BIOMOLECULES & THERAPEUTICS

Review
Biomol Ther 29(1), 31-40 (2021)

Cell Autonomous Circadian Systems and Their Relation to Inflammation

Venkata Prakash Annamneedi[1], Jun Woo Park[1], Geum Seon Lee[2] and Tae Jin Kang[1,*]

[1]Convergence Research Center, Department of Pharmacy and Institute of Chronic Disease, Sahmyook University, Seoul 01795, [2]Department of Counseling and Psychology, Sahmyook University, Seoul 01795, Republic of Korea

Abstract

All living beings on earth have an important mechanism of 24-h periodicity, which controls their physiology, metabolism, and behavior. In humans, 24-h periodicity is regulated by the superchiasmatic nucleus (SCN) through external and environmental cues. Peripheral organs demonstrate circadian rhythms and circadian clock functions, and these are also observed in cultured cell lines. Every cell contains a CLOCK: BMAL1 loop for the generation of circadian rhythms. In this review, we focused on cell autonomous circadian rhythms in immune cells, the inflammatory diseases caused by disruption of circadian rhythms in hormones, and the role of clock genes in inflammatory diseases.

Key Words: Circadian rhythm, BMAL 1, PER2, Atopic dermatitis, Asthma

INTRODUCTION

Every organism on earth needs the ability to know the time of day for anticipating and responding to the changes in the external environments imposed by solar time (Curtis *et al*., 2014). Biological processes and functions are well organized in time, as evidenced by the expression of ultradian (high frequency), circadian (approximately 24-h), circamensual (approximately monthly), and circannual (approximately yearly) rhythms, as well as by the changes that occur with menarche, reproduction, and menopause (Lincoln *et al*., 2006). These circadian rhythms are mainly maintained based on the cyclical changes depending on the light-dark cycle (Haus, 2007). These rhythms and biological signals form a complex network including participation and interaction of the central nervous system (CNS), endocrine glands, peripheral endocrine tissues, and the immune system (Haus, 2007). These endogenous rhythms are flexible and adjust with light-dark, feeding, and temperature cycles as environmental cues (Cermakian *et al*., 2014). The circadian system has adaptability and predictability, and maintains homeostasis in health and well-being (Hastings *et al*., 2007; Nakagawa and Okumura, 2010). Circadian rhythms form a network with the CNS, suprachiasmatic nucleus (SCN), peripheral endocrine system, and the immune system (Fig. 1).

The SCN is located in the small hypothalamic region and is identified as the master pacemaker for regulating circadian rhythms through neuronal, humoral, and behavioral cues (Ralph *et al*., 1990). The SCN receives photic inputs through the retinohypothalamic tract (RHT) and non-photic cues from disparate neural inputs (Rosenwasser, 2009). These inputs converge in the SCN, which integrates both environmental and physiological signals to coordinate downstream brain areas and organ systems through neuronal and endocrine outputs (Guo *et al*., 2006). The SCN generates dissemination signals including several neuropeptides and direct axonal projections for communicating with specific neuronal populations to generate circadian patterns of behavior and physiology (Inouye and Kawamura, 1979; Meyer-Bernstein *et al*., 1999). Circadian oscillators are found in peripheral organs such as the liver, heart, kidney, and skin, as well as in cultured cell lines (Fig. 2) (Yamazaki *et al*., 2000; Lamia *et al*., 2008). Each cell of almost every tissue has independent oscillators; the spleen, lymph nodes, and isolated macrophages contain autonomous cellular oscillators that even operate without systemic time information (Nagoshi *et al*., 2004; Welsh *et al*., 2004). Circadian rhythm generation is a cell autonomous and fundamental mechanism of cell rhythm; it is highly conserved in SCN and peripheral cells.

The simplest form of the cell autonomous system forms as

Open Access **https://doi.org/10.4062/biomolther.2020.215**

Received Nov 29, 2020 **Revised** Dec 5, 2020 **Accepted** Dec 7, 2020
Published Online Jan 1, 2021

***Corresponding Author**
E-mail: kangtj@syu.ac.kr
Tel: +82-2-3399-1608, Fax: +82-2-3399-1617

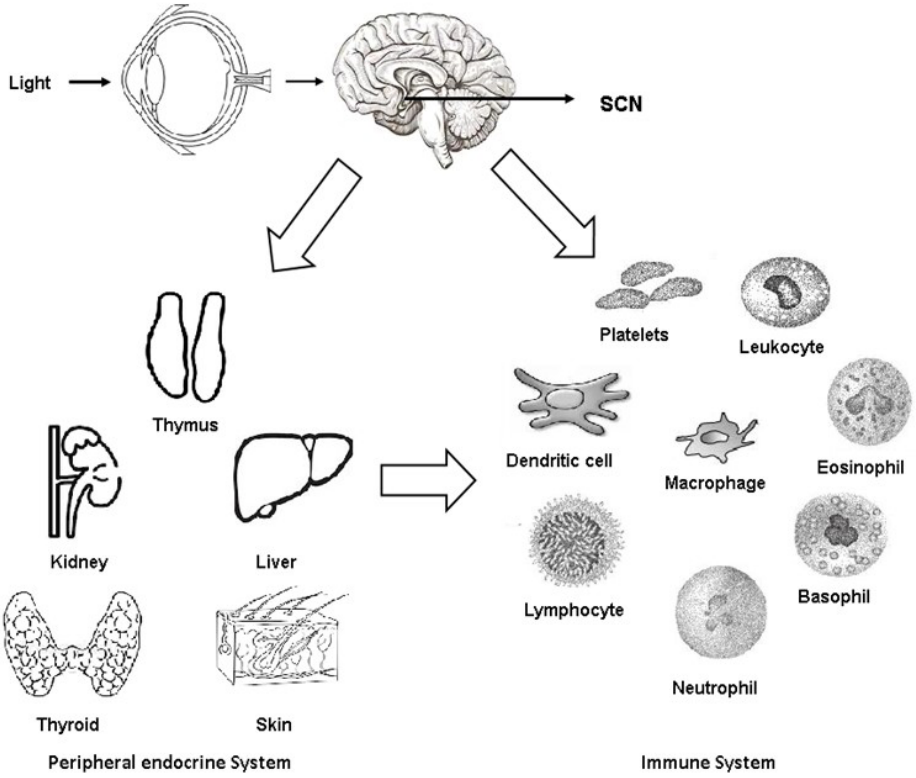

Fig. 1. Schematic for the complex network of the central nervous system, superchiasmatic nucleus (SCN), peripheral endocrine system, and immune system.

a transcription-translation oscillator loop (Fig. 3) (Curtis *et al*., 2014). The core of this oscillator is composed of a heterodimeric partnership of the basic-helix-loop-helix PER-ARNT-SIM (PAS) domain proteins, BMAL1 and CLOCK, which bind E-box sites and induce the expression of the repressors, Period (PER) and Cryptochrome (CRY), which in turn translocate to the nucleus and suppress their own expression by interfering with the BMAL: CLOCK complex. PER and CRY proteins are then degraded gradually and the repression of BMAL1 and CLOCK is relieved; the cycle thus begins freshly for another 24-h cycle (Curtis *et al*., 2014). Further levels of complexity, robustness, and regulation in the basic feedback loop ensues through additional feedback loops, which involve other transcription factors such as the orphan nuclear receptors REV-ERB α, β, and retinoic acid receptor-related orphan receptor (ROR) α, β, and γ (Duguay and Cermakian, 2009). CK1δ and CK1ε play a vital role in the posttranslational modification of the circadian timing system (Lee *et al*., 2009). PER proteins are phosphorylated from CK1δ and CK1ε, leading to their ubiquitin-dependent degradation and thus determine the intrinsic period of the clock. Suppression of CK1δ/ε slows PER protein turnover, decelerates clock progression, and lengthens the circadian period (Lee *et al*., 2009).

BMAL1

BMAL1 is a crucial clock component among all clock genes and proteins. Knock out of this single clock gene results in the loss of all rhythmic behavioral activity (Bunger *et al*., 2000). A span of basal pathologies understates the conditions related to accelerated aging caused by BMAL1 (Kondratov *et al*., 2006). The role of BMAL1 was investigated in myeloid cells, which mediate the clearance of the gram-positive bacterium, Listeria monocytogenes (Nguyen *et al*., 2013). Ly6C^{hi} monocytes are the first line of defense against this bacterium. BMAL1 emphasizes the recruitment of these cells into inflamed tissues, and mice with myeloid BMAL1 depletion show depletion of Ly6C^{hi} cells (Nguyen *et al*., 2013). E-boxes attached to BMAL1 in the promoters of Ccl2, Ccl8, and S100a8, then reduce Ccl2 transcription, and attenuate Ly6C^{hi} monocyte numbers at inflamed sites (Nguyen *et al*., 2013).

Absence of BMAL1 drives chronic inflammation, insulin resistance, hyperglycemia, whereas lack of BMAL1 enhances Ly6C^{hi} monocytosis and their recruitment into metabolically stressed tissues such as fat pads (Nguyen *et al*., 2013). Therefore, BMAL1 acts as an anti-inflammatory molecule in monocytes by repressing Ccl2. When BMAL1 is present at low levels in myeloid cells, excessive inflammation progresses to sepsis (Nguyen *et al*., 2013). LPS-stimulated peritoneal macrophages lacking BMAL1 produce high amounts of IL-6 (Gibbs *et al*., 2012).

Inflammatory responses are controlled by BMAL1 after toll-like receptor 4 (TLR4) activation in macrophages via regulation of the epigenetic state of enhancers. Acetylation of lysine 27 in histones is increased with BMAL1 deletion, along with prolonged activation of NF-kB target genes (Oishi *et al*., 2017). In the immune response, reactive oxygen species (ROS) play

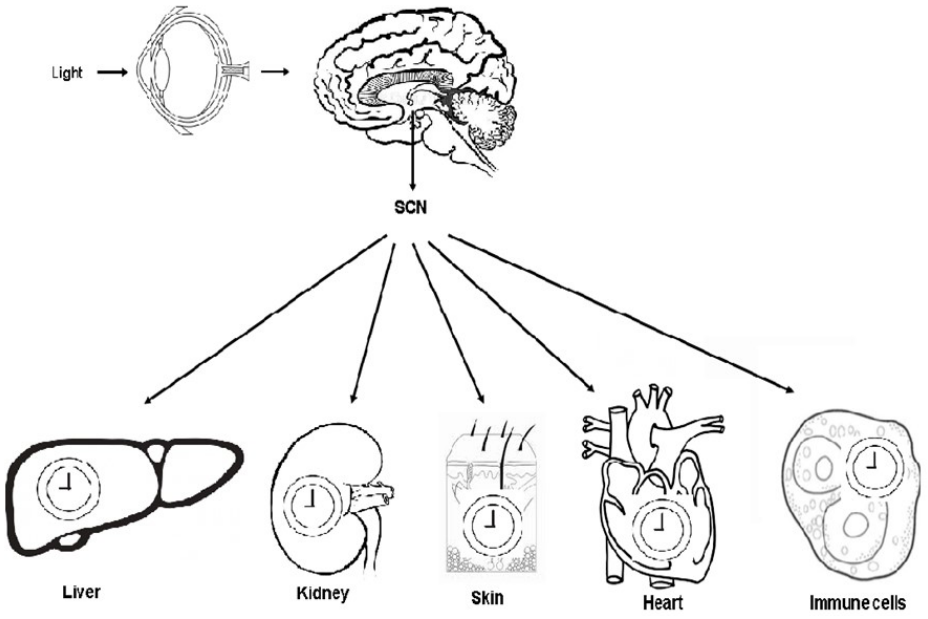

Fig. 2. Coordination of environmental cues and peripheral organs by the superchiasmatic nucleus (SCN) through downstream signals.

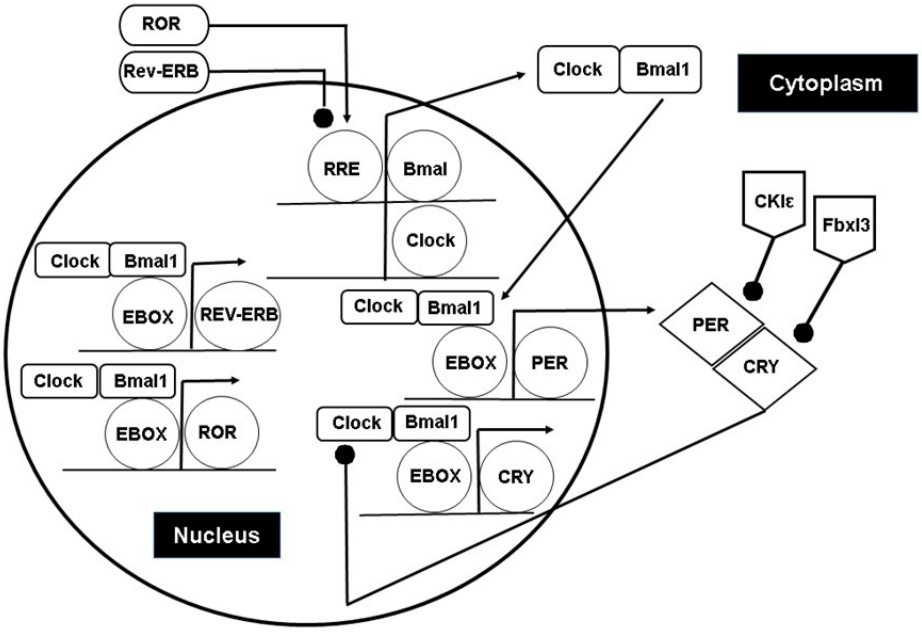

Fig. 3. The cell autonomous transcription-translation oscillator loop (Figure modified from Robinson and Reddy, 2014).

a vital role and are regulated by BMAL1 in multiple tissue types (Mittal *et al*., 2014). Deletion of BMAL1 promotes an advanced aging phenotype, which results in increased oxidative stress and generates a diabetic phenotype in the pancreas due to oxidative stress-induced death of β-cells (Kondratov *et al*., 2009; Lee *et al*., 2013). Neurodegeneration and astrogliosis are enhanced in the brain due to oxidative stress induced by the deletion of BMAL1 (Musiek *et al*., 2013). BMAL1 is known to regulate inflammation by controlling ROS levels and through the direct binding of NRF2 to the IL-1β promoter (Kobayashi *et al*., 2016).

PER2

Per2 is part of the PER clock proteins and modulates inflammation along with CRY. The mutant mouse strain PER2 (*Per2*$^{-/-}$) is an ideal experimental model that is used to reveal

specific characteristics of molecular clock components in the immune system and to peruse the role of PER2 in immune regulatory function (Zheng *et al*., 1999). PER2 mutant mice lose any changes in IFN-γ levels over 24-h periodicity (Arjona and Sarkar, 2006). Per2 plays a pivotal role in the host response and regulates IFN-γ production in NK cells and the spleen, as well as IL-1β production in macrophages (Liu *et al*., 2006). Circadian clock gene oscillations are disturbed in *Per2*$^{-/-}$ mice, which lose their circadian rhythm by LPS-induced shock (Halberg *et al*., 1960). *Per2*$^{-/-}$ mice are resistant to LPS-induced death via reduced production of IFN-γ by NK and NKT cells (Liu *et al*., 2006). IL-1β is mainly secreted from macrophages and the vascular endothelium in response to systemic endotoxin challenge (Liu *et al*., 2006). IL-1β levels were decreased by 30% in LPS-stimulated splenic macrophages and by 95% in the serum of LPS-challenged *Per2*$^{-/-}$ mice. Rather than monocytes/macrophages, *Per2*$^{-/-}$ deficient vascular endothelium shows IL-1β impairment in response to systemic endotoxin (Borish *et al*., 1992; Fabry *et al*., 1993; Bourdoulous *et al*., 1995).

Per2 gene regulates daily IFN-γ production, and *Per2* deficiency leads to immune modulation by changing the rhythm of IFN-γ mRNA in the spleen. In *Per2* mutant mice, there were no significant changes in IFN-γ levels, which were significantly lower than those in wild-type mice (Arjona and Sarkar, 2006). Table 1 shows that clock genes influence inflammation by specific inflammatory mediators.

INFLUENCE OF CIRCADIAN CLOCKS IN INFLAMMATORY DISEASES THROUGH HORMONES

Glucocorticoids

The adrenal cortex produces glucocorticoids (GCs), which are steroid hormones. The SCN plays an important role in rhythmic glucocorticoid synthesis and secretion (Son *et al*., 2011). GC levels are the highest in the early active phase, which means early in the day in humans (Son *et al*., 2011). GCs have an anti-inflammatory nature and a rhythm peak at night, which opposes their pro-inflammatory rhythm (Webster *et al*., 2002). GCs control various cell types in the immune system, and high levels of circulating GCs suppress immune responses leading to higher infection susceptibility (Webster *et al*., 2002). Suppression of GCs also exacerbates inflammatory responses (Webster *et al*., 2002).

GCs are influenced by circadian clocks, as binding elements of GC receptors are found in the promoters of clock genes PER1, PER2, and Rev-erbα (Balsalobre *et al*., 2000). *per1* and *per2* expression is drastically upregulated by GC treatment in cultured cells, *in vitro* cultured lung slices, and in different organs (Fukuoka *et al*., 2005; Gibbs *et al*., 2009; So *et al*., 2009; Cheon *et al*., 2013). GCs contemporizing cellular circadian oscillators *in vitro* and in peripheral tissues *in vivo* (Nagoshi *et al*., 2004; Fukuoka *et al*., 2005) PER2 protein rhythms are based on GC rhythms in the limbic forebrain (Segall and Amir, 2010). GCs are candidates for mediating the peripheral clock reset and regulate behavioral reset under the control of the SCN (Kiessling *et al*., 2010).

Cortisol synthesis shows circadian rhythms as a maximum in the early morning hours at 8:00 AM and steadily declines through the day to reach trough levels after sleep onset at night (Lakatos *et al*., 1995; Haeck *et al*., 2007). Cortisol is an influential endogenous anti-inflammatory substance that is upregulated in the early morning and is related to the inhibition of inflammation during the day; its downregulation in the evening and night time is related to the exacerbation of inflammation during the early morning.

Corticotropin-Releasing Hormone (CRH) regulates cortisol secretion, and fluctuations in cortisol lead to atopic dermatitis (AD). Low nocturnal cortisol levels lead to nocturnal itching in individuals with AD (Patel *et al*., 2007). Circadian dysregulation results in abnormalities in cortisol secretion, which leads to inflammatory diseases such as AD (Vaughn *et al*., 2018). In patients with rheumatoid arthritis (RA), cortisol rhythm is related to disease activity at low to moderate levels. This rhythm is highly distributed in patients with RA when the disease is in the active stage, leading to a flattening of the response curve, and two peaks appear in the morning and afternoon (Neeck *et al*., 1990). High disease activity in patients with RA is related to elevated serum cortisol levels.

Melatonin

The pineal gland synthesizes and secretes melatonin, and this synthesis mainly occurs at night (Maronde and Stehle, 2007). Melatonin is secreted in a diurnal pattern by a steady increase and peak between 2:00 AM to 4:00 AM, followed by a gradual decrease (Delagrange and Guardiola-Lemaitre, 1997). Melatonin has a sedative effect, and promotes sleep by acting on the SCN; it can also decrease the core body temperature (Haldar and Ahmad, 2010). Melatonin exerts anti-oxidative and immunomodulatory effects and plays a role in inflammation as an anti-inflammatory or pro-inflammatory component depending on the cell type and conditions (Schwarz *et al*., 1988; Delagrange and Guardiola-Lemaitre, 1997; Mauriz *et al*., 2013).

Melatonin also influences the diurnal rhythms of leukocyte proliferation, cytokine production, and NK cell activity (del Gobbo *et al*., 1989; Drazen *et al*., 2001). Melatonin administration suppresses nitric oxide synthase by upregulating antioxidant enzymes, cyclooxygenase-1/2 expression, PGE2 levels, and pro-inflammatory cytokine levels in various inflammatory models and also decreased CRY1 protein and *Cry1* mRNA levels in an experimental mouse model of arthritis (Bang *et al*., 2012; Mauriz *et al*., 2013).

Disrupted melatonin secretion induces AD in children as

Table 1. Interactions of clock gene components with inflammatory mediators

Clock genes	Inflammatory mediators	Effect on inflammation
BMAL1	Enhances Ly6C^{hi} monocytosis, supresses the reactive oxygen species, 9ROS)	Anti-inflammatory gene, controls inflammation
PER2	Monitors daily IFN-γ production	Promotes inflammation

reported by Schartz and colleagues (Schwarz *et al*., 1988). Low levels of melatonin lead to AD exacerbation in children whereas higher nocturnal melatonin results in reduced sleep disturbance and less severity of AD symptoms in children (Munoz-Hoyos *et al*., 2007; Chang *et al*., 2014). In a double-blind randomized controlled study, children aged 1-18 years who were administered melatonin had significantly lower SCORing of atopic dermatitis (SCORAD) index scores than those receiving a placebo (Chang *et al*., 2014). Chang and colleagues reported that latency to sleep onset in children with AD was significantly shortened with melatonin supplementation than with the placebo (Chang *et al*., 2016). Melatonin ameliorates AD development in DNFB-treated NC/Nga mice by reducing total serum immunoglobulin E levels and the IL-4 and IFN-γ production by activated $CD4^+$ T cells (Kim *et al*., 2009).

Melatonin levels exhibit a wide plateau lasting 2-3 h in RA (Sulli *et al*., 2002). A study in a northern European country reported that serum melatonin levels were elevated RA patients as compared to those in the controls (Cutolo *et al*., 2005). Melatonin enhances the Th1 immune response and may lead to unwanted increases in related cellular immune phenomena in patients with RA during the night (Cutolo and Maestroni, 2005). Table 2 shows the role of hormones in inflammation according to their circadian rhythmicity.

Circadian clock regulates inflammatory responses by immune cells

Circadian oscillators are present in peripheral organs such as the liver, heart, kidney, and skin, as well as cultured cell lines. The SCN sets the phase of these peripheral clocks that are involved in the regulation of local physiology (Yamazaki *et al*., 2000; Schibler, 2006; Lamia *et al*., 2008). In mammalian physiology including the immune system, many functions and parameters change in a time-of-day dependent manner; these include lymphocyte proliferation, natural killer cell activity, humoral immune responses, absolute and relative numbers of circulating white blood cells, and cytokine levels (Fernandes *et al*., 1976; Kawate *et al*., 1981; Young *et al*., 1995; Esquifino *et al*., 1996; Arjona and Sarkar, 2005). GC regulates the immune system by regulating immune cell number, cytokine concentration, surface marker abundance, and immunological effector functions rhythmically. This has contributed to an enormous progress in our understanding regarding the molecular basis of the circadian clock which is important in both health and disease (Takahashi *et al*., 2008). Peripheral tissues have autonomous circadian clocks, which are important regulators of normal peripheral physiology (Storch *et al*., 2007; Lamia *et al*., 2008).

Circadian clocks, which are fully operational and autonomous, exist in immunological tissues such as the spleen, lymph nodes, and resident peritoneal macrophages (Keller *et al*., 2009). Innate immunity is a crucial feature that is involved in the recognition of pathogens and subsequent initiation of defense strategies related to the macrophage intrinsic clock (Keller *et al*., 2009). Stimulation of macrophages by bacterial endotoxin for pro-inflammatory cytokine production is determined by the circadian phase of the macrophage clock (Krieger, 1975). Peritoneal macrophages have 8% of transcripts that are expressed rhythmically, uncovering multiple possible points in the LPS response pathway that link the macrophage intrinsic circadian clock with important immunological effector functions (Keller *et al*., 2009). Different cell types such as granulocytes, T cells, B cells, and myeloid lineage-derived cell lines in immunological tissues have both time-dependent heterogeneity, and clock gene dynamics that represent an average of many possible distinct clocks in different cell populations (Keller *et al*., 2009). There is some circadian variation in cytokine levels, lytic activity, and phagocytosis in NK cells and macrophages (Arjona and Sarkar, 2005; Hayashi *et al*., 2007).

The circadian system has one major output route of transcriptional regulation; 5-10% of the transcriptome is controlled by the circadian clock in many tissues and most transcripts oscillating in a tissue-specific manner (Schibler, 2007). Peritoneal macrophages have 8% of genes related to circadian modulation (Keller *et al*., 2009). Circadian expression in LPS induces immune pathways at multiple levels ranging from signal reception via signal transduction to response generation and interestingly, gene transcription of AP-1 or TLR4 inhibitory molecules such as CD80 and MD-1, indicating their circadian transcription (Keller *et al*., 2009). The circadian clock plays a regulatory role in many functional aspects including phagocytosis, antigen presentation, and immune regulation. Immune regulatory genes such as Cd59a, Cd69, Cd86, and Cd200r1 are expressed with high amplitude (Keller *et al*., 2009). Macrophages are regulated in a time-of-day-dependent manner in response to bacterial endotoxin. Halberg *et al*. first reported dramatic diurnal variations in a mouse model of endotoxic shock and suggested the biological significance of the timing of immune functions (Halberg *et al*., 1960; Barnes *et al*., 1993; Hrushesky *et al*., 1994). Splenocytes secrete TNF-α and IL-6 in a circadian manner, and T cells, monocyte/macrophages show circadian fluctuations; immune cell trafficking is also under circadian regulation (Keller *et al*., 2009). The circadian clock also plays a regulatory role in many functional aspects including phagocytosis. It thus seems reasonable to assume that cytokine secretion is regulated by the circadian clock in these cells. The circadian system interacts with and regulates the immune system as investigated in $CD11b^+$ macrophages based on global circadian gene expression profiling experiments (Keller *et al*., 2009). The circadian clock plays a regulatory role in many functional aspects including phagocytosis. Among 17,308 genes from peritoneal macrophages, 1,403 genes are rhythmically expressed in a circadian manner (Keller *et al*., 2009). Circadian transcriptional regulation at the

Table 2. Circadian rhythmicity of hormones and their effect on inflammation

Hormones	Relation to circadian rhythmicity	Effect on inflammation
Cortisol	Maximum levels at 8:00 AM, declines throughout the day	Fluctuations leads to atopic dermatitis and rheumatoid arthritis
Melatonin	Maximum levels at 2:00 AM to 4:00 AM. Then decrease gradually	Suppress the nitric oxide synthase, disruption leads to atopic dermatitis

level of LPS-induced immune response has been confirmed in components regulating LPS binding to TLR4 and homodimerization of TLR4 (MD-1 and CD180/RP105), components of the MAPK pathway controlling multiple downstream levels including transcription factor activation, cytokine protein processing, subunits and regulatory components of NF-κB and AP-1 transcription factors involved in proinflammatory cytokine transcription (NFkB1, RELA, IkBα, JUN, FOS), components regulating cytokine mRNA stability and localization (ELAVL1, SFPQ), and protein processing (ADAM17) (Keller *et al*., 2009). According to the nature of the transcriptional circadian clock within this pathway, it is very likely that the observed rhythmic immune functions are at least partly controlled by circadian clocks present in immune cells.

PER2 and Rev-Erbα transcripts from the spleen and lymph nodes showed high-amplitude circadian oscillations with a peak-to-trough ratio of ~4 for Per2 and ~20 for Rev-Erbα. In $CD11b^+$ peritoneal macrophages, high amplitude mRNA rhythms of *Per2* (peak-to-through ratio ~100) and Rev-Erbα (peak-to-through ~300) were observed (Keller *et al*., 2009). Moreover, their expression peaked around circadian time (CT) 6-9 for Rev-Erbα and around CT 12-15 for PER2. These clock proteins are part of an autonomous functional clock, which is manifested in PER2: LUC knockin mice; the spleen and lymph node explants of these mice showed persistent circadian oscillations of bioluminescence for more than a week. These cells showed high-amplitude circadian rhythmicity for more than a week, indicating that the circadian clock can operate autonomously without the requirement of systemic drivers in immune cells (Keller *et al*., 2009).

In *ex vivo* stimulated spleen cells, TNF-α and IL-6 are secreted at the peak levels when the mortality rate of endotoxic shock is the highest and the overall spleen contents as well as absolute numbers of monocytes/macrophages, are at their maximum levels (Halberg *et al*., 1960). The relationship between the circadian clock and immune system is bidirectional; sometimes, immune system parameters can also modulate the circadian clock (Majde and Krueger, 2005; Coogan and Wyse, 2008). LPS can induce a phase-shift in the circadian clock in mice and proinflammatory cytokines can alter circadian neuronal activity in the SCN (Marpegan *et al*., 2005; Kwak *et al*., 2008).

Circadian clock and atopic dermatitis (AD)

The skin shows time-of-day -dependent changes based on environmental conditions such as UV irradiation and temperature, and forms a barrier between the body and the environment. Some studies have reported circadian regulation of metabolic and physiological processes in the skin, time-of-day-dependent variations in human skin function such as barrier recovery, trans-epidermal water loss, sebum secretion, skin temperature, and skin pH (Yosipovitch *et al*., 1998; Le Fur *et al*., 2001; Yosipovitch *et al*., 2004). HaCaT keratinocytes are a model for investigating the effects of temperature cycles on epidermal oscillators (Yosipovitch *et al*., 1998). Temperature is a potent Zeitgeber of HaCaT clocks, synchronizing clock gene expression, and expanding the amplitude of clock-controlled genes (Sporl *et al*., 2011). Clock genes such as *Dbp, Bmal1, Per2*, and *Cry1* are expressed rhythmically after temperature entrainment (Brown *et al*., 2002). Upon temperature paradigm entrainment of the circadian clock in primary epidermal cells, canonical clock genes show high amplitude oscillations in HaCaT keratinocytes (Sporl *et al*., 2011). These observations indicate that primary epidermal keratinocytes possess a cell-autonomous circadian clockwork (Sporl *et al*., 2011). Cholesterol is needed for keratinocyte differentiation and proliferation as well as for cornified envelope formation (Schmidt *et al*., 1991). Keratinocytes form an epidermal lipid barrier by cholesterol. Regulation of cholesterol metabolism in keratinocytes by the circadian clock would imply a strict timing for keratinocyte differentiation and skin barrier function (Yosipovitch *et al*., 1998, 2004).

According to the circadian rhythms, fluctuations occur in skin barrier biophysical parameters (Le Fur *et al*., 2001). Facial sebum production shows oscillations in 8- and 24-h patterns and is the highest in the early afternoon and the lowest at night (Verschoore *et al*., 1993). Night-time pruritus is caused by low production of nocturnal sebum, which forms a hydrolipid film on the skin surface to maintain hydration (Vaughn *et al*., 2018). Generally, in healthy adults, trans-epidermal water loss (TEWL) is highest in the late afternoon and evening (Yosipovitch *et al*., 1998; Le Fur *et al*., 2001; Yosipovitch *et al*., 2004). Circadian clock genes regulate skin hydration at the cellular level. CLOCK gene knockout mice show a lack of stratum corneum (SC) hydration due to dysfunctional aquaporin-3 proteins (AQP3), which are transmembrane channels on keratinocytes that facilitate water and glycerol entry to maintain hydration (Hara-Chikuma and Verkman, 2008).

Immune functions in AD including regulation of circulating leukocyte numbers and types, and cytokine production are also controlled by circadian rhythms (Lange *et al*., 2006). Pro-inflammatory cytokines such as interleukin (IL)-12 and circulating naïve T- cells peak at night in adults, whereas cytotoxic T-cells and anti-inflammatory cytokines such as IL-10 peak during the day *in vitro* (Dimitrov *et al*., 2007; Lange *et al*., 2010). This diurnal pattern of immune function parallels the nocturnal itching and exaggeration of AD (Yosipovitch *et al*., 2002).

Circadian clock and asthma

Asthma is a complex lung disease driven by airway remodeling and involves inflammation, subepithelial fibrosis, smooth muscle proliferation, and goblet cell metaplasia (Bergeron *et al*., 2009). Additionally, an important characteristic of asthma is that patients exhibit symptoms by circadian rhythms (Clark, 1987). Clock genes may also contribute to lung inflammation, fibrosis, glucocorticoid response, and immunity (Silver *et al*., 2012; Gibbs *et al*., 2014; Pekovic-Vaughan *et al*., 2014). Environmental and genetic manipulation of circadian function can also lead to acute and chronic viral airway pathologies. Asthma symptoms frequently show exacerbation in the early hours of the morning, at around 4:00 AM, and sudden death also tends to occur at this time (Litinski *et al*., 2009). One survey including 7,729 patients with asthma reported that 74% awoke at least once per week with asthma symptoms, 64% showed nocturnal asthma symptoms at least three times per week, and approximately 40% of the patients experienced symptoms nightly (Litinski *et al*., 2009). Mice with disruption of circadian rhythms mimic chronic jet lag or shift work, causing alterations in lung mechanisms and clock gene expression in the lung (Hadden *et al*., 2012). Some subjects with nocturnal asthma show circadian variation in the number per unit volume of alveolar eosinophils, with a significantly greater number present at 4:00 AM *vs.* 4:00 PM; alveolar eosinophils

Table 3. Rhythmicity of inflammatory mediators with respect to inflammatory diseases

Inflammatory diseases	Inflammatory mediators to relation circadian rhythmicity
Atopic dermatitis	IL-12 and naïve T cell levels peak at night Low nocturnal sebum production induces pruritus
Asthma	Nocturnal asthma peaks at 4:00 AM *vs.* 4:00 PM Alveolar eosinophils are higher at 4:00 AM *vs.* 4:00 PM Macrophages, neutorophils & CD4 T cell numbers are high at 4:00 AM *vs.* 4:00 PM

increased with nocturnal asthma and correlated with nocturnal decrements in forced expiratory volume 1 (FEV1) (Durrington *et al*., 2014). The bronchoalveolar lavage fluid from patients with asthma contains increased numbers of macrophages, neutrophils, and CD4 T lymphocytes at 4:00 AM *vs.* that at 4:00 PM, and the percentage of CD4 T lymphocytes in the 4:00 AM lavage fluid is inversely correlated with the 4:00 AM FEV1 (Kelly *et al*., 2004; Litinski *et al*., 2009).

The Bmal1 deletion mouse model showed greater susceptibility to bacterial infections and inflammation in response to microbial products such as endotoxin and to sterile agents such as bleomycin and cigarette smoke (Nguyen *et al*., 2013; Gibbs *et al*., 2014; Hwang *et al*., 2014; Pekovic-Vaughan *et al*., 2014). Both eosinophils and mast cells are called harbor circadian clocks and show circadian rhythms (Ehlers *et al*., 2018). *Bmal1*$^{-/-}$ mice exhibit more severe infection by increasing weight loss, mortality, and viral RNA expression (Ehlers *et al*., 2018). *Bmal1*$^{-/-}$ mice develop more extensive airway inflammation than wild-type mice, as evidenced by bronchoalveolar lavage (BAL) protein concentrations and leukocyte counts. Moreover, *Bmal1*$^{-/-}$ mice show increased magnitude of granulocyte-predominant lung inflammation during Sendai virus (SeV) infection (Ehlers *et al*., 2018). Bacterial infection and endotoxin-challenge in *Bmal1*$^{-/-}$ mice have been shown to induce exaggerated inflammation because of the dysregulation of specific chemotactic factors such as CCL2 and CXCL5 (Ehlers *et al*., 2018). Bmal1 deletion enhanced the broad-based increase of pro-inflammatory cytokine expression in response to SeV; for instance, 30 cytokines and chemokines were identified at higher levels in BAL fluid in response to SeV infection in WT animals, and 20 cytokines exhibited significantly greater levels in *Bmal1*$^{-/-}$ mice for at least 2 time points as compared to those in wild-type mice (Ehlers *et al*., 2018). IFN-γ levels are much higher in SeV-infected *Bmal1*$^{-/-}$ mice along with higher levels of CXCL5 and IL-6. The circadian clock gene, *Bmal1*, thus regulates bronchiolitis and asthmatic airway phenotypes (Ehlers *et al*., 2018). Table 3 shows the inflammatory mediators in AD and asthma that follow circadian rhythmicity while upregulating inflammation.

CONCLUSION

Overall, cell-autonomous circadian rhythmicity is generated by a transcription/translational loop, ensued by an additional loop including Rev-Erbα and retinoic acid receptor-related orphan receptors (ROR). Disruption of circadian genes leads to inflammatory diseases. We thus concluded that among clock genes, BMAL1 acts as an anti-inflammatory component, *Per2* gene ensures inflammatory responses, fluctuation in cortisol and melatonin leads to atopic dermatitis. Proinflammatory cytokines such as TNF-α and IL-6, are also secreted rhythmically from immune cells.

CONFLICT OF INTEREST

The authors confirm that they have no conflicts of interest.

ACKNOWLEDGMENTS

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (No. 2019R1F1A1062953).

REFERENCES

Arjona, A. and Sarkar, D. K. (2005) Circadian oscillations of clock genes, cytolytic factors, and cytokines in rat NK cells. *J. Immunol.* **174**, 7618-7624.

Arjona, A. and Sarkar, D. K. (2006) The circadian gene mPer2 regulates the daily rhythm of IFN-gamma. *J. Interferon Cytokine Res.* **26**, 645-649.

Balsalobre, A., Brown, S. A., Marcacci, L., Tronche, F., Kellendonk, C., Reichardt, H. M., Schutz, G. and Schibler, U. (2000) Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science* **289**, 2344-2347.

Bang, J., Chang, H. W., Jung, H. R., Cho, C., Hur, J., Lee, S., Choi, T. H., Kim, S. and Ha, E. (2012) Melatonin attenuates clock gene Cryptochrome1, which may aggravate mouse anti-type II collagen antibody-induced arthritis. *Rheumatol. Int.* **32**, 379-385.

Barnes, P. J., Adcock, I., Spedding, M. and Vanhoutte, P. M. (1993) Anti-inflammatory actions of steroids: molecular mechanisms. *Trends Pharmacol. Sci.* **14**, 436-441.

Bergeron, C., Al-Ramli, W. and Hamid, Q. (2009) Remodeling in asthma. *Proc. Am. Thorac. Soc.* **6**, 301-305.

Borish, L., King, M. S., Mascali, J. J., Johnson, S., Coll, B. and Rosenwasser, L. J. (1992) Transthyretin is an inhibitor of monocyte and endothelial cellinterleukin-1 production. *Inflammation* **16**, 471-484.

Bourdoulous, S., Bensaid, A., Martinez, D., Sheikboudou, C., Trap, I., Strosberg, A. D. and Couraud, P. O. (1995) Infection of bovine brain microvessel endothelial cells with Cowdria ruminantium elicits IL-1 beta, -6, and -8 mRNA production and expression of an unusual MHC class II DQ alpha transcript. *J. Immunol.* **154**, 4032-4038.

Brown, S. A., Zumbrunn, G., Fleury-Olela, F., Preitner, N. and Schibler, U. (2002) Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Curr. Biol.* **12**, 1574-1583.

Bunger, M. K., Wilsbacher, L. D., Moran, S. M., Clendenin, C., Radcliffe, L. A., Hogenesch, J. B., Simon, M. C., Takahashi, J. S. and Bradfield, C. A. (2000) Mop3 is an essential component of the master circadian pacemaker in mammals. *Cell* **103**, 1009-1017.

Cermakian, N., Westfall, S. and Kiessling, S. (2014) Circadian clocks and inflammation: reciprocal regulation and shared mediators. *Arch. Immunol. Ther. Exp.* **62**, 303-318.

Chang, Y., Chou, Y., Lee, J., Lee, P., Dai, Y., Sun, C., Lin, Y., Wang, L., Yu, H., Yang, Y., Chen, C., Wan, K. and Chiang, B. (2014) Atopic dermatitis, melatonin, and sleep disturbance. *Pediatrics* **134**, e397-e405.

Chang, Y., Lin, M., Lee, J., Lee, P., Dai, Y, Chu, K., Sun, C., Lin, Y., Wang, L., Yu, H., Yang , Y., Chen, C., Wan, K. and Chiang, B.

(2016) Melatonin supplementation for children with atopic dermatitis and sleep disturbance: a randomized clinical trial. *JAMA Pediatr.* **170**, 35-42.
Cheon, S., Park, N., Cho, S. and Kim, K. (2013) Glucocorticoid-mediated Period2 induction delays the phase of circadian rhythm. *Nucleic Acids Res.* **41**, 6161-6174.
Clark, T. J. (1987) Diurnal rhythm of asthma. *Chest* **91**, 137S-141S.
Coogan, A. N. and Wyse, C. A. (2008) Neuroimmunology of the circadian clock. *Brain Res.* **1232**, 104-112.
Curtis, A. M., Bellet, M. M., Sassone-Corsi, P. and O'Neill, L. A. (2014) Circadian clock proteins and immunity. *Immunity* **40**, 178-186.
Cutolo, M., Maestroni, G. J., Otsa, K., Villaggio, B., Capellino, S., Montagna, P., Fazzuoli, L., Veldi, T., Peets, T., Hertens, E. and Sulli, A. (2005) Circadian melatonin and cortisol levels in rheumatoid arthritis patients in winter time: a north and south Europe comparison. *Ann. Rheum. Dis.* **64**, 212-216.
Cutolo, M. and Maestroni, G. J. (2005) The melatonin-cytokine connection in rheumatoid arthritis. *Ann. Rheum. Dis.* **64**, 1109-1111.
del Gobbo, V., Libri, V., Villani, N., Calio, R. and Nistico, G. (1989) Pinealectomy inhibits interleukin-2 production and natural killer activity in mice. *Int. J. Immunopharmacol.* **11**, 567-573.
Delagrange, P. and Guardiola-Lemaitre, B. (1997) Melatonin, its receptors, and relationships with biological rhythm disorders. *Clin. Neuropharmacol.* **20**, 482-510.
Dimitrov, S., Lange, T., Nohroudi, K. and Born, J. (2007) Number and function of circulating human antigen presenting cells regulated by sleep. *Sleep* **30**, 401-411.
Drazen, D. L., Bilu, D., Bilbo, S. D. and Nelson, R. J. (2001) Melatonin enhancement of splenocyte proliferation is attenuated by luzindole, a melatonin receptor antagonist. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280**, R1476-R1482.
Duguay, D. and Cermakian, N. (2009) The crosstalk between physiology and circadian clock proteins. *Chronobiol. Int.* **26**, 1479-1513.
Durrington, H. J., Farrow, S. N., Loudon, A. S. and Ray, D. W. (2014) The circadian clock and asthma. *Thorax* **69**, 90-92.
Ehlers, A., Xie, W., Agapov, E., Brown, S., Steinberg, D., Tidwell R., Sajol, G., Schutz, R., Weaver, R., Yu, H., Castro, M., Bacharier, L. B., Wang, X., Holtzman, M. J. and Haspel, J. A. (2018) BMAL1 links the circadian clock to viral airway pathology and asthma phenotypes. *Mucosal Immunol.* **11**, 97-111.
Esquifino, A. I., Selgas, L., Arce, A., Maggiore, V. D. and Cardinali, D. P. (1996) Twenty-four-hour rhythms in immune responses in rat submaxillary lymph nodes and spleen: effect of cyclosporine. *Brain Behav. Immun.* **10**, 92-102.
Fabry, Z., Fitzsimmons, K. M., Herlein, J. A., Moninger, T. O., Dobbs, M. B. and Hart, M. N. (1993) Production of the cytokines interleukin 1 and 6 by murine brain microvessel endothelium and smooth muscle pericytes. *J. Neuroimmunol.* **47**, 23-34.
Fernandes, G., Halberg, F., Yunis, E. J. and Good, R. A. (1976) Circadian rhythmic plaque-forming cell response of spleens from mice immunized with Srbc. *J. Immunol.* **117**, 962-966.
Fukuoka, Y., Burioka N., Takata, M., Ohso, S., Miyata, M., Endo, M. and Shimizu, E. (2005) Glucocorticoid administration increases hPer1 mRNA levels in human peripheral blood mononuclear cells *in vitro* or *in vivo*. *J. Biol. Rhythms* **20**, 550-553.
Gibbs, J. E., Beesley, S., Plumb, J., Singh, D., Farrow, S., Ray, D. W. and Loudon, A. S. I. (2009) Circadian timing in the lung; a specific role for bronchiolar epithelial cells. *Endocrinology* **150**, 268-276.
Gibbs, J., Ince, L., Matthews, L., Mei, J., Bell, T., Yang, N., Saer, B., Begley, N., Poolman, T., Pariollaud, M., Farrow, S., Francesco, D., Hussel, T., Worthen, G. S., Ray, D. and Loudon, A. (2014) An epithelial circadian clock controls pulmonary inflammation and glucocorticoid action. *Nat. Med.* **20**, 919-926.
Gibbs, J. E., Blaikley, J., Beesley, S., Matthews, L., Simpson, K. D., Boyce, S. H., Farrow, S. N., Else, K. J., Singh, D., Ray, D. W. and Loudon, A. S. I. (2012) The nuclear receptor REV-ERBα mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 582-587.
Guo, H., Brewer, J. M., Lehman, M. N. and Bittman, E. L. (2006) Suprachiasmatic regulation of circadian rhythms of gene expression in hamster peripheral organs: effects of transplanting the pacemaker. *J. Neurosci.* **26**, 6406-6412.
Hadden, H., Soldin, S. J. and Massaro, D. (2012) Circadian disruption alters mouse lung clock gene expression and lung mechanics. *J. Appl. Physiol.* **113**, 385-392.
Haeck, I. M., Timmer-de Mik, L., Lentjes, E. G., Buskens, E., Hijnen, D. J., Guikers, C., Bruijnzeel-Koomen, C. A. F. M. and de Bruin-Weller, M. S. (2007) Low basal serum cortisol in patients with severe atopic dermatitis: potent topical corticosteroids wrongfully accused. *Br. J. Dermatol.* **156**, 979-985.
Halberg, F., Johnson, E. A., Brown, B. W. and Bittner, J. J. (1960) Susceptibility rhythm to E. coli endotoxin and bioassay. *Proc. Soc. Exp. Biol. Med.* **103**, 142-144.
Haldar, C. and Ahmad, R. (2010) Photoimmunomodulation and melatonin. *J. Photochem. Photobiol. B Biol.* **98**, 107-117.
Hara-Chikuma, M. and Verkman, A. S. (2008) Roles of aquaporin-3 in the epidermis. *J. Invest. Dermatol.* **128**, 2145-2151.
Hastings, M., O'Neill, J. S. and Maywood, E. S. (2007) Circadian clocks: regulators of endocrine and metabolic rhythms. *J. Endocrinol.* **195**, 187-198.
Haus, E. (2007) Chronobiology in the endocrine system. *Adv. Drug Deliv. Rev.* **59**, 985-1014.
Hayashi, M., Shimba, S. and Tezuka, M. (2007) Characterization of the molecular clock in mouse peritoneal macrophages. *Biol. Pharm. Bull.* **30**, 621-626.
Hrushesky, W. J., Langevin, T., Kim, Y. J. and Wood, P. A. (1994) Circadian dynamics of tumor necrosis factor alpha (cachectin) lethality. *J. Exp. Med.* **180**, 1059-1065.
Hwang, J. W., Sundar, I. K., Yao, H., Sellix, M. T. and Rahman, I. (2014) Circadian clock function is disrupted by environmental tobacco/cigarette smoke, leading to lung inflammation and injury via a SIRT1-BMAL1 pathway. *FASEB J.* **28**, 176-194.
Inouye, S. T. and Kawamura, H. (1979) Persistence of circadian rhythmicity in a mammalian hypothalamic "island" containing the suprachiasmatic nucleus. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5962-5966.
Kawate, T., Abo, T., Hinuma, S. and 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**, 1364-1367.
Keller, M., Mazuch, J., Abraham, U., Eom, G. D., Herzog, E. D., Volk, H., Kramer, A. and Maier, B. (2009) A circadian clock in macrophages controls inflammatory immune responses. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 21407-21412.
Kelly, E. A., Houtman, J. J. and Jarjour, N. N. (2004) Inflammatory changes associated with circadian variation in pulmonary function in subjects with mild asthma. *Clin. Exp. Allergy* **34**, 227-233.
Kiessling, S., Eichele, G. and Oster, H. (2010) Adrenal glucocorticoids have a key role in circadian resynchronization in a mouse model of jet lag. *J. Clin. Invest.* **120**, 2600-2609.
Kim, T. H., Jung, J. A., Kim, G. D., Jang, A. H., Ahn, H. J., Park, Y. S. and Park, C. S. (2009) Melatonin inhibits the development of 2,4-dinitrofluorobenzene-induced atopic dermatitis-like skin lesions in NC/Nga mice. *J. Pineal Res.* **47**, 324-329.
Kobayashi, E. H., Suzuki, T., Funayama, R., Nagashima, T., Hayashi, M., Sekine, H., Tanaka, N., Moriguchi, T., Motohashi, H., Nakayama, K. and Yamamoto, M. (2016) Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. *Nat. Commun.* **7**, 11624.
Kondratov, R. V., Vykhovanets, O., Kondratova, A. A. and Antoch, M. P. (2009) Antioxidant N-acetyl-L-cysteine ameliorates symptoms of premature aging associated with the deficiency of the circadian protein BMAL1. *Aging (Albany N.Y.)* **1**, 979-987.
Kondratov, R. V., Kondratova, A. A., Gorbacheva, V. Y., Vykhovanets, O. V. and Antoch, M. P. (2006) Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock. *Genes Dev.* **20**, 1868-1873.
Krieger, D. T. (1975) Rhythms of ACTH and corticosteroid secretion in health and disease, and their experimental modification. *J. Steroid Biochem.* **6**, 785-791.
Kwak, Y., Lundkvist, G. B., Brask, J., Davidson, A., Menaker, M., Kritensoon, K. and Block, G. D. (2008) Interferon-gamma alters electrical activity and clock gene expression in suprachiasmatic nucleus neurons. *J. Biol. Rhythms* **23**, 150-159.

Lakatos, P., Blumsohn, A., Eastell, R., Tarjan, G., Shinoda, H. and Sterm. P. H. (1995) Circadian rhythm of *in vitro* bone-resorbing activity in human serum. *J. Clin. Endocrinol. Metab*. **80**, 3185-3190.

Lamia, K. A., Storch, K. F. and Weitz, C. J. (2008) Physiological significance of a peripheral tissue circadian clock. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 15172-15177.

Lange, T., Dimitrov, S., Fehm, H. L. and Born, J. (2006) Sleep-like concentrations of growth hormone and cortisol modulate type 1 and type2 in-vitro cytokine production in human T cells. *Int. Immunopharmacol.* **6**, 216-225.

Lange, T., Dimitrov, S. and Born, J. (2010) Effects of sleep and circadian rhythm on the human immune system. *Ann. N. Y. Acad. Sci.* **1193**, 48-59.

Le Fur, I., Reinberg, A., Lopez, S., Morizot, F., Mechkouri, M. and Tschachler, E. (2001) Analysis of circadian and ultradian rhythms of skin surface properties of face and forearm of healthy women. *J. Invest. Dermatol.* **117**, 718-724.

Lee, H., Chen, R., Lee, Y., Yoo, S. and Lee, C. (2009) Essential roles of CKIδ and CKIε in the mammalian circadian clock. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 21359-21366.

Lee, J., Moulik, M., Fang, Z., Saha, P., Zou, F., Xu, Y., Nelson, D. L., Ma, K., Moore, D. D. and Yechoor, V. K. (2013) Bmal1 and β-cell clock are required for adaptation to circadian disruption, and their loss of function leads to oxidative stress-induced β-cell failure in mice. *Mol. Cell. Biol.* **33**, 2327-2338.

Lincoln, G. A., Clarke, I. J., Hut, R. A. and Hazlerigg, D. G. (2006) Characterizing a mammalian circannual pacemaker. *Science* **314**, 1941-1944.

Litinski, M., Scheer, F. A. and Shea, S. A. (2009) Influence of the circadian system on disease severity. *Sleep Med. Clin.* **4**, 143-163.

Liu, J., Mankani, G., Shi, A., Meyer, M., Cunningham-Runddles, S., Ma, X. and Sun, Z. S. (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**, 4750-4756.

Majde, J. A. and Krueger, J. M. (2005) Links between the innate immune system and sleep. *J. Allergy Clin. Immunol.* **116**, 1188-1198.

Maronde, E. and Stehle, J. H. (2007) The mammalian pineal gland: known facts, unknown facets. *Trends Endocrinol. Metab.* **18**, 142-149.

Marpegan, L., Bekinschtein, T. A., Costas, M. A. and Golombek, D. A. (2005) Circadian responses to endotoxin treatment in mice. *J. Neuroimmunol.* **160**, 102-109.

Mauriz, J. L., Collado, P. S., Veneroso, C., Reiter, R. J. and Gonzales-Gallego, J. (2013) A review of the molecular aspects of melatonin's anti-inflammatory actions: recent insights and new perspectives. *J. Pineal Res.* **54**, 1-14.

Meyer-Bernstein, E. L., Jetton, A. E., Matsumoto, S. I., Markuns, J. F., Lehman, M. N. and Bittman, E. L. (1999) Effects of suprachiasmatic transplants on circadian rhythms of neuroendocrine function in golden hamsters. *Endocrinology* **140**, 207-218.

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P. and Malik, A. B. (2014) Reactive oxygen species in inflammation and tissue injury. *Antioxid. Redox Signal.* **20**, 1126-1167.

Munoz-Hoyos, A., Espin-Quirantes, C., Molina-Carballo, A., Uberos, J., Contreras-Chova, F., Narbona-Lopez, E. and Gutierrez-Salmeron, M. J. (2007) Neuroendocrine and circadian aspects (melatonin and beta-endorphin) of atopic dermatitis in the child. *Pediatr. Allergy Immunol.* **18**, 679-686.

Musiek, E. S., Lim, M. M., Yang, G., Bauer, A. Q., Qi, L., Lee, Y., Roh, J. H., Ortiz-Gonzalez, X., Dearborn, J. T., Culver, J. P., Herzog, E. D., Hogenesch, J. B., Wozniak, D. F., Dikranian, K., Giasson, B. I., Weaver, D. R., Holtzman, D. M. and Fitzgerald, G. A (2013) Circadian clock proteins regulate neuronal redox homeostasis and neurodegeneration. *J. Clin. Invest.* **123**, 5389-5400.

Nagoshi, E., Saini, C., Bauer, C., Laroche, T., Naf, F. and Schibler, U. (2004) Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells. *Cell* **119**, 693-705.

Nakagawa, H. and Okumura, N. (2010) Coordinated regulation of circadian rhythms and homeostasis by the suprachiasmatic nucleus. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* **86**, 391-409.

Neeck, G., Federlin, K., Graef, V., Rusch, D. and Schmidt, K. L. (1990) Adrenal secretion of cortisol in patients with rheumatoid arthritis. *J. Rheumatol.* **17**, 24-29.

Nguyen, K. D., Fentress, S. J., Qiu, Y., Yun, K., Cox, J. S. and Chawla, A. (2013) Circadian gene Bmal1 regulates diurnal oscillations of Ly6C(hi) inflammatory monocytes. *Science* **341**, 1483-1488.

Oishi, Y., Hayashi, S., Isagawa, T., Oshima, M., Iwama, A., Shimba, S., Okamura, H. and Manabe, I. (2017) Bmal1 regulates inflammatory responses in macrophages by modulating enhancer RNA transcription. *Sci. Rep.* **7**, 7086.

Patel, T., Ishiuji, Y. and Yosipovitch, G. (2007) Nocturnal itch: why do we itch at night? *Acta Derm. Venereol.* **87**, 295-298.

Pekovic-Vaughan, V., Gibbs, J., Yoshitane, H., Yang, N., Pathiranage, D., Guo, B., Sagami, A., Taguchi, K., Bechtole, D., Loudon, A., Yamamoto, M., Chan, J., van der Horst, G. T., Fukada, Y. and Meng, Q. (2014) The circadian clock regulates rhythmic activation of the NRF2/glutathione-mediated antioxidant defense pathway to modulate pulmonary fibrosis. *Genes Dev.* **28**, 548-560.

Ralph, M. R., Foster, R. G., Davis, F. C. and Menaker, M. (1990) Transplanted suprachiasmatic nucleus determines circadian period. *Science* **247**, 975-978.

Robinson, I. and Reddy, A. B. (2014) Molecular mechanisms of the circadian clockwork in mammals. *FEBS Lett.* **588**, 2477-2483.

Rosenwasser, A. M. (2009) Functional neuroanatomy of sleep and circadian rhythms. *Brain Res. Rev.* **61**, 281-306.

Schibler, U. (2006) Circadian time keeping: the daily ups and downs of genes, cells, and organisms. *Prog. Brain Res.* **153**, 271-282.

Schibler, U. (2007) The daily timing of gene expression and physiology in mammals. *Dialogues Clin. Neurosci.* **9**, 257-272.

Schmidt, R., Parish, E. J., Dionisius, V., Cathelineau, C., Michel, S., Shroot, B., Rolland, A., Brzokewicz, A. and Reichert, U. (1991) Modulation of cellular cholesterol and its effect on cornified envelope formation in cultured human epidermal keratinocytes. *J. Invest. Dermatol.* **97**, 771-775.

Schwarz, W., Birau, N., Hornstein, O. P., Heubeck, B., Schonberger, A., Meyer, C. and Gottschalk, J. (1988) Alterations of melatonin secretion in atopic eczema. *Acta Derm. Venereol.* **68**, 224-229.

Segall, L. A. and Amir, S. (2010) Glucocorticoid regulation of clock gene expression in the mammalian limbic forebrain. *J. Mol. Neurosci.* **42**, 168-175.

Silver, A. C., Arjona, A., Walker, W. E. and Fikrig, E. (2012) The circadian clock controls toll-like receptor 9-mediated innate and adaptive immunity. *Immunity* **36**, 251-261.

So, A. Y., Bernal, T. U., Pillsbury, M. L., Yamamoto, K. R. and Feldman, B. J. (2009) Glucocorticoid regulation of the circadian clock modulates glucose homeostasis. *Proc .Natl. Acad. Sci. U.S.A.* **106**, 17582-17587.

Son, G. H., Chung, S. and Kim, K. (2011) The adrenal peripheral clock: glucocorticoid and the circadian timing system. *Front. Neuroendocrinol.* **32**, 451-465.

Sporl, F., Schellenberg, K., Blatt, T., Wenck, H., Wittern, K., Schrader, A. and Kramer, A. (2011) A circadian clock in HaCaT keratinocytes. *J. Invest. Dermatol.* **131**, 338-348.

Storch, K. F., Paz, C., Signorovitch, J., Raviola, E., Pawlyk, B., Li, T. and Weitz, C. J. (2007) Intrinsic circadian clock of the mammalian retina: importance for retinal processing of visual information. *Cell* **130**, 730-741.

Sulli, A., Maestroni, G. J., Villaggio, B., Hertens, E., Craviotto, C., Pizzorni, C., Briata, M., Seriolo, B. and Cutolo, M. (2002) Melatonin serum levels in rheumatoid arthritis. *Ann. N. Y. Acad. Sci.* **966**, 276-283.

Takahashi, J. S., Hong, H. K., Ko, C. H. and McDearmon, E. L. (2008) The genetics of mammalian circadian order and disorder: implications for physiology and disease. *Nat. Rev. Genet.* **9**, 764-775.

Vaughn, A. R., Clark, A. K., Sivamani, R. K. and Shi, V. Y. (2018) Circadian rhythm in atopic dermatitis—pathophysiology and implications for chronotherapy. *Pediatr. Dermatol.* **35**, 152-157.

Verschoore, M., Poncet, M., Krebs, B. and Ortonne, J. P. (1993) Circadian variations in the number of actively secreting sebaceous follicles and androgen circadian rhythms. *Chronobiol. Int.* **10**, 349-359.

Webster, J. I., Tonelli, L. and Sternberg, E. M. (2002) Neuroendocrine regulation of immunity. *Annu. Rev. Immunol.* **20**, 125-163.

Welsh, D. K., Yoo, S. H., Liu, A. C., Takahashi, J. S. and Kay, S. A. (2004) Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. *Curr. Biol.* **14**, 2289-2295.

Yamazaki, S., Numano, R., Abe, M., Hida, A., Takahashi, R., Ueda, M., Block, G. D., Sakaki, Y., Menaker, M. and Tei, H. (2000) Resetting central and peripheral circadian oscillators in transgenic rats. *Science* **288**, 682-685.

Yosipovitch, G., Xiong, G. L., Haus, E., Sackett-Lundeen, L., Ashkenazi, I. and Maibach, H. I. (1998) Time-dependent variations of the skin barrier function in humans: transepidermal water loss, stratum corneum hydration, skin surface pH, and skin temperature. *J. Invest. Dermatol.* **110**, 20-23.

Yosipovitch, G., Goon, A. T., Wee, J., Chan, Y. H., Zucker, I. and Goh, C. L. (2002) Itch characteristics in Chinese patients with atopic dermatitis using a new questionnaire for the assessment of pruritus. *Int. J. Dermatol.* **41**, 212-216.

Yosipovitch, G., Sackett-Lundeen, L., Goon, A., Huak, C. Y., Goh, C. L. and Haus, E. (2004) Circadian and ultradian (12 h) variations of skin blood flow and barrier function in non-irritated and irritated skin-effect of topical corticosteroids. *J. Invest. Dermatol.* **122**, 824-829.

Young, M. R., Matthews, J. P., Kanabrocki, E. L., Sothern, R. B., Roitman-Johnson, B. and Scheving, L. E. (1995) Circadian rhythmometry of serum interleukin-2, interleukin-10, tumor necrosis factor-alpha, and granulocyte-macrophage colony-stimulating factor in men. *Chronobiol. Int.* **12**, 19-27.

Zheng, B., Larkin, D. W., Albrecht, U., Sun, Z. S., Sage, M., Eichele, G., Lee, C. C. and Bradley, A. (1999) The mPer2 gene encodes a functional componentof the mammalian circadian clock. *Nature* **400**, 169-173.