



Differences in Diurnal Variation of Immune Responses in Microglia and Macrophages: Review and Perspectives

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Abstract

Biological rhythms, especially those that last close to 24 h, better known as circadian rhythms, are highly regulated phenomena, maintained throughout evolution in various organisms which allow organisms to predict, prepare for, and adapt to environmental changes. One of these phenomena that exhibit biological rhythms is the immune response to external agents. Immune cells (neutrophils, lymphocytes, macrophages, among others), as well as their mediators such as cytokines and chemokines, undergo variations in tissue and blood concentrations during the day. These rhythms are still being elucidated in microglia, the resident macrophages of the central nervous system, but since these cells share a common origin with peripheral macrophages, they are expected to behave similarly. In this review, we will discuss the possible differences in the responses between peripheral macrophages and microglia, their relationship with the circadian clock, and whether these rhythms can influence therapeutic choices.

Keywords Circadian rhythm · Immune clock · Macrophages · Microglia

Introduction

Biological rhythms are essential properties of almost all living organisms from bacteria and fungi to plants and animals (Bhadra et al. 2017). These rhythms are considered to be the result of evolutionary pressures and natural selection and enable organisms to predict, prepare for, and adapt to environmental changes through synchronizers, better known as *zeitgebers* (a German word meaning “time giver”). *Zeitgebers* are perceived by the organism, responding to physiological and behavioral changes by altering hormone levels, body temperature, metabolism, and even cognitive processes. However, it is essential to note that these rhythms

are expressed even in the absence of environmental changes. The *zeitgebers* only synchronize the circadian clock. The light–dark cycle is one of the most important and best-studied *zeitgeber*; changes in the intensity of light are detected by a series of hierarchically organized structures found at the hypothalamus (Terzibasi-Tozzini et al. 2017). However, the light–dark cycle is not the only kind of *zeitgeber*, since the organism can also receive synchronization stimuli through food intake (Sahar and Sassone-Corsi 2012; Morton et al. 2014), changes in temperature (Rensing and Ruoff 2002), and physical activity (Schibler and Sassone-Corsi 2002). One of the biological functions regulated by the circadian clock is the immune system (IS) (Scheiermann et al. 2013, 2018). In this review, we focus on the responses of microglia and peripheral macrophages and their relationship with the circadian clock.

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The Central and Peripheral Clocks

In mammals, the changes in light and darkness are detected in the retina, and then the retinohypothalamic tract (RHT) transmits this information to the “central clock”, a region located in the hypothalamus known as the suprachiasmatic nucleus (SCN). SCN is made up of about 10,000 neurons

in mice and up to 50,000 in humans (Videnovic et al. 2014). SCN neurons and virtually all cells of an organism have a molecular clock consisting of a mechanism of transcriptional-translational retro control. The main circuit of such system is regulated by the transcription factors aryl hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL), also known as Brain and Muscle ARNT-Like 1 (BMAL1), as well as the circadian locomotor output cycles kaput (CLOCK). When heterodimerized, both transcription factors bind to the E-box sequences found in the promoters of the so-called clock-controlled genes (CCGs). Among these genes are the proteins period (PER) 1–3, cryptochrome (CRY) 1–2, and nuclear receptors Rev-Erb α and Ror α . PER and CRY inactivate the proteins BMAL1 and CLOCK, generating a transcriptional control cycle of 24 h. Importantly, CCGs also code for a large number of proteins and can generate the physiological rhythms of metabolism and behavior (Takahashi 2017).

On the other hand, peripheral oscillators are usually synchronized with the SCN and have been characterized in various tissues such as the liver (Lamia et al. 2008), kidney (Solocinski and Gumz 2015), skin (Matsui et al. 2016), heart (Durgan and Young 2010), and in immune system cells such as lymphocytes (Druzdz et al. 2017) and macrophages (Keller et al. 2009). This synchronization of the central clock with the peripheral organs occurs through the hypothalamic–pituitary–adrenal efferent pathways and the autonomic nervous system (Curtis et al. 2014).

Circadian Rhythms of the Immune System

The IS differentiates the usual from the strange and protects the organism from harmful agents that come from the environment, such as pathogens (bacteria, viruses, fungi, and parasites) and foreign substances. The IS is divided into two large arms, the innate IS (IIS) and the adaptive (AIS). The IIS is considered the first line of defense that includes physical barriers (skin and mucous membranes), different cell types like nuclear polymorphs (neutrophils, eosinophils, and basophils), mononuclear cells (monocytes, macrophages, and lymphocytes), and soluble factors (complement system and mediators of inflammation, such as cytokines and chemokines).

The IIS allows a rapid, non-specific defense response mainly directed against pathogens or necrotic cells. This is achieved by recognizing molecules denominated as pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides (LPS), as well as damage-associated molecular patterns (DAMPs), such as the high-mobility group box 1 (HMBG1) (Chen and Nuñez 2010).

Finally, the IIS is capable of interacting with and activating the other major branch: the AIS. This is characterized

by a slow, antigen-specific response mediated by T and B lymphocytes that proliferate and differentiate into the effector population after antigen presentation. Thus, the complete elimination of pathogens occurs, as well as the generation of mechanisms of immunological memory (Parkin and Cohen 2001; Cermakian et al. 2013; Labrecque and Cermakian 2015).

It is currently known that the magnitude of IS response could be controlled by circadian signals that could influence the trafficking of immune cells, the host–pathogen interaction, or the activation of innate and adaptive immunity. In mice, a rhythmic pattern of leukocyte recruitment has been described, and more significant traffic of immune cells to tissues was observed during the activity period (dark phase). For example, neutrophils, i.e., cells that are attracted to inflamed tissues early on in the immune reaction and act through phagocytosis and the secretion of antimicrobial substances, vary their concentration in the blood throughout the day (Scheiermann et al. 2012). In humans, this circadian variation has been related to the expression of CLOCK molecular proteins. Although neutrophils express CLOCK genes such as *Per1*, *Dbp*, and *Rev-erba*, the levels of PER2 and BMAL1 were undetectable and minimal, respectively. Therefore, it was concluded that it might be systemic mechanisms and not the autonomous self-oscillator of the cells that determine the diurnal changes in the effector activity of peripheral neutrophils (Ella et al. 2016).

However, with the administration of a pro-inflammatory stimuli such as LPS, the relative expression of clock molecules like *Clock*, *Per3*, *Cry1*, and *Cry2* in peripheral blood leukocytes decreased by 80–90%, suggesting that endotoxin is a potent entrainer of the circadian clock network in circulating inflammatory cells (Haimovich et al. 2010). Furthermore, after trauma or during inflammatory disease, neutrophil migration to tissues is significantly influenced by the endogenous rhythms of endothelial cells due to the circadian expression of adhesion proteins (Scheiermann et al. 2012). This variation may have evolved since phagocytes greatly improve the response to pathogens during periods of activity, during which injuries and infections of microorganisms are more likely to occur (Casanova-Acebes et al. 2013).

Other cellular components of the IIS are macrophages, innate immune cells derived from monocytes, and long-term residents of various tissues. Although initially described as mainly phagocytic cells, macrophages participate in multiple processes and mediate inflammatory phenomena, regulate T lymphocyte activation through antigen presentation, and play a role in tissue repair and regeneration (Snyder et al. 2016). In this manuscript, we describe the functions of these cells in relation to the molecular clock.

Finally, another component of the IIS that varies through the day is soluble factors like cytokines and chemokines. In healthy humans, serum levels of interleukin (IL)-1, IL-6,

and soluble IL-2 receptors peak at 1–4 am (rest period) and are lower to 8–10 am (active period) (Petrovsky 2001). In a cell culture of peritoneal exudate cells (PECs) of mice, the secretion of cytokines like IL-6 and IL-12 (p40) and chemokines such as CXCL1, CCL5, and CCL2 showed a circadian response to LPS administered in an intraperitoneal (i.p.) manner. The highest response to LPS was observed at the transition of the rest and active phases, at circadian time 12 (CT12) (Gibbs et al. 2012).

Circadian Variation in the Immune Response of Macrophages

Studies in mice showed that the ability of peritoneal macrophages (PM) to initiate a defense cascade is strongly regulated by an intrinsic circadian clock. For example, ex vivo PM showed expression of canonical clock genes such as *Bmal1*, *Cry1-2*, *Per1-3*, and *Rev-erba*. However, with the administration of LPS, specifically, pro-inflammatory cytokine such as TNF- α and IL-6 production is determined by the circadian phase of the macrophage clock rather than by systemic circadian modulators such as cortisol levels (Keller et al. 2009). Another study reported that the ability of PM to phagocytize is higher during the day as compared to the night, and the transcription of mRNA pro-inflammatory cytokines and chemokines on it also have the same rhythmicity (Hayashi et al. 2007).

The close relationship of the immune response of macrophages with the circadian clock was affirmed when the function of the *Per2* gene was shown to be linked to the expression of toll-like receptor-9 (TLR9) in mice. In macrophages, *Tlr9* mRNA expression showed a daily rhythm that peaked at *zeitgeber* time 11 (ZT11), the *Per2* mutation causes alterations, such as a decrease in TLR9 expression and a lower secretion of TNF- α and IL-12 after challenge with a ligand for TLR9 (Silver et al. 2012). Subsequently, ex vivo studies of bone marrow-derived macrophages of mice having a complete deletion of the *Arntl*^{-/-} gene (also known as *Bmal1*) showed that it plays an essential role in the overall control of inflammatory gene regulation. This occurs by presenting a more significant response and prolonged activation of inflammatory genes activated by TLR4 as compared to its control group (Oishi et al. 2017). These data correlated with a previous study in which macrophages with deletion of the *Arntl*^{-/-} gene displayed a higher production of cytokines, especially of TNF- α and IL-6 (Curtis et al. 2015).

A study focusing on PECs, specifically macrophages characterized by the surface expression of F4/80 and CD11b, found that the nuclear receptor REV-ERB α , which plays a central role in the control of circadian expression of *Bmal1*, is the main one involved in the modulation of circadian

control of the IIS. In an initial experiment, LPS i.p. induced an increase in the synthesis of cytokines IL-6 and IL-12 with a peak during the transition to the active phase (CT12) as compared with the transition to resting phase (CT0) in control mice. However, in a knockout mouse with a macrophage-specific *Bmal1*^{-/-}, this time-dependent effect on the magnitude of the LPS response was lost. In another experiment, WT mice exposed to LPS i.p. showed higher serum concentrations of IL-6 at CT12 compared to CT0. However, in the *Rev-erb* gene (*rev-erba*^{-/-}) knockout mice, this difference was lost. The marked discrepancy in the cytokine secretion in both experiments demonstrated the essential role of the molecular clock on immune response rhythmicity (Gibbs et al. 2012).

Finally, in an infection model of *Leishmania major*, the authors showed for the first time that the circadian clock of mice bone marrow-derived macrophages is involved in the susceptibility of infection to this parasite. When *Bmal1*^{-/-} knockout mice were infected, no circadian variations were observed in the parasite load or the number of infected macrophages. In contrast, in WT animals it was found that parasitic load, together with the number of CD206⁻ macrophages and CD11b⁺ neutrophils, was higher during the active phase (CT15), (Kiessling et al. 2017).

Microglia

Microglia are the resident macrophages of the central nervous system (CNS) and represent near 20% of the total number of cells in the CNS (Lawson et al. 1990). These cells are distributed through all regions of the brain, and its density varies depending on the area, ranging from only 5% in the corpus callosum or up to 12% in the substantia nigra (Lawson et al. 1990; Savchenko et al. 1997; Tremblay et al. 2011). For a long time, it was believed that microglia played a “static” role within the normal physiology of the CNS; however, in this apparent state of rest it plays a highly dynamic function by continuously sampling their microenvironment with long protrusions and a variety of surface receptors (Nimmerjahn et al. 2005). It is currently known that microglia maintain CNS homeostasis by regulating synapses depending on neuronal activity and the simultaneous interaction with neurons and astrocytes (Tremblay et al. 2011). Actually, microglial cells collaborate during fetal development, childhood, and puberty, thereby being responsible for “synaptic pruning” and the production of neurotrophic factors (Wu et al. 2015; Kierdorf and Prinz 2017).

Furthermore, microglia can actively sense any possible signs of damage coming mainly from neurons and are responsible for initiating the innate immune response to aggression from either microorganism (bacteria, viruses,

etc.), direct acute damage (trauma or cerebral vascular events) or neurodegenerative mechanisms (Brown and Neher 2014; Butovsky et al. 2016; Wolf et al. 2017). For its activation, microglia require a specific pathological stimulus that could generate changes in its morphology as described in several models, for example, in prion disease (Vincenti et al. 2015), traumatic brain injury (TBI) (Morrison et al. 2017), and stroke (Heindl et al. 2018), among others. Therefore, microglia respond to these stimuli by synthesizing a broad spectrum of chemokines, pro-inflammatory and anti-inflammatory cytokines, and other mediators. In fact, depending of the activation profile, microglia can be divided into M1 (classical profile) and M2 (alternative), the latter of which can be further differentiated into three subsets: (1) M2a, focused on repair and regeneration; (2) M2b, an immunoregulatory phenotype; and (3) M2c, an acquired-deactivating phenotype (Kabba et al. 2018).

Circadian Rhythms in Microglia

Although the works of this CNS resident macrophage regarding the presence of an intrinsic biological clock are recent, they have yielded exciting results (Chi-Castañeda and Ortega 2018). For example, Hayashi et al. (2013a) found that the isolated microglia of the cerebral cortex of mice exhibited variations throughout the day in its morphology, thereby finding a greater branching during the darkness, which subsequently decreased during the light phase; also these changes were associated with circadian variations because it was preserved in conditions of constant darkness. These morphological variations were related to the cycles of expression of clock proteins and the induction of purinergic receptors, specifically the P2Y₁₂ type (P2Y₁₂R), a G_{i/o}-coupled ATP receptor exclusively expressed in microglia in CNS (Tozaki-Saitoh et al. 2012), which activate a downstream signaling cascade that could reorganize the cytoskeleton (Hristovska and Pascual 2016). Hayashi et al. generated *clock*-mutant mice and observed that diurnal morphological changes of microglia and the relative mRNA levels of P2Y₁₂R were abolished, both in normal light/dark and under constant-dark conditions (Hayashi et al. 2013a).

In other research, Takayama et al. reproduced the diurnal variation of cortical microglia, they measured the mean total length and the mean number of branch points and found that the significance of both was greater at ZT14 than ZT2. However, in a second experiment, focal administration of *Porphyromona gingivalis* to the somatosensory cortex resulted in a greater significant microglial process extension at ZT2 than at ZT14. Finally, the authors relate the microglial expression of P2y₆R, a G_{q/11} protein that regulates the intracellular Ca²⁺ store and is activated by uridine triphosphate and UDP, with the diurnal variations since a pharmacological blockade

of this receptor caused the microglial process extensions to be significantly attenuated. Furthermore, they showed that the promoter region of the gene *P2ry6* of P2y₆R contains a RORE site, which is the target of Rev-erb α . Therefore, Rev-erb α could suppress the expression of *P2ry6* during the active phase of the mouse (dark phase) (Takayama et al. 2016).

In addition, the molecular machinery of the cortical microglia clock generates the circadian expression of cathepsin S (CatS) (a specific lysosomal cysteine protease). The levels of expression of CatS are low during the light period, correlating with a decrease in the size of the dendritic spines and the synaptic strength and, by elevating the expression of CatS during the dark phase, the dendritic spines and the synaptic strength increase (Hayashi et al. 2013b). Interestingly, CatS has been related to the part of the adaptive immune response that contributes to antigenic presentation, for example, in the formation of specific peptides against foreign proteins (Hsieh et al. 2002) and in the degradation of the invariant chaperone chain associated with the major histocompatibility complex (MHC) class II (Honey and Rudensky 2003; Clark and Malcangio 2013).

Finally, genes of pro-inflammatory factors (IL-1 β , TNF- α , and IL-6) in rat hippocampal microglia showed rhythmicity throughout the day. When LPS was administered during the light phase, there was a significant expression of IL-1 β , TNF- α , and IL-6 of both, the mRNA and protein. In contrast, during the dark phase, the levels of these cytokines were lower regardless of the concentrations of LPS administered. This response seems to be independent of glucocorticoids, since, despite adrenalectomy and the absence of circulating corticosteroids, the circadian rhythms in the microglia were maintained (Fonken et al. 2015).

Summary of the articles discussed is presented in Table 1, and comparison throughout the day of the response of both microglia and macrophages is shown in Fig. 1.

Are There Different Variations in Microglia and Macrophages?

The immune response varies throughout the day and depends on the molecular machinery of the circadian clock (Scheiermann et al. 2013, 2018). Apparently, this physiological variation correlates with immune response, the susceptibility to different agents throughout the day, such as bacteria, and the prognosis after the immune challenge. Extensive reviews describe that the transition from the rest phase to active phase is associated with a higher susceptibility and induction of pro-inflammatory cytokines, as compared to the transition from active phase to rest phase where the susceptibility is lower and with a lower induction of pro-inflammatory cytokine synthesis (Curtis et al. 2014; Geiger et al. 2015).

Table 1 Comparison of different functions in light and dark period macrophages and microglia in murine models

Function	Period	Macrophages	Microglia
Cytokine and chemokine synthesis	Light	<ul style="list-style-type: none"> Increased IL-1β, TNF-α, IL-6 and CCL2 in peritoneal macrophages (Hayashi et al. 2007) Increased secretion of TNF-α and IL-6 by spleen macrophages at CT8-CT12 after LPS stimulation (Keller et al. 2009) Higher induction in serum IL-6, IL-12, CCL2, CXCL1 and CCL5 at CT12 after LPS stimulation (Gibbs et al. 2012) 	<ul style="list-style-type: none"> Higher expression at ZT6 of mRNA and protein of IL-1β, TNF-α and IL-6 in hippocampal microglia after LPS stimulation (Fonken et al. 2015) Higher relative gene expression of hippocampal IL-1β and TNF-α 24h after cardiac in the mid-light murine group (Weil et al. 2009)
	Dark	<ul style="list-style-type: none"> Higher serum TNF-α and IL-6 levels at ZT14 after 80 min of LPS application (Guerrero-Vargas et al. 2014) Decreased secretion of TNF-α and IL-6 by spleen macrophages at CT16 after LPS stimulation. (Keller et al. 2009) Lower induction of IL-10 at ZT12 than at ZT0 (Curtis et al. 2015) 	<ul style="list-style-type: none"> Higher expression at ZT16 of protein of IL-10 in unstimulated hippocampal microglia and after LPS stimulation Fonken et al. 2015
Glucocorticoid influence	Light	<ul style="list-style-type: none"> In adrenalectomized mice TNF-α and IL-6 secretion by spleen macrophages was higher at CT12 after LPS stimulation (Keller et al. 2009) 	<ul style="list-style-type: none"> In rats with adrenalectomy the relative gene expression of IL-1β and TNF-α is higher at ZT6 after LPS stimulation. (Fonken et al. 2015)
Phagocytosis	Dark		
	Light	<ul style="list-style-type: none"> Increased ability of phagocytosis of peritoneal macrophages (Hayashi et al. 2007) 	No published information available
Morphology	Dark		
	Light	No published information available	<ul style="list-style-type: none"> Less branching and length of cell processes in cortical microglia during ZT2 (Hayashi et al. 2013a) Cortical microglia respond more to P. gingivalis exposure at ZT2 (Takayama et al. 2016) Cortical microglia has longer cell processes and more branching points at ZT14 (Takayama et al. 2016) Cortical microglia respond more to ATP exposure at ZT14 (Takayama et al. 2016)
Other immune molecules	Light	<ul style="list-style-type: none"> Higher number of CD11b+/CD14+ spleen macrophages at CT8 (Keller et al. 2009) 	<ul style="list-style-type: none"> Unstimulated hippocampal microglia show higher MHCII levels at ZT6 (Fonken et al. 2015)
	Dark	<ul style="list-style-type: none"> Higher number of inflammatory CD206+ peritoneal macrophages (Kiesling et al. 2017) Higher expression of chemoattractants (<i>Mip2</i>, <i>Mcp1</i>, <i>Mip1α</i>, <i>Mip1β</i> and <i>Trifα</i>) in peritoneal macrophages after L. major infection (Kiesling et al. 2017) 	

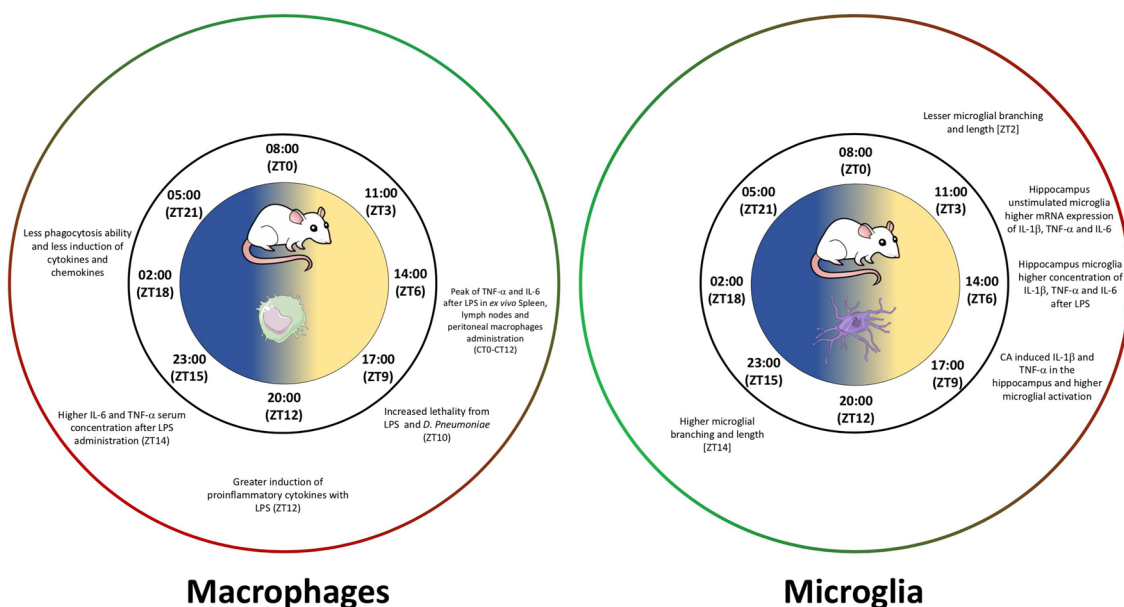


Fig. 1 Comparative immune response between peripheral macrophages and microglia through the day. Several studies highlight the pro-inflammatory immune response of peripheral macrophages during the light–dark transition or in darkness, while microglial studies show this tendency towards daylight hours. ZT is a unit of time based

Guerrero-Vargas et al. showed that intravenous LPS administration at ZT14 (2 h after the beginning of the active phase) induced a higher serum concentration of IL-6 compared to the group with LPS administration at ZT2 (2 h after the beginning of the rest phase) (Guerrero-Vargas et al. 2014). Therefore, it is necessary to consider factors that cause circadian variation in the organism and could influence these variations in immune response; for example, hormones that display typical circadian rhythms, such as growth hormone and prolactin which peak at the rest phase, catecholamines which peak during the active phase or, for example corticosteroids, that in a study with administration of dexamethasone in peripheral oscillators like liver and fibroblast, have been related to a phase-shifting in expression of *Dhb* and *Rev-erba* caused a strong phase delay when it was injected at ZT14 and ZT21 (night phase), whereas injection at ZT1 (light phase) resulted in a phase-advance (Balsalobre et al. 2000). This could explain why the peak at the transition of rest phase to active phase of corticosteroids could be related to a phase-shifting in microglia and macrophages; however, as we previously mentioned, some studies had reported a corticosteroids independent mechanism. Therefore, more studies are necessary to elucidate these mechanisms, considering that there may be several factors that are generating this difference (Fig. 2).

Nevertheless, it is necessary to consider the context of the CNS, which presents structural features of the tissue that differentiate it from the response generated in the rest

on the period of a *zeitgeber*, such as the 12:12 light:dark cycle. In free-running, constant conditions, the onset of activity of day-active organisms is circadian time zero (CT0) and the onset of activity of night-active organisms is CT12 (Karatsoreos and Silver 2017)

of the organism. One such example is the presence of the blood–brain barrier, a morpho-functional structure that separates the CNS from blood and which also presents variations in permeability that depends both on sleep and circadian rhythms (Cuddapah et al. 2019). On the other hand, the glymphatic system was recently described as a glial-dependent perivascular network that subserves a pseudolymphatic function in the brain (Iliff et al. 2012; Plog and Nedergaard 2018). The specific response of the microglia needs to be considered, depending on the time in which an immune challenge is generated, for example, research on CNS of a murine model showed that a lower induction of the pro-inflammatory response during the dark phase is observed compared to the light phase. For example, the hippocampal relative gene expression of IL-1 β and TNF- α was higher in mice exposed to cardiac arrest 6–8 h after turning lights on (mid-light period) than in mice challenged 4–6 h after turning lights off (mid-dark period). These observations correlated with microglial activation that was quantified in different brain areas and found to be higher in the mid-light period compared to the mid-dark period phase (Weil et al. 2009). These results are in agreement with the variations described in the production of pro-inflammatory cytokines (IL-1 β , TNF- α , and IL-6) in the hippocampus 3 and 24 h after the application of LPS at two different time points (ZT6 and ZT16), thereby showing a greater response during the light phase compared to the dark phase (Fonken et al. 2015). In rats subjected to the unavoidable shock test of the tail and

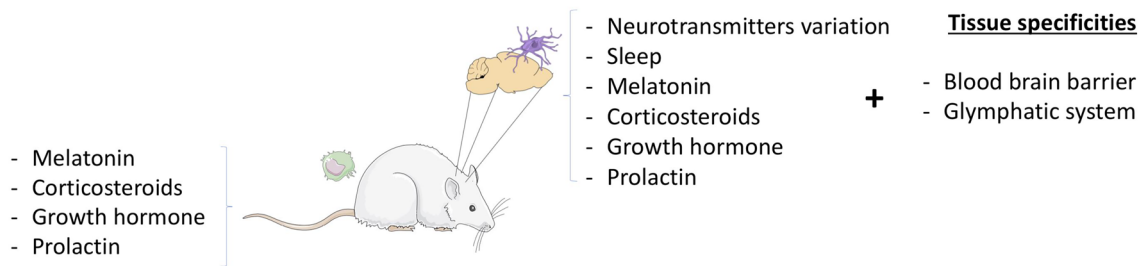


Fig. 2 Factors that can influence the difference in the immune response between macrophages and microglia. The activation peak of macrophages agrees with several studies that indicate that the resting period (i.e., the dark period in humans and the light period in nocturnal rodents) is characterized by maximum levels of pro-inflammatory hormones, such as growth hormone (GH) and prolactin, as well as Th1 or pro-inflammatory cytokines, such as IL-1 and TNF- α . In addition, the numbers of CD4+ T cells and the ability to respond to LPS are also higher during the rest period. While during the active period (i.e., the light period in humans and the dark period in noc-

turnal rodents), the hypothalamus–hypophysis–adrenal axis turns on and cortisol or corticosterone peaks; also, adrenaline released from the adrenal medulla and noradrenaline released from the sympathetic nerve endings increase. These hormones are anti-inflammatory and shut down the pro-inflammatory response induced during the rest period. Indeed, also IL-10, the major anti-inflammatory cytokine peaks in the active period. All these factors coincide with a lower activation of the macrophages (Besedovsky et al. 2012; Cermakian et al. 2013)

subsequently exposed to LPS, the hippocampal induction of pro-inflammatory cytokines (IL-1 β , IL-6, and TNF- α) was higher in the subjects tested during the light period than in rats subjected to stress during the dark period (Fonken et al. 2016). Finally, these works suggest that microglia could be generating an immune-regulatory role, by receiving the immune signals coming from the periphery and initiating a greater pro-inflammatory response in the CNS during the period of light compared to the dark period.

Implications and Future Perspectives

Differences between both responses, central and peripheral seem reasonable when considering tissue macrophages in different circumstances; therefore, the immune profile variation throughout the day would not seem surprising. However, the observations that the CNS presents greater susceptibility during the light period compared to the period of darkness due to a more pronounced pro-inflammatory response may be of clinical relevance since brain therapies should consider these circumstances. For example, in our laboratory, we have demonstrated that rats subjected to TBI during the light period present remarkable damage evaluated through neurobehavioral tests and higher mortality in comparison with rats traumatized during the dark period (Estrada-Rojo et al. 2018). These data are consistent with the previously-mentioned studies.

Nevertheless, whether this difference is due to the variation of the immune response in CNS is open to corroboration. However, we hypothesize that it could be the sleep condition that could be generating a microenvironment that promotes the observed gap between microglia and peripheral macrophages, for example, in previous works it has already

been reviewed that the neurons and astrocytes of areas that are involved in the regulation of sleep–wake behavior as hypothalamus, hippocampus, and brain stem and even cortex are immunoreactive for cytokines such as IL-1 β and TNF- α (Imeri and Opp 2009). This pro-inflammatory microenvironment coincides with the onset of susceptibility during the cycle of light in rats, and in turn would explain the works mentioned in this review. Finally, future experiments will need to consider another variable, the glymphatic system that has a central role in brain clearance and is functional during sleep (Iliff et al. 2012). Other factors to be considered are the vagus nerve regulation of peripheral immune response, melatonin production, and the effects of sleep deprivation among others.

Although macrophage-based cell therapies are novel and promising in the treatment of different pathologies, for example, brain ischemia (Kanazawa et al. 2017), or in the context of an acquired infection or injury, the treatment should take into consideration the time of day that the event occurred. This is because the microenvironment generated by microglia or macrophages is affected by this factor when injury or infection occurs and could determine the levels of pro-inflammatory response, which might affect prognosis.

Conclusion

Conclusion

There is plenty of evidence in the literature suggesting a difference in the response between peripheral macrophages and microglia in CNS throughout the day. In this review, we presented the most representative works oriented to the variation of the immune response in the periphery and CNS, and its relation to macrophages. However, we feel that more studies are necessary to elucidate this hypothesis, and if this

is confirmed, it could imply significant repercussions on the research of various diseases. On the other hand, the time of the day that injury or infection occurs, and whether it occurs in the CNS or the periphery should be considered in future therapies since the outcome may vary.

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Author Contributions RJMT, AC, and LN designed the paper; RJMT wrote the paper and designed the figures; and AC and LN reviewed the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors report no conflict of interest.

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