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Focused Review

Neuroimmunology of the circadian clock

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ABSTRACT

Circadian timekeeping is a ubiquitous feature of all eukaryotes which allows for the imposition of a biologically appropriate temporal architecture on an animal's physiology, behavior and metabolism. There is growing evidence that in mammals the processes of circadian timing are under the influence of the immune system. Such a role for the neuroimmune regulation of the circadian clock has inferences for phenomena such as sickness behavior. Conversely, there is also accumulating evidence for a circadian influence on immune function, raising the likelihood that there is a bidirectional communication between the circadian and immune systems. In this review, we examine the evidence for these interactions, including circadian rhythmicity in models of disease and immune challenge, distribution of cytokines and their receptors in the suprachiasmatic nucleus of the hypothalamus, the site of the master circadian pacemaker, and the evidence for endogenous circadian timekeeping in immune cells.

1. Introduction

Current understanding is that the central nervous system is by no means the "immune-privileged" site it was once considered to be (Lucas et al, 2006). Indeed the brain and spinal cord show signs of inflammation in response to injury/infection, and this immune-response is coordinated by a number of mediators, including cytokines and prostaglandins, which may be produced by, and act on, glial and neuronal cells. Systemically produced inflammatory mediators may also cross the blood–brain barrier and act on specific receptors expressed on neuronal and glial cells (for review see Pollmacher et al., 2002).

Such is the wide spectrum of actions of neuroimmune signalers in the mammalian CNS that a syndrome of behavioral alterations that occur during infection, termed "sickness behavior" has been ascribed to the central action of immune signalers (Dantzer, 2006). The physiological and behavioral alterations that characterize sickness behavior during the acute phase of the immune response (lethargy, weakness, listlessness, decreased food intake, altered sleep/wake patterns and a tendency towards depression) have previously been described to have a circadian component (e.g. Wirz-Justice 2006). Circadian rhythms are recurring patterns in behavioral, endocrine and physiological parameters that exhibit periodicities of approximately 24 h (Levi and Schibler, 2007). In mammals the master circadian pacemaker is localised to the suprachiasmatic nucleus (SCN) of the anterior, ventral hypothalamus (Ralph et al., 1990), although several other central and peripheral sites are now understood to express quasi-autonomous circadian clocks (Guilding and Piggins, 2007; Levi and Schibler, 2007). The molecular basis for

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Abbreviations: AVP, arginine-vasopressin; CLC, cardiotrophin-like cytokine; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; SCN, suprachiasmatic nucleus; SOCS, suppressor of cytokine signaling; TGF, tansforming growth factor; TNF, tumour necrosis factor

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circadian rhythm generation involves interlocking feedback/feedforward transcriptional loops involving a panel of "Clock" genes (*Clock*, *Bmal1*, *Per1*, *2*, *3*, *Cry1*, *2*, *Rev-erb-α*, *Dec 1*, *2*) which in turn can control the expression of a number of clock-controlled genes. Some estimates have put the percentage of transcripts being under a measure of circadian control as high as 99% (Ptitsyn et al., 2007). Output from the SCN to target sites is via both humoral and neuronal connections, and clock output is known to have significant influence on the architecture of the sleep/wake cycle, food intake, attention, learning and memory and other cognitive functions (Kalsbeek et al., 2006). A number of afferent or SCN-intrinsic neurotransmitters/neuropeptides are also found to alter SCN neuronal function and play roles in phase-setting, illustrating the sensitivity of the SCN clock to neurochemical input (e.g. Coogan et al., 2001).

Thus, the question arises, to what extent the behavioral effects of centrally acting cytokines and other immune mediators are mediated via the circadian clock. In this review, we will further examine the current evidence for neuroimmune modulation of the circadian clock, and the modulation of immune function by the core circadian clockworks, and look towards the future in what promises to be an important and exciting topic in chronobiology and neuroimmunology.

2. Do immune mediators affect the circadian clock?

There are a number of lines of evidence that suggest that an animal's immune-status can influence circadian timekeeping processes, and likewise that cytokines and other immune mediators can also influence these processes. Treatment of hamsters with systemic lipopolysaccharide (LPS), a bacterial endotoxin that stimulates the inflammatory response both centrally and in the periphery, was found both to induce phase-delays in free-running hamsters in a manner that was not additive with photic-induced phase-shifts (Marpegan et al., 2005) and also to alter the photic-induction of c-Fos in the SCN of mice in a reversible fashion (Palomba and Bentivoglio, 2007). LPS treatment has also been shown to induce the expression of the circadian clock gene *Per1* in the paraventricular nucleus of the hypothalamus (Takahashi et al., 2001). More recently, LPS treatment has also been shown to suppress the SCN expression of *Per2* and the clock-controlled gene *dbp* on the first day following treatment, with rhythmicity then being restored from day 2 onwards, whilst in the liver levels of *Per1* and *Per2*, along with a number of clock-controlled genes, were suppressed at day 1, but normal expression was restored from the second day post-treatment (Okada et al., 2008). *In vitro*, treatment of SCN slice cultures with LPS leads to an increase in arginine-vasopressin (AVP) production, suggesting that SCN derived neuroendocrine output can be directly modified by immune challenge (Nava et al., 2000).

If immune stimulation by LPS or other infectious agents and autoimmune processes can alter circadian timing, it is likely that they will do so through cytokine and other immune-mediator regulation. It is well established that peripheral LPS or pro-inflammatory cytokines will lead to the central upregulation of pro-inflammatory mediators such as ineterleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α), perhaps via actions at the circumventricular organs (Parnet et al., 2002). Thus, it is important to ascertain the extent to which such immune mediators can directly influence circadian timekeeping processes, and a number of studies to date have addressed this issue. A recent study by Cavadini et al. (2007) has shown that treatment of fibroblasts with TNF-α downregulates the rhythmic expression of *Per1*, *2* and *3* (but not *Bmal1*) as well as that of the PARbZIP transcription factors that function in clock output (*tef*, *hif* and *dbp*) and IL-1β was found to significantly attenuate the expression of *Per3* and *dbp*. This suppression was found to be mediated via E-box elements on the promoter sequences of these clock associated genes. Furthermore, subcutaneous administration of TNF-α was found to reduce the expression of *Per1*, *2*, *3*, *tef*, *hif* and *dbp*, as well as *rev-erb-α* in the livers of treated animals and downregulated the expression of *dbp* in the SCN. This finding suggests that the effects of peripheral cytokines can be translated across the blood–brain barrier to affect SCN function. Finally in this study, the authors report that peripheral TNF-α treatment, at a dose that does not elicit sickness behavior, caused significant decreases in the amount of nocturnal activity, whilst having no effect on the free-running period.

In an electrophysiological study of the action of cytokines in the SCN, a cocktail of TNF-α, LPS and interferon (IFN)-γ has been reported to cause a decrease in the frequency of both excitatory and inhibitory currents (Lundkvist et al., 2002). Similarly, this cocktail of inflammatory mediators ablated the circadian rhythm in spontaneous neuronal firing frequency of the SCN, reducing firing frequency to nadir levels across the cycle. Interestingly, this flattening of the SCN firing rate rhythm by these cytokines is similar to that seen in aged mice and hamsters (Nygard et al., 2005; Watanabe et al., 1995), indicating that cytokine action in the SCN may play a role in age-related circadian dysfunction, as they are proposed to do in other brain regions (Godbout and Johnson, 2006). IL-1β, TNF-α and LPS treatment of SCN derived astrocytes all result in activation of the NFκB signaling pathway (Leone et al., 2006). Also, i.c.v injection of TNF-α/IFN-γ induced marked glial activation in both young and old mice, although the aged group showed a more exaggerated response to the cytokine treatment (Bentivoglio et al., 2006).

Sadki et al. (2007) have examined the effect of TNF-α/IFN-γ i.c.v microinjection on SCN expressed c-Fos. Treatment of young animals with these cytokines during the subjective night was found to induce c-Fos expression in the "shell" region of the SCN, a region known to rhythmically express clock genes and produce humoral clock output signals such as arginine-vasopressin (Antle and Silver, 2005). In young animals treated with TNF-α and IFN during the early subjective night, c-Fos was induced, but in the "core" region of the SCN, an area known to directly responsive to photic stimulation. This temporal regional variation in c-Fos induction by TNF-α and IFN-γ was not seen in the SCN of aged mice, in which induction of c-Fos occurred in the shell region during both the lights on and off periods. These findings suggest that the effects of cytokines on the SCN are age-dependent, and may mediate the changes in circadian timekeeping that accompany old age.

As well as the above-mentioned studies, a number of studies have examined the effects of interferons on circadian

timekeeping, and have indicated important considerations for the field of chronotherapeutics. The interferons are a highly conserved group of molecules that play roles in the immune response as well as physiological processes such as development and metabolism (Campbell et al., 1999). The interferons are broadly classed into type I interferons (IFN-α and IFN-β principal amongst these), molecules with immunomodulatory properties, and type II interferons (IFN-γ), which are not structurally related to the type I interferons, but also have immunoregulatory and immunoactivating properties (Takaoka and Yanai, 2006). It has been shown that i.c.v. microinjection of IFN-γ in hamsters leads to phase-advances when applied during the subjective day, but not during the subjective night (Boggio et al., 2003). This type of phase-dependency suggests that IFN-γ may reset the SCN pacemaker in a non-photic manner, although a more comprehensive phase-response curve is required prior to making any firm deductions. IFN-α has also been reported to have a number of effects on the circadian system. There are diurnal variations in the efficacy and tolerance of IFN-α immunotherapy (Abrams et al., 1985; Koren and Fleischmann, 1993), and these variations may be due to temporal variations in the number of receptors expressed on IFN-α responsive lymphocytes and the metabolism of IFN-α (Ohdo et al., 2001). Similar diurnal variation in receptor expression may also account for the variability of IFN-β's antiviral properties (Takane et al., 2002). The role of interferon treatment on the SCN clock was then examined, as alterations in the central pacemaker could, via endocrine signalers, lead to alterations in peripheral immunity. Dosing of mice with a single treatment of IFN-α at "dusk" (Zeitgeber Time (ZT) 12) led to significant blunting of the SCN rhythm in *Per1*, 2, 3 and *Bmal1* expression, whilst dosing at "dawn" (ZT0) had no such effect (Ohdo et al., 2001). Dosing of mice with IFN-γ for 6 days also significantly depressed SCN *Per* expression, when the time of treatment was ZT12 but not ZT0. Repeated dosing with IFN-α at ZT12 was also found to attenuate the induction of *Per* by a nocturnal light-pulse (Ohdo et al., 2001). These changes in gene expression profiles following IFN-α treatment at ZT12 were mirrored in the blunting of locomotor and body temperature rhythms. Thus, these authors have proposed a chronological schedule for dosing with IFN that could minimize the effects on the circadian pacemaker, which in turn might contribute to the adverse CNS side-effects that such treatment often entails.

Further, treatment with IFN-α was then described as down-regulating the expression of *Clock* and *Bmal1* in a human hepatocyte cell line, in a STAT1 dependent mechanism (Koyanagi and Ohdo, 2002). Continuous infusion of IFN-α via an osmotic minipump also downregulated the Clock and Bmal-1 immunoreactivity in the SCN (Koyanagi and Ohdo, 2002). This continuous application of IFN-α also blunts the SCN rhythms in the core clock genes *Per1*, *Per2*, *Cry1*, and the outputs *avp* (SCN) and *dbp* (liver). The locomotor rhythm of mice receiving this infusion of IFN-α became fragmented under a light/dark schedule, with decreased nocturnal activity accompanied by an increase in activity during the lights on period (Koyanagi and Ohdo, 2002).

To date, the impact of immune mediators in circadian timekeeping has been assessed in classical rodent models which are nocturnal. What has yet to be ascertained is whether diurnal rodents (e.g. the Nile rat *Arvicanthis niloticus*, the ground squirrel *Spermophilus citellus*) show altered sensitivity to such mediators and challenges, and shed light onto the appropriateness of the use of nocturnal species for translation research into another diurnal species, the human.

3. How does infection alter circadian rhythms?

Models of infection and disease states have also been used to demonstrate chronobiological effects. A recent study has demonstrated altered circadian rhythms in body temperature and locomotor activity, both rhythms that are SCN dependent, in monkeys infected with simian immunodeficiency virus (Huitron-Resendiz et al., 2007). Trypanosomiasis, also known as African sleeping sickness, alters the induction of c-Fos in the SCN in response to photic stimulation in mice (Peng et al., 1994), as well as spontaneous c-Fos expression in the SCN (Bentivoglio et al., 1994). Lundkvist et al. (2002) examined the effects of trypanosomiasis on the neurophysiological properties of the SCN, and found that infection leads to a decrease in the frequency, but not amplitude, of excitatory post-synaptic currents, indicating a decrease in transmitter release probability. Circadian rhythms are disrupted in models of chronic peripheral inflammation, such as monoarthritis (Millecamps et al., 2005). In humans, diseases with neuroinflammatory components, such as Alzheimer's disease, are accompanied by marked circadian dysregulation (Hatfield et al., 2004).

4. Is there physiological neuroimmune modulation of the circadian system under basal conditions?

The above-discussed lines of evidence point to the ability of the immune system to affect circadian timekeeping processes during disease states and pharmacological treatment. However, evidence from other systems, such as sleep regulation, point to roles of cytokines in normal physiological processes (Opp, 2005). Thus, we ask the question as to whether there is evidence to suggest that neuroimmune regulation plays a role in normal circadian processes.

A number of immunohistochemical studies have described the presence of Il-1β and TNF-α in and around the SCN (Lechan et al., 1990; Breder et al., 1993), although subsequently considerable reservations have been raised as to the specificity of the antisera used in these studies. Intriguingly, brain levels of IL-1β and TNF-α have been reported to display diurnal variation, with hypothalamic levels of IL-1β being higher during the lights on period (Taishi et al., 1997) and expression of TNF-α mRNA showing a diurnal variation that parallels the amount of non-REM sleep (Bredow et al., 1997), with levels across the forebrain, diencephalons and brainstem higher at ZT2 than at ZT11 (Cearley et al., 2003).

Expression of receptors for TNF-α has been demonstrated in the SCN by means of RT-PCR, with expression levels showing a diurnal rhythm in both young and aged mice (Sadki et al., 2007). The SCN also exhibits rhythmic expression of suppressor of cytokine signaling (SOCS) 1 and 3, molecules

that constitute an intracellular inhibitory feedback mechanism on cytokine signaling (Sadki et al., 2007). To date there are no published reports of IL-1β receptor expression in the SCN, although diurnal rhythms in heart-rate, body temperature and locomotor activity, all processes known to have a strong control from the SCN, are altered in mice doubly knocked-out for IL-1α and β, suggesting that interleukin-1 plays a role in the normal, physiological circadian modulation of these parameters (Furuzawa et al., 2002).

Prevot et al. (2000) have described SCN expression of receptors for the anti-inflammatory cytokine transforming growth factor (TGF)-β1, although the physiological significance of this observation is not clear, as TGF-β1 has not been investigated in a chronobiological context.

The SCN has been shown to express class II interferon receptors (responsible for binding IFN-γ), and this expression in the SCN is regulated by development stage and exposure to the light/dark cycle (Lundkvist et al., 1998, 1999). The diurnal pattern of the receptor in the SCN was mirrored by the levels of JAK1 and JAK2, signal transducers for the interferon system, as well as STAT1 (Lundkvist et al., 1998). The presence of receptors for IFN-γ is in agreement with the above-discussed effects of IFN-γ, when applied with TNF-α and/or LPS, in inducing changes in gene expression and electrophysiological parameters (Lundkvist et al., 2002; Sadki et al., 2007). IFN-γ receptor mRNA ceases to show significant rhythmicity in its SCN expression in aged mice, suggesting a modification of the system with increasing age that may contribute to altered circadian timekeeping in senescence (Sadki et al., 2007). That type I interferons (which include IFN-α and IFN-β) might play a role in circadian timekeeping is argued against by the findings of Bohnet et al. (2004) who reported that mice knocked-out for type I IFN receptors show normal sleep and temperature rhythms.

Recently, a non-classical cytokine, cardiotrophin-like cytokine (CLC) has been postulated to act as SCN locomotor inhibitory output (Kraves and Weitz, 2006). This cytokine signals through the GP130 complex, which has been described in the periventricular region of the hypothalamus, an area known to be important in regulating locomotor rhythms. CLC was then found to be expressed in a rhythmic manner by a subset of SCN neurons, and when infused i.c.v. into the third ventricle it was found to depress locomotor activity. Ciliary neurotrophic factor, another cytokine that signals through the same GP130 complex as CLC also caused this profound attenuation of motor output, although IL-6 and IL-11, which signal through different GP130 containing receptor complexes, did not (Kraves and Weitz, 2006). These effects of CLC on locomotor rhythms are the first brain-actions of this cytokine described, and appear to be separated from any immune/inflammatory role CLC may play, either in the periphery or the brain. This finding suggests that other cytokines may be found to have physiological effects on the circadian system that are independent of their immune functions.

Thus, there is evidence that the SCN in the resting state expresses receptors for a number of cytokine systems. However, much work needs to be done to investigate the roles these systems may play in setting and maintaining circadian phase and output. Further to this, it is now recognized that the circadian timekeeping system in the brain is distributed and involves a number sites that are putative semi-autonomous oscillators, such as the olfactory bulb, the habenula, the arcuate nucleus of the hypothalamus and the dosomedial hypothalamus, proposed site of the food-entrainable circadian oscillator (Guilding and Piggins, 2007). Thus, it is possible that cytokines alter circadian timekeeping by acting at extra-SCN components of the system. Table 1 summarises the localisation of cytokines and their receptors in the SCN and extra-SCN sites, although much work needs to be done to delineate the consequence of neuroimmune interaction on biological timekeeping at these sites.

Table 1 – Expression of cytokines, and their receptors, in the master SCN pacemaker and central, semi-autonomous oscillators of the circadian system of the rodent brain

Cytokine/receptor	Location	Method of detection	Reference
Il-1β	SCN	Immunohistochemistry	Lechan et al. (1990)
TNF-α	SCN	Immunohistochemistry	Breder et al. (1993)
TNF-α	SCN	RT-PCR	Sadki et al. (2007)
IL-6	SCN	*In situ* hybridisation	Gonzalez-Hernandez et al. (2006)
TGF-β1 receptor	SCN	*In situ* hybridisation	Prevot et al. (2000)
IFN-γ receptor	SCN	Immunohistochemistry	Lunkvist et al. (1998, 1999)
IFN-γ	SCN	RT-PCR	Sadki et al. (2007)
CLC	SCN	*In situ* hybridisation	Kraves and Weitz (2006)
IL-2/15	Habenula	*In situ* hybridisation	Petitto and Huang (2001)
IL-18	Habenula	*In situ* hybridisation/immunohistochemistry	Sugama et al. (2002)
IL-6/IL-6 receptor	Habenula	*In situ* hybridisation	Schobitz et al. (1992)
IL-6/IL-6 receptor	Dorsomedial hypothalamus	*In situ* hybridisation	Schobitz et al. (1992)
IL-1 type I receptor	Dorsomedial hypothalamus	Immunohistochemistry	Hassanain et al. (2005)
IL-2 receptor	Dorsomedial hypothalamus	Immunohistochemistry	Bhatt et al. (2005)
IL-2/IL-2 receptor	Arcuate nucleus	*In situ* hybridisation	Lapchak (1992)
Il-1 type 1 receptor	Arcuate nucleus	*In situ* hybridisation	Ericsson et al. (1995)
IL-2	Arcuate nucleus	PCR	Tanebe et al. (2000)
TGF-β receptor	Arcuate nucleus	*In situ* hybridisation	Prevot et al. (2000)
IL-1β	Olfactory bulb	*In situ* hybridisation	Bandtlow et al. (1990)
IL-1β	Olfactory bulb	*In situ* hybridisation	Lim and Brunjes (1999)
IL-2/IL-2 receptor	Olfactory bulb	RT-PCR/immunohistochemistry	Wang et al. (2001)

5. Dual roles of cytokines in sleep and circadian regulation?

A potentially important interaction with regards to understanding the roles of neuroimmune regulation of circadian rhythms is the link between the circadian timekeeper and sleep processes. In the two process model of sleep regulation, the circadian drive to sleep (process C) interacts with the homeostatic drive to sleep (process S) (Borbely, 1982). Sleep status can also feedback onto the circadian pacemaker — for example, sleep-deprivation in hamsters elicits non-photic phase-shifts of wheel-running rhythms (Antle and Mistlberger, 2000). Cytokines such as IL-1β, TNF-α and IL-6 have a number of roles ascribed to them in the physiological and regulation of both REM and non-REM sleep (Opp, 2005). For example, injection of IL-1β into the dorsal raphe nucleus increases non-REM (Manfridi et al., 2003). Injection of TNF-α into the pre-optic area also increases non-REM sleep and diminishes REM sleep (Kubota et al., 2002), whilst mice doubly knocked-out for the TNF receptor and the IL-1 type I receptor show altered REM and non-REM sleep following sleep-deprivation when compared to controls (Baracchi and Opp, 2008). Levels of these cytokines exhibit diurnal rhythms in the brain that mimic timing of non-REM sleep (Bredow et al., 1997; Taishi et al., 1997). There are also extensive, if sometimes indirect, neuroanatomical connections between the SCN and other regions associated with homeostatic sleep regulation (e.g. pre-optic hypothalamus, dorsal raphe; Saper et al., 2005). Thus, there is the distinct possibility that results examining circadian rhythmicity in response to cytokine treatment may be an indirect consequence of altered sleep. Likewise, such a consideration must be taken into account when considering circadian disturbance during neuroinflammatory diseases (Bentivoglio and Kristensson, 2007). At present there is a distinct lack of studies that examine electrophysiologically determined sleep parameters in concert with measures of circadian rhythmicity. Until this knowledge gap is addressed, we can only make tentative assumptions about the direct roles of cytokines in the *in vivo* regulation of circadian timekeeping.

6. Are there intrinsic circadian clocks in the peripheral immune system?

There is a growing body of evidence that suggests that peripheral immune cells possess endogenous circadian clocks and display rhythmic expression of clock genes. Circadian rhythmicity is an established feature of many immune parameters and significant diurnal variation was demonstrated in circulating levels of all human peripheral blood mononuclear cell (PBMC) subsets (Born et al., 1997), and in serum cytokine and cytokine receptors including IL-2, IL-10, GM-CSF, IL-1β, IL-6, TNF-α, MCP-1JE, CCR2, IFN-γ, and IFN receptors (Young et al., 1995; Lundkvist et al., 1998; Takane et al., 2002; Hayashi et al., 2007). Furthermore, circadian oscillation in PBMCs was shown to occur independently of the sleep–wake cycle (Born et al., 1997), corroborating the endogenous origin of these rhythms. Interestingly, circadian rhythms in circulating T-lymphocytes persisted despite constant-light mediated desynchronization and suppression of circadian rhythms in catecholamine release, locomotor activity and body temperature in rats, suggesting that the putative immune oscillator may operate independently of the other major outputs of the circadian clock (Depres-Brummer et al., 1997). The circadian features of the immune system are most likely mediated, in part at least, by autoregulatory oscillations of the canonical clock genes and their protein products — a mechanism originally characterized in the master clock in the SCN, and since found to be common to all peripheral oscillators. The clock gene, *Per1*, shows daily oscillatory expression in immune cells (Kusanagi et al., 2004; Arjona and Sarkar, 2005), and circadian patterns of expression of *Bmal1*, *Clock* and *Per2* in murine peritoneal macrophages were similar to those shown in other peripheral oscillators (Hayashi et al., 2007). Furthermore, circadian oscillation in *Per2* expression and in cytokine release was shown to persist in NK cells taken from rats kept in constant darkness (Arjona and Sarkar, 2005), supporting the endogenous origin of these rhythms. LPS-induced systemic inflammation caused upregulation of peripheral blood clock genes (*Per2* and *Bmal1*) in horses (Murphy et al., 2007). In the mouse jejunum, there is rhythmic expression of a panel of clock genes as well as circadian expression of a number of toll-like receptors, the sensors of the innate immune system for bacterial and viral antigens (Froy and Chapnik, 2007).

Further insight into the regulation of immune function by circadian mechanisms has been derived from studies with clock gene knockout mice. *Clock*-mutant mice showed phase-delayed circadian patterns in lymphocyte numbers, and a bimodal pattern of plasma neutrophils, compared with wild-type mice (Oishi et al., 2006). *Per2* mutant mice did not show a circadian rhythm in IFN-γ mRNA expression that characterized wild-type control mice (Arjona and Sarkar, 2005). Furthermore, *Per2*-deficient mice also showed aberrations in cytokine production in response to LPS challenge, most significant of which was dramatically reduced levels of IFN-γ and IL-10 (Liu et al., 2006). *Per2* mutant mice also demonstrated loss of a circadian pattern of resistance to LPS challenge that was well-defined in the wild-type mice, and the *Per2*-deficient mice were more resistant to LPS-induced death (Liu et al., 2006). These effects were attributed to defective production of IFN-γ and IL-10 by NK and NKT cells, and provide evidence that *Per2* may mediate the production of IFN-γ and IL-10 following an immune challenge (Liu et al., 2006; Arjona and Sarkar, 2005). Knockdown of *Bmal1* expression by RNA interference suppressed NF-kB activity and MCP-1JE mRNA expression in macrophages (Hayashi et al., 2007). The fact that immune cells exhibit circadian variation in function and cytokine production, along with concurrent expression and oscillation of clock genes, illustrates the potential significance of clock gene mediated control of cytokine production.

7. Bidirectional communication between the circadian and immune systems?

So far we have described the evidence that the master circadian pacemaker located to the SCN is amenable to neuroimmune modulation, and that the peripheral immune system contains semi-autonomous timekeeping mechanisms. The

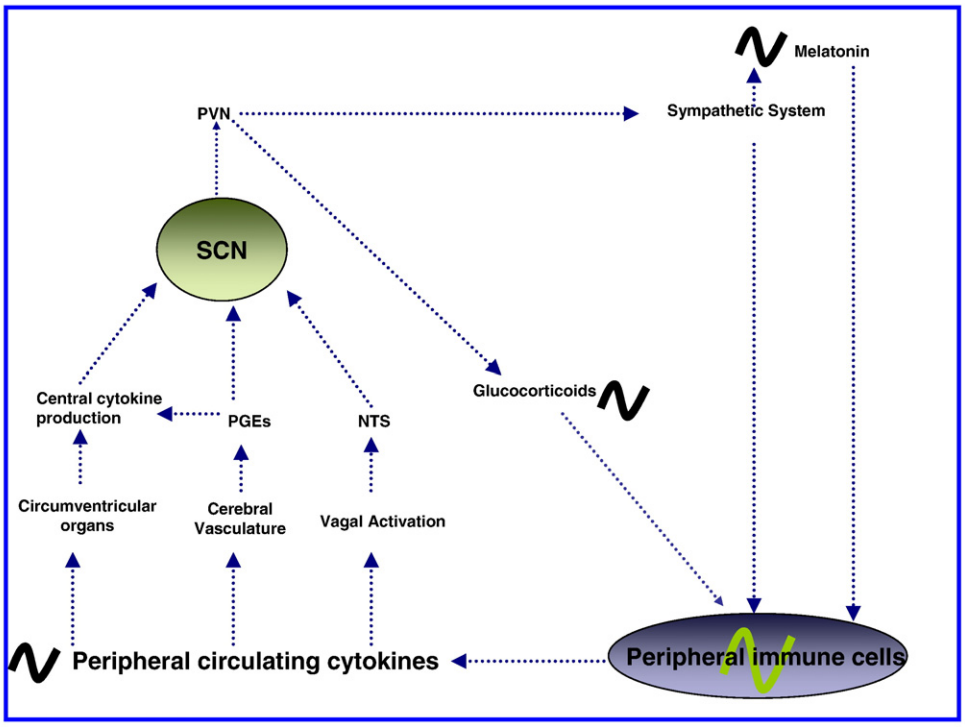

Fig. 1 – Schematic illustrating some putative pathways by which circadian output from the SCN may be influenced, and in turn influence, levels of peripheral and central cytokines. SCN output via the autonomic system, hypothalamic–pituitary axis activation or melatonin secretion (all involving SCN projections to the paraventricular nucleus (PVN) of the hypothalamus) impacts on cytokine production by peripheral immune cells, some of which have also been shown to possess an endogenous clock. Circulating cytokines may then feedback to the SCN via induction of prostaglandins (PGEs) in the cerebral vasculature, activation at the circumventricular organs or via brainstem pathways (involving the nucleus of the solitary tract (NTS)) following vagal afferent activation.

question arises as to how these processes may interact with each other. Fig. 1 illustrates a number of putative pathways by which this may occur. Given the interplay between peripheral and central cytokine expression, it is possible that the master SCN pacemaker receives feedback from the peripheral immune system's circadian clock(s) via rhythms in circulating cytokines. For example circulating cytokines may act by stimulating circumventricular organs, in turn stimulating central cytokine production that may act in the SCN, or by prostaglandin production from the cerebral vasculature. Conversely, circulating cytokines might activate vagal afferents signaling to brainstem viscerosensory areas such as the nucleus of the solitary tract (NTS) which may in turn influence SCN activity (Dantzer, 2006). Prostaglandins have been shown to alter clock gene expression in mice (Tsuchiya et al., 2005) and the clock gene response to LPS treatment in horses is attenuated by cyclooxygenase inhibition (Murphy et al., 2007). Conversely, the master circadian clock may communicate with peripheral immune clocks via endocrine regulation and/or via regulation of autonomic outflow. For example, the paraventicular nucleus of the hypothalamus (PVN), a key regulatory site for both the endocrine and autonomic outflow, is also recognized as a key afferent area for SCN derived projections (Kalsbeek et al., 2006). Regulation of glucocorticoid production, via the PVN, may have a marked influence on the intrinsic circadian mechanisms of peripheral immune cells, as it has on other peripheral pacemakers (Levi and Schibler, 2007). This tentative schematic also indicates a number of pathways by which the circadian timekeeping system may modulate immune function, either directly or indirectly, and the putative roles played by these require careful future investigation.

A major endocrine output of the circadian system is the nocturnal pineal hormone, melatonin. Melatonin, whose expression is gated by the SCN through the sympathetic system, has well established effects on the immune system (Guerrero and Reiter, 2002), and is likely to have an important role in the circadian and seasonal modulation of cytokine production. Likewise, there is evidence that sepsis and TNF-α can alter the expression of melatonin (Fernandes et al., 2006; Mundigler et al., 2002), indicating another level of complexity within the circadian-immune system.

8. Conclusion

As the literature currently stands, there is good reason to imagine that future studies will reveal exciting new details of the interplay of the circadian and immune systems. However, there is much work still to do. By and large there is little anatomical data regarding the expression of cytokines and their receptors and signalers in the SCN, under both basal and immune-challenged conditions. Likewise, there needs to be

further, comprehensive studies of the impact of various cytokines on SCN neuronal function and ultimately on the integrity of the SCN circadian oscillator. There also needs to be mechanistic studies by which we can understand how cytokine systems can affect timekeeping processes at the molecular and neurophysiological levels. And finally there needs to be more study of circadian rhythms in disease, both in patients and animal models, as circadian dysfunction can have very significant negative impact on quality of life indices.

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