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REVIEW

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Time is on the Immune System's Side, Yes it is

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From bacteria to mammals, nearly all organisms have adapted their physiology and behavior to a daily rhythm. These circadian (daily) rhythms influence virtually all aspects of physiological architecture (i.e., from gene expression to organismal behavior). Therefore, it is not surprising that several features of the immune response are regulated in a time-of-day dependent manner. The field of chrono-immunology has expanded tremendously over the past decade. In this abridged review, we present studies from the past five years that have revealed new parameters of the immune system that demonstrate daily variations in the control of pathogens and response to microbial components. These studies analyzed how the disruption of circadian rhythms impairs immune function, how microbial components alter the circadian clock, and how immune responses demonstrate daily variations in human subjects. Further elucidating the intricate connections between the circadian clock and the immune system will hopefully provide opportunities for chrono-immunotherapy in disease treatment and prevention.

INTRODUCTION

In order to optimize their survival, organisms have adapted their physiology and behavior (*e.g.,* sleep-wake cycle and feeding behavior) to anticipate daily environmental changes. These physiological processes and activities that oscillate over the course of the daily 24-h period, are referred to as circadian rhythms [1]. While environmental stimuli such as changes in temperature or food intake can entrain the master clock, the primary entrainer is sunlight, which is sensed by ganglion cells in the retina. This information is then sent via the retinohypothalamic tract to the master clock, which is located in the suprachiasmatic nucleus (SCN†) in the hypothalamus [2]. The master clock regulates peripheral clocks throughout the body [3,4]. On a molecular level, the clock is comprised of no less than three interlocking feedback loops, which consist of the core clock genes, *Cryptochrome* (*Cry1-2*), *Period* (*Per1-3*), *Clock*, and *Bmal1*, with genes such as *Rev-erbα* that from ancillary feedback loops regulating the core clockwork [5].

Since circadian rhythms regulate nearly all aspects

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†Abbreviations: SCN, suprachiasmatic nucleus; OCT3, organic cation transporter 3; TLR, toll-like receptor; PAMP, pathogen-associated molecular pattern; LPS, lipopolysaccharide; RSV, respiratory syncytial virus; CLP, Cecal ligation and puncture; PIV3, parainfluenza virus type 3; VSV, vesicular stomatitis virus; CCL, circulating chemokine ligand.

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of physiology and behavior, it is not surprising that research, especially over the past decade, has revealed several aspects of the immune system that fluctuate in a daily fashion. It is plausible that our immune system has evolved to peak when we are most likely to encounter pathogens, nadir when we are at rest in order to repair tissue damage from inflammation, and/or fluctuate in order to conserve energy and not be in a constant state of heightened immune awareness. Cytokine expression, immune cell trafficking, and phagocytosis have all been shown to exhibit daily rhythmicity [6-8]. In addition, functional molecular clocks have been described in numerous immune cells such as natural killer cells, B cells, dendritic cells, and macrophages [6,7,9].

Recently, several reviews have addressed the current state of research involving the circadian-immune connection. In addition to highlighting the various ways in which the circadian clock coordinates the immune system over the course of a day, authors have also focused on how the circadian clock initiates immune responses against pathogens as well as its role in inflammatory and autoimmune diseases [10-16]. Here, we discuss findings within the past five years that focus on the circadian influence over immune parameters and control of pathogens, as well as how conserved microbial components disrupt the circadian clock and how clock disruptions impair the immune response.

CIRCADIAN CONTROLLED IMMUNE PARAMETERS

The circadian clock has been shown to influence numerous immune cells at both the molecular and cellular levels. Maturation of neutrophils, the most common leukocytes found in human blood, is rhythmic and their antimicrobial activity functions in a time-of-day dependent manner [17]. In addition, neutrophils produce significantly more superoxide and phagocytosed considerably more *Staphylococcus aureus ex vivo*, at 1 a.m. compared to 1 p.m. [17]. The circadian clock was also shown to play a role in the mast cell immune response. While mast cells have numerous immune functions, they are most well known for their role in allergic reactions and release of histamine [18]. IgE-mediated mast cell responses were shown to demonstrate time-of-day dependent variation via FcεRI, the IgE receptor [19]. Additionally, the circadian clock within mast cells contributes to the regulation of homeostatic plasma histamine levels via organic cation transporter 3 (*Oct3*), which plays a role in cellular transport of histamine [20]. Furthermore, CLOCK binds the promoter regions of both *Oct3* and *FcεRIβ*, encoding one of three subunits of FcεRI [19,20]. Environmental disturbances, such as stress and abnormal light-dark patterns, result in a loss of rhythm within these cells, which resulted in plasma histamine levels to lose their time-of-day variation [20].

Circadian oscillations of T and B cell trafficking resulted in time-of-day differences in the adaptive immune response weeks after immunization to induce experimental autoimmune encephalomyelitis [21]. However, a separate study concluded that cell-intrinsic clocks do not play a role in adaptive responses of T and B cells [22]. A potential explanation could be that in the latter study, additional time points were warranted over the course of the day in order to detect time-dependent variations.

It was previously shown that the molecular clock directly modulates expression and function of Toll-like receptor (TLR) 9 [23]. TLRs are immune recognition receptors located either on the cell surface or in endosomal compartments. Their recognition and subsequent binding of pathogen-associated molecular patterns (PAMPs), conserved microbial motifs (*e.g.,* bacterial surface lipoproteins and viral RNA), triggers both the innate and adaptive immune response [24]. A recent study revealed rhythmic expression of several *Tlrs* in splenic macrophages and within a subpopulation of splenocytes, which predominately consisted of B cells, DCs, and macrophages [25]. Additionally, they revealed time-of-day dependent variations in responsiveness to several PAMPs, such as Pam3CSK4, heat-killed *Listeria monocytogenes*, poly(I:C), lipopolysaccharide (LPS), FLA, FSL-1, ssRNA, and Imiquimod, in *ex vivo* challenges of splenocytes [25].

CIRCADIAN CONTROL OF PATHOGENS

Viral Pathogens

The circadian clock has been implicated in controlling various aspects of microbial infection as studies have observed the importance of a functional molecular clock on viral infection. Clock disruption has been induced at the molecular and organismal levels, via clock gene deletion mutants and disruption of the light-dark cycle, respectively. For example, chronic diurnal disruption in mice, mimicking that of shift-work (Table 1), followed with an acute inflammatory challenge via LPS, led to an increase in latent viral reactivity and in viral load in a murine Epstein-Barr virus model [26]. *In vitro*, *Bmal1* deficient immortalized lung fibroblasts were shown to be more susceptible to infection by respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV3), both members of the Paramyxoviridae family of viruses [27]. Moreover, these results were recapitulated *in vivo* for PIV3 using *Bmal1* deficient mice [27]. Using the vesicular stomatitis virus (VSV) as a model for encephalitis, mice were shown to have a 95 percent mortality rate when infected with an LD_{50} at the start of their rest

Table 1. Models of murine shiftwork.

Day of the week	Hours of dark (D): Hours of light (L)	Sleep disruption	Reference
Model 1			
Day 1 (work week)	20D:4L	Not pertinent in this model	[26]
Day 2 - 5 (work week)	8L:12D:4L		
Day 6 - 7 (weekend)	12D:12L		
Model 2			
Day 1 - 5 (work week)	12L:12D	8 h of sleep disruption during light phase	[38]
Day 6 - 7 (weekend)	12L:12D	Undisturbed	

phase, compared to 40 percent mortality when infected at the start of their active phase [28]. This time-dependent difference in survival rate was attributed to an overactive immune response, which was linked to the REV-ERBα-mediated inhibition of circulating chemokine ligand 2 (CCL2) [28]. This was consistent with the finding that in macrophages, REV-ERBα was shown to bind to the *Ccl2* promoter and inhibit its expression, which led to diminished macrophage adhesion and migration via ERK and p38-signaling pathways [29]. Mice infected with Murid Herpesvirus at the start of the rest phase presented with higher viral loads compared to mice infected 2 hours prior to the start of their active phase [30]. This time-of-day variation in infection was abolished in *Bmal1* deficient mice, while viral replication and dissemination were enhanced [30].

Eukaryotic Pathogens

Circadian control of immune responses also play a role in controlling protozoan and multicellular pathogens. *Leishmania major* was used to demonstrate that parasite load varied based on the circadian time of infection [31]. The time-of-day variation in infection was linked to daily changes in chemokine levels and immune cell (*i.e.*, neutrophil and macrophage) recruitment after the subsequent parasite challenge [31]. The daily changes in infection efficiency were most likely attributed to the higher number of immune cells present at the infection site for the intracellular parasite to invade [31]. Additionally, mice infected towards the beginning of the day (*i.e.,* rest phase) with the helminth, *Trichuris muris,* were observed to have lower parasitic worm burdens compared to mice infected at the start of the active phase [32]. This time-of-day variation in worm expulsion efficiency was eliminated with the deletion of *Bmal1* [32]. Furthermore, it was revealed that a functional clock in dendritic cells has an essential role in determining the efficiency at which the parasite is cleared [32].

Sepsis

Sepsis, an infection that leads to an exacerbated immune response, can lead to organ dysfunction and potentially death. Cecal ligation and puncture (CLP) is a well-established model for studying sepsis. Recently, it was demonstrated that mice experienced a worse sepsis phenotype when CLP was induced during their active phase when compared to their rest period [33]. This time-dependent difference was abolished in *Per2*-mutant mice [33]. Furthermore, serum entrained mouse macrophages challenged with the TLR2 ligand, demonstrated rhythmic fluctuations in IL-6 production and *Tlr2*-mutant mice no longer displayed a time-dependent sepsis phenotype, suggesting TLR2 plays a role in daily variations in sensitivity to sepsis [33]. A subsequent study revealed that *Bmal1* deficient mice display a heightened susceptibility to CLP-induced sepsis, where BMAL1 has an inhibitory role in an immune pathway in macrophages that ultimately results in septic death [33]. In an additional study, in the presence of LPS, BMAL1 levels were inhibited by the up-regulation of the proinflammatory microRNA, miR-155 [34]. Revealing that the circadian control of the inflammatory response to LPS is regulated by BMAL1 via miR-155 [34]. Another link between BMAL1 and the inflammatory response was observed in macrophages through BMAL1 regulation of *Nrf2*, which is involved in the repression of reactive oxygen species and proinflammatory cytokines [35].

DISRUPTED CIRCADIAN RHYTHMS LEAD TO IMPAIRED IMMUNE FUNCTION

It is clear that the circadian clock controls numerous aspects of the immune system, therefore, it is not surprising that disruption of circadian rhythms results in an altered immune response. This was recently demonstrated when dysregulation of circadian rhythms in the form of circadian deletion mutants (*Per1-3* and *Bmal1*) led to altered macrophage behavior (*e.g.*, altered balance between

nitric oxide and reactive oxygen species, and increased adhesion and migration), which in turn led to a heightened inflammatory state [36]. A separate study showed that deletion of *Per1* in Kupffer cells, resident liver macrophages, results in increased macrophage recruitment, elevated pro-inflammatory cytokine release, and subsequently, a greater incidence of lethality post-LPS challenge [37]. This was due to the interaction between PER1 and peroxisome proliferator-activated receptor gamma, which together, inhibited expression of CC chemokine receptor 2 [37]. A murine model of shift work (Table 1) revealed that circadian disruption affected the inflammatory response to LPS *in vivo* and *in vitro*, which produced elevated cytokine levels [38]. Interestingly, when food, a non-photic entrainer of the clock, was restricted to the end of working protocol, (*i.e.*, closer to their normal active phase) it alleviated the elevated inflammatory response after LPS challenge [38]. Furthermore, day-fed mice had an impaired immune response post-LPS challenge in the form of reduced cytokine levels and decreased bactericidal capacity when compared to mice fed at night or *ad libitum* [39].

IMPACT OF MICROBIAL COMPONENTS ON CIRCADIAN RHYTHMS

Since Halberg *et al*. first demonstrated a susceptibility rhythm to endotoxin in mice in 1960 [40], several studies have revealed the various levels (*e.g.,* gene expression and cellular) of circadian control that are disrupted by microbial components (*i.e.,* PAMPs). The influence of LPS has been the most extensively studied. For example, when observing the effects of endotoxin treatment within the murine lung, not only did treatment disrupt rhythmic gene expression but it also induced rhythmic expression in non-rhythmic genes as well as altered the timing of leukocyte trafficking [41]. LPS disrupts circadian gene expression in peritoneal macrophages, liver macrophages, splenocytes, ovaries, and SCN [36,42-44]. Bacterial endotoxin was also shown to affect circadian rhythms at the organismal level, as rats recovered locomotion activity faster when challenged during the day compared to the night [43]. While endotoxin use has been the gold standard in assessing time-dependent inflammatory responses, other microbial components activate similar immune pathways. One study explored the effect of PAMPs other than LPS on the molecular clock. These included heat-killed *Listeria monocytogenes*, Pam3CSK4, flagellin, poly(I:C), ssRNA40, and ODN1826. All of these PAMPs were shown to alter expression of at least one circadian clock gene in mouse splenocytes via *ex vivo* challenge [45]. Additionally, poly(I:C) was shown to inhibit expression of all four clock genes examined (*Per2*, *Bmal1*, *Rev-erbα*, and *Dbp*) in an *in vivo* challenge [45].

IMMUNE RESPONSES DEMONSTRATE DAILY VARIATIONS IN HUMAN SUBJECTS

A limitation of current literature is its heavy reliance on mouse-based research, which has an uneven record of accurately modeling human immunology. However, a handful of human-based studies suggest that the circadian influence on immunology that is seen in mice appears generally applicable to humans. Recently, circadian rhythmicity of cytokine and chemokine levels were observed after an *ex vivo* LPS stimulation from whole blood isolated from human subjects who were under constant environmental and behavioral conditions [46]. In a subsequent study, a heightened inflammatory response was observed when healthy volunteers were challenged with LPS at night, compared to the day [47]. Further linking circadian disruption to immune impairment in human volunteers, it was shown that detection of early disruption of the daily rhythm of cortisol in trauma patients was correlated with an increased incidence of sepsis during their ICU stay [48].

CONCLUSIONS AND OUTLOOK

Over the past fifteen years, it has become increasingly clear that numerous immune parameters and responses exhibit time-of-day dependent variation. Identifying the role of the molecular clock in various immune cells as well as the time of peak responsiveness, could have great ramifications in disease treatment and prevention. Scientists have begun to exploit the daily rhythms of the immune system in order to achieve maximum vaccine efficacy. For example, it was previously shown that TLR9 expression and responsiveness fluctuates over the daily light-dark cycle and that mice immunized using the TLR9 ligand as adjuvant, demonstrated a significantly more robust adaptive immune response weeks later compared to mice immunized twelve hours earlier [23]. Additionally, influenza vaccine demonstrated time-of-day dependent efficacy when administered in an elderly population [49]. Conversely, many inflammatory diseases demonstrate daily fluctuations in the severity of symptoms, which correlate with circadian rhythms of immune parameters such as leukocyte recruitment and cytokine production. For example, rheumatoid arthritis patients experience the most severe joint pain and stiffness in the morning, which follows the peak in proinflammatory serum cytokine levels (*e.g.,* IL-6 and TNF) [50,51]. As a result, the chronotherapeutic administration of the anti-inflammatory steroid prednisone was shown to improve the morning symptoms associated with rheumatoid arthritis [52]. As it currently stands, a majority of the top 100 best-selling drugs in the United States target circadian genes and that over a third of the murine genome is influenced by the

circadian clock [53], thereby demonstrating the importance of time administered prophylaxis. Therefore, as more links between the circadian and immune systems are elucidated, the opportunities for chrono-immunotherapy will expand.

Despite the advances in recent years, there is still a great deal to be uncovered in the field of chrono-immunology. While researchers have revealed circadian oscillations of immune parameters, in many regards, the molecular mechanism linking the clock and immune system is yet to be elucidated. Molecular clock proteins such as CLOCK, BMAL1, and REV-ERBα have all been shown to directly modulate transcription of various immune components [23,54-56] while the circadian transcription of other immune genes could be controlled indirectly, for example by clock controlled transcription factors or regulatory RNAs.

The circadian clock controls several aspects of the immune system and conversely, the immune response feeds back and influences the clock. Infection and inflammation disrupt the circadian architecture at both the gene expression and organismal levels [36,42,43,45]. While environmental disturbances (*e.g.*, shift work or jet lag) that lead to circadian dysregulation have been shown to negatively impact immune regulation by leading to exacerbated immune responses [38,39]. Understanding the molecular mechanisms linking these disturbances to circadian dysregulation could provide novel targets for immunotherapy.

Although exciting breakthroughs in the field of chrono-immunology are constantly being made, a major hurdle, perhaps more so than in other fields, will be the application of these findings in clinical research. While investigations need to be made into determining if the immune clockworks in mice are consistent with those in humans, there are several layers of complexity that also need to be taken into account. For example, since rodents are nocturnal animals, their active and rest phases are opposite of ours, however, many of the immune factors measured to date, show consistency between mouse and human active phases [12]. One must also take into account that several different inbred rodent models have been used when assessing the circadian-immune connection, therefore, there could be species specificity between different animal lines. Another confounding factor is in regard to experimental setup, and whether diurnal rhythms, which are subjected to environmental stimuli (*e.g.,* light or food), or true circadian rhythms, which are daily rhythms that occur in the absence of an external cue are being assessed. Finally, since circadian rhythms can differ between individuals due to intrinsic (*e.g.*, age, sex, or underlying medical condition) and extrinsic stimuli (*e.g.*, eating behavior, exercise, and night-time electronic usage), ideally, in the future, when it comes to the clinical application of chrono-immunotherapy, a personalized approach will be taken.

While daily variations in the immune response have been observed for over half a century, recent findings, such as the literature described in this review, have not only begun to uncover the molecular mechanisms that link the clock to the immune system, but have also made significant progress in discovering the extent to which circadian rhythms regulate various facets of the immune response. For example, the clock has a vital role in orchestrating the immune response to diverse pathogens (*e.g.*, bacteria, viruses, and protozoa) while also contributing to daily variations in several inflammatory disorders. Over the years there have been numerous reports chronicling circadian regulation of the innate arm of the immune system, however, recent studies suggest adaptive immunity is also controlled by the clock. Taken together, all of the aforementioned discoveries have led to the application of this knowledge in clinical research, which has enhanced the development of chrono-immunotherapy to treat inflammatory diseases and improve vaccine efficacy.

REFERENCES

1. Bell-Pedersen D, Cassone VM, Earnest DJ, Golden SS, Hardin PE, Thomas TL, et al. Circadian rhythms from multiple oscillators: lessons from diverse organisms. Nat Rev Genet. 2005;6(7):544–56.
2. Mohawk JA, Green CB, Takahashi JS. Central and Peripheral Circadian Clocks in Mammals. Annu Rev Neurosci. 2012;35(1):445–62.
3. Yamazaki S. Resetting Central and Peripheral Circadian Oscillators in Transgenic Rats. Science. 2000;288(5466):682–5.
4. Yoo SH, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED, et al. PERIOD2:LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. Proc Natl Acad Sci USA. 2004;101(15):5339–46.
5. Lowrey PL, Takahashi JS. Genetics of circadian rhythms in mammalian model organisms. Adv Genet. 2011;74:175–230.
6. Arjona A, Sarkar DK. Circadian Oscillations of Clock Genes, Cytolytic Factors, and Cytokines in Rat NK Cells. J Immunol. 2005;174(12):7618–24.
7. Keller M, Mazuch J, Abraham U, Eom GD, Herzog ED, Volk HD, et al. A circadian clock in macrophages controls inflammatory immune responses. Proc Natl Acad Sci USA. 2009;106(50):21407–12.
8. Hriscu ML. Modulatory factors of circadian phagocytic activity. Ann N Y Acad Sci. 2005;1057:403–30.
9. Silver AC, Arjona A, Hughes ME, Nitabach MN, Fikrig E. Circadian expression of clock genes in mouse macrophages, dendritic cells, and B cells. Brain Behav Immun. 2012;26(3):407–13.
10. Tognini P, Thaiss CA, Elinav E, Sassone-Corsi P. Circadian Coordination of Antimicrobial Responses. Cell Host Microbe. 2017;22(2):185–92.

11. Curtis AM, Bellet MM, Sassone-Corsi P, O'Neill LA. Circadian Clock Proteins and Immunity. Immunity. 2014;40(2):178–86.
12. Geiger SS, Fagundes CT, Siegel RM. Chrono-immunology: progress and challenges in understanding links between the circadian and immune systems. Immunology. 2015;146(3):349–58.
13. Nobis CC, Labrecque N, Cermakian N. From immune homeostasis to inflammation, a question of rhythms. Curr Opin Physiol. 2018;5:90–8.
14. Scheiermann C, Gibbs J, Ince L, Loudon A. Clocking in to immunity. Nat Rev Immunol. 2018;18(7):423–37.
15. Zhuang X, Rambhatla SB, Lai AG, McKeating JA. Interplay between circadian clock and viral infection. J Mol Med (Berl). 2017;95(12):1283–9.
16. Man K, Loudon A, Chawla A. Immunity around the clock. Science. 2016;354(6315):999–1003.
17. Ella K, Csépányi-Kömi R, Káldi K. Circadian regulation of human peripheral neutrophils. Brain Behav Immun. 2016;57:209–21.
18. da Silva EZ, Jamur MC, Oliver C. Mast Cell Function: A New Vision of an Old Cell. Vol. 62. J Histochem Cytochem. 2014:698–738.
19. Nakamura Y, Nakano N, Ishimaru K, Hara M, Ikegami T, Tahara Y, et al. Circadian regulation of allergic reactions by the mast cell clock in mice. J Allergy Clin Immunol. 2014;133(2):568–75.
20. Nakamura Y, Ishimaru K, Shibata S, Nakao A. Regulation of plasma histamine levels by the mast cell clock and its modulation by stress. Sci Rep. 2017;7(January):1–12.
21. Druzd D, Matveeva O, Ince L, Harrison U, He W, Schmal C, et al. Lymphocyte Circadian Clocks Control Lymph Node Trafficking and Adaptive Immune Responses. Immunity. 2017;46(1):120–32.
22. Hemmers S, Rudensky AY. The Cell-Intrinsic Circadian Clock Is Dispensable for Lymphocyte Differentiation and Function. Cell Rep. 2015;11(9):1339–49.
23. Silver AC, Arjona A, Walker WE, Fikrig E. The Circadian Clock Controls Toll-like Receptor 9-Mediated Innate and Adaptive Immunity. Immunity. 2012;36:251–61.
24. Kawai T, Akira S. review The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Publ Gr. 2010;11(5):373–84.
25. Silver AC, Buckley SM, Hughes ME, Hastings AK, Nitabach MN, Fikrig E. Daily oscillations in expression and responsiveness of Toll-like receptors in splenic immune cells. Heliyon. 2018:e00579.
26. Trammell RA, Toth LA. Effects of chronic diurnal disruption and acute inflammatory challenge on mice with latent murine gammaherpesvirus infection. Comp Med. 2016;66(6):445–54.
27. Majumdar T, Dhar J, Patel S, Kondratov R, Barik S. Circadian transcription factor BMAL1 regulates innate immunity against select RNA viruses. Innate Immun. 2017;23(2):147–54.
28. Gagnidze K, Hajdarovic KH, Moskalenko M, Karatsoreos IN, McEwen BS, Bulloch K. Nuclear receptor REV-ERBα mediates circadian sensitivity to mortality in murine vesicular stomatitis virus-induced encephalitis. Proc Natl Acad Sci USA. 2016;113(20):5730–5.
29. Sato S, Sakurai T, Ogasawara J, Takahashi M, Izawa T, Imaizumi K, et al. A Circadian Clock Gene, Rev-erb, Modulates the Inflammatory Function of Macrophages through the Negative Regulation of Ccl2 Expression. J Immunol. 2014;192(1):407–17.
30. Edgar RS, Stangherlin A, Nagy AD, Nicoll MP, Efstathiou S, O'Neill JS, et al. Cell autonomous regulation of herpes and influenza virus infection by the circadian clock. Proc Natl Acad Sci USA. 2016;113(36):10085–90.
31. Kiessling S, Dubeau-Laramee G, Ohm H, Labrecque N, Olivier M, Cermakian N. The circadian clock in immune cells controls the magnitude of Leishmania parasite infection. Sci Rep. 2017;7(1):1–11.
32. Hopwood TW, Hall S, Begley N, Forman R, Brown S, Vonslow R, et al. The circadian regulator BMAL1 programmes responses to parasitic worm infection via a dendritic cell clock. Sci Rep. 2018;8(1):1–13.
33. Heipertz EL, Harper J, Lopez CA, Fikrig E, Hughes ME, Walker WE. Circadian Rhythms Influence the Severity of Sepsis in Mice via a TLR2-Dependent, Leukocyte-Intrinsic Mechanism. J Immunol. 2018;201(1):193–201.
34. Curtis AM, Fagundes CT, Yang G, Palsson-McDermott EM, Wochal P, McGettrick AF, et al. Circadian control of innate immunity in macrophages by miR-155 targeting Bmal1. Proc Natl Acad Sci USA. 2015;112(23):7231–6.
35. Early JO, Menon D, Wyse CA, Cervantes-Silva MP, Zaslona Z, Carroll RG, et al. Circadian clock protein BMAL1 regulates IL-1β in macrophages via NRF2. Proc Natl Acad Sci USA. 2018;115(36):201800431.
36. Wang Y, Pati P, Xu Y, Chen F, Stepp DW, Huo Y, et al. Endotoxin disrupts circadian rhythms in macrophages via reactive oxygen species. PLoS One. 2016;11(5).
37. Wang T, Wang Z, Yang P, Xia L, Zhou M, Wang S, et al. PER1 prevents excessive innate immune response during endotoxin-induced liver injury through regulation of macrophage recruitment in mice. Cell Death Dis. 2016;7(4):e2176–11.
38. Guerrero-Vargas NN, Guzmán-Ruiz M, Fuentes R, García J, Salgado-Delgado R, Basualdo MD, et al. Shift work in rats results in increased inflammatory response after lipopolysaccharide administration. J Biol Rhythms. 2015;30(4):318–30.
39. Cissé YM, Borniger JC, Lemanski E, Walker WH, Nelson RJ. Time-Restricted Feeding Alters the Innate Immune Response to Bacterial Endotoxin. J Immunol. 2018;200(2):681–7.
40. Halberg F, Johnson EA, Brown BW, Bittner JJ. Susceptibility rhythm. Proc Soc Exp Biol Med. 1960;(103):142–4.
41. Haspel JA, Chettimada S, Shaik RS, Chu JH, Raby BA, Cernadas M, et al. Circadian rhythm reprogramming during lung inflammation. Nat Commun. 2014;5(May):4753.
42. Shimizu T, Watanabe K, Anayama N, Miyazaki K. Effect of lipopolysaccharide on circadian clock genes Per2 and Bmal1 in mouse ovary. J Physiol Sci. 2017;67(5):623–8.
43. Moravcová S, Pačesová D, Melkes B, Kyclerová H, Spišská V, Novotný J, et al. The day/night difference in the circadian clock's response to acute lipopolysaccharide and the rhythmic Stat3 expression in the rat suprachiasmatic nucleus. PLoS One. 2018;13(9).

44. Silver AC. The use of mouse splenocytes to assess pathogen-associated molecular pattern influence on clock gene expression. J Vis Exp. 2018;(137):1–6.
45. Silver AC. Pathogen-associated molecular patterns alter molecular clock gene expression in mouse splenocytes. PLoS One. 2017:12–5.
46. Rahman SA, Castanon-Cervantes O, Scheer FA, Shea SA, Czeisler CA, Davidson AJ, et al. Endogenous circadian regulation of pro-inflammatory cytokines and chemokines in the presence of bacterial lipopolysaccharide in humans. Brain Behav Immun. 2015;47:4–13.
47. Alamili M, Bendtzen K, Lykkesfeldt J, Rosenberg J, Gögenur I. Pronounced inflammatory response to endotoxaemia during nighttime: A randomised cross-over trial. PLoS One. 2014;9(1).
48. Coiffard B, Diallo AB, Culver A, Mezouar S, Hammad E, Vigne C, et al. Circadian rhythm disruption and sepsis in severe trauma patients. Shock. 2018.
49. Long JE, Drayson MT, Taylor AE, Toellner KM, Lord JM, Phillips AC. Morning vaccination enhances antibody response over afternoon vaccination: A cluster-randomised trial. Vaccine. 2016;34(24):2679–85.
50. Kirwan JR, Clarke L, Hunt LP, Perry MG, Straub RH, Jessop DS. Effect of novel therapeutic glucocorticoids on circadian rhythms of hormones and cytokines in rheumatoid arthritis: Annals of the New York Academy of Sciences. Ann N Y Acad Sci. 2010;1193:127–33.
51. Cutolo M. Circadian rhythms in RA. Ann Rheum Dis. 2003;62(7):593–6.
52. Buttgereit F, Mehta D, Kirwan J, Szechinski J, Boers M, Alten RE, et al. Low-dose prednisone chronotherapy for rheumatoid arthritis: A randomised clinical trial (CAPRA-2). Ann Rheum Dis. 2013;72(2):204–10.
53. Zhang R, Lahens NF, Ballance HI, Hughes ME, Hogenesch JB. A circadian gene expression atlas in mammals: implications for biology and medicine. Proc Natl Acad Sci USA. 2014;111(45):16219–24.
54. Gibbs JE, Blaikley J, Beesley S, Matthews L, Simpson KD, Boyce SH, et al. The nuclear receptor REV-ERB α mediates circadian regulation of innate immunity through selective regulation of in fl ammatory cytokines. Proc Natl Acad Sci USA. 2012;109(2):582–7.
55. Spengler ML, Kuropatwinski KK, Comas M, et al. Core circadian protein CLOCK is a positive regulator of NF-κB-mediated transcription. Proc Natl Acad Sci U S A. 2012;109(37):E2457–E2465.
56. Nguyen KD, Fentress SJ, Qiu Y, Yun K, Cox JS, Chawla A. Circadian gene Bmal1 regulates diurnal oscillations of Ly6C(hi) inflammatory monocytes. Science. 2013;341(6153):1483–8.