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Crosstalk between the circadian clock circuitry and the immune system

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Abstract

Various features, components, and functions of the immune system present daily variations. Immunocompetent cell counts and cytokine levels present variations according to the time of day and the sleep-wake cycle. Moreover, different immune cell types, such as macrophages, natural killer cells, and lymphocytes, contain a circadian molecular clockwork. The biological clocks intrinsic to immune cells and lymphoid organs, together with inputs from the central pacemaker of the suprachiasmatic nuclei via humoral and neural pathways, regulate the function of cells of the immune system, including their response to signals and their effector functions. Consequences of this include, for example, the daily variation in the response to an immune challenge (e.g.,

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bacterial endotoxin injection) and the circadian control of allergic reactions. The circadian-immune connection is bidirectional, because in addition to this circadian control of immune functions, immune challenges and immune mediators (e.g., cytokines) were shown to have strong effects on circadian rhythms at the molecular, cellular, and behavioral levels. This tight crosstalk between the circadian and immune systems has wide-ranging implications for disease, as shown by the higher incidence of cancer and the exacerbation of autoimmune symptoms upon circadian disruption.

Keywords

Adaptive immune response; allergic reaction; circadian rhythm; clock gene; cytokine; innate immune response

INTRODUCTION: THE CIRCADIAN SYSTEM

Various aspects of life vary rhythmically over the day with a period of approximately 24 h. Many of these rhythms, called circadian, persist even in the absence of any timing cues from the environment. Circadian rhythms are an adaptation of living organisms to the daily variations of the environment (Dunlap et al., 2004). They also allow physiological and cellular functions to be coordinated in time, both for energy conservation purposes and for the time segregation of opposing processes. Circadian rhythmicity characterizes also the function of the immune system, with variations of immunocompetent cells and cytokines, and assuring a well-timed anticipatory organization of biological processes that play a critical role in the preservation of body homeostasis and defense.

Circadian rhythms are generated by biological clocks. The master circadian clock in mammals is located in the suprachiasmatic nuclei (SCN), in the anterior hypothalamus (Antle & Silver, 2005). However, circadian oscillators are also present in other brain regions as well as in most peripheral tissues, including in lymphoid organs and white blood cells, as described below (Schibler et al., 2003). Circadian clock function is cell autonomous: for example, isolated SCN neurons can exhibit circadian rhythms of electrical activity, metabolic activity, and gene expression for extended periods of time (Antle & Silver, 2005). Non-neuronal cell types such as fibroblasts can also show circadian rhythms in a cell-autonomous manner (Schibler et al., 2003). However, in order for clocks to sustain circadian rhythms at the tissue level over the long term, there need to be synchronizing cues. The SCN maintains robust circadian rhythmicity as a consequence of the tight coupling of its neurons (Welsh et al., 2010). Peripheral tissues, on the other hand, require synchronizing signals, and the SCN plays a major role in this respect, either through neuronal output via the autonomic nervous system, rhythmic humoral cues (such as circadian oscillation of cortisol and melatonin levels in the blood), and systemic cues such as temperature cycles (Barclay et al., 2012).

At the intracellular level, circadian clocks are based on a set of *clock genes*. Mutation of these genes can lead to the loss of behavioral and physiological circadian rhythms, or to alteration in the period, phase, or amplitude of the rhythms (for review, see Ko & Takahashi, 2006; Duguay & Cermakian, 2009). In short, the mechanism is based on interlocked

autoregulatory feedback loops: in the main loop, the transcription factors CLOCK and BMAL1 activate the expression of genes encoding the PERIOD (PER) 1–3 and CRYPTOCHROME (CRY) 1/2 proteins, which in turn repress CLOCK/BMAL1 transcriptional activity, and thus their own expression. This leads to rhythms in the levels of the mRNAs and proteins from the *Per* and *Cry* genes. CLOCK/BMAL1 also rhythmically control the expression of nuclear receptors, such as REV-ERBα/β (reverse transcript of erythroblastosis gene) and RORα/β/γ (retinoic acidrelated (RAR) orphan receptor), which in turn repress and activate *Bmal1* expression, respectively, conferring amplitude and robustness to the oscillations in the molecular clockwork. These transcription factors, as well as others directly controlled by the clock, such as albumin gene D-sitebinding protein (DBP), thyrotroph embryonic factor (TEF), hepatic leukemia factor (HLF), adenoviral E4 proteinbinding protein (E4BP4), and DEC1/2 (differentially expressed in chondrocyte proteins 1), drive the circadian rhythm of expression of hundreds of *clock-controlled genes*, via E-boxes, D-boxes, and ROREs (ROR response element) in their promoters. This leads to rhythmic variations of cellular functions in different tissues and to circadian orchestration of regulatory processes that maintain body homeostasis (Bozek et al., 2009; Mazzoccoli et al., 2012b).

CIRCADIAN RHYTHMS OF COMPONENTS OF THE IMMUNE SYSTEM

Different white blood cell (leukocyte) populations present variations in abundance in the blood of humans and rodents (Figure 1). In the human blood, higher counts of lymphocytes, T lymphocytes, and B lymphocytes have been consistently observed in the night time (Abo et al., 1981; Ackermann et al., 2012; Born et al., 1997; Dimitrov et al., 2009; Haus & Smolensky, 1999; Lange et al., 2010;Mazzoccoli et al., 2011a). When T lymphocyte subsets are considered, $CD4^+$ (T-helper)− and $CD8^+$ (cytotoxic)− naive, central memory, and effector memory T lymphocytes show peak numbers in the night, whereas $CD4^+$ effector T cells show no rhythm and $CD8^+$ effector T cells show a low-amplitude rhythm with a peak in the day (Dimitrov et al., 2009; Mazzoccoli et al., 2011c). As discussed below, whereas T and B lymphocytes are involved in the adaptive (i.e., antigen-specific) immune response, other immune cell types such as granulocytes, monocytes, and natural killer (NK) cells mainly belong to the innate (i.e., not antigen-specific) immune system. In most studies, peak levels in the counts of these innate immune system cells were found in the daytime or late day (Ackermann et al., 2012; Born et al., 1997; Haus & Smolensky, 1999; Mazzoccoli et al., 2010d). Rodent studies describing white blood cell subset levels across the day are scarcer, but the available literature showed higher numbers of total leukocytes and of lymphocytes in the day (Hriscu, 2005; Kawate et al., 1981; Pelegri et al., 2003; Oishi et al., 2006). Thus, in both diurnal humans and nocturnal rodents, highest lymphocyte counts are observed during the rest period. Peaks of other cell types (granulocytes, neutrophils, monocytes) were found in the day in rats, whereas the highest NK cell numbers were observed at the end of the night (Hriscu, 2005; Pelegri et al., 2003). In rodents, B lymphocytes, T lymphocytes, and T lymphocyte subsets were also studied in lymph nodes, with daily rhythms found in rat submaxillary lymph nodes under a light-dark (LD) cycle (Esquifino et al., 2004a, 2004b), and no rhythm in mouse superficial lymph nodes under constant darkness (Fortier et al., 2011).

The levels of cytokines and other effector molecules were also shown to present variation across the day. Serum levels and in vitro production of interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2,IL-6, and IL-12 were all shown to present a rhythm in humans, with a peak generally observed at night or in the early morning (Born et al., 1997; Dimitrov et al., 2006, 2007; Haus & Smolensky, 1999; Petrovsky, 2001). Experiments on lymphoid organs of rats (spleen, lymph nodes) have also shown diurnal rhythmicity of IFN-γ (Arjona et al., 2004; Esquifino et al., 2004a, 2004b).

What leads to these 24-h rhythms in blood cells counts and cytokine levels? Many of the studies were performed on human subjects or animals kept in LD and sleep-wake conditions, which makes it impossible to separate the contributions of the circadian system versus the effects of sleep. However, a few studies on human subjects have attempted to distinguish between these influences. Born and colleagues (Born et al., 1997) compared samples collected during a sleep-wake cycle and during a day of sleep deprivation. The data showed that the rhythmic immune variables were differentially regulated by the sleep-wake cycle. B cell and T cell counts peaked at night, and these rhythms were only slightly, but significantly affected by the sleep conditions. So, both subsets were phase advanced and the amplitude of the T cell rhythm was reduced by sleep in comparison with sleep deprivation. On the contrary, the night trough of NK cells and monocytes was increased and the amplitude reduced under sleep deprivation. As for cytokines, the amplitude of the rhythm was reduced under sleep deprivation for IL-2. Another study showed a rhythm of IL-7 under sleep deprivation condition, but increased levels under a regular sleep-wake cycle, especially in the night (Benedict et al., 2007). In a recent study, the rhythm of granulocyte cell counts was shown to have a lower amplitude under sleep deprivation (Ackermann et al., 2012).

There is very little information available on the mechanisms behind the rhythms of immune variables. Numerous studies correlated hormone rhythms (e.g., cortisol, norepinephrine) with such immune rhythms, but very few showed a causal relationship. A study in mice showed that the rhythm of lymphocyte counts was lost upon adrenalectomy (Kawate et al., 1981). More recently, Dimitrov and colleagues (Dimitrov et al., 2009) showed in humans that rhythms of naive, central memory, and effector memory T cell counts are regulated by cortisol, whereas numbers of $CD8^+$ effector T cells follow changes in endogenous epinephrine (see below). In contrast to these studies, though, another group showed that in rats subjected to constant light conditions, which abolished rhythms of locomotor activity and catecholamines, the rhythms of lymphocyte counts were preserved (Deprés-Brummer et al., 1997). Another study presented data supporting a circadian regulation via the sympathetic nervous system of leukocyte recruitment in the bone marrow and skeletal muscle, via the control of endothelial cell adhesion molecules and chemokines (Scheiermann et al., 2012). The circadian system may also be important for immune cell development, as *Bmal1* knockout (KO) mice exhibit an impaired development of B lymphocytes (Sun et al., 2006)

The remainder of this review article will give an overview of the bidirectional regulation between the circadian and immune systems. We will describe the presence of clocks in immune cells and lymphoid organs, and rhythms in the functions of cells within the immune system. The other hand of the clock-immune crosstalk will be illustrated by the effects of

immune responses and mediators on behavioral and molecular circadian rhythms. Finally, this will be put in the context of impacts on human health and disease.

CIRCADIAN CONTROL OF THE INNATE IMMUNE SYSTEM

The circadian clock circuitry plays a role in the control of innate immune system function, as evidenced by studies performed in animal models with clock gene mutations, showing varied immune-related phenotypes. This section will focus on the circadian control of the innate immune system, i.e., the response not specific to an antigen.

Molecular and cellular links between the circadian clocks and innate immunity

Exposure to pathogens might be higher at certain times of the day, and organismal response must anticipate and address properly this time-related challenge. Accordingly, the molecular clockwork controls the expression and function of pattern recognition receptors for pathogen-associated molecules, such as Toll-like receptor 9 (TLR9), a receptor for viral and bacterial DNA (Silver et al., 2012b). In mouse models, susceptibility to lipopolysaccharide (LPS)-induced endotoxic shock was found to be dependent on the administration time in the 24-h LD cycle and linked to different time-dependent patterns of cytokine secretion (Marpegan et al., 2009). Chronic jet lag in animal models leads to altered or abolished rhythms in the expression of clock genes in the central clock, liver, thymus, and peritoneal macrophages, with exaggerated shock induced by LPS, and increased mortality when compared with unshifted control mice (Castanon-Cervantes et al., 2010). Moreover, the circadian regulation of leukocyte recruitment is a key factor in determining the survival to an LPS-induced septic shock (Scheiermann et al., 2012). Finally, a circadian modulation of neuroendocrine responses induced by endotoxemic challenge was also demonstrated (Kalsbeek et al., 2012).

What could be the mechanisms underlying these rhythms in the response to immune challenges? Melatonin was proposed to modulate the response to LPS, through the regulation of nuclear factor kappa B (NF-κB) and inducible nitric oxide synthase (iNOS) expression, downstream of LPS activation of TLR4 (Markus et al., 2007; Tamura et al., 2009). Animal and human studies have shown that the clock gene machinery is present in peripheral blood leukocytes (Boivin et al., 2003; Ebisawa et al., 2010; James et al., 2007; Kusanagi et al., 2008; Mazzoccoli et al., 2011d; Teboul et al., 2005) and controls the expression of transcription factors involved in immune cell differentiation and functioning, including signal transducer and activator of transcription 3 and 5 (STAT3, STAT5) and NF-κB (Bozek et al., 2009; Keller et al., 2009). Macrophages were shown to bear a circadian clock, which was proposed to control phagocytosis and LPS-induced cytokine secretion by these cells (Gibbs et al., 2012; Hayashi et al., 2007; Keller et al., 2009). One candidate for the interface between the clock and innate immune functions is CRY2, which seems to transcriptionally regulate STAT3 (Hoffman et al., 2009), and to hinder activation of NF-κB signaling by negatively regulating the cyclic adenosine phosphate–protein kinase A (cAMP-PKA) pathway (Narasimamurthy et al., 2012). Daily variations of NF-κB–mediated transcription in response to various immunomodulators involves the participation of CLOCK, which is found in protein complexes with the p65 subunit of NF-kB. This

mechanism is independent of the usual heterodimerization of CLOCK with BMAL1 (Spengler et al., 2012). Besides, the REV-ERB and ROR nuclear receptors regulate immune cell function by recruiting and interacting with coactivators and corepressors of transcription (Jetten, 2009). RORa restrains nuclear entry of NF-κB, hampering cytokine secretion (Delerive et al., 2001; Dzhagalov et al., 2004), and REV-ERBα may control cytokine expression, as evidenced by enhanced cytokine response to lipopolysaccharide (LPS; or endotoxin) (Bechtold et al., 2010) and loss of temporal gating of cytokine production and innate immune response to endotoxin in *Rev-erbα* KO mice (Gibbs et al., 2012).

Circadian control of natural killer cell function

NK cells are critical for immune surveillance against fungal, bacterial, and viral infections. They also play a vital role in cellular resistance to malignancy and tumor metastasis. NK cells are a subset of lymphocytes capable of lysing certain tumor cells and virus-infected cells without prior sensitization (Yokoyama, 1999; Yokoyama et al., 2004). NK precursor cells with no cytolytic activity are formed in the bone marrow, whereas mature peripheral cells are found primarily in the blood, spleen, liver, and uterus. NK cells bear no antigen receptor and therefore belong to the innate immune system; however, they share several features with highly differentiated T lymphocytes (e.g., $CD45RA^+$ $CD8^+$ effector T cells, NK T cells), such as a high tissue migratory potential and the production of granzyme B and perforin, IFN-γ, TNF-α, and granular macrophage cell-stimulating factor, allowing immediate cytotoxic effector defense in the periphery (Colucci et al., 2003; Janeway & Medzhitov, 2002; Kim et al., 2002; Levy et al., 2011; Sun et al., 2011; Vivier et al., 2011).

Perturbations of daily rhythms caused by external and internal stressors may compromise the first line of defense against infections and cancer by disrupting the circadian NK cell function. A number of studies have found relationships between longer light exposure and cancer progression (Garland et al., 1990; Stevens et al., 2011). Women in occupations that expose them to light at night do experience a higher risk of breast cancer (Dickerman & Liu, 2012). The mechanism by which light at night influences cancer progression may involve changes in the rate of cancer cell proliferation, which occurs in a circadian manner (Fu & Lee, 2003), as well the cytolytic function of innate immune cells including NK cells, which are involved in tumor surveillance (Arjona et al., 2004; Terao et al., 2002). Furthermore, suppression of NK cell count and cytolytic function has been shown to be a reliable marker of more rapid metastatic breast cancer progression (Sephton et al., 2000). Both empirical and epidemiological data also yield the possibility that NK cell function is subject of circadian regulation. Conclusive experimental evidence supporting this hypothesis is discussed in the remainder of the present section.

Control of NK cell function by clock genes

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 in the spleen of rats and mice (Arjona & Sarkar, 2005; Logan & Sarkar, 2012). In addition, rhythmic changes in NK cells were detected in blood samples from healthy men (Mazzoccoli et al., 2011c). Similarly to other peripheral tissues, Period (Per1, 2) and *Clock/Bmal1* genes in NK cells are expressed in antiphase, peaking during the

subjective day and night, respectively (Arjona & Sarkar, 2005). Dbp also oscillates in phase with Per1 in NK cells, suggesting that the clock regulates immune cellular pathways by promoting expression of clock-controlled genes containing D-box promoter elements (Arjona & Sarkar, 2005). In these cells, expression of cytokines (IFN-γ and TNF-α)and cytolytic factors (granzyme B and perforin) are highly synchronized, peaking approximately during the middle of the active period in rats (Arjona et al., 2006). Interestingly, NK cell cytotoxic activity peaks at similar circadian phases (Arjona & Sarkar, 2006b). Similarly, NK cytotoxicity is maximal during periods of wakefulness in humans (Kronfol et al., 1997).

Clock genes are intricately involved with NK cell function. In rat-derived RNK16 NK cells, knockdown of *Per2* or *Bmal1* differentially alters gene expression of IFN-γ, TNF-α, granzyme B, and perforin (Arjona & Sarkar, 2006a). Moreover, protein levels of granzyme B and perforin are altered following knockdown of *Per2* or *Bmal1*, without any effect on IFN-γ and TNF-α (Arjona & Sarkar, 2008; Liu et al., 2006), suggesting that disruption of the molecular clock alters the coordinated expression of NK cell cytolytic factors. Also, mice carrying a mutation in the *Per2* gene displayed significantly altered rhythms of IFN-γ, granzyme B, and perforin, accompanied by changes in the rhythm of *Bmal1* and *Per2* (Logan et al., 2012): IFN-γ expression remains low throughout the day in *Per2* mutant mice (Arjona & Sarkar, 2006a), and the decreased levels in proinflammatory cytokines confer them relative resistance to bacterial LPS-induced endotoxic shock (Liu et al., 2006).

The SCN pacemaker, the sympathetic nervous system, and circadian NK cell function

In accordance with a hierarchical model for the modulation of mammalian rhythms, NK cell circadian function relies on molecular clock mechanisms within these cells, which are coordinated by the SCN via neural and hormonal cues. The timing information from the SCN master pacemaker to the spleen, a lymphoreticular organ that plays a fundamental role during the immune response, has been recently studied. The splenic nerve contains about 98% of sympathetic nerve fibers (Elenkov et al., 2000) and secrete norepinephrine (Shimizu et al., 1994). Splenic lymphocytes express β-adrenergic receptors that regulate their function upon norepinephrine exposure (Kohm & Sanders, 2001). Shakhar & Ben-Eliyahu (1998) showed an in vivo suppression of NK cell activity and tumor clearance after β-adrenergic stimulation. In vitro, norepinephrine and β-adrenergic agents inhibit NK cell function parameters such as cytokine secretion, target binding, cytolytic function, and programming for cytotoxicity (Dokur & Sarkar, 2004; Gan et al., 2002).

The circadian signal from the SCN to the spleen appeared to be conveyed through norepinephrine, since the norepinephrine content in this tissue shows a distinct circadian profile, which is abolished or shifted following splenic sympathectomy. Additionally, splenic sympathectomy altered the daily variations of *Bmal1* and *Per2* mRNAs and proteins, and of TNF-α, granzyme B, and perforin in spleen NK cells (Logan et al., 2011). However, the treatment did not affect the rhythmic expression of IFN-γ, suggesting non-neural entrainment cues may also be necessary to regulate specific immune factors. Collectively, these results demonstrated that rhythmic norepinephrine input to the spleen acts as an entrainment cue to modulate the molecular clock in NK cells in the spleen, which in turn regulates cytokine production and cytolytic function of these cells. In addition to the

sympathetic nervous system, it is also evident that NK cell circadian function can be coordinated by the SCN via humoral cues (Atanackovic et al., 2002).

CIRCADIAN CONTROL OF THE ADAPTIVE IMMUNE SYSTEM

T and B lymphocytes represent the adaptive immune system that mounts an antigen-specific immune response and is capable of memory formation. Antigen-presenting cells (APCs) activate naïve $CD4^+$ T-helper cells bearing the cognate T cell receptor in secondary lymphatic tissues (e.g., lymph nodes) to proliferate and differentiate into effector populations that are termed according to their cytokine profile (e.g., IFN-γ–producing Th1 cells). These cells then support $CD8^+$ cytotoxic T cells and macrophages to eliminate the pathogen and B cells to produce antibodies. Some T and B lymphocytes become memory cells that can react faster and more efficiently upon reencounter of the antigen.

Opposing rhythms in numbers of lymphocyte subsets

First mentioned in 1946 (Elmadjian & Pincus, 1946), a rhythm in circulating lymphocyte numbers peaking during early sleep was replicated in numerous animal and human studies (Haus & Smolensky, 1999; Kawate et al., 1981; Oishi et al., 2006). This rhythm is pronounced in total T cells, and $CD4^+$ T cells and B cells, but less consistent or absent in $CD8^+$ T cells (Born et al., 1997; Haus & Smolensky, 1999; Pelegri et al., 2003). Other investigators even reported an opposing rhythm in the percentage of $CD8^+$ T cells, with a peak during the activity period that is likewise observed with high amplitude in NK cell counts (Dimitrov et al., 2007; Mazzoccoli et al., 1997, 2011c; Pelegri et al., 2003; Suzuki et al., 1997). Glucocorticoids, catecholamines, and the parasymphathetic nervous system were discussed as potential mediators of these antiphasic rhythms (Deprés-Brummer et al., 1997; Oishi et al., 2006; Suzuki et al., 1997). Recent experiments in humans revealed that rhythm acrophase and amplitude of a given lymphocyte subpopulation are determined by its stage of differentiation and its cytotoxicity (Lange et al., 2010).

So, T cells at early stages of differentiation such as naïve and central memory $CD4^+$ and $CD8^+$ T cells show a high-amplitude rhythm peaking during early sleep that is governed by the antiphasic rhythm of cortisol. In light of earlier studies on steroid-induced decreases in T cell counts (Fauci, 1975), it is assumed that naïve and central memory T cells are depleted from the blood in the morning hours due to the rise in endogenous cortisol that redirects these cells to the bone marrow by enhancing their CXCR4 expression (Dimitrov et al., 2009). In contrast, cytotoxic effector cells such as NK cells, NK T cells, and $CD45RA^+$ $CD8^+$ effector T cells peak during the activity period, as they are mobilized from the marginal pool into the circulation due to increases in endogenous epinephrine (Dimitrov et al., 2009, 2010; Mazzoccoli et al., 1997, 2011c; Suzuki et al., 1997). Until now animal studies that could prove such distinct time-dependent traffic patterns of lymphocyte subsets have been lacking. However, like naïve and central memory T cells, murine and human hematopoietic stem cells show peak numbers in peripheral blood during early sleep, when CXCR4 expression on these cells and bone marrow levels of the respective ligand CXCL12 are lowest (Lucas et al., 2008; Mendéz-Ferrer et al., 2008). Hence, the rhythmic expression of CXCR4 seems to be a common signal that allows the release of cells from the bone

marrow into the circulation during the resting period and their retention within the bone marrow during the activity period.

Cytotoxic effector functions are fostered during the activity period

The epinephrine-induced mobilization of cytotoxic effector cells from the marginal pool into the circulation is interpreted as an enhanced immunosurveillance that allows an efficient recruitment of these cells into peripheral tissues (Benschop et al., 1996; Campbell et al., 2009). As such, peak numbers of cytotoxic effector cells during the activity period could represent an immediate defense system against pathogens that are more likely encountered during wakefulness. In line with this assumption, NK cell activity follows a parallel rhythm with a peak during wakefulness in many animal and human studies (Arjona & Sarkar, 2008; Lévi et al., 1991; Moldofsky et al., 1986; see also preceding section) and the same pattern of activity is evident for NK T cells in the liver (Mocchegiani et al., 2007).

Sleep supports the immunological synapse between APC and T cell in lymph nodes

It is presently unknown if naïve and central memory T cells rhythmically migrate to the bone marrow to receive survival signals and to be kept in a quiescent state, such as has been described for hematopoietic stem cells (Benvenuto et al., 2007; Lapidot & Kollet, 2010). Following such a line of argumentation, the activity of these cells should peak during early sleep, when they are released from the bone marrow and can be redistributed to their sites of action. Naïve and central memory T cells recirculate through lymph nodes in search of their antigen and several lines of evidence indeed indicate that lymph node homing and T cell activation by APCs take place during sleep (Auzeby et al., 1988; Haus & Smolensky, 1999; Lange et al., 2010). So, early sleep is characterized by a unique endocrine constellation with lowest levels of cortisol that otherwise impedes T cell entry into the lymph node (Cox & Ford, 1982) and highest levels of hormones that seem to facilitate this traffic route such as growth hormone (GH) (Smaniotto et al., 2011) and aldosterone (Besedovsky et al., 2012a). Matching temporal changes in the percentage of $CD4^+$ T cells in lymph nodes with a peak during the rest period are described in some (Bonacho et al., 2001; Esquifino et al., 2004a, 2004b), but not all (Fortier et al., 2011; Griffin & Whitacre, 1991; Kurepa et al., 1992;), animal studies.

Rhythms in T cell activity were assessed in numerous animal and human experiments (Haus & Smolensky 1999). Studies on rhythmic T cell proliferation show overall discrepant results (Bollinger et al., 2009; Cardinali & Esquifino, 2003; Esquifino et al., 2004a, 2004b; Fortier et al., 2011; Hiemke et al., 1995; Lévi et al., 1991; Moldofsky et al., 1986). In one report it was proposed that T cell proliferative response is under the control of the circadian clock (Fortier et al., 2011). These authors showed that the circadian rhythm in T cell proliferation in response to T cell receptor (TCR) triggering was paralleled by a rhythm of the tyrosine kinase ZAP-70 (zeta-chain–associated protein of 70 kDa), which is a key signaling molecule downstream of the TCR. Very consistent acrophases at the beginning of the rest period are found for the production of Th1 cytokines such as IFN-γ ex vivo (Bollinger et al., 2010a; Hohagen et al., 1993; Kirsch et al., 2012; Petrovsky & Harrison, 1995, 1997) and in vivo in lymph nodes of rats (Esquifino et al., 2004a, 2004b). Again, these rhythms seem to follow endocrine changes, as early sleep is associated with peak levels of proinflammatory Th1-

supporting hormones such as melatonin, GH, prolactin, and leptin, whereas anti-inflammatory hormones such as cortisol and catecholamines reach nadir levels (Besedovsky et al., 2012b; Esquifino et al., 2004a; Lange et al., 2006, 2010). This proinflammatory hormonal boost during sleep acts like an adjuvant, presumably on APCs, which show a sleep-dependent production of IL-12, which in turn supports Th1 lineage commitment (Dimitrov et al., 2007; Lange et al., 2006, 2011). The regulatory role of APCs mediating the sleep-associated peak of T cell cytokine production is substantiated by the fact that the rhythm in cytokine production of isolated $CD4^+$ T cells is shifted by several hours (Bollinger et al., 2011).

Rhythms in adaptive immune responses

To summarize this section, adaptive immune functions are organized in time and space. Immediate cytotoxic effector defense in peripheral tissues is fostered during the activity period (Dimitrov et al., 2010; Mazzoccoli et al., 1997, 2011c; Suzuki et al., 1997). In contrast, the initiation of a Th1 immune response that evolves more slowly requiring the interaction between APC and T cell in lymph nodes, protein biosynthesis, and cell proliferation is supported during sleep. The Th1 response is energy consuming and involves the release of proinflammatory mediators associated with pain, immobility, and malaise; therefore, its timing to the sleep period seems reasonable (Petrovsky & Harrison, 1995; Straub et al., 2010). B and T cells harbor an intrinsic clock (Bollinger et al., 2011; Fortier et al., 2011; Silver et al., 2012a), but additional regulatory influences of sleep, locomotor activity, hormonal changes, and APC activity entrain the rhythm of lymphocyte numbers and functions. In many, but not all (Silver et al., 2012b), in vivo studies, adaptive immune responses such as the delayed-type hypersensitivity reaction (Pöllmann & Pöllmann, 1988), as well as the T cell and antibody response to immunizations (Fernandes et al., 1976; Fortier et al., 2011; Lange et al., 2011), benefit from sleep. Such a time-limited boost of lymphocyte activity may serve immune homeostasis and maximize efficiency of the immune system (Bollinger et al., 2010b; Lange et al., 2010). The knowledge of these rhythms in adaptive immunity will help to understand time-dependent phenomena in rheumatoid arthritis (Straub & Cutolo, 2007), asthma bronchiale (Haus & Smolensky, 1999), and graft rejection (Knapp et al., 1979) and to optimize the timing of immunomodulatory hormones (Buttgereit et al., 2008; Keisari et al., 1999). Moreover, these studies might help optimizing vaccination by appropriately selecting the time of immunization (Feigin et al., 1967; Fortier et al., 2011; Lange et al., 2011; Langlois et al., 1995).

CIRCADIAN CONTROL OF ALLERGIC RESPONSES

The prevalence of allergic diseases such as asthma, eczema, rhinitis, and food intolerance has reached pandemic proportions in industrialized countries, and the already high costs to public health and the economy are continuing to grow (Holgate & Polosa, 2008). Although numerous studies have attempted to elucidate allergic diseases, significant puzzles that hinder the development of effective cures and prevention persist.

One such puzzle is that many symptoms in allergic diseases exhibit prominent 24-h variation (Maurer et al., 2009; Smolensky et al., 2007). For instance, in most allergic rhinitis patients,

symptoms worsen overnight or early in the morning (“morning attack”) and often compromise nighttime sleep, which results in poor daytime quality of life (Gelfand, 2004). Such phenomena were actually recognized long before the birth of chronobiology. For example, Aurelianus Caelius (4th or 5th century AD) noted the frequent nocturnal occurrence of asthma attacks (Caelius, 1709). However, the precise mechanisms remain enigmatic.

Recent studies are beginning to reveal the role of the circadian clock in the daily variations and the severity of allergic reactions. In this section, we briefly review relevant data emerging from new studies and conclude by highlighting key questions that must be answered to translate fundamental knowledge into novel strategies for the prevention and treatment of allergic diseases.

The circadian clock temporally controls IgE/Mast cell–mediated allergic responses

A classic allergy is caused by an immune reaction to harmless environmental antigens (allergens) that is mediated by immunoglobulin (Ig) E (Galli et al., 2005; Gould & Sutton, 2008). Genetically susceptible individuals synthesize allergen-specific IgE when allergens (such as interior allergens [e.g., house dust mite], pollen, and food proteins) make contact with the mucosal surface (sensitization) and when IgE binds to its high affinity receptor (FcεRI) on mast cells and basophils. Reexposure to allergens in a sensitized individual leads to cross-linking of the IgE/FcεRI complex, which causes mast cell and basophil activation. Activated mast cells and basophils release preformed chemical mediators such as histamine (“degranulation”), and newly synthesized cytokines and chemokines, thereby initiating and maintaining allergic inflammation.

As described earlier, most IgE-mediated allergic responses have been reported to show circadian variation (Maurer et al., 2009; Smolensky et al., 2007). This is also the case in mice; Miller and Church found anaphylactic sensitivity in the pinna of a mouse to be subject to diurnal variation (Miller & Church, 1976). Although rhythmic humoral signals from the adrenal glands, such as cortisol, or the rhythmic activity of the autonomic nerves is implicated in diurnal variation, the precise molecular mechanisms have remained unclear for decades.

Recently, murine bone marrow–derived mast cells (BMMCs) cultured in vitro were shown to have an intrinsic clock, as indicated by circadian expression of core clock genes such as *Per1*, *Per2*, *Bmal1*, *Rev-erba*, and *Dbp* (Wang et al., 2010). Importantly, serum shock-synchronized BMMCs displayed circadian rhythms in *IL*-13 and *IL*-6 mRNA expression when stimulated via FcεRI at different intervals. These in vitro findings suggested a circadian regulation of mast cell function, which may be associated with the circadian pathophysiology of allergic diseases.

Consistently, the circadian clock temporally controls IgE/mast cell–mediated allergic reaction in vivo. The time of day–dependent variation observed in IgE-mediated immediate-type allergic reaction in the skin of wild-type (WT) mice (passive cutaneous anaphylactic reaction) was absent in Per2 mutant mice, adrenalectomized mice, and aged mice in association with an aberrant daily variation of serum corticosterone levels (Nakamura et al.,

2011). In addition, the *Per2* mutation decreased the sensitivity of mast cells to the inhibitory effects of glucocorticoid both in vitro and in vivo. Collectively, these results suggest that the key clock gene *Per2* is involved in the temporal regulation of IgE-mediated allergic reaction by controlling the rhythmic secretion of glucocorticoids from the adrenal glands and/or by gating the glucocorticoid response of mast cells to certain times of day. The redundancy of such dual controls of glucocorticoid levels and sensitivity by *Per2* might enhance the robustness of the circadian control of the IgE-mediated mast cell response. These findings suggest that the circadian clock drives daily rhythms in IgE/mast cell–mediated allergic reactions, which may underlie the diurnal symptoms observed in patients with allergic diseases typified by IgE-mediated reactions such as asthma, allergic rhinitis, and urticaria. However, the relative contribution of systemic (e.g., neuronal and endocrine) clocks and local (e.g., mast cell) clocks to the temporal regulation of IgE/mast cell–mediated allergic reactions remains unclear.

Dysfunction of the circadian clock exacerbates skin contact hypersensitivity

Recently, the circadian clock has been shown to control not only the daily variations of allergic reactions, but also their severity. The circadian clock impacts the severity of T cell–mediated allergic reactions and skin contact hypersensitivity (CHS), which is IgE independent (Takita et al., 2013). In this study, mice were sensitized with 2,4,6-trinitro-1-chlorobenzene (TNCB) on the abdominal skin on day 0 and challenged with TNCB on the ears on day 5. This resulted in a more severe inflammation in Clock mutant mice compared with the response in WT mice, as indicated by ear swelling, serum IgE level, and skin mast cell number. The serum corticosterone levels were lower in Clock mutant mice than in WT mice, with loss of daily rhythmicity. Additionally, adrenalectomy markedly worsened TNCB-induced CHS in WT mice but not in *Clock* mutant mice. Moreover, dramatic dexamethasone-induced protection against CHS was observed in *Clock* mutant mice compared with WT mice. The results suggest that dysfunction of the circadian *clock* enhances CHS via altered corticosterone rhythmicity and/or levels. These findings lead to the suggestion that following a proper day/night lifestyle (e.g., waking up early and not oversleeping) may be beneficial for reducing allergic symptoms. They also suggest that altered environmental or internal conditions that disrupt circadian clock function such as jet lag and shiftwork and metabolic abnormalities such as obesity (Bechtold et al., 2010) may affect the severity of symptoms in patients with allergic diseases. In fact, obesity is known to be a predisposing factor for allergy, although the precise mechanisms remain unknown (Stream & Sutherland, 2012).

Future challenges

The recent findings summarized here strongly suggest that the circadian clock is an important regulator of allergic immune responses. The precise mechanisms of the circadian clock with respect to the control of allergic reactions remain unclear. In particular, to clarify the roles of the intrinsic clockwork in mast cell or other immune cell (e.g., T cell) or nonimmune cell (e.g., bronchial epithelial cells; Gibbs et al., 2009) in the regulation of allergic responses will be important. Such studies are crucial for elucidating the relative contribution of systemic circadian influences (e.g., neuronal, endocrine, and metabolic) versus peripheral cells and tissues to the temporal regulation of allergic responses.

Furthermore, the molecular pathways linking the core clock machinery and specific allergic outputs (e.g., mast cell degranulation) remain to be elucidated.

The translation of novel findings on the circadian control of allergic response into the field of medicine will be a significant challenge. It will also be interesting to test whether several new chemicals that affect the circadian clock and rhythmic physiology (e.g., sleep-wake cycles) (Isojima et al., 2009, Solt et al., 2012) also influence allergic responses. New chronotherapy for allergic diseases using conventional drugs must also be developed based on updated knowledge.

Given these examples, the critical question is: “Why has circadian control of allergic responses evolved in mammals?” Conventional wisdom holds that allergic responses are the body’s first line of defense against macroparasites such as helminth worms and biting arthropods and that these stimuli have been an evolutionary driving force in allergic responses (Pulendran & Artis, 2012). Many parasites and biting arthropods, as well as many plants and other animals, exhibit circadian rhythms in their behavior and physiology (Hawkins, 1975). Therefore, the circadian control of allergic responses has most likely evolved to deal with parasites and biting arthropods with circadian rhythms. This notion can be tested in experiments that assess the host response to infection when clock gene–mutant or –deficient mice are infected with parasites or biting arthropods that have circadian physiology.

RESPONSE OF THE CIRCADIAN SYSTEM TO IMMUNE CHALLENGES

The crosstalk between the circadian pathways and the immune system is bidirectional: besides the circadian control of immune function described in the previous sections, recent data indicate that immune factors are able to affect the circadian timing system. IL-1β and TNF-α levels and specific receptor density show nycthemeral oscillation in the central nervous system and particularly in areas of the brain involved in the control of behavioral cycles (sleep-wake, rest-activity, fasting-feeding cycles) (Beynon & Coogan, 2010; Cearley et al., 2003). The influence of the immune factors on the circadian system is particularly evident under activated conditions, as evidenced by the modulatory effect played on the biological clock by LPS or cytokines, which determine different phase changes of the circadian rhythms depending on time of administration (Coogan & Wyse, 2008; Marpegan et al., 2005). This section describes these effects of immune challenges on the circadian system, at the behavioral, cellular, and molecular levels.

Effects of immune challenges on sleep regulation

There is extensive evidence supporting the interaction between immune challenges and sleep. In particular, infection affects the amount and quality of sleep, both in humans and in several animal models of disease (Bryant et al., 2004; Fernandez Alfonso et al., 2003). Sleep alterations have also been proposed as markers of sepsis development (Baracchi et al., 2011). In particular, endotoxin (LPS) and proinflammatory cytokine challenges regulate diverse sleep stages (Clinton et al., 2011; Jewett & Krueger, 2012). Most proinflammatory cytokines seem to be somnogenic, presumably acting through NF-κB (Bryant et al., 2004), whereas most anti-inflammatory cytokines are not (Krueger, 2008; Krueger et al., 2011a,

2011b). These data indicate that the immune system is able to modify sleep in pathological and physiological conditions.

Effects of inflammation and cytokines on circadian rhythms

Other than sleep, proinflammatory immune challenges affect the circadian clock and its outputs, both under pathological or physiological situations. For example, high doses of LPS or pathological states (sepsis or infection with blood-borne parasites) severely affect circadian output such as locomotor activity or body temperature (Bauhofer et al., 2001, 2002; Bentivoglio et al., 1994; Ebong et al., 1999a, 1999b). Moreover, chronic effects of LPS-induced sepsis have been recently described in mice, including changes in light-induced phase shifts and up-regulation of microglial markers in the SCN (O'Callaghan et al., 2012).

On the other hand, administration of low, subpyrogenic doses of LPS can be used as a stimulus to evaluate the physiological effects of immune factors on clock-controlled locomotor activity rhythms. Indeed, LPS induces phase shifts in mice when delivered in the early subjective night (Marpegan et al., 2005) and affects vasopressin release from the SCN (Nava et al., 2000). As for the pathway for LPS-induced circadian phase shifts, the role of the classic TLR4 has been demonstrated with the use of *Tlr4* KO mice, which do not show this response to the challenge (Paladino et al., 2010).

Experiments to address the mechanisms underlying these effects showed that the SCN are able to respond to central proinflammatory cytokines in vivo (such as TNF-α and IL-1β), producing phase shifts in locomotor activity rhythms, and that central inhibition of TNF-α blocks the effects of LPS on circadian activity (Leone et al., 2012). The circadian effect of proinflammatory cytokines might be mediated by changes in central receptors, including those at the SCN level. Indeed, IL-1 receptor was found to be expressed in a circadian manner in the SCN and is increased following LPS challenges (Beynon & Coogan, 2010). Cytokines also affect electrical activity in the SCN. Indeed, a cocktail of cytokines phase-shifted circadian rhythms of firing rate (Lundkvist et al., 2002) and IFN-γ treatment altered electrical properties and clock gene expression in SCN neurons (Kwak et al., 2008). Moreover, IFN-γ is also able to phase-shift behavioral rhythms during the subjective day (Boggio et al., 2003) and its receptor is expressed in the SCN (Lundkvist et al., 1998, 1999). LPS challenge was also shown to affect melatonin synthesis via the action of TNF-α on pinealocytes, and subsequent activation of NF-κB activity and iNOS expression (Carvalho-Sousa et al., 2011), and down-regulation of *arylalkylamine N-acetyltransferase gene* transcription (Fernandes et al., 2006) (*aa-nat* gene encodes the rate-limiting enzyme in the melatonin synthesis pathway).

In addition to cytokines, proinflammatory agents such as prostaglandin E_2 (PGE_2) can also reset the clock in cultured fibroblasts in vitro and in peripheral tissues in vivo (Tsuchiya et al., 2005). Chemokines might also be involved in the circadian response to immune challenges. For example, monocyte chemoattractant protein-1 expression is under the control of BMAL1 in mouse macrophages, and its induction upon a LPS challenge is time dependent (Hayashi et al., 2007; Leone et al., 2012). Also, a circadian phenotype was found

in mice KO for the glycan-binding protein galectin-1, suggesting a yet to be defined role in the circadian system (Casiraghi et al., 2010).

There are more cues on the action of immune challenges on circadian responses. Chronic inflammation also affects circadian responses, including photic entrainment (Palomba & Bentivoglio, 2008). An addtional indication of the role of immune challenges in photic-like phase-shifting behavior comes from data showing that acute immunosuppression with cyclosporine or tacrolimus blocks the circadian effects of light on the clock and is also able to induce nonphotic phase shifts of locomotor activity rhythms when administered during the subjective day (Katz et al., 2008).

Effects of inflammation and cytokines on circadian clock genes

In vitro results showed that proinflammatory factors such as IL-6 can trigger changes in the expression from clock gene promoters (Motzkus et al., 2002). This suggests that inflammation can act on the molecular clockwork. This is indeed the case in a mouse model of experimental arthritis, where clock gene expression was strongly altered in the joints and in the spleen (Hashiramoto et al., 2010). In addition, inflammatory stressors (such as LPS administration) increased clock gene expression in the hypothalamic paraventricular nucleus, thereafter acting on a putative circadian output (Takahashi et al., 2001).

LPS can also transiently suppress clock genes in peripheral tissues of rodents, which might be related to both its peripheral and central effects (Okada et al., 2008; Yamamura et al., 2010), and disturbs their expression in the SCN (Okada et al., 2008). Similarly, in a rat model of localized inflammation, turpentine oil injection induced gene, tissue-, and time-specific effects on clock gene expression in peripheral tissues (Westfall, Cermakian, and colleagues, unpublished data).

Treatment with cytokines also alters significantly clock gene expression, which might underlie the disruptive effect of LPS challenge or cytokines themselves on circadian rhythms. For example, *Clock* and *Bmal1* expression levels in a cell line and in vivo in the SCN and liver are reduced after IFN-α treatment (Koyanagi & Ohdo, 2002). TNF-α treatment was shown to have acute and gene-specific effects on the expression of clock genes in cultured cells (Cavadini et al., 2007; Perez-Aso et al., 2013) and in the liver in vivo (Cavadini et al., 2007). A mechanism might be through the increase of CRY1 protein levels, which seems to involve the deubiquitinating enzyme ubiquitin-specific peptidase 2 (USP2) (Tong et al., 2012). USP2 is a circadian-controlled deubiquitinase that interacts with clock proteins including PER1, CRY1, and BMAL1 and enhances their stability and/or activity via deubiquitination (Scoma et al., 2011; Tong et al., 2012; Yang et al., 2012). Effects of cytokines on clock genes in central and peripheral clocks might also operate through NF-κB pathways (Marpegan et al., 2005), reducing gene expression, neuronal firing, and neuropeptide signaling in the SCN, and modulating hormone and neural outputs (Beynon & Coogan, 2010).

Cellular and molecular interfaces for immune modulation of circadian rhythms

Molecular interactions between immune factors and the circadian system might reside in transcriptional regulation, where NF-κB might play a key role, as indicated by its role in

sleep (Kuo et al., 2010) and its activity in the SCN (Marpegan et al., 2004). Indeed, NF-κB inhibition blocked both LPS-and light-induced phase shifts of circadian rhythms; it has been suggested that this transcription factor might serve as an interface for immune and photic effects on the circadian system (Marpegan et al., 2004). Moreover, the relB subunit of the NF-kB complex acts as a repressor of circadian transcription by interaction with the molecular clockwork (Bellet et al., 2012).

The specific pathway for the circadian effect of TNF-a has been recently worked out, indicating that this cytokine uses a p38 mitogen-activated protein kinases (MAPK) and calcium-dependent mechanism and suppresses E-box–mediated clock gene expression (Cavadini et al., 2007; Petrzilka et al., 2009).

As for the cellular interface between immune signaling and the circadian system, recent evidence suggests that glia might play a key role, since SCN astrocytes and microglia respond to immune challenges (Deng et al., 2010). SCN glia exhibit a circadian rhythm in glial fibrillary acidic protein (GFAP) (Lavialle & Serviere, 1993) and respond to cytokine stimulation, suggesting that these cells can mediate input signals to the mouse circadian system coming from the immune system (Leone et al., 2006).

Immune effects on the clock: roles and consequences

Finally, it is interesting to speculate on the putative role of these immune effects on the biological clock. On the one hand, regarding pathological states, alterations of circadian activity by the immune system might constitute both a strategy for infectious agents or a factor promoting recovery after infections. For example, endotoxin suppresses clock gene expression in human leukocytes (Haimovich et al., 2010), which might initially decrease immune function but ultimately synchronize cellular activity and promote additional responses.

However, a more intriguing consequence of this interaction might reside in its physiological role. The biological clock resides on feedback mechanisms from its controlled rhythms in order to fine-tune its circadian phase and general activity. Locomotion, temperature, melatonin secretion, and other rhythmic variables have been demonstrated to exert such feedback onto the SCN through specific pathways and/or receptors. It is possible that immune humoral factors might serve a similar purpose: with their abundance and activity controlled by the clock, these factors might interact with SCN mechanisms and subtly adjust entrainment and adapation to environmental cues. In such scenario, immune disruption by infection or inflammation might interfere with the circadian system; the corollary is that readjusting chronobiological parameters should aid in the recovery of disease.

CIRCADIAN RHYTHMICITY OF IMMUNE SYSTEM FUNCTION IN HEALTHY AGING AND DISEASE

The proper synchronization among the many components of the circadian timing system and the neuroendocrine-immune system plays a crucial role in the maintenance of body homeostasis and preservation of health. Accordingly, the alteration of the spatiotemporal arrangement of the numerous anatomical and humoral elements implicated in the

functioning of organ systems is involved in the biological aging and in the pathophysiological basis of metabolic, immune-related, and neoplastic diseases.

Crosstalk between the circadian timing system and the immune system in the aging process

The contribution of the immune system to healthy aging and longevity is a crucial issue and immunosenescence is a process that affects all cell compartments of the immune system, accompanied by a remodeling of the immune function. In aged mice, chronic jet lag induces increased mortality rates, possibly related to sleep deprivation and disruption of the immune system (Davidson et al., 2006). In humans, the innate compartment of the immune system is relatively preserved, whereas changes in circadian rhythmicity and response reduction occur in the adaptive immune system during aging, accompanied by expansion of memory T cells and elevated levels of proinflammatory cytokines (Alberti et al., 2006; Born et al., 1995; Borrego et al., 1999; Cayetanot et al., 2009; Ginaldi et al., 2000; Sansoni et al., 2008; Solana & Mariani, 2000; Touitou et al., 1997). A low-grade chronic inflammatory condition in the periphery and brain characterizes the aging process, and inflammatory signals may target SCN neurons, with altered response of the biological clock to such signals during senescence (Bentivoglio et al., 2006).

As stated above, IFN-γ can affect the function of the SCN (Kwak et al., 2008), and an aging-related link between plasma IFN-γ levels and changes in circadian rhythms has been evidenced in the nocturnal mouse lemur (*Microcebus murinus*), a nonhuman primate species. In the aged animals, an increase of IFN-γ levels was evidenced, positively correlated with alterations of biological rhythms (high percentage of diurnal activity, anticipation of the activity onset relative to lights-off, short free-running period, and delayed occurrence of minimal body temperature), and inversely correlated with lifetime duration, suggesting that plasma IFN-γ levels may predict survival and the degree of circadian rhythm alterations during aging (Cayetanot et al., 2009).

The total number of T cells is increased in the elderly, but the $CD4^+$ T cells do not show circadian rhythmicity, which may represent an important alteration for immune system function during aging, since this subset has a fundamental regulatory function in adaptive immunity (Mazzoccoli et al., 2011a). Besides, elderly subjects have decreased levels of $CD20^+$ cells (B lymphocytes), higher levels of $CD25^+$ cells (activated T lymphocytes with expression of the α chain of IL-2 receptor) and the circadian rhythmicity of these lymphocyte subsets is severely altered (Mazzoccoli et al., 2010b).

The circadian system drives neuroendocrine secretion, and the hypothalamus-pituitary axis plays an immunomodulating role and influences cellular immune responses by releasing various hormones and neuropeptides with direct modulatory action on the immune effectors into the blood, or by regulating the hormonal secretion of peripheral endocrine glands (Besedovsky & del Rey, 1996; Dhabhar et al., 1995; Dimitrov et al., 2009; Kronfol et al., 1997; Mazzoccoli et al., 1997, 2010a; Miller et al., 1994; Ottaway & Husband, 1994; Petrovsky, 2001). The loss of circadian variation of $CD4^+$ T lymphocytes may consequently cause loss of timed window of interaction with the neuroendocrine hormones that play an immunomodulatory role: cortisol, melatonin, prolactin, thyrotropin-releasing hormone

(TRH), thyroid-stimulating hormone (TSH), and the GH/insulin-like growth factor 1 (IGF1) axis (Arlt & Hewison, 2004). The nervous, endocrine, and immune systems are connected by shared neurotransmitters, hormones, and cytokines (Petrovsky, 2001), and a number of age-related changes in the 24-h hormonal rhythms have been found in older human beings (Moser & Loetscher, 2001; Schwartz, 2003; Touitou & Haus, 1994, 2000; Touitou et al., 1997; von Andrian & Mempel, 2003).

As described in previous sections, levels and function of different T cell subsets as well as NK cells are regulated across the day by rhythmic humoral mediators such as cortisol, epinephrine, and norepinephrine. In addition, cortisol is generally thought to have immunosuppressive function (Balow et al., 1975; Fauci, 1975). Another important humoral output of the SCN master pacemaker is melatonin, which is secreted by the pineal gland, with a circadian rhythm under the control of the SCN. Melatonin was shown to modulate the function of the immune system (Arendt, 2006; Markus et al., 2007). Melatonin is directly involved in immunomodulation by opiatergic ways and stimulates activated $CD4^+$ T lymphocytes to produce opioid agonists and cytokines (IL-2 and IL-4) (Maestroni et al., 1987). Serum levels of β-endorphin, an endogenous μ-opioidergic agonist derived from the precursor hormone proopiomelanocortin, show circadian variation with a nadir around midnight (Straub & Cutolo, 2007). The immunomodulatory role of melatonin may also be exerted by an influence on the thymic function mediated by TRH and TSH, able to counteract in experimental conditions thymic involution induced by prednisolone, an effect that seems to be thyroid independent and not correlated to thyroxine levels (Maestroni, 1995; Maestroni & Conti, 1990; Maestroni et al., 1988).

Aged subjects have altered cortisol secretion with higher cortisol serum levels in the afternoon and during the night, melatonin serum levels and rhythmicity of secretion are altered in the elderly, and there is disturbance of TSH/TSH axis function and reduction of GH/IGF1 axis activity with increasing age (Bauer, 2008; Cardinali et al., 2008; Corpas et al., 1993; Gusenoff et al., 2001; Karasek, 2004; Mazzoccoli et al., 2010b; Morrhaye et al., 2009; Rehman & Masson, 2001; Russell-Aulet et al., 2001; Toogood, 2003). Aging-associated alterations of daily rhythmicity of hormone secretion reveal the disturbances in the aged circadian system, and may hamper communications among the neuro-endocrine-immune system components, thus underlying pathophysiological mechanisms (Hertoghe, 2005; Mazzoccoli et al., 2011b).

Crosstalk between the circadian timing system and the immune system in disease

Regarding the crosstalk between the circadian clock circuitry and the immune system in pathological conditions, alterations of the biological clock of immune components and alterations of circadian rhythmicity of immune function have been evaluated particularly in metabolic, immune-related, and neoplastic diseases. The expression in peripheral blood cells of numerous genes involved in circadian rhythmicity, inflammation, and oxidative stress has been found significantly correlated with visceral fat accumulation (Yamaoka et al., 2012). Besides, in patients affected by type 2 diabetes mellitus the rhythmicity of mRNA expression levels of clock genes in leukocytes from the peripheral blood was dampened, and

the decrease of expression was positively correlated to the severity of metabolic disturbances (Ando et al., 2009).

Cytokine levels exhibit a marked rhythmicity and in the case of immune-related diseases, such as rheumatoid arthritis, this leads to circadian variations in the intensity of disease-related symptoms. Studies on the human monocytic THP-1 cell line have revealed that adenosine A(2A) receptors (R) and TNF-α regulate the intrinsic circadian clock in these cells, providing a possible link between the daily variation of rheumatoid arthritis symptoms (e.g., higher stiffness in the joints in the morning) and the molecular circadian clockwork (Perez-Aso et al., 2013). Besides, absence of circadian rhythmicity of clock gene expression was observed in primary synovial fibroblasts obtained from patients affected by rheumatoid arthritis (Haas & Straub, 2012). This is reminiscent of the suppression of *Per2* gene expression in joints and spleen, and deregulation of other clock genes, in mice with induced arthritis (Hashiramoto et al., 2010). Interestingly, in this model, the intensity of the symptoms and of TNF-α response was increased in *Cry1/Cry2* double KO mice (Hashiramoto et al., 2010).

PER3 single-nucleotide polymorphisms have been associated with circadian disruption and altered secretion of cytokines involved in chronic inflammation. In particular, the rs2797685 variant of the *PER3* gene has been found to be associated with disease aggressivity and onset age of Crohn's disease and ulcerative colitis, two inflammatory bowel diseases resulting from the interaction among genetic and environmental/microbial factors and the intestinal immune system (Mazzoccoli et al., 2012a).

Epidemiological studies in human populations have put in evidence that prolonged exposure to rotating-shift work with unstable work schedules or exposure to light at night with intentional sleep reduction is associated with chronic circadian disruption and with pathological conditions, in particular metabolic, cardio-vascular, and neoplastic diseases (Monsees et al., 2012; Pan et al., 2011; Sigurdardottir et al., 2012). Accordingly, the results obtained from experimental animal models through chronic shift-lag paradigm have shown alteration of NK cell circadian function and cytolytic activity accompanied by altered expression of the clock gene machinery promoting tumor growth (Logan et al., 2012). There are controversial results about the time-dependent profiles of T cytotoxic lymphocytes and NK cells in human subjects affected by neoplastic disease (Lissoni & Rovelli, 2012). In any cases, in lung cancer patients, severe alterations of relative percentages and circadian rhythmicity of lymphocyte subsets have been documented (Mazzoccoli et al., 2012c, 2012d). Altered 24-h periodicity is observed for the daily variations of $CD8^+$-, $CD8^{bright}$-, and γδTCR-expressing cells, which are diminished, whereas NK and $CD25^+$ cells are increased in lung cancer patients, as well as in several other cancer types, and this increase is paralleled by an increase of IL-2 serum levels (Mazzoccoli et al., 2010c). Furthermore, a severe alteration of blood levels with loss of normal circadian oscillation is observed for a number of immunomodulatory hormones (cortisol, TRH, TSH, GH/IGF1 axis) (Mazzoccoli et al., 2012d). These alterations are related to the stage of disease and suggest that important disturbances in the circadian system, with modification of the rhythmic patterns of neuroendocrine and immune system components, may underlie neoplastic disease and may influence disease onset and progression.

CONCLUSION

Immune system function is characterized by daily variations of immunological parameters and by self-sustained biological clocks ticking in the immunocompetent cells, driven by a master pacemaker located in the hypothalamic SCN. The bidirectional interactions between central and peripheral clocks are hardwired with neural circuits and conveyed through humoral mediators. The synchronization among the single elemnts of these multicomponent systems provides tight coupling among behavioral cycles, circadian rhythmicity of neuroendocrine pathways, and periodicity of immune activation. This allows anticipatory cooperation and proper trafficking of immune cells between the circulation, lymphoid organs, and peripheral tissues, with the aim to supply appropriate immune responses. The temporal dimension of the biological processes underlying immune responses must be addressed by therapeutic strategies involving chronomodulation of drug administration, and taking into account the bidirectional crosstalk between the circadian clock circuitry and the immune system.

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REFERENCES

Abo T, Kawate T, Itoh K, Kumagai K. (1981). Studies on the bioperiodicity of the immune response. I. Circadian rhythms of human T, B, and K cell traffic in the peripheral blood. J Immunol, 126, 1360–3. [PubMed: 6970770]

Ackermann K, Revell VL, Lao O, et al. (2012). Diurnal rhythms in blood cell populations and the effect of acute sleep deprivation in healthy young men. Sleep, 35, 933–40. [PubMed: 22754039]

Alberti S, Cevenini E, Ostan R, et al. (2006). Age-dependent modifications of Type 1 and Type 2 cytokines within virgin and memory $CD4^+$ T cells in humans. Mech Ageing Dev, 127, 560–6. [PubMed: 16516272]

Ando H, Takamura T, Matsuzawa-Nagata N, et al. (2009). Clock gene expression in peripheral leucocytes of patients with type 2 diabetes. Diabetologia, 52, 329–35. [PubMed: 18974966]

Antle MC, Silver R. (2005). Orchestrating time: arrangements of the brain circadian clock. Trends Neurosci, 28, 145–51. [PubMed: 15749168]

Arendt J (2006). Melatonin and human rhythms. Chronobiol Int, 23, 21–37. [PubMed: 16687277]

Arjona A, Sarkar DK. (2005). Circadian oscillations of clock genes, cytolytic factors, and cytokines in rat NK cells. J Immunol, 174, 7618–24. [PubMed: 15944262]

Arjona A, Sarkar DK. (2006a). The circadian gene mPer2 regulates the daily rhythm of IFN-gamma. J Interferon Cytokine Res, 26, 645–9. [PubMed: 16978068]

Arjona A, Sarkar DK. (2006b). Evidence supporting a circadian control of natural killer cell function, Brain Behav. Immun, 20, 469–76. [PubMed: 16309885]

Arjona A, Sarkar DK. (2008). Are circadian rhythms the code of hypothalamic-immune communication? Insights from natural killer cells. Neurochem Res, 33, 708–18. [PubMed: 17965936]

Arjona A, Boyadjieva N, Sarkar DK. (2004). Circadian rhythms of granzyme B, perforin, IFN-gamma, and NK cell cytolytic activity in the spleen: effects of chronic ethanol. J Immunol, 172, 2811–17. [PubMed: 14978081]

Arjona A, Boyadjieva N, Kuhn P, Sarkar DK. (2006). Fetal ethanol exposure disrupts the daily rhythms of splenic granzyme B, IFN-gamma, and NK cell cytotoxicity in adulthood. Alcohol Clin Exp Res, 30, 1039–44. [PubMed: 16737463]

Arlt W, Hewison M. (2004). Hormones and immune function: implications of aging. Aging Cell, 3, 209–16. [PubMed: 15268754]

Auzéby A, Bogdan A, Krosi Z, Touitou Y. (1988). Time-dependence of urinary neopterin, a marker of cellular immune activity. Clin Chem, 34, 1866–7. [PubMed: 3416434]

Atanackovic D, Brunner-Weinzierl MC, Kröger H, et al. (2002). Acute psychological stress simultaneously alters hormone levels, recruitment of lymphocyte subsets, and production of reactive oxygen species. Immunol Invest, 31, 73–91. [PubMed: 12148950]

Balow JE, Hurley DL, Fauci AS. (1975). Immunosuppressive effects of glucocorticosteroids: differential effects of acute vs chronic administration on cell-mediated immunity. J Immunol, 114, 1072–6. [PubMed: 803535]

Baracchi F, Ingiosi AM, Raymond RM Jr, Opp MR. (2011). Sepsis-induced alterations in sleep of rats. Am J Physiol Regul Integr Comp Physiol, 301, R1467–78. [PubMed: 21900639]

Barclay JL, Tsang AH, Oster H. (2012). Interaction of central and peripheral clocks in physiological regulation. Progr Brain Res, 199, 163–81.

Bauer ME. (2008). Chronic stress and immunosenescence: a review. Neuroimmunomodulation, 15, 241–50. [PubMed: 19047801]

Bauhofer A, Witte K, Celik I, et al. (2001). Sickness behaviour, an animal equivalent to human quality of life, is improved in septic rats by G-CSF and antibiotic prophylaxis. Langenbecks Arch Surg, 386, 132–40. [PubMed: 11374046]

Bauhofer A, Witte K, Lemmer B, et al. (2002). Quality of life in animals as a new outcome for surgical research: G-CSF as a quality of life improving factor. Eur Surg Res, 34, 22–9. [PubMed: 11867897]

Bechtold DA, Gibbs JE, Loudon AS. (2010). Circadian dysfunction in disease. Trends Pharmacol Sci, 31, 191–8. [PubMed: 20171747]

Bellet MM, Zocchi L, Sassone-Corsi P. (2012). The RelB subunit of NFkappaB acts as a negative regulator of circadian gene expression. Cell Cycle, 11, 3304–11. [PubMed: 22894897]

Benedict C, Dimitrov S, Marshall L, Born J. (2007). Sleep enhances serum interleukin-7 concentrations in humans. Brain Behav Immun, 21, 1058–62. [PubMed: 17524612]

Benschop RJ, Rodriguez-Feuerhahn M, Schedlowski M. (1996). Catecholamine-induced leukocytosis: early observations, current research, and future directions. Brain Behav Immun, 10, 77–91. [PubMed: 8811932]

Bentivoglio M, Grassi-Zucconi G, Peng ZC, et al. (1994). Trypanosomes cause dysregulation of c-fos expression in the rat suprachiasmatic nucleus. Neuroreport, 5, 712–14. [PubMed: 8199344]

Bentivoglio M, Deng XH, Nygård M, et al. (2006). The aging suprachiasmatic nucleus and cytokines: functional, molecular, and cellular changes in rodents. Chronobiol Int, 23, 437–49. [PubMed: 16687317]

Benvenuto F, Ferrari S, Gerdoni E, et al. (2007). Human mesenchymal stem cells promote survival of T cells in a quiescent state. Stem Cells, 25, 1753–60. [PubMed: 17395776]

Besedovsky HO, del Rey A. (1996). Immune-neuro-endocrine interactions: facts and hypotheses. Endocr Rev, 17, 64–102. [PubMed: 8641224]

Besedovsky L, Born J, Lange T. (2012a). Blockade of mineralocorticoid receptors enhances naïve T-helper cell counts during early sleep in humans. Brain Behav Immun, 26, 1116–21. [PubMed: 22884414]

Besedovsky L, Lange T, Born J. (2012b). Sleep and immune function. Pflugers Arch, 463, 121–37. [PubMed: 22071480]

Beynon AL, Coogan AN. (2010). Diurnal, age, and immune regulation of interleukin-1beta and interleukin-1 type 1 receptor in the mouse suprachiasmatic nucleus. Chronobiol Int, 27, 1546–63. [PubMed: 20854134]

Boggio VI, Castrillón PO, Perez Lloret S, et al. (2003). Cerebroventricular administration of interferon-gamma modifies locomotor activity in the golden hamster. Neurosignals, 12, 89–94. [PubMed: 12876403]

Boivin DB, James FO, Wu A, et al. (2003). Circadian clock genes oscillate in human peripheral blood mononuclear cells. Blood, 102, 4143–5. [PubMed: 12893774]

Bollinger T, Bollinger A, Skrum L, et al. (2009). Sleep-dependent activity of T cells and regulatory T cells. Clin Exp Immunol, 155, 231–8. [PubMed: 19040608]

Bollinger T, Bollinger A, Naujoks J, et al. (2010a). The influence of regulatory T cells and diurnal hormone rhythms on T helper cell activity. Immunology, 131, 488–500. [PubMed: 20646075]

Bollinger T, Bollinger A, Oster H, Solbach W. (2010b). Sleep, immunity, and circadian clocks: a mechanistic model. Gerontology, 56, 574–80. [PubMed: 20130392]

Bollinger T, Leutz A, Leliavski A, et al. (2011). Circadian clocks in mouse and human $CD4^+$ T cells. PLoS ONE, 6, e29801. [PubMed: 22216357]

Bonacho MG, Cardinali DP, Castrillón P, et al. (2001). Aging-induced changes in 24-h rhythms of mitogenic responses, lymphocyte subset populations and neurotransmitter and amino acid content in rat submaxillary lymph nodes during Freund's adjuvant arthritis. Exp Gerontol, 36, 267–82. [PubMed: 11226742]

Born J, Uthgenannt D, Dodt C, et al. (1995). Cytokine production and lymphocyte subpopulations in aged humans. An assessment during nocturnal sleep. Mech Ageing Dev, 84, 113–26. [PubMed: 8788239]

Born J, Lange T, Hansen K, et al. (1997). Effects of sleep and circadian rhythm on human circulating immune cells. J Immunol, 158, 4454–64. [PubMed: 9127011]

Borrego F, Alonso MC, Galiani MD, et al. (1999). NK phenotypic markers and IL2 response in NK cells from elderly people. Exp Gerontol, 34, 253–65. [PubMed: 10363791]

Bozek K, Relógio A, Kielbasa SM, et al. (2009). Regulation of clock-controlled genes in mammals. PLoS ONE, 4, e4882. [PubMed: 19287494]

Bryant PA, Trinder J, Curtis N. (2004). Sick and tired: does sleep have a vital role in the immune system? Nat Rev Immunol, 4, 457–67. [PubMed: 15173834]

Buttgereit F, Doering G, Schaeffler A, et al. (2008). Efficacy of modified-release versus standard prednisone to reduce duration of morning stiffness of the joints in rheumatoid arthritis (CAPRA-1): a double-blind, randomised controlled trial. Lancet, 371, 205–14. [PubMed: 18207016]

Caelius A (1709). De Morbis acuitis et chronicis. Amsterdam: Wetsteniand.

Campbell JP, Riddell NE, Burns VE, et al. (2009). Acute exercise mobilises CD8þ T lymphocytes exhibiting an effector-memory phenotype. Brain Behav Immun, 23, 767–75. [PubMed: 19254756]

Cardinali DP, Esquifino AI. (2003). Circadian disorganization in experimental arthritis. Neurosignals, 12, 267–82. [PubMed: 14739557]

Cardinali DP, Esquifino AI, Srinivasan V, Pandi-Perumal SR. (2008). Melatonin and the immune system in aging. Neuroimmunomodulation, 15, 272–8. [PubMed: 19047804]

Carvalho-Sousa CE, da Silveira Cruz-Machado S, Tamura EK, et al. (2011). Molecular basis for defining the pineal gland and pinealocytes as targets for tumor necrosis factor. Front Endocrinol, 2, 10.

Casiraghi LP, Croci DO, Poirier F, et al. (2010). "Time sweet time": circadian characterization of galectin-1 null mice. J Circadian Rhythms, 8, 4. [PubMed: 20403179]

Castanon-Cervantes O, Wu M, et al. (2010). Dysregulation of inflammatory responses by chronic circadian disruption. J Immunol, 185, 5796–805. [PubMed: 20944004]

Cavadini G, Petrzilka S, Kohler P, et al. (2007). TNF-alpha suppresses the expression of clock genes by interfering with E-box-mediated transcription. Proc Natl Acad Sci U S A, 104, 12843–8. [PubMed: 17646651]

Cayetanot F, Nygård M, Perret M, et al. (2009). Plasma levels of interferon-gamma correlate with age-related disturbances of circadian rhythms and survival in a non-human primate. Chronobiol Int, 26, 1587–601. [PubMed: 20030542]

Cearley C, Churchill L, Krueger JM. (2003). Time of day differences in IL1beta and TNFalpha mRNA levels in specific regions of the rat brain. Neurosci Lett, 352, 61–3. [PubMed: 14615050]

Clinton JM, Davis CJ, Zielinski MR, et al. (2011). Biochemical regulation of sleep and sleep biomarkers. J Clin Sleep Med, 7, S38–S42. [PubMed: 22003330]

Colucci F, Caligiuri MA, Di Santo JP. (2003). What does it take to make a natural killer? Nat. Rev Immunol, 3, 413–25. [PubMed: 12766763]

Coogan AN, Wyse CA. (2008). Neuroimmunology of the circadian clock. Brain Res, 1232, 104–12. [PubMed: 18703032]

Corpas E, Harman SM, Blackman MR. (1993). Human growth hormone and human aging. Endocr Rev, 14, 20–39. [PubMed: 8491152]

Cox JH, Ford WL. (1982). The migration of lymphocytes across specialized vascular endothelium. IV. Prednisolone acts at several points on the recirculation pathways of lymphocytes. Cell Immunol, 66, 407–22. [PubMed: 6279318]

Davidson AJ, Sellix MT, Daniel J, et al. (2006). Chronic jet-lag increases mortality in aged mice. Curr Biol, 16, R914–R916. [PubMed: 17084685]

Delerive P, Monte D, Dubois G, et al. (2001). The orphan nuclear receptor ROR alpha is a negative regulator of the inflammatory response. EMBO Rep, 2, 42–8. [PubMed: 11252722]

Deng XH, Bertini G, Palomba M, et al. (2010). Glial transcripts and immune-challenged glia in the suprachiasmatic nucleus of young and aged mice. Chronobiol Int, 27, 742–67. [PubMed: 20560709]

Deprés-Brummer P, Bourin P, Pages N, et al. (1997). Persistent T lymphocyte rhythms despite suppressed circadian clock outputs in rats. Am J Physiol, 273, R1891–9. [PubMed: 9435642]

Dhabhar FS, Miller AH, McEwen BS, Spencer RL. (1995). Effects of stress on immune cell distribution: dynamics and hormonal mechanisms. J Immunol, 154, 5511–27. [PubMed: 7730652]

Dickerman B, Liu J. (2012). Does current scientific evidence support a link between light at night and breast cancer among female night-shift nurses? Review of evidence and implications for occupational and environmental health nurses. Workplace Health Saf, 60, 273–81. [PubMed: 22658734]

Dimitrov S, Lange T, Benedict C, et al. (2006). Sleep enhances IL-6 trans-signaling in humans. FASEB J, 20, 2174–6. [PubMed: 16912152]

Dimitrov S, Lange T, Nohroudi K, Born J. (2007). Number and function of circulating human antigen presenting cells regulated by sleep. Sleep, 30, 401–11. [PubMed: 17520784]

Dimitrov S, Benedict C, Heutling D, et al. (2009). Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. Blood, 113, 5134–43. [PubMed: 19293427]

Dimitrov S, Lange T, Born J. (2010). Selective mobilization of cytotoxic leukocytes by epinephrine. J Immunol, 184, 503–11. [PubMed: 19949113]

Dokur M, Sarkar DK. (2004). Catecholaminergic control of NK cell cytolytic activity regulatory factors in the spleen. J Neuroimmunol, 151, 148–57. [PubMed: 15145613]

Duguay D, Cermakian N. (2009). The crosstalk between physiology and circadian clock proteins. Chronobiol Int, 26, 1479–513. [PubMed: 20030537]

Dunlap JC, Loros JJ, DeCoursey PJ, eds. (2004). Chronobiology: biological timekeeping. Sunderland, MA: Sinauer Associates, 406pp.

Dzhagalov I, Zhang N, He YW. (2004). The roles of orphan nuclear receptors in the development and function of the immune system. Cell Mol Immunol, 1, 406–7.

Ebisawa T, Numazawa K, Shimada H, et al. (2010). Self-sustained circadian rhythm in cultured human mononuclear cells isolated from peripheral blood. Neurosci Res, 66, 223–7. [PubMed: 19883700]

Ebong S, Call D, Nemzek J, et al. (1999a). Immunopathologic alterations in murine models of sepsis of increasing severity. Infect Immun, 67, 6603–10. [PubMed: 10569781]

Ebong SJ, Call DR, Bolgos G, et al. (1999b). Immunopathologic responses to non-lethal sepsis. Shock, 12, 118–26. [PubMed: 10446892]

Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. (2000). The sympathetic nerve—an integrative interface between two super-systems: the brain and the immune system. Pharmacol Rev, 52, 595–638. [PubMed: 11121511]

Elmadjian F, Pincus G. (1946). A study of the diurnal variations in circulating lymphocytes in normal and psychotic subjects. J Clin Endocrinol Metab, 6, 287–94. [PubMed: 21025121]

Esquifino AI, Alvarez MP, Cano P, et al. (2004a). 24-hour pattern of circulating prolactin and growth hormone levels and submaxillary lymph node immune responses in growing male rats subjected to social isolation. Endocrine, 25, 41–8. [PubMed: 15545705]

Esquifino AI, Chacon F, Cano P, et al. (2004b). Twenty-four-hour rhythms of mitogenic responses, lymphocyte subset populations and amino acid content in submaxillary lymph nodes of growing male rats subjected to calorie restriction. J Neuroimmunol, 156, 66–73. [PubMed: 15465597]

Fauci AS. (1975). Mechanisms of corticosteroid action on lymphocyte subpopulations. I. Redistribution of circulating T and B lymphocytes to the bone marrow. Immunology, 28, 669–80. [PubMed: 1080130]

Feigin RD, Jaeger RF, McKinney RW, Alevizatos AC. (1967). Live, attenuated Venezuelan equine encephalomyelitis virus vaccine.II. Whole-blood amino-acid and fluorescent-antibody studies following immunization. Am J Trop Med Hyg, 16, 769–77. [PubMed: 4864056]

Fernandes G, Halberg F, Yunis EJ, Good RA. (1976). Circadian rhythmic plaque-forming cell response of spleens from mice immunized with SRBC. J Immunol, 117, 962–6. [PubMed: 986413]

Fernandes PA, Cecon E, Markus RP, Ferreira ZS. (2006). Effect of TNF-alpha on the melatonin synthetic pathway in the rat pineal gland: basis for a 'feedback' of the immune response on circadian timing. J Pineal Res, 41, 344–50. [PubMed: 17014691]

Fernandez Alfonso T, Celentano AM, Gonzalez Cappa SM, Golombek DA. (2003). The circadian system of Trypanosoma cruzi-infected mice. Chronobiol Int, 20, 49–64. [PubMed: 12638690]

Fortier EE, Rooney J, Dardente H, et al. (2011). Circadian variation of the response of T cells to antigen. J Immunol, 187, 6291–300. [PubMed: 22075697]

Fu L, Lee CC. (2003). The circadian clock: pacemaker and tumour suppressor. Nat Rev Cancer, 3, 350–61. [PubMed: 12724733]

Galli SJ, Kalesnikoff J, Grimbaldeston MA, et al. (2005). Mast cells as "tunable" effector and immunoregulatory cells: recent advances. Annu Rev Immunol, 23, 749–86. [PubMed: 15771585]

Gan X, Zhang L, Solomon GF, Bonavida B. (2002). Mechanism of norepinephrine-mediated inhibition of human NK cytotoxic functions: inhibition of cytokine secretion, target binding, and programming for cytotoxicity. Brain Behav Immun, 16, 227–46. [PubMed: 12009684]

Garland FC, White MR, Garland CF, et al. (1990). Occupational sunlight exposure and melanoma in the U.S. Navy. Arch Environ Health, 45, 261–7. [PubMed: 2256710]

Gelfand EW. (2004). Inflammatory mediators in allergic rhinitis. J Allergy Clin Immunol, 114, S135–138. [PubMed: 15536444]

Gibbs JE, Beesley S, Plumb J, et al. (2009). Circadian timing in the lung: a specific role for bronchial epithelial cells. Endocrinology, 150, 268–76. [PubMed: 18787022]

Gibbs JE, Blaikley J, Beesley S, et al. (2012). The nuclear receptor REV-ERBa mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines. Proc Natl Acad Sci U S A, 109, 582–7. [PubMed: 22184247]

Ginaldi L, De Martinis M, Modesti M, et al. (2000). Immunophenotypical changes of T lymphocytes in the elderly. Gerontology, 46, 242–8. [PubMed: 10965179]

Gould HJ, Sutton BJ. (2008). IgE in allergy and asthma today. Nat Rev Immunol, 8, 205–17. [PubMed: 18301424]

Griffin AC, Whitacre CC. (1991). Sex and strain differences in the circadian rhythm fluctuation of endocrine and immune function in the rat: implications for rodent models of autoimmune disease. J Neuroimmunol, 35, 53–64. [PubMed: 1955572]

Gusenoff JA, Harman S, Veldhuis JD, et al. (2001). Cortisol and GH secretory dynamics, and their interrelationships, in healthy aged women and men. Am J Physiol Endocrinol Metab, 80, E616–E625.

Haas S, Straub RH. (2012). Disruption of rhythms of molecular clocks in primary synovial fibroblasts of patients with osteoarthritis and rheumatoid arthritis, role of IL-1β/TNF. Arthritis Res Ther, 14, R122. [PubMed: 22621205]

Haimovich B, Calvano J, Haimovich AD, et al. (2010). In vivo endotoxin synchronizes and suppresses clock gene expression in human peripheral blood leukocytes. Crit Care Med, 38, 751–8. [PubMed: 20081528]

Hashiramoto A, Yamane T, Tsumiyama K, et al. (2010). Mammalian clock gene Cryptochrome regulates arthritis via proinflammatory cytokine TNF-alpha. J Immunol, 184, 1560–5. [PubMed: 20042581]

Haus E, Smolensky MH. (1999). Biologic rhythms in the immune system. Chronobiol Int, 16, 581–622. [PubMed: 10513884]

Hawkins F (1975). Circadian and other rhythms of parasites. Adv Parasitol, 13, 123–82. [PubMed: 1094812]

Hayashi M, Shimba S, Tezuka M. (2007). Characterization of the molecular clock in mouse peritoneal macrophages. Biol Pharm Bull, 30, 621–6. [PubMed: 17409491]

Hertoghe T (2005). The “multiple hormone deficiency” theory of aging: is human senescence caused mainly by multiple hormone deficiencies? Ann N Y Acad Sci, 1057, 448–65. [PubMed: 16399912]

Hiemke C, Brunner R, Hammes E, et al. (1995). Circadian variations in antigen-specific proliferation of human T lymphocytes and correlation to cortisol production. Psychoneuroendocrinology, 20, 335–42. [PubMed: 7777661]

Hoffman AE, Zheng T, Stevens RG, et al. (2009). Clock-cancer connection in non-Hodgkin’s lymphoma: a genetic association study and pathway analysis of the circadian gene cryptochrome 2. Cancer Res, 69, 3605–13. [PubMed: 19318546]

Hohagen F, Timmer J, Weyerbrock A, et al. (1993). Cytokine production during sleep and wakefulness and its relationship to cortisol in healthy humans. Neuropsychobiology, 28, 9–16. [PubMed: 8255417]

Holgate ST, Polosa R. (2008). Treatment strategies for allergy and asthma. Nat Rev Immunol, 8, 218–30. [PubMed: 18274559]

Hriscu ML. (2005). Modulatory factors of circadian phagocytic activity. Ann N Y Acad Sci, 1057, 403–30. [PubMed: 16399910]

Isojima Y, Nakajima M, Ukai H, et al. (2009). CKIepsilon/delta-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. Proc Natl Acad Sci U S A, 106, 15744–9. [PubMed: 19805222]

James FO, Boivin DB, Charbonneau S, et al. (2007). Expression of clock genes in human peripheral blood mononuclear cells throughout the sleep/wake and circadian cycles. Chronobiol Int, 24, 1009–34. [PubMed: 18075796]

Janeway CA Jr, Medzhitov R. (2002). Innate immune recognition. Annu Rev Immunol, 20, 197–216. [PubMed: 11861602]

Jetten AM. (2009). Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism. Nucl Recept Signal, 7, e003. [PubMed: 19381306]

Jewett KA, Krueger JM. (2012). Humoral sleep regulation; interleukin-1 and tumor necrosis factor. Vitam Horm, 89, 241–57. [PubMed: 22640617]

Kalsbeek A, Liu J, Lei J, et al. (2012). Differential involvement of the suprachiasmatic nucleus in lipopolysaccharide-induced plasma glucose and corticosterone responses. Chronobiol Int, 29, 835–49. [PubMed: 22823867]

Karasek M (2004). Melatonin, human aging, and age-related diseases. Exp Gerontol, 39, 1723–9. [PubMed: 15582288]

Katz ME, Simonetta SH, Ralph MR, Golombek DA. (2008). Immunosuppressant calcineurin inhibitors phase shift circadian rhythms and inhibit circadian responses to light. Pharmacol Biochem Behav, 90, 763–8. [PubMed: 18590756]

Kawate T, Abo T, Hinuma S, Kumagai K. (1981). Studies of the bioperiodicity of the immune response. II. Co-variations of murine T and B cells and a role of corticosteroid. J Immunol, 126, 1364–7. [PubMed: 6970771]

Keisari Y, Cincotta E, Meier AH, Cincotta AH. (1999). Timed daily administration of prolactin and corticosteroid hormone reduces murine tumor growth and enhances immune reactivity. Chronobiol Int, 16, 315–33. [PubMed: 10373101]

Keller M, Mazuch J, Abraham U, et al. (2009). A circadian clock in macrophages controls inflammatory immune responses. Proc Natl Acad Sci U S A, 106, 21407–12. [PubMed: 19955445]

Kim S, Iizuka K, Kang HS, et al. (2002). In vivo developmental stages in murine natural killer cell maturation. Nat Immunol, 3, 523–8. [PubMed: 12006976]

Kirsch S, Thijssen S, Alarcon Salvador S, et al. (2012). T-cell numbers and antigen-specific T-cell function follow different circadian rhythms. J Clin Immunol, 32, 1381–9. [PubMed: 22797814]

Knapp MS, Cove-Smith JR, Dugdale R, et al. (1979). Possible effect of time on renal allograft rejection. Br Med J, 1, 75–7. [PubMed: 367500]

Ko CH, Takahashi JS. (2006). Molecular components of the mammalian circadian clock. Hum Mol Genet, 15, R271–R277. [PubMed: 16987893]

Kohm AP, Sanders VM. (2001). Norepinephrine and beta 2-adrenergic receptor stimulation regulate $CD4^+$ T and B lymphocyte function in vitro and in vivo. Pharmacol Rev, 53, 487–525. [PubMed: 11734616]

Koyanagi S, Ohdo S. (2002). Alteration of intrinsic biological rhythms during interferon treatment and its possible mechanism. Mol Pharmacol, 62, 1393–9. [PubMed: 12435807]

Kronfol Z, Nair M, Zhang Q, et al. (1997). Circadian immune measures in healthy volunteers: relationship to hypothalamic-pituitary-adrenal axis hormones and sympathetic neurotransmitters. Psychosom Med, 59, 42–50. [PubMed: 9021865]

Krueger JM. (2008). The role of cytokines in sleep regulation. Curr Pharm Des, 14, 3408–16. [PubMed: 19075717]

Krueger JM, Clinton JM, Winters BD, et al. (2011a). Involvement of cytokines in slow wave sleep. Prog Brain Res, 193, 39–47. [PubMed: 21854954]

Krueger JM, Majde JA, Rector DM. (2011b). Cytokines in immune function and sleep regulation. Handb Clin Neurol, 98, 229–40. [PubMed: 21056190]

Kuo TH, Pike DH, Beizaeipour Z, Williams JA. (2010). Sleep triggered by an immune response in Drosophila is regulated by the circadian clock and requires the NFkappaB Relish. BMC Neurosci, 11, 17. [PubMed: 20144235]

Kurepa Z, Rabatić S, Dekaris D. (1992). Influence of circadian light-dark alternations on macrophages and lymphocytes of CBA mouse. Chronobiol Int, 9, 327–40. [PubMed: 1394604]

Kusanagi H, Hida A, Satoh K, et al. (2008). Expression profiles of 10 circadian clock genes in human peripheral blood mononuclear cells. Neurosci Res, 61, 136–42. [PubMed: 18336944]

Kwak Y, Lundkvist GB, Brask J, et al. (2008). Interferon-gamma alters electrical activity and clock gene expression in suprachiasmatic nucleus neurons. J Biol Rhythms, 23, 150–9. [PubMed: 18375864]

Lange T, Dimitrov S, Fehm HL, et al. (2006). Shift of monocyte function toward cellular immunity during sleep. Arch Intern Med, 166, 1695–700. [PubMed: 16983046]

Lange T, Dimitrov S, Born J. (2010). Effects of sleep and circadian rhythm on the human immune system. Ann N Y Acad Sci, 1193, 48–59. [PubMed: 20398008]

Lange T, Dimitrov S, Bollinger T, et al. (2011). Sleep after vaccination boosts immunological memory. J Immunol, 187, 283–90. [PubMed: 21632713]

Langlois PH, Smolensky MH, Glezen WP, Keitel WA. (1995). Diurnal variation in responses to influenza vaccine. Chronobiol Int, 12, 28–36. [PubMed: 7750155]

Lapidot T, Kollet O. (2010). The brain-bone-blood triad: traffic lights for stem-cell homing and mobilization. Hematology Am Soc Hematol Educ Program, 2010, 1–6. [PubMed: 21239763]

Lavialle M, Serviere J. (1993). Circadian fluctuations in GFAP distribution in the Syrian hamster suprachiasmatic nucleus. Neuroreport, 4, 1243–6. [PubMed: 8219021]

Leone MJ, Marpegan L, Bekinschtein TA, et al. (2006). Suprachiasmatic astrocytes as an interface for immune-circadian signalling. J Neurosci Res, 84, 1521–7. [PubMed: 16955486]

Leone MJ, Marpegan L, Duhart JM, Golombek DA. (2012). Role of proinflammatory cytokines on lipopolysaccharide-induced phase shifts in locomotor activity circadian rhythm. Chronobiol Int, 29, 715–23. [PubMed: 22734572]

Lévi F, Canon C, Dipalma M, et al. (1991). When should the immune clock be reset? From circadian pharmacodynamics to temporally optimized drug delivery. Ann N Y Acad Sci, 618, 312–29. [PubMed: 2006792]

Levy EM, Roberti MP, Mordoh J. (2011). Natural killer cells in human cancer: from biological functions to clinical applications. J Biomed Biotechnol, 2011, 676198. [PubMed: 21541191]

Lissoni P, Rovelli F. (2012). Principles of psychoneuroendocri-noimmunotherapy of cancer. Immunotherapy, 4, 77–86. [PubMed: 22150002]

Liu J, Malkani G, Shi X, et al. (2006). The circadian clock Period 2 gene regulates gamma interferon production of NK cells in host response to lipopolysaccharide-induced endotoxic shock. Infect Immun, 74, 4750–6. [PubMed: 16861663]

Logan RW, Sarkar DK. (2012). Circadian nature of immune function. Mol Cell Endocrinol, 349, 82–90. [PubMed: 21784128]

Logan RW, Arjona A, Sarkar DK. (2011). Role of sympathetic nervous system in the entrainment of circadian natural-killer cell function. Brain Behav Immun, 25, 101–9. [PubMed: 20816749]

Logan RW, Zhang C, Murugan S, et al. (2012). Chronic shift-lag alters the circadian clock of NK cells and promotes lung cancer growth in rats. J Immunol, 188, 2583–91. [PubMed: 22308312]

Lucas D, Battista M, Shi PA, et al. (2008). Mobilized hematopoietic stem cell yield depends on species-specific circadian timing. Cell Stem Cell, 3, 364–6. [PubMed: 18940728]

Lundkvist GB, Robertson B, Mhlanga JD, et al. (1998). Expression of an oscillating interferon-gamma receptor in the suprachiasmatic nuclei. Neuroreport, 9, 1059–63. [PubMed: 9601667]

Lundkvist GB, Andersson A, Robertson B, et al. (1999). Light-dependent regulation and postnatal development of the interferon-gamma receptor in the rat suprachiasmatic nuclei. Brain Res, 849, 231–4. [PubMed: 10592307]

Lundkvist GB, Hill RH, Kristensson K. (2002). Disruption of circadian rhythms in synaptic activity of the suprachiasmatic nuclei by African trypanosomes and cytokines. Neurobiol Dis, 11, 20–7. [PubMed: 12460543]

Maestroni GJM. (1995). T-helper-2 lymphocytes as a peripheral target of melatonin. J Pineal Res, 18, 84–9. [PubMed: 7629695]

Maestroni GJM, Conti A. (1990). The pineal nehurohormone melatonin stimulates activated CD4+, Thy-1 cells to release opioid agonist(s) with immunoenhancingþand anti-stress properties. J Neuroimmunol, 28, 167–76. [PubMed: 1972943]

Maestroni GJM, Conti A, Pierpaoli W. (1987). The pineal gland and the circadian, opiatergic, immunoregolatory role of melatonin. Ann N Y Acad Sci, 496, 67–77. [PubMed: 3474997]

Maestroni GJM, Conti A, Pierpaoli W. (1988). Melatonin antagonizes the immunosoppressive effect of acute stress via an opiatergic mechanism. Immunology, 63, 465–9. [PubMed: 3350581]

Markus RP, Ferreira ZS, Fernandes PA, Cecon E. (2007). The immune-pineal axis: a shuttle between endocrine and paracrine melatonin sources. Neuroimmunomodulation, 14, 126–33. [PubMed: 18073503]

Marpegan L, Bekinschtein TA, Freudenthal R, et al. (2004). Participation of transcription factors from the Rel/NF-kappa B family in the circadian system in hamsters. Neurosci Lett, 358, 9–12. [PubMed: 15016422]

Marpegan L, Bekinschtein TA, Costas MA, Golombek DA. (2005). Circadian responses to endotoxin treatment in mice. J Neuroimmunol, 160, 102–9. [PubMed: 15710463]

Marpegan L, Leone MJ, Katz ME, et al. (2009). Diurnal variation in endotoxin-induced mortality in mice: correlation with proinflammatory factors. Chronobiol Int, 26, 1430–42. [PubMed: 19916840]

Maurer M, Ortonne JP, Zuberbier T. (2009). Chronic urticaria: an internet survey of health behaviours, symptom patterns and treatment needs in European adult patients. Br J Dermatol, 160, 633–41. [PubMed: 19014398]

Mazzoccoli G, Correra M, Bianco G, et al. (1997). Age-related changes of neuro-endocrine-immune interactions in healthy humans. J Biol Regul Homeost Agents, 11, 143–7. [PubMed: 9582614]

Mazzoccoli G, De Cata A, Greco A, et al. (2010a). Circadian rhythmicity of lymphocyte subpopulations and relationship with neuro-endocrine system. J Biol Regul Homeost Agents, 24, 341–50. [PubMed: 20846482]

Mazzoccoli G, Giuliani F, Inglese M, et al. (2010b). Chronobiologic study of the GH-IGF1 axis and the aging immune system. J Appl Biomed, 8, 213–26.

Mazzoccoli G, Vendemiale G, De Cata A, et al. (2010c). Altered time structure of neuro-endocrine-immune system function in lung cancer patients. BMC Cancer, 10, 314. [PubMed: 20565977]

Mazzoccoli G, Vendemiale G, La Viola M, et al. (2010d). Circadian variations of cortisol, melatonin and lymphocyte subpopulations in geriatric age. Int J Immunopathol Pharmacol, 23, 289–96. [PubMed: 20378015]

Mazzoccoli G, Carughi S, Sperandeo M, et al. (2011a). Alteration of circadian rhythmicity of CD3 CD4 lymphocyte subpopulation in healthy aging. J BiolþRegulþ Homeost Agents, 25, 405–16.

Mazzoccoli G, Inglese M, De Cata A, et al. (2011b). Neuroendocrine-immune interactions in healthy aging. Geriatr Gerontol Int, 11, 98–106. [PubMed: 20618451]

Mazzoccoli G, Sothern RB, De Cata A, et al. (2011c). A timetable of 24-hour patterns for human lymphocyte subpopulations. J Biol Regul Homeost Agents, 25, 387–95. [PubMed: 22023763]

Mazzoccoli G, Sothern RB, Greco G, et al. (2011d). Time-related dynamics of variation in core clock gene expression levels in tissues relevant to the immune system. Int J Immunopathol Pharmacol, 24, 869–79. [PubMed: 22230394]

Mazzoccoli G, Palmieri O, Corritore G, et al. (2012a). Association study of a polymorphism in clock gene PERIOD3 and risk of inflammatory bowel disease. Chronobiol Int, 29, 994–1003. [PubMed: 22881285]

Mazzoccoli G, Pazienza V, Vinciguerra M. (2012b). Clock genes and clock-controlled genes in the regulation of metabolic rhythms. Chronobiol Int, 29, 227–51. [PubMed: 22390237]

Mazzoccoli G, Sothern RB, Parrella P, et al. (2012c). Comparison of circadian characteristics for cytotoxic lymphocyte subsets in non-small cell lung cancer patients versus controls. Clin Exp Med, 12, 181–94. [PubMed: 21910027]

Mazzoccoli G, Vinciguerra M, Muscarella LA, et al. (2012d). Hormone and cytokine circadian alteration in non small cell lung cancer patients. Int J Immunopathol Pharmacol, 25, 691–702. [PubMed: 23058019]

Méndez-Ferrer S, Lucas D, Battista M, Frenette PS. (2008). Haematopoietic stem cell release is regulated by circadian oscillations. Nature, 452, 442–7. [PubMed: 18256599]

Miller P, Church MK. (1976). Pinnal anaphylaxis in the mouse. Mediating antibodies and rhythmic variations in the response. Clin Exp Immunol, 25, 177–9. [PubMed: 991449]

Miller AH, Spencer RL, Hassett J. (1994). Effects of selective type I and II adrenal steroid agonists on immune cell distribution. Endocrinology, 135, 1934–44. [PubMed: 7956914]

Mocchegiani E, Giacconi R, Muti E, et al. (2007). Zinc-bound metallothioneins and immune plasticity: lessons from very old mice and humans. Immun Ageing, 4, 7. [PubMed: 17903270]

Moldofsky H, Lue FA, Eisen J, et al. (1986). The relationship of interleukin-1 and immune functions to sleep in humans. Psychosom Med, 48, 309–18. [PubMed: 3487810]

Monsees GM, Kraft P, Hankinson SE, et al. (2012). Circadian genes and breast cancer susceptibility in rotating shift workers. Int J Cancer, 131, 2547–52. [PubMed: 22473669]

Morrhaye G, Kermani H, Legros JJ, et al. (2009). Impact of growth hormone (GH) deficiency and GH replacement upon thymus function in adult patients. PLoS ONE, 4, E5668. [PubMed: 19479077]

Moser B, Loetscher P. (2001). Lymphocyte traffic control by chemokines. Nat Immunol, 2, 123–8. [PubMed: 11175804]

Motzkus D, Albrecht U, Maronde E. (2002). The human PER1 gene is inducible by interleukin-6. J Mol Neurosci, 18, 105–9. [PubMed: 11931340]

Nakamura Y, Harama D, Shimokawa N, et al. (2011). The circadian clock gene Period2 regulates a time of day-dependent variation in cutanenous anaphylactic reaction. J Allergy Clin Immunol, 127, 1038–45. [PubMed: 21458659]

Narasimamurthy R, Hatori M, Nayak SK, et al. (2012). Circadian clock protein cryptochrome regulates the expression of proinflammatory cytokines. Proc Natl Acad Sci U S A, 109, 12662–7. [PubMed: 22778400]

Nava F, Carta G, Haynes LW. (2000). Lipopolysaccharide increases arginine-vasopressin release from rat suprachiasmatic nucleus slice cultures. Neurosci Lett, 288, 228–30. [PubMed: 10889349]

O'Callaghan EK, Anderson ST, Moynagh PN, Coogan AN. (2012). Long-lasting effects of sepsis on circadian rhythms in the mouse. PLoS ONE, 7, e47087. [PubMed: 23071720]

Oishi K, Ohkura N, Kadota K, et al. (2006). Clock mutation affects circadian regulation of circulating blood cells. J Circadian Rhythms, 4, 13. [PubMed: 17014730]

Okada K, Yano M, Doki Y, et al. (2008). Injection of LPS causes transient suppression of biological clock genes in rats. J Surg Res, 145, 5–12. [PubMed: 18279697]

Ottaway CA, Husband AJ. (1994). The influence of neuroendocrine pathways on lymphocyte migration. Immunol Today, 15, 511–17. [PubMed: 7802920]

Paladino N, Leone MJ, Plano SA, Golombek DA. (2010). Paying the circadian toll: the circadian response to LPS injection is dependent on the Toll-like receptor 4. J Neuroimmunol, 225, 62–7. [PubMed: 20554031]

Palomba M, Bentivoglio M. (2008). Chronic inflammation affects the photic response of the suprachiasmatic nucleus. J Neuroimmunol, 193, 24–7. [PubMed: 17936367]

Pan A, Schernhammer ES, Sun Q, Hu FB. (2011). Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women. PLoS Med, 8, e1001141. [PubMed: 22162955]

Pelegrí C, Vilaplana J, Castellote C, et al. (2003). Circadian rhythms in surface molecules of rat blood lymphocytes. Am J Physiol Cell Physiol, 284, C67–76. [PubMed: 12388092]

Perez-Aso M, Feig JL, Aranzazu M, Cronstein BN. (2013). Adenosine A(2A) Receptor and TNF-alpha regulate the circadian machinery of the human monocytic THP-1 cells. Inflammation, 36, 152–62. [PubMed: 22923002]

Petrovsky N (2001). Towards a unified model of neuroendocrine-immune interaction. Immunol Cell Biol, 79, 350–7. [PubMed: 11488982]

Petrovsky N, Harrison LC. (1995). Th1 and Th2: swinging to a hormonal rhythm. Immunol Today, 16, 605. [PubMed: 8579755]

Petrovsky N, Harrison LC. (1997). Diurnal rhythmicity of human cytokine production: a dynamic disequilibrium in T helper cell type 1/T helper cell type 2 balance? J. Immunol, 158, 5163–8. [PubMed: 9164932]

Petrzilka S, Taraborrelli C, Cavadini G, et al. (2009). Clock gene modulation by TNF-alpha depends on calcium and p38 MAP kinase signaling. J Biol Rhythms, 24, 283–94. [PubMed: 19625730]

Pöllmann L, Pöllmann B. (1988). [Untersuchungen zum Tuberkulin-Stempeltest] In: Hofmann F, Stössel U, eds. Arbeitsmedizin im Gesundheitsdienst. Stuttgart: Gentner Verlag, 183–8.

Pulendran B, Artis D. (2012). New paradigms in type 2 immunity. Science, 337, 431–5. [PubMed: 22837519]

Rehman HU, Masson EA. (2001). Neuroendocrinology of ageing. Age Ageing, 30, 279–87. [PubMed: 11509304]

Russell-Aulet M, Dimaraki EV, Jaffe CA, et al. (2001). Aging-related growth hormone (GH) decrease is a selective hypothalamic GH-releasing hormone pulse amplitude mediated phenomenon. J Gerontol A Biol Sci Med Sci, 56, M124–9. [PubMed: 11213276]

Sansoni P, Vescovini R, Fagnoni F, et al. (2008). The immune system in extreme longevity. Exp Gerontol, 43, 61–5. [PubMed: 17870272]

Scheiermann C, Kunisaki Y, Lucas D, et al. (2012). Adrenergic nerves govern circadian leukocyte recruitment to tissues. Immunity, 37, 290–301. [PubMed: 22863835]

Schibler U, Ripperger J, Brown SA. (2003). Peripheral circadian oscillators in mammals: time and food. J Biol Rhythms, 18, 250–60. [PubMed: 12828282]

Schwartz RS. (2003). Diversity of the immune repertoire and immunoregulation. N Engl J Med, 348, 1017–26. [PubMed: 12637612]

Scoma HD, Humby M, Yadav G, et al. (2011). The de-ubiquitinylating enzyme, USP2, is associated with the circadian clockwork and regulates its sensitivity to light. PLoS ONE, 6, e25382. [PubMed: 21966515]

Sephton SE, Sapolsky RM, Kraemer HC, Spiegel D. (2000). Diurnal cortisol rhythm as a predictor of breast cancer survival. J Natl Cancer Inst, 92, 994–1000. [PubMed: 10861311]

Shakhar G, Ben-Eliyahu S. (1998). In vivo beta-adrenergic stimulation suppresses natural killer activity and compromises resistance to tumor metastasis in rats. J Immunol, 160, 3251–8. [PubMed: 9531281]

Shimizu N, Hori T, Nakane H. (1994). An interleukin-1 beta-induced noradrenaline release in the spleen is mediated by brain corticotropin-releasing factor: an in vivo microdialysis study in conscious rats. Brain Behav Immun, 8, 14–23. [PubMed: 8003768]

Sigurdardottir LG, Valdimarsdottir UA, Fall K, et al. (2012). Circadian disruption, sleep loss, and prostate cancer risk: a systematic review of epidemiologic studies. Cancer Epidemiol Biomarkers Prev, 21, 1002–11. [PubMed: 22564869]

Silver AC, Arjona A, Hughes ME, et al. (2012a). Circadian expression of clock genes in mouse macrophages, dendritic cells, and B cells. Brain Behav Immun, 26, 407–13. [PubMed: 22019350]

Silver AC, Arjona A, Walker WE, Fikrig E. (2012b). The circadian clock controls toll-like receptor 9-mediated innate and adaptive immunity. Immunity, 36, 251–61. [PubMed: 22342842]

Smaniotto S, Martins-Neto AA, Dardenne M, Savino W. (2011). Growth hormone is a modulator of lymphocyte migration. Neuroimmunomodulation, 18, 309–13. [PubMed: 21952682]

Smolensky MH, Lemmer B, Reinberg AE. (2007). Chronobiology and chronotherapy of allergic rhinitis and bronchial asthma. Adv Drug Deliv Rev, 59, 852–82. [PubMed: 17900748]

Solana R, Mariani E. (2000). NK and NK/T cells in human senescence. Vaccine, 18, 1613–20. [PubMed: 10689137]

Solt LA, Wang Y, Banerjee S, et al. (2012). Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. Nature, 485, 62–8. [PubMed: 22460951]

Spengler ML, Kuropatwinski KK, et al. (2012). Core circadian protein CLOCK is a positive regulator of NF-kB-mediated transcription. Proc Natl Acad Sci U S A, 109, E2457–65. [PubMed: 22895791]

Stevens RG, Hansen J, Costa G, et al. (2011). Considerations of circadian impact for defining ‘shift work’ in cancer studies: IARC Working Group Report. Occup Environ Med, 68, 154–62. [PubMed: 20962033]

Straub RH, Cutolo M. (2007). Circadian rhythms in rheumatoid arthritis: implications for pathophysiology and therapeutic management. Arthritis Rheum, 56, 399–408. [PubMed: 17265475]

Straub RH, Cutolo M, Buttgereit F, Pongratz G. (2010). Energy regulation and neuroendocrine-immune control in chronic inflammatory diseases. J Intern Med, 267, 543–60. [PubMed: 20210843]

Stream AR, Sutherland ER. (2012). Obesity and asthma disease phenotypes. Curr Opin Allergy Clin Immunol, 12, 76–81. [PubMed: 22157152]

Sun Y, Yang Z, Niu Z, et al. (2006). MOP3, a component of the molecular clock, regulates the development of B cells. Immunology, 119, 451–60. [PubMed: 16925591]

Sun JC, Lopez-Verges S, Kim CC, et al. (2011). NK cells and immune “memory.” J. Immunol, 186, 1891–7. [PubMed: 21289313]

Suzuki S, Toyabe S, Moroda T, et al. (1997). Circadian rhythm of leucocytes and lymphocytes subsets and its possible correlation with the function of the autonomic nervous system. Clin Exp Immunol, 110, 500–8. [PubMed: 9409657]

Takahashi S, Yokota S, Hara R, et al. (2001). Physical and inflammatory stressors elevate circadian clock gene mPer1 mRNA levels in the paraventricular nucleus of the mouse. Endocrinology, 142, 4910–17. [PubMed: 11606459]

Takita E, Yokota S, Tahara Y, et al. (2013). Biological clock dysfunction exacerbates contact hypersensitivity in mice. Br J Dermatol, 168, 39–46. [PubMed: 22834538]

Tamura EK, Cecon E, Monteiro AW, et al. (2009). Melatonin inhibits LPS-induced NO production in rat endothelial cells. J Pineal Res, 46, 268–74. [PubMed: 19215575]

Teboul M, Barrat-Petit M-A, Mei Li X, et al. (2005). Atypical patterns of circadian clock gene expression in human peripheral blood mononuclear cells. J Mol Med, 83, 693–9. [PubMed: 16096739]

Terao K, Suzuki J, Ohkura S. (2002). Circadian rhythm in circulating CD16-positive natural killer (NK) cells in macaque monkeys, implication of palsma cortisol levels. Primates, 43, 329–38. [PubMed: 12426466]

Tong X, Buelow K, Guha A, et al. (2012). USP2a protein deubiquitinates and stabilizes the circadian protein CRY1 in response to inflammatory signals. J Biol Chem, 287, 25280–91. [PubMed: 22669941]

Toogood AA. (2003). Growth Hormone (GH) Status and body composition in normal ageing and in elderly adults with GH deficiency. Horm Res, 60, 105–11.

Touitou Y, Haus E. (1994). Aging of the human endocrine and neuroendocrine time structure. Ann N Y Acad Sci, 719, 378–97. [PubMed: 8010608]

Touitou Y, Haus E. (2000). Alterations with aging of the endocrine and neuroendocrine circadian system in humans. Chronobiol Int, 17, 369–90. [PubMed: 10841211]

Touitou Y, Bogdan A, Haus E, Touitou C. (1997). Modifications of circadian and circannual rhythms with aging. Exp Gerontol, 32, 603–14. [PubMed: 9315460]

Tsuchiya Y, Minami I, Kadotani H, Nishida E. (2005). Resetting of peripheral circadian clock by prostaglandin E2. EMBO Rep, 6, 256–61. [PubMed: 15723041]

Vivier E, Raulet DH, Moretta A, et al. (2011). Innate or adaptive immunity? The example of natural killer cells. Science, 331, 44–9. [PubMed: 21212348]

von Andrian UH, Mempel TR. (2003). Homing and cellular traffic in lymph nodes. Nat Rev Immunol, 3, 867–78. [PubMed: 14668803]

Wang X, Reece SP, Van Scott MR, Brown JM. (2010). A circadian clock in murine bone marrow-derived mast cells modulates IgE-dependent activation in vitro. Brain Behav Immun, 25, 127–34. [PubMed: 20854894]

Welsh DK, Takahashi JS, Kay SA. (2010). Suprachiasmatic nucleus: cell autonomy and network properties. Annu Rev Physiol, 72, 551–77. [PubMed: 20148688]

Yamamura Y, Yano I, Kudo T, Shibata S. (2010). Time-dependent inhibitory effect of lipopolysaccharide injection on Per1 and Per2 gene expression in the mouse heart and liver. Chronobiol Int, 27, 213–32. [PubMed: 20370466]

Yamaoka M, Maeda N, Nakamura S, et al. (2012). A pilot investigation of visceral fat adiposity and gene expression profile in peripheral blood cells. PLoS ONE, 7, e47377. [PubMed: 23091619]

Yang Y, Duguay D, Bedard N, et al. (2012). Regulation of behavioral circadian rhythms and clock protein PER1 by the deubiquitinating enzyme USP2. Biol Open, 1, 789–801. [PubMed: 23213472]

Yokoyama WM. (1999). Natural killer cells In: Paul WE, ed. Fundamental immunology. New York: Lippincott-Raven, 573–603.

Yokoyama WM, Kim S, French AR. (2004). The dynamic life of natural killer cells. Annu Rev Immunol, 22, 405–29. [PubMed: 15032583]

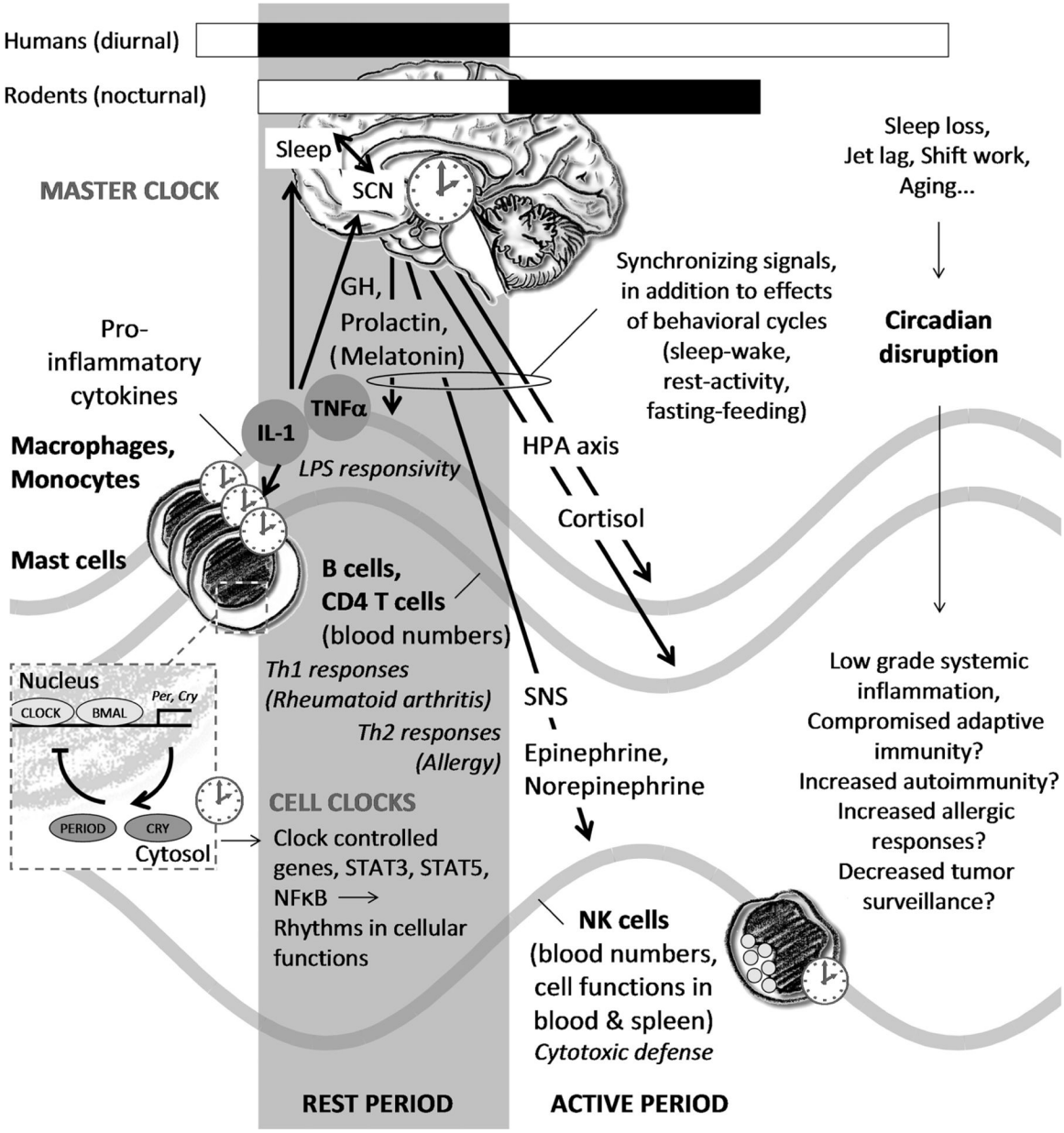

FIGURE 1.

Schematic view of the crosstalk between the circadian system and different aspects of the immune system. Immune cells such as macrophages, monocytes, mast cells, B cells, and $CD4^+$ T cells harbor intrinsic cell clocks (small clock symbols) that base on interlocked autoregulatory feedback loops of clock genes and their transcripts (see enlarged insert). This molecular clock in turn regulates clock-controlled genes and transcription factors, leading to rhythmic variations of cellular functions. Cellular immune rhythms are synchronized by the mammalian master clock (large clock symbol) that is located in the suprachiasmatic nuclei (SCN) in the anterior hypothalamus via time-dependent changes in the activity of the sympathetic nervous system (SNS), in the release of hormones (growth hormone [GH], prolactin, melatonin, cortisol) and in behavior that is linked to the sleep-wake cycle. As such

the rest period (i.e., the dark period in humans and the light period in nocturnal rodents) is characterized by peak levels of proinflammatory hormones such as GH, prolactin (and melatonin in humans), and proinflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α. In parallel, numbers of $CD4^+$ T cells are highest, and on a functional level the responsivity to lipopolysaccharide (LPS) as well as Th1 and Th2 responses are likewise highest during sleep. During the active period, the hypothalamus pituitary adrenal (HPA) axis becomes activated and cortisol suppresses proinflammatory cytokine production, $CD4^+$ T cell numbers, and allergic reactions. In contrast, natural killer (NK) cell numbers and functions peak during the active period and this rhythm is mainly regulated by the SNS and the release of epinephrine and norepinephrine. Disruption of this temporal organization of the immune system can lead to immunodeficiency (e.g., decreased tumor surveillance) and overshooting immune reactions (e.g., low-grade systemic inflammation). In addition to the circadian control of immune functions, immune mediators such as cytokines feed back to cellular clocks, the SCN, and sleep regulatory centers.