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Circadian influence on inflammatory response during cardiovascular disease

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Abstract

Circadian rhythms follow a 24 h day and night cycle, regulate vital physiological processes, and are especially relevant to cardiovascular growth, renewal, repair, and remodeling. A recent flurry of clinical and experimental studies reveals a profound circadian influence on immune responses in cardiovascular disease. The first section of this review summarizes the importance of circadian rhythms for cardiovascular health and disease. The second section introduces the circadian nature of inflammatory responses. The third section combines these to elucidate a new role for the circadian system, influencing inflammation in heart disease, especially myocardial infarction. Particular focus is on circadian regulation of the NACHT, LRR, and PYD domains–containing protein 3 inflammasome, neutrophils, monocytes/macrophages, and T cells involved in cardiac repair. A role for biological sex is noted. The final section explores circadian influences on inflammation in other major cardiovascular conditions. Circadian regulation of inflammation has profound implications for benefitting the diagnosis, treatment, and prognosis of patients with cardiovascular disease.

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Introduction

The circadian system underlies the behavior and physiology of virtually all life on earth. The mechanistic molecular underpinnings of the circadian system were recognized by the 2017 Nobel Prize in Physiology or Medicine [1]. The circadian system and its relevance to human biology has been extensively reviewed [2–4]. Briefly, the circadian system is primarily set by light input received by dedicated cells in the retina, which then transmit signals to the master pacemaker in the hypothalamic suprachiasmatic nucleus to set time. This time-of-day information is subsequently processed via neural and hormonal outputs from the suprachiasmatic nucleus to the different organ systems of the body, thus coordinating time of day in the peripheral systems as well. At a cellular level, these signals impinge on a molecular clock mechanism comprised of a 24 h mRNA and protein transcription/translation loop, leading to the temporal regulation of key biological processes in the body's organ systems. This circadian clock mechanism involves the factors circadian locomotor output cycles kaput (CLOCK), brain and muscle Arnt-like protein-1 (BMAL1), periods (PER1 and PER2), cryptochromes (CRY1 and CRY2), the orphan nuclear-receptors REV-ERB and ROR, and regulated gene and protein outputs, and it is present in almost every cell type in the body including the cardiomyocytes [5–10].

Over the past several decades, numerous clinical and experimental studies have investigated the role of the circadian system in cardiovascular health and disease (reviewed by Martino and Sole [11], Sole and Martino [12], Alibhai et al. [13], Martino and Young [14], Reitz and Martino [15], Tsimakouridze et al. [16], Martino and Young [17], Mistry [18], and Rabinovich-Nikitin et al. [19]). Almost all cardiovascular parameters that have been assessed over 24-h diurnal cycles exhibit a temporal pattern, including heart rate, blood pressure,

catecholamines, vascular function, and thrombolytic activity. These daily fluctuations in cardiovascular parameters are considered to be beneficial in healthy individuals. However, they also underlie the timing of onset, severity, and outcomes of many adverse cardiovascular events. One of the best studied examples of this is myocardial infarction (MI) (heart attack), a leading cause of morbidity and mortality in the Western world [20–23]. An early morning peak in the timing of onset of acute MI was initially reported in the New England Journal of Medicine in 1985 [24]. This pattern of morning onset of MI persists despite changes in lifestyle and advances in medicine over many decades [25,26]. Clinical studies also reveal temporal patterns in other major cardiovascular conditions such as stroke [27,28], angina [29], tachyarrhythmias [30–32], defibrillation energy requirements [33], ventricular refractoriness [34], and sudden cardiac death [35–37]. The circadian mechanism has been linked to a variety of internal and external factors driving this timing [38–45]. A classic internal factor is the circadian mechanism in cardiomyocytes, which has been demonstrated to regulate diurnal variation in infarct size in rodents subjected to myocardial ischemia reperfusion (I/R) [46]; findings of time-of-day variation in infarct size have also been clinically observed (e.g. Suarez-Barrientos et al. [47] and Reiter et al. [48]). However external factors also play key roles, and this review will especially focus on the novelty of the circadian influence on immune responses in MI.

Although many studies have identified inflammation as a critical pathogenic factor of MI (e.g. reviewed by Epelman et al. [49], Swirski and Nahrendorf [50], Nahrendorf et al. [51,52], Nahrendorf and Swirski [53], Nian et al. [54], Frangogiannis et al. [55], Frangogiannis [56], and Prabhu and Frangogiannis [57]), not much is known about the role of the circadian mechanism in regulating these inflammatory processes in the heart. Numerous studies have shown that the composition and rupture of atherosclerotic plaques, and subsequent obstruction of a coronary artery by a thrombotic clot, are mediated in part by inflammatory factors. Moreover, blockage of the coronary arteries resulting in infarcted myocardium then triggers a sterile immune response aimed at clearing away tissue debris in the infarct zone and helping to repair the heart. In addition, thrombolytic therapy or percutaneous coronary intervention, common medical procedures to re-establish blood flow to the damaged myocardium to reduce infarct size after MI, paradoxically trigger an adverse inflammatory response referred to as reperfusion injury [58,59]. However, even though many studies have investigated inflammation in MI, only recently has a critical role for the circadian system in regulating these responses come to light. This review will focus on the following:1) the circadian nature of inflammatory responses; and 2) recent studies elucidating a role for the circadian mechanism in regulating the critical, orderly, and temporal sequence of inflammatory processes involved in cardiac repair, especially in MI; 3) a role for the circadian mechanism in regulating cardiac immune responses in both male and female biological sexes is considered; 4) the circadian immune component of other major cardiovascular conditions is also briefly discussed. These and future studies will lead to a much deeper understanding of the pathophysiology of cardiovascular diseases. Moreover, given that the mammalian myocardium has limited regenerative capacity, it seems likely that these and future studies will also give rise to novel circadian immune–based therapies to benefit patients with cardiovascular disease.

The circadian nature of inflammatory responses

It has been known since the 1950s that circulating leukocytes exhibit diurnal rhythmicity. One of the earliest studies to demonstrate this in humans was by Bartter and Delea [60] , who collected blood samples over 30-h periods from healthy individuals, and showed diurnal rhythms in the abundance of circulating eosinophils, lymphocytes, and polymorphonuclear leukocytes. This day/night rhythm in circulating lymphocytes in healthy humans coincides inversely with plasma cortisol levels [61]. Moreover, not just cell abundance but also the rate of DNA synthesis of human lymphocytes was shown to vary in a 24-h cycle, by thymidine-2-^{14}C radio-incorporation assays *in vitro* [62]. These and other early studies on the rhythmicity of immune cells in humans were corroborated by extensive research using a wide variety of animal models (e.g. Halberg et al. [63], Pritchett and Reddy et al. [64], and Brown and Dougherty [65]).

Over the next several decades, investigators interrogated the rhythmic patterns of specific blood-cell subpopulations over 24-h cycles and their relationship to cellular function. For example, percentages of human blood mononuclear cells, T cells, and B cells fluctuate with a clear peak at night, followed by a depression in levels in the morning, as plasma cortisol levels start to rise [66]. Conversely, antibody-dependent cytotoxicity of purified lymphocytes shows a reverse profile that peaks in the day and troughs at night, a pattern which correlates with the 24-h fluctuations of lymphocytes with Fc receptors in the blood [66]. Rodents also exhibit a clear circadian rhythm in the levels of subpopulations of peripheral blood cells; however, notably, their rhythmic light (sleep):dark (wake) cycle is the reverse of that in humans, which is to be expected as these animals are nocturnal and humans are diurnal [67].

Many physiologic factors likely influence the diurnal rhythmicity of immune cell levels, including recruitment signals from endothelial cells, retention signals in the bone marrow and others. By the late 1990s, Born et al. [68] were using models of disrupted sleep to

better understand the factors that modulate immune cell cycling patterns. These studies revealed that sleep deprivation profoundly alters the rhythmic profiles of immunocytes in the blood and their production of various cytokines. Moreover, it was suggested that the altered abundance profiles resulted from redistribution of the number of cells between blood and extravascular tissue compartments and, intriguingly, that this could have significant implications for human health and disease [68]. Indeed, the daily rhythmic nature of the immune system and changes in rhythmicity in response to stimuli is now known to have many significant clinical implications, as was first extensively discussed by Haus and Smolensky [69]. Collectively, these and related observations set off a flurry of research over the next three decades, leading to many discoveries highlighting a critical role for circadian rhythms in immune system homeostasis and, especially, in areas with high clinical translational potential [70]. Research by our group and others has focussed specifically on the circadian influence of immune responses in MI, as is described in detail below.

Molecular circadian mechanism in immune cells

Although time-dependent variations of immune system parameters are now well-known and pervasive aspects of immunology, two important questions are asked: 1) whether individual immune cells or cell subtypes possess their own circadian regulatory mechanisms and 2) whether this affects disease pathophysiology. Keller et al. [71] addressed these questions by demonstrating that murine spleen, lymph nodes, and peritoneal macrophages contained intrinsic autonomous circadian clockwork mechanisms and showed that the rhythmicity underlies innate immune functions such as timing of elaboration of cytokines such as TNFα and IL6 . Moreover, Hayashi et al. [72] showed that macrophage functions such as phagocytosis and cytokine/chemokine production displayed a circadian rhythm that was regulated by autonomous circadian molecular machinery. To date, it has now been extensively described that immune responses are tightly controlled by the circadian mechanism and act as critical regulators of infectious and sterile inflammatory responses that drive disease outcomes (e.g. reviewed by Orozco-Solis and Aguilar-Arnal [73], Scheiermann et al. [74], and others). The followingsections propose in more detail how rhythmicity in immune cell function specially impacts cardiac repair after MI.

Circadian regulation of the NACHT, LRR, and PYD domains–containing protein 3 inflammasome following MI

MI is a leading cause of death worldwide, and this important clinical condition is especially dependent on inflammatory responses to mitigate outcomes. That MI is associated with profound inflammatory responses, some of which are beneficial to healing and some of which adversely exacerbate remodeling, has been extensively reviewed [49–57]. One of the adverse inflammatory responses that occurs shortly after MI involves the formation of a multiprotein complex termed the NACHT, LRR, and PYD domains–containing protein 3 (NLRP3) inflammasome [75]. This early humoral immune response acts as a sensor for damage signals following myocardial injury; however, NLRP3 inflammasome activation also leads to production of the cytokines interleukin (IL)-1β and IL-18, which collectively amplify immune responses in the heart leading to exacerbated myocardial damage [76–78]. Indeed, studies identifying selective NLRP3 inhibitors are eagerly awaited to reduce the adverse inflammasome activity; however, none are clinically available at the present time [79].

Intriguingly, the NLRP3 inflammasome and its constituent cytokine IL-1β are under the transcriptional regulation of REV-ERB [80–82] — a core component of the circadian clock mechanism. In recent studies, we have shown that pharmacologically targeting REV-ERB downregulates the adverse inflammasome activity and improves outcomes after MI [83]. That is, we administered the REV-ERB agonist SR9009, leading to decreased inflammasome activation, which in turn reduced inflammation in the infarcted myocardium, and resulted in virtually no scar formation.

Notably, chronotherapeutically targeting the inflammasome by administering SR9009 shortly after daytime infarction (zeitgeber time 06, ZT06, 6 h after lights on, rodent sleep time) had the greatest benefit on healing, consistent with the notion that the timing of SR9009 administration corresponded to the peak expression timing of its circadian mechanism target REV-ERB. Treatment for just one day was sufficient to abate the inflammasome — although administered once at ZT06 if the infarct occurred in the daytime and twice (ZT18, then ZT06) if the infarct occurred at night. Additional key findings of the study were that the drug could be administered *in vivo* to mice after MI, and alongside conventional reperfusion therapy.

Importantly, these studies introduce the notion that pharmacologically targeting the circadian mechanism, or "drugging the clock" can reduce the adverse inflammasome triggered early after MI, while leaving the reparative immune responses intact, thereby improving outcomes [83]. Ultimately, reduction of the inflammasome and its adverse cytokine storm can create a more favorable healing environment and better (reduced) remodeling after MI (e.g. reviewed by Nian et al. [54]).

Circadian rhythms in neutrophils in MI

There are circadian features of neutrophil biology that are fundamentally important to their cell structure and function and, especially, the rapid turnover of neutrophils in the blood. During granulopoiesis, oscillatory signals in chemokines and their receptors tightly regulate retention in the bone marrow and timed release of immature neutrophils to the bloodstream (reviewed by Aroca-Crevillen et al. [84]). As the neutrophils mature, they exhibit rhythmic patterns of cell-surface markers such as highly expressing CD62L which then progressively declines during the day, and the chemokine receptor CXCR4 which increases prior to clearance of neutrophils from the bloodstream into the body tissues [85].

Neutrophils display a time-dependent expression of core circadian mechanism components that is characteristically different than in other immunocytes and likely helps regulate pathomechanistic inflammatory responses [86].

Neutrophils are an essential part of the early innate immune response to MI injury, infiltrating ischemic myocardium within the first day after MI and influencing cardiac healing by removal of cell debris, and by promoting fibrotic scar formation (reviewed by Silvestre-Roig et al. [87] and Puhl and Steffens [88]). Intriguingly, the circadian mechanism may play a direct role in coordinating neutrophil responses after MI, as indicated from studies where deletion of the core gene *Bmal1* in neutrophils leads to altered temporal trafficking from the bone marrow to blood and tissues and impaired responsiveness in disease models [89]. Moreover, neutrophils accumulate more slowly and persist for a longer time in the myocardium of circadian mutant $Clock^{\Delta 19/\Delta 19}$ MI hearts than wild-type MI hearts [90], consistent with the notion that the circadian mechanism is a key factor regulating their infiltration to infarcted myocardium.

Importantly, biological sex appears to mitigate the circadian influence on neutrophils during MI. That is, the temporal neutrophil response differs in experimental studies of female versus male MI. For example, female mice given an MI during their wake time (zeitgeber time, ZT13) have greater neutrophil infiltration into infarcted hearts, and worse outcomes, as compared to female mice when MI was induced during their sleep time (ZT05) [91]. Intriguingly, our group showed a different neutrophil response phenotype in male versus female mice, that is, male mice given an MI during their sleep time had less neutrophil infiltration and reduced survivorship than the male mice given an MI during their wake time [92]. This is especially important because for a long time it has not been well recognized that cardiovascular pathophysiology differs in women in comparison to men. Indeed, we now know that women's heart health has been under-researched, under-recognized, under-diagnosed, and under-treated [93]. These experimental findings by our group and others on neutrophils reveal that males and females can exhibit differences in time-of-day inflammatory responses important for remodeling after MI. In light of these findings, it is worth highlighting that sex and gender should be considered in circadian based immunotherapies for cardiovascular disease (reviewed in Pyle et al. [94]).

Circadian rhythms in monocytes/macrophages in MI

The monocytes (in the blood) and macrophages (in the tissues) are important regulators of many cardiovascular disease pathologies, as has been recently reviewed (e.g. Fayad et al. [95], Lavine et al. [96], Moore et al. [97], Williams et al. [98]). Following MI, distinct populations of monocyte/macrophage cells infiltrate the heart over the first week, scavenging dead myocardium and mediating cardiac remodeling.

Efferocytosis — efficient clearance of apoptotic cells by monocytes — is considered a key factor in inflammatory resolution and tissue repair after MI [99]. The cellular circadian mechanism likely plays a key role, as studies have shown that macrophages exhibit robust expression of circadian genes such as *Bmal1*, and the circadian mechanism appears to drive their phagocytotic activity, elaboration of monocyte chemoattractant proteins, and temporal expression of cytokine mediators like IL-1b, IL-6, and TNFa [72]. From a cardiac repair perspective, $Ly6C^{high}$ monocytes exhibit diurnal oscillations in the blood regulated by the core circadian mechanism gene *Bmal1*, which acts as a transcriptional repressor of cell-surface chemokines that are involved in monocyte trafficking [100]. These diurnal variations in monocytes with cell-surface chemokine biomarkers appear important for MI healing, as mice given an MI at wake time have higher levels of cell-surface chemokine receptor 2 on circulating monocytes compared to noninfarcted controls, higher levels of CC chemokine ligand 2 protein, and more pronounced $Ly6C^{high}$ monocytes recruited to the infarcted myocardium than the hearts of mice infarcted during sleep time [101]. That is, circadian regulation of monocyte infiltration of infarcted myocardium likely influences cardiac repair and outcomes.

The time-dependant cascade of early inflammatory responses in cardiac repair is particularly relevant to healing after MI in modern hospital intensive- and coronary-care environments. Critically ill patients are often placed in multibedded rooms, and subject to frequent patient−staff interactions, noise, and light — especially at night. These disruptions affect circadian

rhythms and sleep and can impose an adverse effect on healing [102,103]. We recently showed in the murine MI model that short-term circadian disruption, for just the first few days after MI, alters immune cell recruitment (neutrophils, macrophages) to infarcted hearts, leading to increased infarct expansion and worse outcomes [90]. The circadian mechanism influenced their recruitment to the myocardium, as was demonstrated using circadian mutant $Clock^{\Delta 19/\Delta 19}$ mice [90]. Cardiomyocyte-specific loss of BMAL1, a binding partner of CLOCK, has also been shown to influence inflammation and modulate cardiovascular disease outcomes [104]. The key message is that maintaining normal circadian rhythms in the first few days after MI can promote better temporal inflammatory processes and improve healing.

It's important to note that the intrinsic clock in the cardiomyocyte cells, (not just the immune infiltrators), can also influence the degree of cardiac injury [46]. For example, MI tolerance exhibits a time-of-day dependence, which is mediated by the circadian clock mechanism, and influences infarct size and cardiac function after MI [46,105]. Thus there is a complex interplay between the intrinsic circadian clock in the heart cells, and the inflammatory cells such as neutrophils and macrophages recruited to the infarcted myocardium, that conspire together to set the foundation for scar formation after MI.

Circadian rhythms in adaptive immune responses in MI

T cells also play an important role in healing after MI. Experimental studies in rodents show that T cells are recruited to the infarcted myocardium peak by days 5–7 in permanent MI and by day 3 in ischemia reperfusion [106]. Differences in T-cell abundance are mirrored in clinical studies in humans, which reveal that ischemia followed by reperfusion via percutaneous coronary intervention after MI increases T-cell counts [107]. Interestingly, not only is T-cell abundance clinically relevant, but also the spatial recruitment of T cells to the infarcted versus the remote myocardium after acute MI [108].

However, to date there is little information about how the circadian mechanism influences T-cell responses. T cells do appear to possess rhythmic circadian mechanisms which may help to regulate their functions. For example, human CD4+ T cells demonstrate robust rhythms in circadian gene expression *in vitro* along with robust cytokine expression following stimulation and circadian luciferase reporter activity in murine CD4+ cells and in thymic sections; these findings are consistent with the notion that the T-cell endogenous circadian mechanism helps to orchestrate immune responses in a time-dependent manner [109].

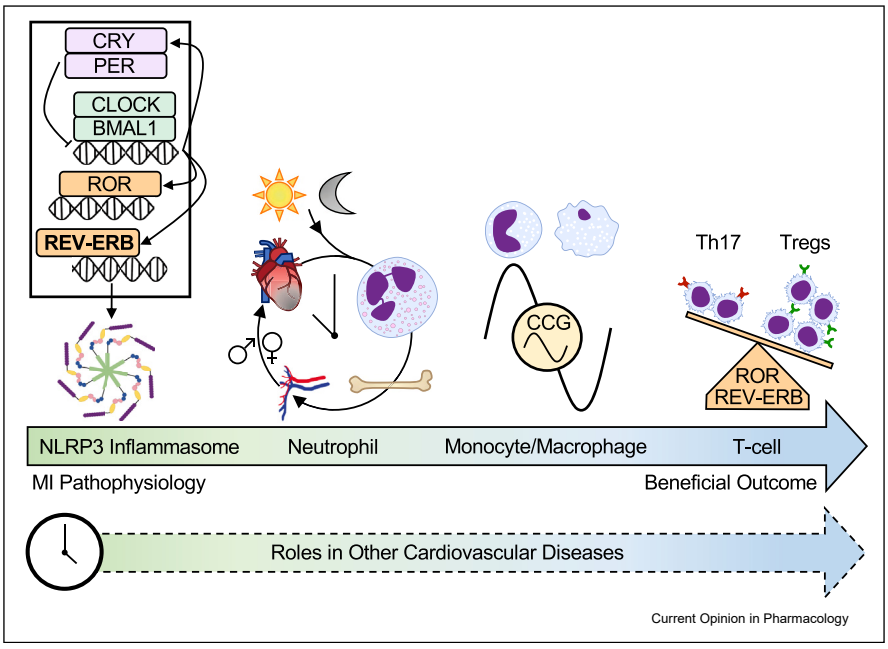
One way in which the circadian mechanism may link to T-cell pathophysiologic responses important for MI is via adrenergic pathways. For example, time-of-day may influence T lymphocyte β2-adrenergic receptor density in patients with coronary disease, with possible implications for inflammatory responses after MI [110]. It has also been demonstrated that the circadian mechanism–regulated nuclear factors REV-ERB/ROR are master regulators for CD4+ proinflammatory T-helper (Th17) cell development. The nuclear receptors orchestrate differentiation of Th17 cells from naïve CD4+ T cells in mice [111,112] and in humans [113].

It is tempting to speculate that drugs targeting the circadian mechanism are a new frontier for modulating the T-cell phenotypes and improving outcomes after MI. Consistent with this notion, the synthetic circadian mechanism ROR inverse agonist SR1001 inhibits Th17 cell differentiation and suppresses the clinical severity of autoimmune disease in mice [114]. In addition, the synthetic ROR ligand SR1555 has been shown to target both suppression of Th17 cells, while stimulating T-regulatory cells [115]. These findings are clinically intriguing for therapeutic application to MI, as it is thought that the composition of T-cell phenotypes in the infarcted myocardium can influence outcomes. Indeed, a deficiency in the Th17 axis improves outcomes in mice after MI [116], while increasing T-regulatory cells benefits wound healing after MI [117]. Future studies targeting the circadian nature of T-cell responses seem warranted, and, with the recent discovery of synthetic drugs targeting the circadian mechanism and T-cell biology, this could be a promising new circadian medicine approach to improve outcomes after MI.

Future perspectives

Although this review focusses on cardiac repair after MI, there are many other cardiovascular disease conditions in which a circadian influence on inflammation could also play a critical pathophysiological role; several relevant conditions are summarized herein. 1) Atherosclerosis [118], vascular biology [119], stroke [120], and chronic heart failure [121] all have inflammatory processes that are fundamentally important to their disease processes. Mounting evidence suggests that the circadian system is also involved (e.g. reviewed by Steffens et al. [122]). 2) Viral myocarditis is a leading cause of acquired heart disease in children and commonly progresses to dilated cardiomyopathy and heart transplant in adults. The disease pathophysiology is a complex interplay involving coxsackievirus B3 activation of an adverse immune response that is damaging to the heart (e.g. reviewed by Martino et al. [123], Liu et al. [124], and Ayach et al. [125]). A circadian rhythm in susceptibility of mice to coxsackievirus B3 infection has been previously reported [126], and it seems likely that

Figure 1



The circadian mechanism influences humoral, innate, and adaptive immune responses after MI. The NLRP3 inflammasome is under transcriptional regulation of REV-ERB — a core component of the circadian mechanism. The neutrophils exhibit time-of-day patterns during granulopoiesis in the bone marrow, upon release into the bloodstream, and of migration into the myocardium after MI. Biological sex mitigates the circadian influence on neutrophil responses. Monocyte/macrophage recruitment to infarcted myocardium is influenced by the circadian mechanism and clock-controlled genes (CCG). The recruitment of circadian regulated T-cells can influence outcomes by promoting more reparative phenoytpes. This review highlights the circadian influence on immune responses during MI; however, the circadian mechanism likely directs inflammatory processes vital for other major cardiovascular conditions as well. MI - myocardial infarction; NLRP3 - NACHT, LRR and PYD domains-containing protein 3; REV-ERB - nuclear receptor subfamily 1 group D member 1/2; CCG - clock controlled genes.

circadian modulation of inflammatory responses could underlie this periodicity and would be worthy of further investigation. 3) Obstructive sleep apnea (OSA) is a common night-time respiratory disorder that when untreated is frequently associated with cardiovascular disease and adverse outcomes. The current literature suggests that inflammation plays a role in the disease pathophysiology (e.g. reviewed by Alibhai et al. [13], Martino and Young [14], Ayas et al. [127], Bradley and Floras [128], Floras [129], and Somers [130]). It is interesting that nocturnal treatment of patients with OSA — continuous positive airway pressure therapy at night — alters inflammatory profiles and improves outcomes. Future experimental studies are needed to demonstrate a direct link between the circadian mechanism and inflammation in OSA; these investigations could lead to significant benefits for patients with sleep disorders and, especially, those with comorbid cardiovascular disease. 4) Shift work is a common occupational disruption of circadian rhythms [131], with mechanistic underpinnings related to inflammation that increase cardiovascular disease risk and worsen outcomes (e.g. Refs. [40,132–136]). Indeed, simulated night-shift work [137], or long-term exposure to night-shift work [138], is associated with dysregulation of the immune system in humans. It is thought that one consequence of the compromised immune responsiveness is that it may make shift workers more susceptible to disease. Relevant to current events, it has even been postulated that shiftwork may increase susceptibility to the 2019 novel coronavirus (COVID-19) [139], a pathogen associated with profound cardiovascular complications [140].

Summary

This review highlights a novel role for the circadian mechanism in regulating inflammatory processes critical to the pathogenesis and pathophysiology of cardiovascular disease (Figure 1). We focussed on circadian aspects of the humoral, innate, and acquired immune responses to MI, a leading cause of morbidity and mortality worldwide. We also explored a role for circadian regulation of inflammation in other major cardiovascular diseases. It seems very likely that future investigations will shed new understanding on cardiovascular health and disease. Moreover, future studies will give rise to promising novel circadian medicine therapies for clinical cardiology, helping patients live longer and healthier lives.

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