors, RORs and REV-ERBs (shades of green), comprise an auxiliary loop that provides stability and robustness to the clock, but also regulates immune function. The NF-κB signaling pathway is involved in immune regulation by inducing gene expression and provides connections to the molecular clock. Potentially, BMAL1 regulates immune-related gene expression by modulating activity of NF-κB, such as phosphorylation (P) of IκB. NF-κB signaling could also modulate Per and Cry gene expression, although the direct connection has not been made. RORs interact with IκB to lift suppression of NF-κB, activating nuclear translocation and gene expression. NF-κB increases the stability of REV-ERBs increasing the chances of nuclear translocation and modulation of gene expression.

of epigenetics in the interactions between molecular clocks, cellular immune response and disease development.

The use of clock mutant mice in immune studies has shed light on the role of clock genes in immune function. We have reported *Per2* mutant mice have lowered expression of IFN-γ throughout the day (Arjona and Sarkar, 2006a,b). These findings are extended by others reporting *Per2* may be critical for initiating particular immune responses, as *Per2* mutant mice are relatively resistant to LPS-induced endotoxic shock, in part due to decreases in proinflammatory cytokines, IFN-γ and IL-1β, while other cytokines, TNF-α, IL-6, and IL-10, were similar to controls (Liu et al., 2006). Other clock mutant mice have different immune-related phenotypes. For example, mice deficient in BMAL1 have poor B-cell development, an essential component of the adaptive immune response (Sun et al., 2006), while *Clock* mutants fail to show rhythmic expression of a number of immunoregulatory genes in the liver (Oishi et al., 2003). A crictical limitation of using clock mutant mice is lack of specificity of immune phenotypes, such that it is difficult to determine if circadian disruption at the systemic level or clock genes within specific immune cells alters immune function (Arjona and Sarkar, 2006a,b). Further studies using cell specific manipulation of clock gene expression, such as knockdown or overexpression techniques, will help clarify specific roles of these genes under normal and challenged immune conditions.

3.2. Clock-controlled genes involved with immune function

The molecular clock is able to regulate tissue function with high specificity by controlling the expression of numerous downstream genes, deemed clock-controlled genes (CCGs). An estimated 10–15% of genes display circadian patterns of expression without considerable overlap between tissues (Akhtar et al., 2002; Oishi et al., 2003,2005; Panda et al., 2002; Storch et al., 2002). Molecular clock components CLOCK, BMAL1, REV-ERB, ROR, and clock-controlled transcription factors, such as albumin gene D-site binding protein (DBP), bind to specific motifs, including, not limited to, E-boxes, ROREs, and D-boxes, to induce transcription of CCGs. Moreover, CCGs with specific regulatory motifs vary depending on tissue and also circadian phase, strongly suggesting the molecular clock operates with some tissue specificity (Bozek et al., 2009). The clock regulates several transcription factors involved with the immune system, including signal transducer and activator of transcription 3 and 5 (*stat3 & stat5*), early growth response gene 1 (*egr1*), nuclear factor kappa B (NF-κB) (Bozek et al., 2009). Both STAT3 and STAT5 are primarily involved in intracellular cytokine signaling pathways of innate and adaptive immune responses (Alexander and Hilton, 2004; Fu, 2006), and activation of STAT3 is associated with immune suppression and cancer progression (Levy and Inghirami, 2006). Recently, it was shown STAT3 may be under transcriptional regulation by *Cry2* (Hoffman et al., 2009), suggesting the STAT pathway may be a potential interface between immune and clock pathways. Clocks may also regulate the early response of innate and adaptive immune systems by modulating the production of cytokines and other factors through direct control of NF-κB signaling pathways (Fig. 2; Bozek et al., 2009; Hayden et al., 2006; Scheidereit, 2006).

Other CCGs, such as REV-ERBs and RORs, are candidates linking the molecular clock to immune function. CLOCK/BMAL1 heterodimers directly regulate the transcription of *REV-ERBs* and *RORs*. REV-ERBs and RORs are also able to feedback to *Bmal1, Clock,* and

Cry1 to repress and activate transcription (Akashi and Takumi, 2005; Crumbley and Burris, 2011; Ko and Takahashi, 2006). Furthermore, these nuclear receptors are able to regulate immune cell function by recruiting and interacting with coactivators and corepressors of transcription (Jetten, 2009). For example, RORα is involved in cytokine secretion, probably by suppressing nuclear entry of NF-κB (Delerive et al., 2001; Dzhagalov et al., 2004). REV-ERBα may repress cytokine expression, similar to its action on *Bmal1* (Fig. 2). *Rev-erb*α mutant mice demonstrate a dramatically enhanced cytokine response to LPS, whereas activation of REV-ERBα in human aveolar macrophages reduces LPS-stimulated IL-6 release (Bechtold et al., 2010; Gibbs et al., 2009). Also, during immune activation, RORs can promote gene transcription in immune cells. However, there is little evidence directly connecting these components to circadian regulation of immunity. The promoting and repressing action of RORs and REV-ERBs within normal clock function may drive cyclical expression of immune factors and ready an immune cell for maximal response.

3.3. Clock genes and immune cells: evidence from natural-killer cells and macrophages

As critical mediators of the innate immune response, NK cells are the first line of defense against aberrant cells (Janeway and Medzhitov, 2002), including the rejection of tumor cells (Colucci et al., 2003). NK cells are quite different than T- and B-cells in that they are capable of lysing tumor and viral-infected cells without prior sensitization and can also modulate the adaptive immune system (Vivier et al., 2011; Yokoyama and Kim, 2006). NK cells are able to destroy infected and malignant cells by recruitment of pro-inflammatory cytokines and calcium-dependent release of cytolytic granules by activation of death receptors on the target cell via tumor necrosis factor (TNF) mediated pathways (Arjona and Sarkar, 2008; Austin Taylor et al., 2000; Biron, 1999). Among these, the release of granzymes, in particular GZMB and perforin (PERF), is the primary killing mechanism of the target cell (Barry and Bleackley, 2002; Raja et al., 2002). NK cells also produce cytokines, IFN-γ and TNF-α, and granular macrophage cell stimulating factor, which are critical for antiviral and antitumor defense, cytotoxicity, and promoting responses of macrophages and dendritic cells (Baxevanis et al., 2000; Biron and Brossay, 2001; Trinchieri, 1989; Vivier et al., 2008). We and others have shown many of these functions of NK cells are under circadian regulation ultimately by direct involvement of the molecular clock.

Under both entrained and constant darkness conditions, circadian expression of negative and positive components of the molecular clock, as well as cytokines and cytolytic factors, are evident in NK cells and other cells of the spleen (Arjona and Sarkar, 2005,2006a,b). Similar to other peripheral tissues, *Per1,2* and *Clock/Bmal1* are expressed in antiphase, peaking during the subjective day and night, respectively (Preitner et al., 2002). Also, *Dbp* oscillates in phase with *Per1* in NK cells, suggesting the clock regulates immune cellular pathways by promoting expression of CCGs containing D-box promoter elements (Arjona and Sarkar, 2005). Although the exact interactions and functions are unknown, it is hypothesized coordination of immune factors in order to promote response efficiency is under direct regulation by the molecular clock. In NK cells, expression of cytokines, IFN-γ and TNF-α and cytolytic factors, GZMB and PERF, are highly synchronized – peaking approximately during the middle of the active period in rats (Arjona and Sakar, 2006a,b). Interestingly, NK cell cytotoxic activity peaks at similar circadian phases (Arjona and Sarkar, 2006a,b). Similarly, NK cytotoxicity is maximal during periods of wakefulness in humans (Angeli, 1992; Gatti et al., 1987; Kronfol et al., 1997). Thus, it is plausible coordination of NK cell function by clock mechanisms provides an anticipatory state during times when individuals are more likely to be exposed to infection and disease. Clock genes are intricately involved with NK cell function. In rat-derived RNK16 NK cells, knock-down of *Per2* or *Bmal1* differentially alters gene expression of IFN-γ, TNF-α, GZMB, and PERF (Arjona and Sarkar, 2006a,b). Moreover, protein levels of GZMB and PERF are increased and decreased, respectively, following knockdown of *Per2* or *Bmal1*, without any effect on IFN-γ and TNF-α (Arjona and Sarkar, 2006a,b, 2008), suggesting disruption of the molecular clock alters the coordinated expression of NK cell cytolytic factors. Further, the seemingly opposite effects of *Per2* and *Bmal1* knockdown on cytolytic factors in NK cells could reflect the opposite roles of PER and BMAL1 in the molecular clock.

Other immune cells contain molecular clock machinery, including macrophages, B- and T-cells (Hayashi et al., 2007; Keller et al., 2009). In mouse peritoneal macrophages, inflammatory factors, IL-1β, IL-6 and TNF-α, display moderate circadian expression, whereas the rhythm of MCP-1/JE, an attractant and activator of monocytes and macrophages, is more pronounced (Hayashi et al., 2007). Interactions between BMAL1 and NF-κB are involved in driving the circadian expression of MCP-1/JE. MCP-1/JE oscillates closely with phagocytic activity, suggesting a clock driven anticipatory state in macrophages (Hayashi et al., 2007). Indeed, LPS stimulated release of TNF-α and IL-6 by macrophages depends on the phase of the molecular clock rather than circulating glucocorticoids (Keller et al., 2009). In addition, of the transcripts found in macrophages that are expressed in a circadian manner, a majority are involved at each level of LPS-induced immune response, including toll-like receptor (TLR) expression (Keller et al., 2009). Therefore, LPS-activated pathways in macrophages are under tight circadian regulation.

4. Back talk to the circadian system: immune factors act on central and peripheral clocks

The interactions between circadian and immune systems are bidirectional, such that immune factors are able to influence circadian timing by acting on the biological clock in the SCN and clocks in peripheral tissues (Fig. 1). In the brain, cytokine and corresponding receptor expression show diurnal variations. For example, levels of TNF-α in the brainstem, hypothalamus, and forebrain vary across the day (Bredow et al., 1997; Cearley et al., 2003; Taishi et al., 1997). In the hypothalamus, IL-1β peaks at similar times (Taishi et al., 1997). Also, TNF-α and IL-1β are found within and surrounding the SCN, where their receptors are also expressed rhythmically (Beynon and Coogan, 2010; Breder et al., 1993; Lechan et al., 1990; Sadki et al., 2007). The precise role of daily variations of cytokines in the brain and how they may be involved in circadian timing is unclear. Mice deficient of IL-1α/β and type I IFN receptors display unaltered heart rate, body temperature, and locomotor activity rhythms (Bohnet et al., 2004; Furuzawa et al., 2002). The apparent effects of immune on circadian physiology may be more evident under activated conditions. There is growing evidence administration of LPS, cytokines, or other factors directly modulate biological clocks. Acute inflammation only transiently synchronizes clock gene expression in peripheral blood (Murphy et al., 2007). Also, particular immune factors differentially modulate the circadian phase of the SCN – producing phase delays and advances depending on time of administration (Boggio et al., 2003; Marpegan et al., 2005; Sadki et al., 2007). The ability of specific immune factors to phase shift the circadian clock may be due to selective action on particular clock genes. For example, in response to systemic administration of LPS, *Per2* and *Dbp* expression is transiently repressed in the SCN (Cavadini et al., 2007; Okada et al., 2008), whereas IFN-α reduces CLOCK and BMAL1 lev-

els in the SCN (Koyanagi and Ohdo, 2002). In SCN slices, IFN-γ treatment blunted the rhythmic expression of *Per1* (Kwak et al., 2008). The suppression of clock genes *Per1,2,3*, *Clock, Bmal1, Rev-erb-α*, and *Dbp* in response to these treatments were also observed in the liver (Cavadini et al., 2007; Koyanagi and Ohdo, 2002; Okada et al., 2008) and may depend on timing of treatment (Shinohara et al., 2008). Immune repression of clock genes in the central and peripheral clocks is likely mediated through NF-κB pathways (Beynon and Coogan, 2010; Marpegan et al., 2005), implicating an immune-related feedback loop within the clock. In general, immune activation appears to dampen the activity of the clock by repressing gene expression, neuronal firing and neuropeptide signaling in the SCN, as well as influencing autonomic and endocrine outputs (Beynoon and Coogan, 2010; Coogan and Wyse, 2008; Frenois et al., 2007; Koyanagi and Ohdo, 2002; Kwak et al., 2008; Leak et al., 1999; Leak and Moore, 2001; Lundkvist et al., 2002; Nava et al., 2000).

5. Implications on health: the relationship between circadian disruption in immune function and disease

A number of studies have found associations between disruptions in circadian clocks and disease development and progression. Epidemiological studies in shift-workers suggests working during the night is a risk factor for several types of cancers, including non-Hodgkin's lymphoma (Lahti et al., 2008), breast (Davis et al., 2001; Hansen, 2001; Schernhammer et al., 2001), endometrial (Viswanathan and Schernhammer, 2009), prostate (Conlon et al., 2007; Kloog et al., 2009; Kubo et al., 2006), and colon (Kloog et al., 2009; Schernhammer et al., 2003) cancers. Moreover, a significant positive relationship was found between the frequency of rotating night shifts an individual had worked and the risk for developing breast or colon cancer (Schernhammer et al., 2001,2003). Also, significant predictors of survival in patients with breast and colon cancers are robustness of circadian rhythms in salivary cortisol and rest/activity cycles (Mormont et al., 2000; Sephton et al., 2000). Other diseases show high prevalence rates among shift-workers, including obesity (Chaput et al., 2006; Karlsson et al., 2001), diabetes (Morikawa et al., 2005), and cardiovascular problems (Haupt et al., 2008; Tenkanen et al., 1998; Tuchsen et al., 2006). Although the mechanisms underlying the association between shift-work and disease are unknown, it is becoming more apparent alterations in common circadian and immune pathways may promote disease development.

In particular, animal models have provided initial insights into the mechanistic link between circadian disruption and cancer. In mice, circadian disruption by ablating the SCN or by experimental manipulation through chronic jet lag (CJL), produced alterations in the circadian rhythms in circulating lymphocyte numbers, which was associated with an increased rate of tumor growth (Filipski et al., 2003, 2006). However, the effects of CJL-induced decreases in lymphocyte numbers on cancer progression have not been systematically addressed. Other mechanisms have received more attention. Alterations in cell proliferation, DNA repair, and apoptotic mechanisms have been found following CJL and in clock mutant mice, which are correlated with increased tumor growth (Filipski et al., 2006; Fu and Lee, 2003; Miyazaki et al., 2010; Sukumaran et al., 2010). Moreover, it is fairly well established the effects of circadian disruption on cancer progression is through down-regulation of clock gene expression, which are known regulators of particular tumor suppressor proteins, in the affected tissue and tumor cells (Filipski et al., 2006; Fu and Lee, 2003). Intriguingly, when *normal* circadian phase of clock gene expression is restored in peripheral tissues, the rate of tumor growth is slowed (Yasuniwa et al., 2010). Another study has reported CJL disrupts the balance of sympathetic output to peripheral tissues, and increases oncogenic activation of tumor cell proliferation pathways (Lee et al., 2010). Thus, dysregulation of circadian sympathetic input and clock genes appears to be a major pathway by which chronic shift-work leads to an increased risk of cancer.

It is obvious much attention has been placed on the effects of circadian disruption on intracellular pathways governing tumor cell proliferation, rather than the effects of immune function. Several studies are worth highlighting in order to gain some insight into the underlying mechanisms of circadian disruption and immune function. As briefly described before, evidence from clock mutant mice indicates clock gene disruption regulates specific immune parameters. The mutation of *Clock* suppressed circadian rhythms in circulating leukocytes and multiple immune-related genes (Oishi et al., 2003,2006). The mutation of *Per2* leads to a loss of daily rhythm of IFN-γ (Arjona and Sarkar, 2006a,b), and resistance to LPS-induced endotoxic shock (Liu et al., 2006). Further, the relative phase coherence of GZMB and PERF expression is governed by PER2 (Arjona and Sarkar, 2006a,b). In addition, circadian disruption of rhythmic sympathetic input to the spleen alters circadian rhythms of cytokines and cytolytic factors in immune cells by disrupting the core components of the molecular clock (Logan et al., 2011), supporting the view circadian disruption desynchronizes central and peripheral immune clocks. These changes in circadian rhythms of NK cell function may be particularly important in cancer progression, as these cells are involved in tumor surveillance (Terao et al., 2002), and suppression of NK cytolytic activity is associated with increased rapidity of metastatic breast cancer (Sephton et al., 2000). It would be necessary to provide experimental evidence that activity of NK cells are susceptible to experimental models of circadian disruption, such as CJL, especially in promoting tumor growth.

A common underlying risk factor for many diseases is inflammation. In a recent study by Davidson and colleagues (Castanon-Cervantes et al., 2010), circadian desynchronization by experimental jet-lag resulted in uncoordinated inflammatory responses to LPS challenge that led to increased mortality in these mice. Additionally, several key immune factors were upregulated in serum of shifted mice, including IL-6, macrophage inflammatory protein-2 (MIP-2), and leukemia inhibitory factor (LIF). Shifted mice also exhibited an exacerbated innate immune response to LPS, which was evident by significant increases in activating components, IL-1β, IL-12, IL-13, and granulocyte macrophage colony-stimulating factor (GM-CSF), and by decreases in IL-10, an anti-inflammatory mediator. These changes were due to cumulative circadian disruptions, as significant changes in immune response were only evident in mice undergoing multiple shifts, and were independent of changes in melatonin rhythms, stress response, and sleep-wake cycles (Castanon-Cervantes et al., 2010). This study is the first direct evidence that chronic circadian disruption produces profound disruptions in immune response. It is reasonable to hypothesize immune function is particularly sensitive to circadian disruption, however the extent to which immune function is impaired and how this may lead to disease requires more comprehensive study. It has been proposed circadian coordination among innate and adaptive immune system may provide a critical state of anticipation and readiness if a challenge arises (Arjona and Sarkar, 2005; Castanon-Cervantes et al., 2010), and upon desynchronization causes particular vulnerabilities to infection and disease.

6. Conclusion

Many immune cells and functions are under circadian control from central and peripheral clocks. The bidirectional interactions

between circadian and immune systems is a dynamic process involved in providing coordination among organ systems, especially during immune challenge. This conceptual model suggests during disease states circadian functioning is greatly impacted. During immune response, including inflammation, biological clocks are dysregulated. This disruption in circadian timing may lead to further progression of the disease. Research elucidating these relationships is critical for understanding disease manifestation, progression, and most importantly, in order to develop novel therapeutic strategies. It is obvious more research is required to understand the basic roles of circadian rhythms, from individual clock genes to SCN signaling outputs, in immune function, and how alterations in both of these may negatively impact health outcomes.

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