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Review

Circadian Coordination of Antimicrobial Responses

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Microbial infection poses a threat to organismal homeostasis and therefore must be efficiently counteracted by host defense mechanisms. It has been recently demonstrated that the immune system may anticipate an emerging pathogenic exposure through a heightened inflammatory state. Such anticipatory responses to fluctuating environmental conditions are typically orchestrated by the circadian clock, an intrinsic time-keeping system that adapts tissue physiology to diurnal variations in external influences. Here, we review current knowledge about the interplay between the circadian clock and antimicrobial responses. We summarize the molecular strategies employed by the circadian system against specific pathogens, the core-clock proteins as well as cells in which they are expressed that mediate host defense, and the consequences of circadian variations on immune function. Furthermore, we highlight the possible implications of such circadian gating in immune reactions against pathogenic infections for the chronopharmacology of antibacterial and antiviral therapies.

Introduction

The rotation of our planet around its axis is responsible for the alternation of day and night. Every living being on Earth experiences this 24 hr cycle and synchronizes to this daily rhythm thanks to an endogenous timekeeper called the circadian clock (from Latin "circa" = around and "diem" = day) (Peek et al., 2015). The endogenous clock enables organisms to anticipate daily fluctuations in the surrounding environment, ensuring an appropriate physiological adaptation. Indeed, many fundamental biological processes function under a circadian control (Abbott et al., 2015; McLoughlin et al., 2015; Tsang et al., 2016). For instance, in humans, sleep/wake cycle, blood pressure, hormonal levels, and metabolic reactions are governed by highly specialized clocks (Gerber et al., 2015; Schibler and Sassone-Corsi, 2002).

The so-called master clock is located in the suprachiasmatic nucleus (SCN) in the hypothalamus and is mainly entrained by light signals, transduced by specific photoreceptors present in the retina (Doyle and Menaker, 2007). Interestingly, studies in the last 20 years have revealed that circadian clocks are present in virtually every tissue of the body and that their proper function is necessary to regulate and preserve the physiology of the specific tissue (Mohawk et al., 2012). Furthermore, hormonal and electrical signals from the SCN clock orchestrate the cycling in peripheral clocks, although the underlying mechanisms are poorly understood.

Regulation of Anticipatory Responses by the Circadian Clock

From a molecular point of view, circadian rhythmicity in gene expression is controlled by a complex transcriptional-translational feedback loop (Figure 1). The heart of the molecular clock comprises the transcriptional activators CLOCK (circadian locomotor output cycles kaput) and BMAL1 (brain and muscle ARNT-like 1), and the repressor proteins Cryptochrome (CRY1 and CRY2) and Period (PER1, PER2, and PER3), which control the daily transcription of clock-controlled genes (CCGs) (Masri et al., 2015; Takahashi, 2015). Moreover, the nuclear receptors RORs (related orphan receptors) and REV-ERB-alpha/beta represent additional layers of circadian regulation through the control of *Bmal1* rhythmicity (Guillaumond et al., 2005). Finally, posttranslational modifications, such as phosphorylation and acetylation of histone and non-histone proteins, are involved in fine-tuning the function and accuracy of the molecular clock system (Aguilar-Arnal and Sassone-Corsi, 2013).

As such, the circadian clock anticipates changes in the external world to optimize physiology. An environmental challenge of major relevance during the evolution of mammals is their potentially life-threatening encounter with pathogens. During the phase of activity and feeding, the risk of infection with foodborne pathogens is higher, thus necessitating anticipatory mechanisms that synchronize the magnitude of immune responses to time of maximal pathogen exposure. Indeed, immune functions have been demonstrated to be under circadian influence (Roberts, 2000). Several studies both in mouse models and humans have shown diurnal variation in many immune-related processes, such as cytokine and chemokine expression (Hayashi et al., 2007; Rahman et al., 2015), response to antigen presentation (Fortier et al., 2011), lymphocyte proliferation and trafficking (Druzd et al., 2017; Fortier et al., 2011; Hiemke et al., 1995), and leukocyte tissue recruitment (Scheiermann et al., 2012). Remarkably, the manifestation of many human diseases displays diurnal features. For instance, ischemic and hemorrhagic stroke (Elliott, 1998; Gupta and Shetty, 2005), myocardial infarction (Cohen et al., 1997; Muller et al., 1997), and vaso-occlusive crisis (Auvil-Novak et al., 1996) are more frequent at dawn. In contrast, chronic obstructive pulmonary disease and asthma exhibit more severe exacerbation during night and early morning (Sundar et al., 2015), while patients with rheumatoid arthritis experience the most painful joint stiffness in the morning, which is often ascribed to joint fluid viscosity, but is also associated with a peak in pro-inflammatory cytokine blood level at the same time of the day (Gibbs and Ray, 2013).

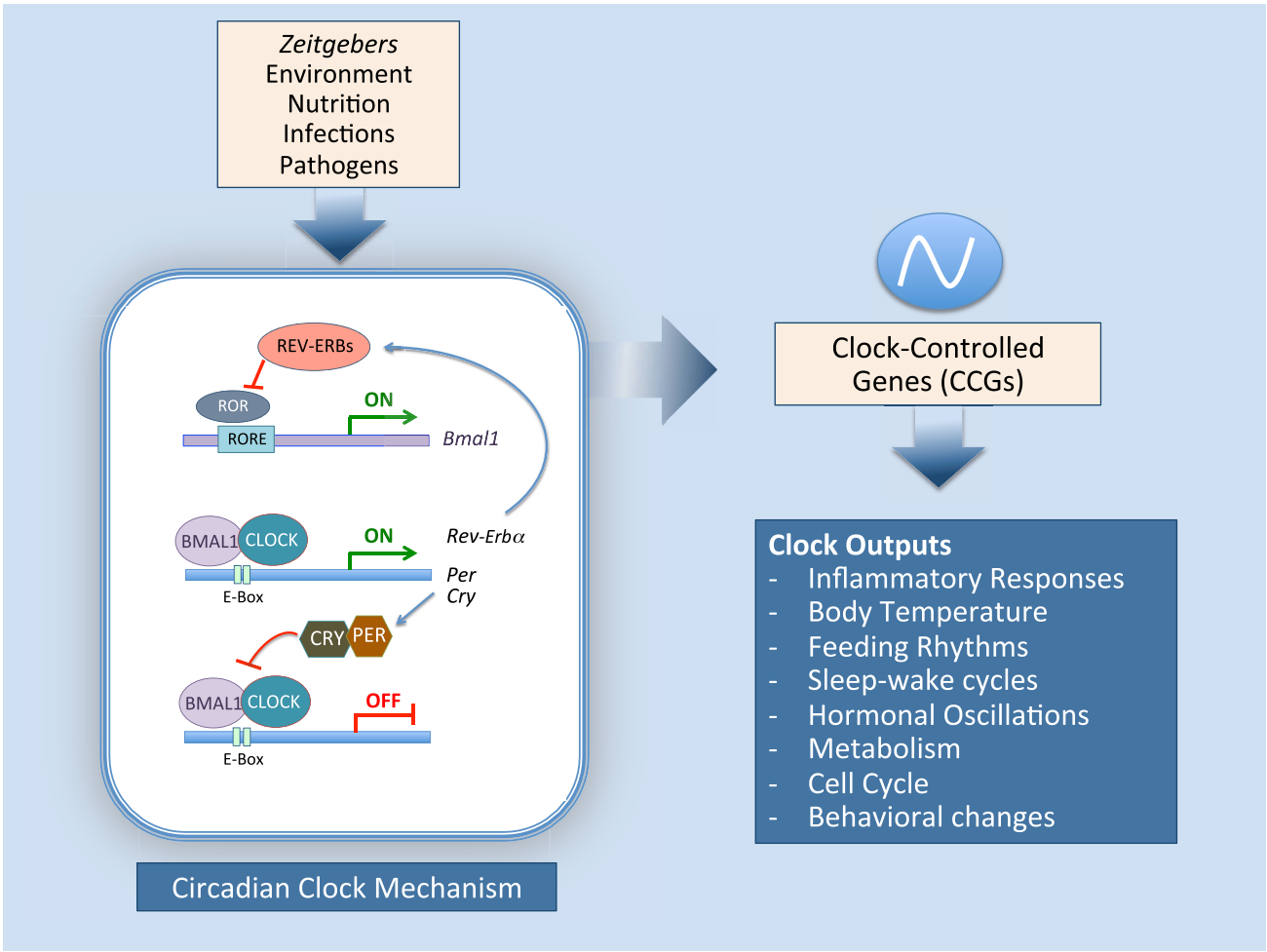

Figure 1. The Molecular Clock Modulates a Variety of Physiological Processes Including Immune Responses
The core-clock transcriptional-translational feedback loop composed of the transcriptional activators CLOCK and BMAL1 as well as the repressor proteins Cryptochrome (CRY) and Period (PER) drives the daily rhythmic gene expression of clock-controlled genes (CCGs). Environmental cues such as the alternation of light and darkness, type of nutrients and time of food intake, or exposure to pathogens may impact the clock molecular machinery and thereby influence its function. The circadian clock governs diurnal oscillation in gene expression and impinges on whole organismal homeostasis and fundamental physiological processes, including immune system functions and inflammatory responses.

Immune cells and tissues contain a functional molecular clock machinery (Arjona and Sarkar, 2005; Curtis et al., 2014; Keller et al., 2009), and this cell-autonomous clock, together with circadian neuronal and endocrine signals coming from the SCN through the modulation of autonomic neurons (Logan and Sarkar, 2012), plays a critical role in tuning the host immune response. Recently, several studies have highlighted the importance of a functional clock system to cope with a variety of infectious agents. Here, we will examine the mechanisms underlying the interaction between the circadian clock and specific pathogenic bacteria or viruses. Additionally, we will discuss how diurnal variations in the host immune response are generated, and how the clock and the infectious state reciprocally influence each other.

The Role of the Circadian Clock in Host Defense against Infection

A tight circadian regulation of the host immune system is emerging as a crucial strategy to fight different infectious agents, and distinct immune cells and clock proteins have been implicated in the response to specific pathogens. Moreover, various aspects of the immune response, such as trafficking of leukocytes, the activation of innate and adaptive immunity, and host-pathogen interactions, have been demonstrated to oscillate in a diurnal manner in both mice and humans. Thus, the effect of pathogen encounter and the subsequent host response in a time-of-day-dependent fashion is of particular interest. Its study will lead to further understanding of how the circadian clock regulates immune activation in order to optimize a cost-efficient and timely anti-pathogen response and thereby maximize survival. Below we review recent findings on the role of the circadian clock in mediating host defense reactions against specific bacterial and viral pathogens, subdivided based on their route of infection (Table 1).

Foodborne Pathogens

The onset of activity and feeding increases the probability of being exposed to an infectious agent, in particular by contact with

Table 1. Overview of Specific Immune Cells and Clock Components Involved in Circadian Host-Pathogen Interactions

Pathogen	Immune Cell/Tissue Analyzed	Genetic/Pharmacological Manipulation	Outcome of the Manipulation
Listeria monocytogenes (Nguyen et al., 2013)	Ly6C^{hi} monocytes in peritoneum	myeloid-specific Bmal1 KO	loss of rhythmic tissue trafficking in Ly6C^{hi} monocytes
Salmonella Typhimurium (Bellet et al., 2013)	caecal neutrophil influx	clock mutant mice	loss of time-of-the-day difference in bacterial colonization
Streptococcus pneumonia (Gibbs et al., 2014)	pulmonary neutrophils	*Bmal1* bronchiolar epithelial cell-specific KO	abnormal CXCL5 production and enhancement in neutrophil recruitment
Vesicular stomatitis virus (Gagnidze et al., 2016)	inflammatory monocytes	specific REV-ERB-alpha inhibitor	decreased survival rate and upregulation of *Ccl2* mRNA
Herpes and influenza viruses (Edgar et al., 2016)	nose and superficial cervical lymph nodes	*Bmal1* KO mouse model	enhancement of acute infection independently of the time of the day of the viral contact
Sendai virus (Ehlers et al., 2017)	macrophage and neutrophils in the airways	total and tamoxifen-inducible *Bmal1* KO mouse models	increased magnitude of granulocyte-predominant lung inflammation

microorganisms present in food. As shown through studies in rodents highlighted below, the endogenous clock anticipates this threat, poising the immune system for a possible foodborne pathogenic assault.

Listeria monocytogenes. Ly6C^{hi} monocytes are an important defense line against *L. monocytogenes* infection, and their recruitment to sites of infection is mediated by the chemokine CCL2. Mice infected at zeitgeber time 0 (ZT0) (“light on” and beginning of the resting phase in rodents) featured a higher number of bacteria in their spleen, liver, and peritoneum than animals treated at ZT8 (4 hr prior to the beginning of their active phase at ZT12, “light off”). Furthermore, Ly6C^{hi} monocyte recruitment to the peritoneum displayed diurnal rhythmicity, with monocyte numbers that were higher at ZT8 than at ZT0. Remarkably, impairment of the clock system in myeloid-specific *Bmal1* knockout (KO) mice completely disrupted the circadian tissue trafficking of Ly6C^{hi} monocytes. *Ccl2* gene oscillation in bone marrow-derived macrophages was BMAL1 mediated through the recruitment of the Polycomb repressive complex 2 (PRC2) to the promoter of this cytokine gene, suggesting that a synergy between the clock and histone methylation changes is responsible for sustaining circadian variation in chemokine levels and consequently in Ly6C^{hi} monocytes (Nguyen et al., 2013). Further studies aimed at unveiling the cascade of molecular events responsible for these effects are necessary to identify possible targets for future therapeutic applications.

Salmonella Typhimurium. In a mouse model of infectious colitis, animals exhibited distinct responses to acute *Salmonella* Typhimurium infection in a time-of-day-dependent manner. Bacterial colonization in the colon was higher in mice inoculated at ZT4 (daytime, resting phase) than in mice inoculated at ZT16 (nighttime, active phase), as was the level of inflammation in the cecum. Importantly, in *Clock* mutant mice, there was no difference in colonization between daytime and nighttime infection and there was a significant decrease in overall proinflammatory gene expression, indicating that a functional clock is necessary to coordinate the host response to *Salmonella* (Bellet et al., 2013). Future investigations will reveal the cell-type-specific clocks involved in the timing regulation of the immune response to *Salmonella* and the potential crosstalk of the intestinal clock with other peripheral clocks during the infection. These findings are likely to provide important notions toward novel approaches to treat infectious colitis.

Airborne Pathogens

The active phase of an organism does not only include feeding time but also other activities related to shelter seeking, mating, and protection and nurturing of offspring; defense against natural enemies; and many others. During this time period, mammals are in contact with several environmental microorganisms, including intranasal pathogens. Interestingly, it has recently emerged that the clock coordinates the immune response to intranasal attacks in a circadian manner, as demonstrated by a variety of animal studies.

Streptococcus pneumoniae. The circadian clock in bronchiolar epithelial cells has been demonstrated to control the magnitude of pulmonary inflammation and its diurnal fluctuation (Gibbs et al., 2014). The mechanism responsible for the daily inflammatory response operates through the oscillation of CXCL5 in the lung. This chemokine is a powerful neutrophil chemo-attractant and was shown to be under circadian regulation *in vivo*, through rhythmic glucocorticoid receptor (GR)-driven repression, a common mechanism through which GR exerts immunosuppressive and anti-inflammatory actions attenuating cytokine-mediated pathways (Necela and Cidlowski, 2004). This is consistent with findings showing that infection with the Gram-positive bacteria *S. pneumoniae*, which is partially controlled by neutrophil-mediated killing, was more aggressive when the mice where infected at ZT0 than at ZT12, showing a higher bacterial burden in the lung and blood 48 hr after infection. However, diurnal GR binding to the *Cxcl5* promoter was disrupted by bronchiolar specific *Bmal1* ablation, causing abnormal CXCL5 production and an enhancement in neutrophil recruitment upon *S. pneumoniae* infection. Finally, targeted ablation of *Bmal1* in bronchiolar epithelial cells resulted in absence of circadian gating (the control by a 24 hr rhythmicity driven by the endogenous clock) in the immune response to bacterial infection (Gibbs et al., 2014).

This suggests that the local clock, together with systemic glucocorticoid signals, plays a key role in the time-of-day-dependent synchronization of pulmonary innate immunity, minimizing vulnerability to the pathogenic attack when it is most likely to occur. Thus, the results of this study highlight the tight link between lung clock function, glucocorticoid signals, and innate immunity. Several inflammatory pulmonary diseases, such as chronic obstructive pulmonary disorder and asthma, display a diurnal variation in their symptoms (Sundar et al., 2015). Local pharmacological targeting of the endogenous clock in the lung might turn out to be beneficial to alleviate the severity of these diseases. On the other hand, the data suggest that variability among patients in the efficacy of steroid treatments might be ascribed to alteration of circadian rhythms.

Vesicular Stomatitis Virus. Intranasal vesicular stomatitis virus (VSV) administration is a well-known model of encephalitis in mice. Recently, it has been shown that VSV infection outcome displayed a strong circadian rhythmicity. Mice infected at the beginning of the resting phase (ZT0) had a lower survival rate than animals infected at the start of the active phase (ZT12). This was associated with an increase in blood CCL2 and higher levels of inflammatory monocytes in the olfactory bulb. In order to dissect the molecular mechanisms behind the diurnal gating of the survival rate upon VSV infection, the authors investigated daily changes in core-clock genes in the olfactory bulb. *Rev-erb-alpha* gene expression displayed a significant difference between ZT0 and ZT12, identifying this transcriptional repressor as a possible regulator of the rhythmic VSV effects *in vivo*. Notably, the acrophase of *Nr1d1* (the gene encoding REV-REB-alpha) expression (ZT12) coincided with the time of greater survival rate after VSV treatment, and REV-ERB-alpha-driven repression of CCL2 was identified as the mechanism underlying this specific neuro-inflammatory state and mediating the diurnal effect on mortality (Gagnidze et al., 2016). Thus, the nuclear receptor REV-ERB-alpha emerges as a molecular candidate to modulate the expression of important inflammatory factors during virus-dependent encephalitis. Investigations in human cohorts are needed to validate whether this molecular pathway has a translational power for future applications in the context of clinical chronopharmacology.

Herpes and Influenza Viruses. Similar to VSV, murid herpes virus 4 (MuHV-4) and herpes simplex virus 1 (HSV-1) featured higher virulence in mice infected at ZT0 compared to mice infected at ZT12 (Edgar et al., 2016). This effect was abolished in the *Bmal1* KO mouse model, which exhibited high levels of viral replication when infected at either time of the day. Interestingly, latent infection was not affected by the circadian disruption in *Bmal1* KO mice, suggesting that the clock is primarily involved in modulating and anticipating the first encounter with the pathogens and thus the acute phase of viral infection.

Importantly, herpes virus infection resulted in alterations of the core-clock machinery through an enhancement in *Bmal1* transcription and a reduction in *Cry1* and *Per2* expression at specific circadian times in cultured cells. However, the precise molecular mechanisms underlying these transcriptional changes remain elusive. Nonetheless, it is worth noting that the regulatory protein ICP0 of HSV-1 physically and functionally interacts with BMAL1 *in vitro*, enhancing specific gene expression (Kawaguchi et al., 2001). Similarly, *in vitro* analyses showed that CLOCK is a component of the HSV-1 transcriptional machinery and its acetyl-transferase activity might contribute to chromatin remodeling and viral gene expression (Doi et al., 2006; Kalamvoki and Roizman, 2011). How these interactions are diurnally modulated has not been elucidated to date.

Interestingly, influenza A virus (IAV) does not exploit the host cell transcriptional machinery as herpes viruses do, yet it displayed an increase in viral protein production in arrhythmic cells, which was linked to modification in the levels of specific enzymes implicated in protein biosynthesis (Edgar et al., 2016). Therefore, different types of viruses seem to crosstalk with the molecular clock through distinct interactions with core-clock proteins (Kalamvoki and Roizman, 2011; Kawaguchi et al., 2001) and their gene expression (Edgar et al., 2016), or indirectly through secondary mechanisms of circadian cell physiology. The exact mechanisms notwithstanding, these examples clearly show that the host immune response, and in particular the cellular reaction to viral attack, is profoundly linked to circadian rhythmicity by a functional clock.

Sendai Virus. Sendai virus (SeV) infection causes acute bronchiolitis followed by chronic airway changes that resemble the manifestation of asthma. In a recent study, wild-type (WT), *Bmal1* KO, and tamoxifen-inducible *Bmal1* KO mice were challenged with SeV (Ehlers et al., 2017). Both KO animals displayed greater susceptibility to infection characterized by higher mortality rate, viral RNA expression, and viral load. Moreover, the altered antiviral response caused by *Bmal1* deletion exacerbated chronic lung inflammation. Similar effects were observed in jetlagged mice after SeV inoculation. Interestingly, decreased levels of *Bmal1* and other CCGs were found in airway cells of patients with SeV-associated asthma, indicating a strong connection between circadian disruption and lung pathology (Ehlers et al., 2017). Further investigations are necessary to dissect the molecular mechanisms underlying the clock antiviral activity and to understand how circadian rhythm disruptions deteriorate tissue pathology upon SeV viral challenge. Although the time-of-day-dependent severity of the immune response was not explored, this study confirms the importance of a functional clock and a correct circadian rhythmicity for optimal host immune responses to pathogenic insults.

Coordination of the Inflammatory Response by the Circadian Clock

The above studies demonstrate an intimate relationship between clock function and the host immune response to various pathogens in multiple tissues. A common feature emerging from all these examples is that the immune system reacts more efficiently to the infection immediately before or during the host active phase independently of the type of infectious agent (Figure 2). In fact, as early as 1960, this principle had been highlighted after treatment of mice with *Escherichia coli* endotoxin at different times of the day (Halberg et al., 1960). Thus, one can hypothesize that the circadian gating of inflammatory responses is built to maximize the host fitness against a multitude of pathogens, especially when the probability of an inflammatory flare-up is higher because of the increased contact with the external environment, i.e., during the phase of activity.

It is worth noting that many aspects of the inflammatory response are characterized by distinct acrophases along the

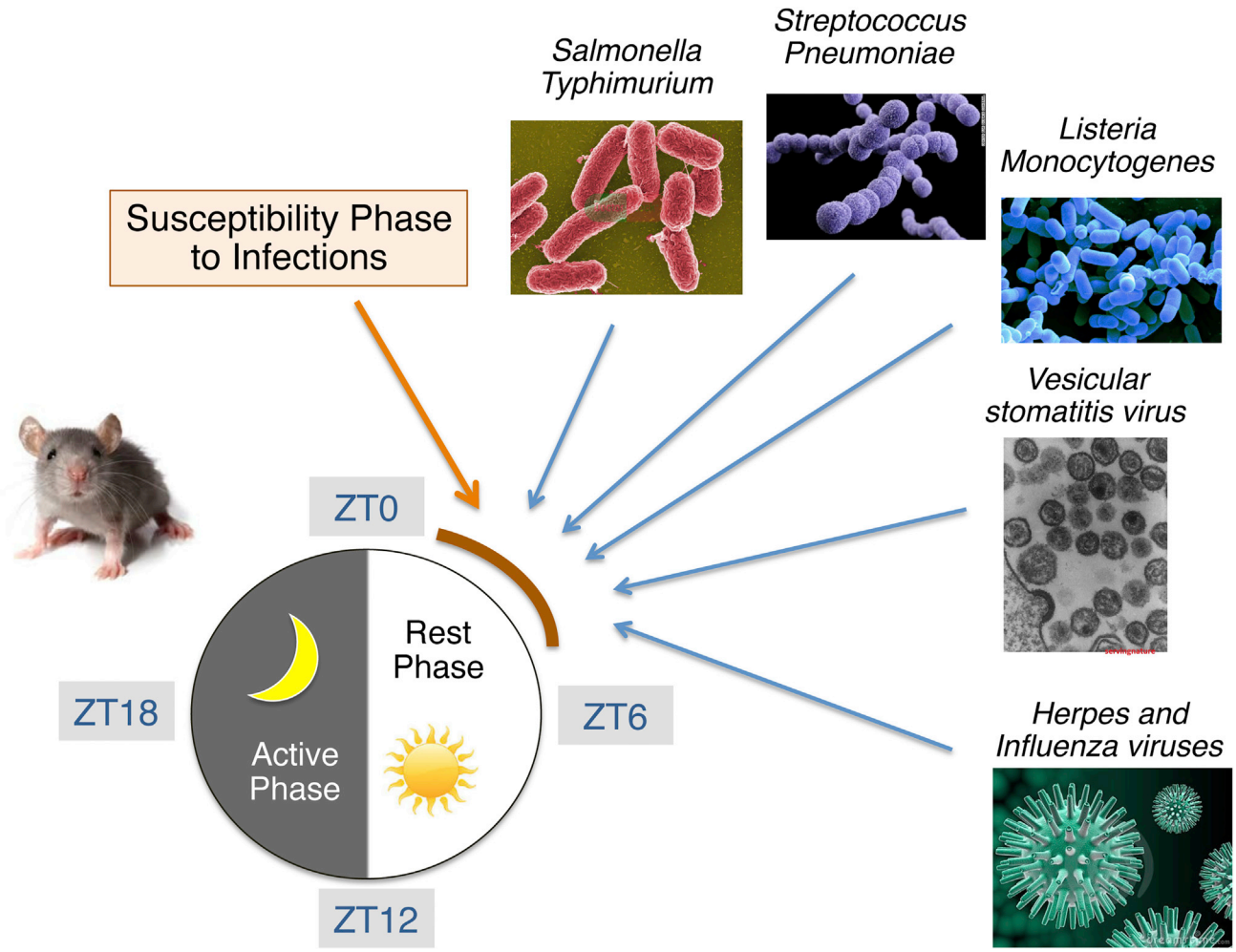

Figure 2. Diurnal Signature of Host Sensitivity to Infections
The endogenous clock anticipates daily fluctuation in the external environment in order to maximize physiological fitness. Susceptibility of mice to a variety of pathogens and to inflammatory cytokines displays circadian features. During the active/feeding phase, the encounter with pathogens is more likely. Indeed, the clock is ready to anticipate the threat by optimizing the immune response. In contrast, susceptibility to infections is higher at the beginning of the resting phase and the inflammation levels are massively increased. This circadian coordination of the host response has been demonstrated for a variety of pathogens, as highlighted in the figure.

circadian cycle, such as blood and splenic Ly6C^{hi} monocytes (ZT8) (Nguyen et al., 2013), leukocyte recruitment to tissues (ZT13) and blood (ZT5) (Scheiermann et al., 2012), lung cytokines (ZT6) and chemokines (ZT0–ZT18) after lipopolysaccharide (LPS) treatment, *Cxcl5* expression in bronchiolar epithelial cells (ZT0) (Gibbs et al., 2014), expression of the innate immune receptor Toll-like receptor 9 (*Tlr9*) in splenic tissue (ZT19), macrophages (ZT11) and B cells (ZT15–ZT19) (Silver et al., 2012), and number of lymphocytes (ZT5 in blood, ZT13 in lymph nodes) (Druzd et al., 2017), among many others. Furthermore, the zenith in the expression of core-clock genes involved in the inflammatory reaction does not overlap. Why is the rhythm in the immune components so asynchronous? The daily variations in these peaks may represent a protection mechanism against abnormal immune responses as in the case of sepsis (Man et al., 2016), a life-threatening state in which an infection provokes an overt systemic inflammatory response inciting injuries to host tissues. In many cases, this immunopathology ultimately leads to demise, rather than the initial infectious stimuli themselves. Indeed, severity in sepsis outcome is directly linked to circadian fluctuation in splenic *Tlr9* expression, a highly conserved pattern recognition receptor (PRR) that recognizes pathogen-associated molecular patterns (PAMPs) from both viruses and bacteria. Mice subjected to cecal ligation and puncture (CLP) at ZT19, when TLR9 was elevated, experienced exacerbated sepsis and earlier mortality than mice operated at ZT7 (Silver et al., 2012). Remarkably, *clock*-deficient mice, a model characterized by lack of rhythmicity in peripheral oscillators (DeBruyne et al., 2007), had a higher survival rate, lower serum levels of inflammatory cytokines, and finally, a better outcome after polymicrobial sepsis regardless of the CLP circadian time (Wang et al., 2016). While the molecular mechanisms have not yet been fully explored, it is notable that CLOCK directly binds the promoter of *Tlr9* gene, activates its expression (Silver et al., 2012), and positively

controls the mRNA levels of NF-κB, a key transcription factor involved in the innate immune response (Spengler et al., 2012), indicating a possible dampening of the excessive inflammatory reaction driven by sepsis with a consequently less severe outcome upon clock disruption.

Circadian Rhythms and the Microbiome

Recent data on the circadian variation in the composition and function of intestinal commensal bacteria (Liang et al., 2015; Thaiss et al., 2014; Zarrinpar et al., 2014), as well as the consequences of diurnal fluctuations in the microbiota on the host (Leone et al., 2015; Mukherji et al., 2013; Murakami et al., 2016; Thaiss et al., 2016), indicate that the role of the circadian clock in host defense against pathogenic infection might represent only a small fraction of the entire repertoire of functions by which the circadian clock orchestrates host-microbial interactions. The daily oscillations in the community structure and metabolic activity of the intestinal microbiome comprise rhythmic variations in bacterial localization, metabolite secretion, and proliferation (Korem et al., 2015; Leone et al., 2015; Thaiss et al., 2016). Since the commensal microbiota fulfills a critical role in providing colonization resistance against invading pathogens, these diurnal rhythms may have important implications for the host defense against intestinal infection. On the one hand, competition between indigenous commensals and pathogens for colonization niches might not be stable over the course of a day, but cooperate with diurnal rhythms in host immunity to provide a heightened state of antimicrobial alertness during the active phase (Thaiss et al., 2015). On the other hand, the mechanisms used by the host to interact with the rhythmic microbiota, albeit largely elusive, may be particularly important in the case of pathogenic infection, as many mechanisms involved in the regulation of host-microbiome interactions are likewise critically important for mucosal host defense against pathogens (Sonnenberg et al., 2011). Thus, orally ingested pathogens encounter a rhythmically fluctuating host defense system, consisting of both microbial oscillations in niche occupancy and circadian variations in host immunity. Future studies are warranted to elucidate the principles underlying this tripartite interaction between colonization resistance, anti-microbial immunity, and pathogenic niche occupation, with the goal of enhancing time-of-day-specific host-microbiome interactions that prevent foreign members from entering the intestinal ecosystem.

Future Directions: From Cell-Type Specificity to Chronopharmacology

Despite growing evidence for the circadian gating of anti-microbial host defense and an intuitive evolutionary teleology for anticipatory immune responses, many outstanding questions remain unsolved. A major gap in our knowledge about the function of the circadian clock in mediating anti-pathogen responses relates to the cell-type specificity of how the clock is linked to immune functions in a cell-autonomous manner. Studies of conditional KO mice have yielded promising insights (Gibbs et al., 2014; Nguyen et al., 2013), but the large majority of cell-intrinsic roles for clock components in the immune response remain to be deciphered. Likewise, it is currently unclear how circadian clocks and the downstream CCGs in different cell types communicate during the course of an infection. One may envision a scenario in which distinct effector functions of the anti-bacterial or anti-viral immune response are synchronized by the circadian clock activity in different immune cells, but such a model awaits experimental proof. The same applies for the circadian clock-mediated communication across tissues, in which temporal coordination of tissue-specific antimicrobial function may accompany the tissue tropism of different phases of pathogenic infection. Future studies using a combination of conditional clock-deficient mice, coupled with timed interventions to light and food exposure, are warranted to address this model.

A major purpose of clock-governed immune responses might be the partitioning of energy-intensive immune functions over the course of a day. The circadian clock and cellular metabolism are strongly intertwined, and numerous mechanisms have been elucidated by which metabolites influence clock activity and by which circadian clock outputs, in turn, modulate metabolic functions (Asher and Sassone-Corsi, 2015; Bass, 2012). Antimicrobial effector functions of immune cells are strongly linked to the orchestration of intracellular energy metabolism (Pearce and Pearce, 2013), thereby enabling the adaptation of cellular homeostasis to the energy-intensive processes involved in immune cell activity, such as the rapid large-scale production of proteins. Focusing these costly processes to the time of the day at which the likelihood for pathogen encounter is maximal may provide an economical way of maintaining immune cell metabolic homeostasis. A direct corollary of this hypothesis is that the circadian clock may facilitate such daily partitioning of immune cell functions through its effect on intracellular energy metabolism. A cell-specific circadian analysis of metabolic pathway activation, coupled with single-cell analysis of resultant immune function, might help to understand how the clock gates cellular energetics and engages particular metabolic cascades to boost a rhythmic immune response in order to optimally defeat a pathogenic attack.

In addition, an involvement of the circadian clock has so far only been demonstrated in the case of rapidly multiplying bacteria and viruses, and it remains to be investigated whether the clock is similarly important for immune responses against slowly replicating multicellular parasites or fungi, or whether its role is limited to the fine-tuning of immune responses on the scale of hours. This is especially important with respect to the timing of the infection. For instance, infections caused by the parasite *Trypanosoma cruzi* are spread primarily through bites from insects of the Triatominae family, which are usually active at night, i.e., the time of reduced immune system activity in humans. Whether there are differential anticipatory responses against foodborne or airborne versus blood-borne pathogens, and how they are timed on a circadian scale, is a fascinating topic for future studies.

Finally, investigations in humans have shown that different types of leukocytes oscillate in a diurnal manner, and that sleep consolidates this rhythmicity by controlling oscillating levels of cortisol and epinephrine (Lange et al., 2010). Moreover, circadian disruptions associated with modern lifestyle, including shift work or frequent trans-oceanic flights, often cause deterioration of the sleep/wake cycle, which has been strongly associated with an increased susceptibility to infectious diseases (Almeida and Malheiro, 2016; Patel et al., 2012; Prather et al., 2015). Thus, the insights gained from preclinical studies of daily oscillations in

host-microbial interactions may inform the chronopharmacology of antimicrobial and anti-inflammatory therapy, i.e., the time-of-day-specific administration of pharmacologic agents aimed at modulating the immune response. For instance, immunosuppressive interventions might best be applied at times of maximal leukocyte recruitment and cytokine production in a given tissue. Likewise, antibiotic therapy may benefit from a deeper understanding of the circadian orchestration of immunity, in order to facilitate the optimal daily timing and dose of administration. Such developments will require a better insight into the diurnal gating of immune responses and the role of the circadian clock in the host response against a wide spectrum of microbial pathogens. Nonetheless, the first studies in this field provide the ground for the systemic elucidation of the interplay between the molecular clock and anti-pathogen immunity, and may open new avenues for harnessing the "time of day" as a critical factor in antimicrobial therapy.

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