Circadian rhythms in adaptive immunity

DOI:
10.1111/imm.13167

Document Version
Accepted author manuscript

Immunology
The journal of cells, molecules, systems and technologies
British Society for immunology

Circadian rhythms in adaptive immunity

Journal:	*Immunology*
Manuscript ID	IMM-2019-5345.R1
Wiley - Manuscript type:	Review
Date Submitted by the Author:	n/a
Complete List of Authors:	Downton, Polly; The University of Manchester, Division of Diabetes, Endocrinology and Gastroenterology, School of Medical Sciences, Faculty of Biology Medicine and Health Early, James; The University of Manchester, Division of Diabetes, Endocrinology and Gastroenterology, School of Medical Sciences, Faculty of Biology Medicine and Health; Royal College of Surgeons in Ireland, School of Pharmacy and Biomolecular Sciences Gibbs, Julie; The University of Manchester, Division of Diabetes, Endocrinology and Gastroenterology, School of Medical Sciences, Faculty of Biology Medicine and Health
Key Words:	T cell, B Cell, Vaccination, Trafficking

Circadian rhythms in adaptive immunity

Downton P*, Early JO* and Gibbs JE

*These authors contributed equally

Abstract

The circadian clock provides organisms with the ability to track time of day, allowing them to predict and respond to cyclical changes in the external environment. In mammals this clock consists of multiple auto-regulatory feedback loops generated by a network of circadian clock proteins. This network provides the fundamental basis for rhythms in behaviour and physiology. This clockwork manchinery exists in most cells, including those of the immune system. In recent years evidence has emerged highlighting the important role of molecular clocks in dictating the response of immune pathways. While initial work highlighted the effect of the clock in the 'first line of defence', the innate immune system, it has become increasingly apparent that it also plays a role in the more tailored, later stage adaptive immune response. This review provides an overview of the role of the circadian cycle in the adaptive immune response. We interrogate the depth of knowledge on cell intrinsic clocks within adaptive immune cells and how these cells may be temporally directed by extrinsic rhythmic signals. We discuss the role of the circadian clock in diseases associated with adaptive immunity such as multiple sclerosis, asthma, and parasitic infection. We also discuss the current knowledge on timing of vaccination, and implications this may have on how we can harness and modulate temporal gating of the adaptive immune response in a clinical setting.

The circadian clock

The vertebrate circadian clock permits an organism to entrain its cellular and physiological processes to regular environmental changes, allowing it to adapt behaviour predictively to confer a survival advantage. Numerous physiological processes are regulated by internal clocks, including body temperature, sleep-wake cycles, feeding and metabolism. Circadian processes oscillate with a period of circa 24 hours, and can continue to oscillate in the absence of entraining stimuli. The mammalian clock is most commonly entrained by light, via input to the hypothalamic suprachiasmatic nucleus (SCN), or 'master clock', from the retina[1]. Cell autonomous peripheral clocks have been identified in numerous different cell and tissue types; the SCN signals through the autonomic nervous system and hormonal secretion to entrain the peripheral clocks and ensure a synchronised response to the diurnal environment[2]. Temperature, food intake and other regularly varying factors can also entrain circadian behaviour[3]. When constrained by an external entraining stimulus, such as light, times experienced by an organism are described in terms of 'zeitgeber time' (ZT). Under standard diurnal 12 hour light:12 hour dark cycles, ZT0 refers to the time at which lights are turned on and ZT12 refers to the time at which lights are turned off. Diurnal

animals, such as humans, are typically most active during the 'daytime' phase after ZT0, whereas nocturnal animals are most active during the 'nighttime' phase after ZT12. If the components of the circadian clock are intact, mammals are able to maintain robust circadian oscillations in activity and physiological processes for long periods of time even in the absence of entraining stimuli.

The molecular basis of the mammalian cellular clock is encoded by several transcription/translation feedback loops which, when intact, regulate the periodic expression of downstream target genes [4]. The core clock consists of several genes which encode transcription factors, including the activators BMAL1 and CLOCK, and the repressors PER1, PER2, CRY1 and CRY2. BMAL1 serves as the master regulator of the circadian cycle and is the only single gene deletion that results in complete ablation of rhythmic circadian activity[5]. BMAL1 and CLOCK bind as a heterodimer to promoters containing E-box sequence elements, including the promoters of the repressive *Cry*, *Per* and *Nr1d1/2 (Rev-erb* α/β*)* genes. These proteins accumulate then translocate into the nucleus, where they act to repress transcription of *Bmal1* and *Clock,* removing the positively-acting input into their own transcription. Over time these proteins are targeted for degradation, resulting in release of repressive control. This interaction network sets up oscillatory transcription of the positive and negative regulators, with different clock transcripts and proteins showing peaks of expression at different times during the circadian cycle[6].

Both the positive and negative arms of the molecular clock have numerous genomic targets, linking the clock to the regulation of rhythmic cellular processes. These include DNA damage repair, redox, signalling and cellular metabolic pathways[7]. Analysis of circadian transcription factor binding and chromatin landscape has identified rhythmic changes in transcriptional architecture[8], and genome-wide profiling has found a substantial proportion of the genome to be under circadian regulation at the transcriptional and/or post-transcriptional level [9-12]. The timing of expression depends upon the specific combination of circadian inputs acting upon the promoters and enhancers of the target genes. Integration with regulation by other signalling systems, such as inputs from the neuroendocrine axis via the glucocorticoid and other hormone receptors [13, 14], or tissue-specific transcription factors, adds a further layer of complexity to circadian target regulation to allow specific tuning of cell function and physiology.

The mammalian immune response

The immune system is a complex organisation of many biological structures, such as the lymph system, bone marrow, thymus, spleen, and a wide variety of immune cell types that each possess their own function and localisation within the body. The immune system protects against infectious agents and aids wound healing. It can be separated into two core aspects: innate and adaptive immunity. The innate immune system has evolved to

rapidly respond in a non-specific manner to a wide array of pathogens; however, the range of molecular patterns it can detect is limited [15]. This is further complicated by the ability of pathogens to rapidly mutate to avoid detection. This has led to a constant struggle between host and pathogen, driving the evolution of the adaptive immune system in response to increased variation in microorganisms [15]. While the recognition receptors of the innate immune response are encoded in their mature functional form in the germline genome, the adaptive receptors are created and tailored to specific antigens via somatic recombination of gene segments [15]. Upon successful interaction with a pathogen, these new receptors are stored and can persist in the organism for life. This allows for immunogenic memory and the ability to respond rapidly to future re-exposure [16].

The cells of the adaptive immune system consist of two forms of highly mobile lymphocytes, derived from haematopoietic stem cells in the bone marrow (Figure 1). T lymphocytes mature in the thymus and are the effector cells of the adaptive immune response. B lymphocytes mature in the bone marrow and produce antibodies [15]. Following maturation, lymphocytes migrate to secondary lymphoid organs, such as the lymph nodes and spleen. For effective activation of the adaptive immune response, naïve T cells must be activated by antigen presenting cells (APCs). Infection of a tissue leads to the stimulation of pattern recognition receptors (PRRs), resulting in the generation of cytokines, which results in maturation of APCs including dendritic cells (DCs). Mature DCs then migrate to the secondary lymphoid organs where they can interact with naïve T cells [17]. Following successful interaction, the naïve T cell rapidly proliferates and differentiates into effector CD4+ T lymphocytes (Figure 1). These cells produce multiple cytokines that activate immune cells such as macrophages and CD8+ T cells to destroy the infection. They also prime B cells to generate antibodies to target pathogens for destruction [17]. After effective pathogen clearance, some B and T cells undergo differentiation into memory cells, which persist for a long period of time in lymph nodes and peripheral circulation, to confer protection against future reinfection, rapidly differentiating into effector cells to provide a more efficient and tailored response [18].

Circadian rhythms in the adaptive immune response

While we now know that clock genes exist within T and B cells [19, 20], very few studies have been carried out to fully characterise their influence on immune function. Clock genes are rhythmically expressed in mouse lymph nodes [21, 22], and this rhythmicity is lost in circadian mutant mice [21]. Circadian clock proteins have been shown to be important in the differentiation of T cells. Knockout of RORα or RORγ impairs T_h17 cell development. Double knockout results in ablation of T_h17 development [23, 24]. CD4+ T cells purified from human blood over the course of the circadian day have been shown to display rhythmicity in clock gene expression [20]. Rhythmic clock gene expression can be maintained in CD4+ T

cells cultured *in vitro*, and CD4+ T cells isolated from PER2::luciferase mouse spleen and thymus display rhythmicity in bioluminescence [20]. Finally, B cells isolated from the spleens of mice housed under a 12:12 light dark cycle or under constant darkness show rhythmicity in clock gene expression [19]. Whilst work has been carried out on CD4+ T cells and B cells, it is unknown whether CD8+ T cells express independently rhythmic clock components, nor whether individual subsets of T and B lymphocytes exhibit variation in the robustness of their rhythms

It has previously been shown that many leukocytes display variation in number and localisation across the circadian day in both mouse and human. In humans, B and T cells are found to be increased in the circulation at night, dropping in level throughout the day as they undergo extravasation [25-28]. The decrease in T cells during the morning has been linked to the rise in cortisol typically experienced at this time. It also coincides with increased CXCR4 expression within the T cells [26, 28]. CXCR4 is a receptor which binds the chemokine CXCL12. Blocking cortisol decreases T cell expression of CXCR4, inhibiting extravasation of T cells from the blood vessels and preventing the morning decline in circulating T cell number [26]. The clock and CXCR4-mediated migration has also been studied in the context of humanised mouse models, in which immune-compromised mice are transplanted with immune cells of human origin (CD45+ leukocytes, including the subsets CD3+ T cells and CD19+ B cells) [29]. The circulation of mouse and human immune cells displayed opposite rhythms in these mice, with mouse CD45+ cells peaking at ZT7 and human CD45+ cells peaking at ZT19, indicating a prominent role of a cell intrinsic molecular clock to allow these cells to differentially regulate their circadian rhythms in the same *in vivo* environment.

A recent study examining rhythmic homing and egress of lymphocytes through lymph nodes has shown an alternative mechanism for lymphocyte trafficking [30]. While CD4+, CD8+ and B cells peak in circulation at approximately ZT5 in mice, they reach their highest number in lymph nodes at ZT13 [30]. Adoptive transfers of ZT5 or ZT13 lymphocytes into animals at opposite times resulted in a dampening of rhythmicity in lymphocyte trafficking, highlighting the importance of both cell intrinsic and microenvironment states. A screen from the same paper found that CCR7 displayed rhythmicity in T and B cells, peaking at ZT13, while CXCR4 displayed significant rhythms in CD4+ T cells, but not in CD8+ or B cells. Analysis in lymph nodes also showed mRNA of the CCR7 ligand, CCL21 cycling, but not the CXCR4 ligand CXCL12. *Ccr7*$^{-/-}$ mice exhibited no oscillations in lymph node cell count and *Ccr7*$^{-/-}$ cells do not display rhythmic lymph node homing. Rhythmic expression of CCR7 was lost in *Bmal1* deficient CD4+ T cells. Overall we see that both microenvironment and intrinsic lymphocyte clocks play a key role in lymphocyte cell trafficking [30, 31]. More recently, the same group has found the rhythmic expression of pro-migratory molecules by leukocytes is dependent upon lineage and environment, and confirmed inverse rhythms in leukocyte homing in humans compared to mice [32].

Given the clear role of circadian proteins in regulating cells of the adaptive immune response, there is a need to characterise the extent of this effect in disease. This is a growing area of research and some of the implications and applications of recent discoveries in this area are discussed in the following sections.

Vaccination timing and adaptive immunity

Vaccinations take advantage of the adaptive immune system to generate a T cell mediated response to a specific target antigen. Vaccination introduces a small amount of the foreign antigen, typically in a dead or attenuated form, to deliberately stimulate an immune response and generate a cache of memory T cells capable of responding swiftly to the antigen during future encounters. This introduces protection for the vaccinated individual by allowing the immune system to mount a rapid secondary response when needed.

As the cellular components mediating adaptive immunity are subject to circadian regulation, effective activation of a response to vaccination may also be under the control of the clock (Figure 2). Effective induction of memory cell development in response to antigen treatment is more likely to occur if the cells involved are numerous and located in close proximity to necessary interacting factors. Response magnitude over several weeks correlates with the population size of naïve CD4+ cells, meaning activation of more cells can result in a stronger response[33]. Several studies have now found evidence that both T cell and B cell response to vaccination varies with time of day, although some discrepancy exists between the optimal time for inoculation in different experimental models. Injection of DCs loaded with ovalbumin (OVA) into mice at ZT6 or ZT18, followed by quantification of the number of OVA-specific induced memory T cells seven days later, found that around twice as many CD8+ cells were found in the spleen following daytime injection [21]. The proportion of these cells that were activated and producing IFNγ was also increased. More recent experiments have found that both follicular T helper (Tfh) cells and germinal centre (GC) B cells appear to be induced more strongly by night time (active phase) immunisation protocols. Intradermal immunisation of mice with a soluble antigen at ZT5 or ZT17 found antigen-specific antibody titres to be higher in the weeks following treatment at ZT17, and also resulted in higher GC B cell and Tfh cell numbers in lymph nodes after one to two weeks [34]. Similar results were found following immunisation of mice with OVA at either ZT4 or ZT16 [35]. In both cases, one mechanism of circadian regulation may be modulation of the signalling environment. Suzuki *et al*. found that differential response in their system was dependent upon intact adrenergic enervation, and could be abrogated by depletion of peripheral adrenergic nerves with 6-hydroxydopamine or knockout of the β_2 adrenergic receptor. The circadian differential effect seen in response to OVA immunisation by Shimba *et al.* was impaired by CD4+ cell-specific knockout of the glucocorticoid receptor (GR), which

also seemed to regulate differentiation of naïve T cells into Th1 and Th2 memory T cell subsets.

Innate immunity interacts with adaptive immunity in a number of ways, including through PRR-mediated upregulation of components needed for efficient antigen presentation and recognition [36]. This effect can be harnessed to improve vaccine efficacy. The most notable method of doing this is by including an innate immunity potentiator as an adjuvant in the vaccine preparation [37]. Toll-like receptor 9 (TLR9), a PRR expressed by innate immune cells which recognises viral and bacterial DNA including CpG oligodeoxynucleotides (CpG ODN), is under circadian regulation and can act as an adjuvant to enhance adaptive immune responses [38]. Mice immunised with OVA in combination with CpG ODN (to activate TLR9) at either ZT7 or ZT19 showed enhanced lymphocyte proliferation four weeks later if immunisation occurred at ZT19. IFNγ production was also increased. This effect was specific to TLR9-activating ligands.

These differences in vaccine response, even weeks after administration, have implications for efficacy and best clinical practice but remain relatively unstudied. The majority of the mouse studies discussed find enhanced adaptive immune responses to vaccination when administered during the early/mid active phase for mice (dark phase; ZT16 to ZT19, depending upon study). This would correspond to immunisation of human subjects in the morning. To date, few formal clinical trials have compared the benefits of morning verses afternoon vaccine administration in humans. One study has suggested antibody titre is increased by around twofold when measured four weeks after influenza vaccination or hepatitis A vaccination administered in the morning compared to the afternoon, but only for some groups of participants [39]. A larger trial conducted on adults aged 65 or older also found increased antibody titre one month after influenza vaccination when it was administered during a morning clinic compared to an afternoon clinic [40]; cortisol level was higher in blood samples taken at the time of morning vaccination, highlighting at least one potential source of difference between vaccination conditions. Several studies have found reduced vaccination efficacy in individuals suffering from chronic insomnia [41] and those subjected to sleep deprivation protocols [42], and a number of immune parameters show sleep-dependent circadian oscillation [43]. This further supports the idea that the interaction of the clock with immune response may modulate the induction of an adaptive response, and suggests clinical considerations for optimising vaccination efficacy.

Parasites and the clock

Parasite infections stimulate multiple immune defence mechanisms, both antibody and cell mediated. Antigen presenting cells detect the presence of parasites, mediated via PRRs. This early event shapes the phenotype of the adaptive immune response, driving CD4+ cells to mature into different subtypes (see Figure 1). These subtypes (T_h1, T_h2, T_h17, T_{reg}, Th_9

and Th_{22}) secrete different networks of cytokines, to allow effective resolution of disease. For example, in the case of the gastro-intestinal helminth *Trichuris*, effective expulsion from the host relies on polarisation of CD4+ T cells towards a T_h2 immune response, promoting production of parasite-specific IgG1. Recent work has implicated the involvement of the circadian clock within DCs to drive T cell polarisation in the most appropriate direction [44].

Hopwood and colleagues described how the time of day at which a mouse is inoculated with *Trichurs muris* eggs affects antigen production and efficiency of expulsion several weeks later. Inoculation at ZT0 was associated with higher circulating levels of parasite specific IgG1 and IL-13 (typical markers of a T_h2 response) and more rapid expulsion. Inoculation at ZT12 resulted in slower expulsion and a higher worm burden. Deletion of *Bmal1* in CD11c+ DCs rendered them arrhythmic and resulted in loss of circadian control over expulsion kinetics, suggesting a critical role for the DC clock in polarising T cells. RNA-seq data from circadian synchronised DCs stimulated with parasite antigen identified time of day differences between the expression of genes associated with T_h1 polarisation (*Il12b* and *Tnfsf15*). These pathways were down-regulated in the absence of *Bmal1*, suggesting BMAL1 may be critical for the establishment of DC-derived T_h1 promoting cytokines.

Robust rhythmic circadian behaviour persisted in mice infected with *Trichurs muris* [44]. In contrast, infection with *Trypanosoma brucei*, a unicellular parasite which invades the brain and causes Sleeping sickness, has profound effects on circadian behaviour in mice and humans [45]. Mice infected with *Trypanosoma brucei* show shortening of the period of their circadian rhythms, with a significant increase in daytime activity and peak body temperature during the day. Intriguingly, addition of trypanosomes to explants of healthy (non-inflamed) murine tissue shortens the period, potentially a consequence of a secreted factor from the parasite. These data reveal for the first time evidence that parasites may directly interact with the clockwork machinery, and thus the parasites themselves (rather than infection associated host responses) may be responsible for the alterations in sleep behaviour that are characteristic of Sleeping sickness.

Malaria infection has also been shown to disrupt host circadian rhythms in behaviour and physiology [46]. Mice infected with the murine malaria parasite *Plasmodium chabaudi* exhibit notable reductions in both locomotor acitivy and body temperature during the nightime. Interestingly, the extent of this disruption is dependent on the genotype of the parasite. Prior and colleagues propose that the effects of malaria infection on circadian rhythms could be a consequence of several factors, including direct or indirect effects on the SCN; alterations in the perception of zeitgebers; or a consequence of reduced resources (e.g. energy) leading to inability to follow instructions provided by the clock.

Further interaction between malaria parasites and host circadian rhythms have been identified. Malaria parasites develop synchronously within the hosts, with each

developmental stage occurring at a specific time of day. Cycles in *P. chabaudi* development last 24h with the burst of progeny into the host blood timed to coincide with the middle of the night when mice are most active. Studies carried out by Prior *et al* [47] and Hirako *et al* [48] demonstrate that these cycles in asexual replication are driven by host feeding entrained rhythms (rather than light-entrained rhythms). Inverting the feeding schedule so that it becomes out of phase with the light-dark cycle results in inversion of development rhythms of the parasites. Both studies suggest that parasites respond to the rapid increase in blood glucose levels which occurs soon after the onset of feeding. Thus parasites may use daily fluctuations in glucose availability as a zeitgeber to time cycles in their development to the host's endogenous rhythms.

Autoimmune disease

While the adaptive immune system is a crucial element in the battle against invading pathogens, it can also give rise to autoimmunity. In such cases, an abnormal host immune response is generated against a normal system or body part. The causes of such disorders remain largely unknown; however, the strongest influences appear to be genetics, infection, and environmental factors [31]. Interestingly, circadian rhythms, or their disruption, have been detected in several autoimmune disorders such as rheumatoid arthritis, asthma, and multiple sclerosis [49].

Multiple sclerosis

Multiple sclerosis (MS) is the most common immune-mediated disorder to affect the CNS [50]. The disease is mediated by T cells which mount an immune response against myelin [51]. While there are several types of the disease, they all feature demyelination of the neurons in the brain and spinal cord. After the initial response against myelin, other immune cells are activated, resulting in breakdown of the blood brain barrier, cytokine release and immune cell infiltration. This increases loss of myelin and dampens neuronal signalling [51]. Interestingly, several studies have linked the pathology of MS to circadian and seasonal cycles. It has been shown that cases of relapse of the disease occur more frequently in spring and summer, perhaps attributable to lower levels of melatonin, a clock regulated hormone [52]. Prevalence of MS varies with geographic latitude and geographically associated SNPs have been found in *Bmal1* and *Clock* that may increase the risk of MS [53]. A population study approach has revealed that teenagers who have experienced circadian disruption through shift work have increased likelihood of developing MS in later life [54].

MS and T cell mediated autoimmune disease can be studied using the experimental autoimmune encephalomyelitis (EAE) model of brain inflammation. This has been particularly useful in isolating the circadian components linked to this disease (Figure 3). Initial experiments in BMAL1-deficient lymphocytes found no significant alterations in the

differentiation, function or inflammatory output of these cells, such that T cell specific *Bmal1* deletion had no impact on EAE susceptibility or disease severity [55]. However, these experiments were not carried out at different times of day. A more recent study displayed increased severity of EAE and increased demyelination when mice were induced at ZT8 versus ZT20 [30]. This result correlated with increased IL-2 expression as well as increased counts of IL17+ VLA-4+ and CD4+ T cells in lymph nodes. Interestingly, in T cell specific *Bmal1*$^{-/-}$ mice, time of day variation in EAE disease severity was lost. Similarly, there was a loss of diurnal alteration in CD4^{+} and CD8^{+} T cell counts in lymph nodes following two days of EAE induction. While there was no alteration to overall inflammation with loss of *Bmal1*, the loss of circadian input to the disease affected severity and cell trafficking, highlighting the importance of testing multiple time points when carrying out experiments on circadian clock proteins [30].

Conversely, myeloid *Bmal1* deletion does appear to influence EAE severity. A time of day variation was noted in severity of EAE, with mice induced at ZT6 displaying greater severity than those injected at ZT18 [56]. Possible circadian disruption was detected in mice undergoing EAE, as *Bmal1* and *Nr1d1* expression were downregulated in the spinal cords of EAE mice, inversely correlating with increased expression of proinflammatory cytokines and chemokines. When *Bmal1* was deleted in myeloid cells, a loss in diurnal variation was noted and the myeloid *Bmal1* knockout mice presented with increased disease severity [56]. Loss of BMAL1 resulted in a more pro-inflammatory environment in the CNS, facilitating increased infiltration of IL-1β secreting CD11b+Ly6C^{hi} monocytes and resulting in increased pathogenic IL17+/IFNY+ T cells in the CNS [56]. Deletion of *Bmal1* in T cells appears to attenuate EAE pathology and deletion in myeloid cells exacerbates this response, highlighting the possibility that BMAL1 in the myeloid lineage is more specifically protective for auto-immunity. Other recent studies have also found increased EAE severity in REV-ERBα knock-out mice, with increased infiltration of CD4+ T cells into the CNS at the peak of disease [57]. Treatment with REV-ERBα agonists reduced the severity of EAE progression. Double knockout of of RORα and RORγ protects mice from EAE by ablating Th17 cell development [23].

Asthma

Asthma is a pulmonary inflammatory disease which causes airway hyperresponsiveness and obstruction. It is associated with T_h2 cells, which orchestrate inflammation by releasing pro-inflammatory cytokines that promote mast cell and eosinophil maturation and survival, enhance basophil recruitment and promote B cell isotype switching to IgE synthesis [58]. Differentiation of naïve CD4+ cells to pro-inflammatory T_h2 cells is a consequence of presentation of aeroallergens (such as those derived from house dust mites, animal fur and pollens) to airway DCs.

Established asthma is well known to show diurnal variation in disease symptoms [59]. Symptoms often become more exacerbated in the early morning, and circadian variation in lung function becomes more pronounced in patients with nocturnal asthma. There is evidence that markers of airway inflammation show circadian variation, with numbers of alveolar macrophages, eosinophils, neutrophils, and CD4+ T cells heightened during the early morning [59-61]. Recent work by Durrington *et al*. [61] highlights the critical importance of fully mapping these daily changes in disease biomarkers, such as sputum eosinophils, as they are often used as indicators of disease severity, and thus can dictate application of therapeutics. The cause of circadian oscillations in inflammatory cell numbers within the asthmatic lung is unclear, but murine studies show that *Bmal1* deletion in cells of a myeloid lineage results in enhanced eosinophil recruitment in an ovalbumin model of allergic asthma [62], suggesting the clock within myeloid cells may be involved.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease causing inflammation and destruction of the joints. Like asthma, patients often exhibit diurnal variation in their symptoms, with joint pain and stiffness heightened in the morning [63]. This correlates with increased levels of circulating pro-inflammatory cytokines [64]. Circadian changes to circulating metabolites are also observed[65]. This diurnal rhythmicity is mirrored in a murine model of inflammatory arthritis, collagen induced arthritis, where joint inflammation is actively repressed during the night-time [66]. Furthermore, animals lacking a functional clock (*Cry1*$^{-/-}$ *Cry2*$^{-/-}$ mice) exhibit a more pronounced phenotype in a collagen antibody driven model of inflammatory arthritis [67]. Joint inflammation in RA results from complex interactions between cells of the adaptive immune system, the innate immune system (including macrophages, neutrophils and DCs) and resident joint cells (fibroblast-like synoviocytes and osteoclasts). The cellular source(s) of the rhythmic inflammatory signal has yet to be elucidated, and the contribution of innate immune cells to this diurnal variation is currently unclear.

While more work is required to tease apart the interactions between innate and adaptive immune clocks, it is clear from the literature that the clock is heavily involved in the adaptive immune response.

Conclusions

The molecular clock has considerable control over the immune system. Whilst work on circadian inflammation to date has focused on the more rapid innate immune response, it is becoming clear that clock proteins also exert considerable influence over the adaptive immune response, either directly through cell intrinsic clocks, or through circadian regulation by the surrounding microenvironment. Knowledge of these processes provides us

with the tools to understand chronic inflammation and provide clinical benefit, by aiding the immune response to clear pathogens, improving the efficacy of interventions such as vaccination, and uncovering potential mechanisms that give rise to autoimmune diseases such as multiple sclerosis. With the emergence of new methods to study circadian rhythms, and with the field itself becoming more influential and openly discussed in mainstream immunology, it is likely many more useful findings will be uncovered, furthering our knowledge of circadian mediated regulation of adaptive immunity.

Figure legends

Figure 1: Cells of the adaptive immune system. Cells of the adaptive immune system are derived from haematopoietic stem cells in the bone marrow. B cells mature in the bone marrow, whilst T cells mature in the thymus. T cells can be classified as $CD8^+$ (cytotoxic) or $CD4^+$ (helper). $CD4^+$ T cells can be further classified into subsets (including T_h1, T_h2, T_h17 and Tregs) according to protein expression and function. After an encounter with an antigen, T cells may transform into long-lived memory T cells. B cells and $CD4^+$ T cells have been shown to possess intrinsic clockwork machinery.

Figure 2: Circadian regulation of immunisation efficacy. Vaccination efficacy depends upon how well T cells are activated by APCs following exposure to the target antigen. Activation occurs when the target antigen is presented to a T cell expressing an appropriate recognition receptor in complex with the MHCII receptor and secondary signaling via CD28 and CD80/86. Oscillatory changes in the signals driving cell migration to the lymph nodes result in circadian differences in the number of T cells and APCs present during active and rest phases of the day, meaning such interactions are more or less likely to occur effectively. When effective activation occurs, it results in the proliferation of helper (CD4+) T cells, stimulation of a specific adaptive response by T cells and B cells, and the production of memory cells to protect against future infection.

Figure 3: Diurnal variation in EAE severity. Time of EAE induction has a profound effect on immune parameters and function.

Table 1: Adaptive immune phenotype of circadian mutant mice.

Abbreviations

APC antigen presenting cell; GC germinal centre; GR glucocorticoid receptor; OVA ovalbumin; PRR pattern recognition receptor; SCN suprachiasmatic nucleus; TfH T follicular helper; TLR toll Like receptor; ZT zeitgeber time.

Acknowledgments

JEG is a Career Development Fellow Versus Arthritis

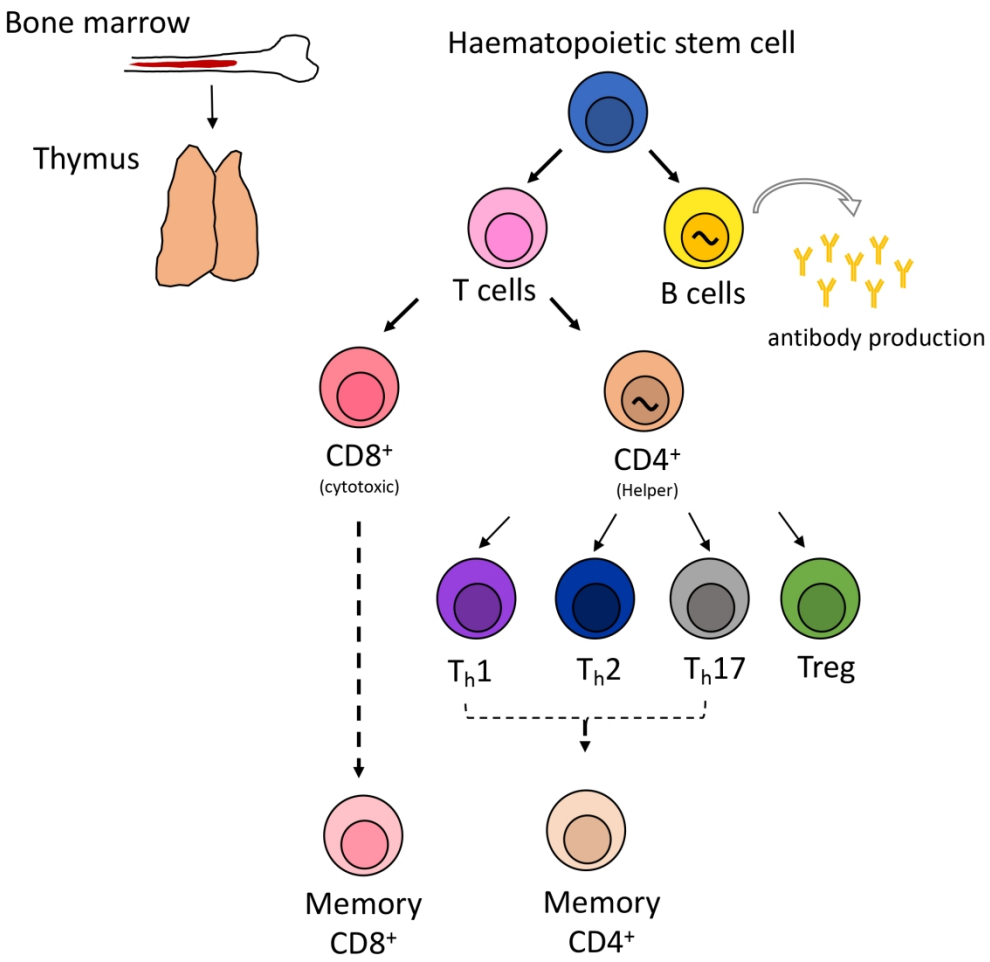
References

1. Brown, T.M. and H.D. Piggins, *Electrophysiology of the suprachiasmatic circadian clock.* Prog Neurobiol, 2007. **82**(5): p. 229-55.
2. Buijs, F.N., et al., *The Circadian System: A Regulatory Feedback Network of Periphery and Brain.* Physiology (Bethesda), 2016. **31**(3): p. 170-81.
3. Reinke, H. and G. Asher, *Crosstalk between metabolism and circadian clocks.* Nat Rev Mol Cell Biol, 2019.
4. Takahashi, J.S., *Transcriptional architecture of the mammalian circadian clock.* Nat Rev Genet, 2017. **18**(3): p. 164-179.
5. Bunger, M.K., et al., *Mop3 is an essential component of the master circadian pacemaker in mammals.* Cell, 2000. **103**(7): p. 1009-1017.
6. Narumi, R., et al., *Mass spectrometry-based absolute quantification reveals rhythmic variation of mouse circadian clock proteins.* Proc Natl Acad Sci U S A, 2016. **113**(24): p. E3461-7.
7. Sulli, G., et al., *Training the Circadian Clock, Clocking the Drugs, and Drugging the Clock to Prevent, Manage, and Treat Chronic Diseases.* Trends Pharmacol Sci, 2018. **39**(9): p. 812-827.
8. Koike, N., et al., *Transcriptional architecture and chromatin landscape of the core circadian clock in mammals.* Science, 2012. **338**(6105): p. 349-54.
9. Zhang, R., et al., *A circadian gene expression atlas in mammals: implications for biology and medicine.* Proc Natl Acad Sci U S A, 2014. **111**(45): p. 16219-24.
10. Robles, M.S., S.J. Humphrey, and M. Mann, *Phosphorylation Is a Central Mechanism for Circadian Control of Metabolism and Physiology.* Cell Metab, 2017. **25**(1): p. 118-127.
11. Wang, J., et al., *Nuclear Proteomics Uncovers Diurnal Regulatory Landscapes in Mouse Liver.* Cell Metab, 2017. **25**(1): p. 102-117.
12. Ruben, M.D., et al., *A database of tissue-specific rhythmically expressed human genes has potential applications in circadian medicine.* Sci Transl Med, 2018. **10**(458).
13. Caratti, G., et al., *REVERBa couples the circadian clock to hepatic glucocorticoid action.* J Clin Invest, 2018. **128**(10): p. 4454-4471.
14. Dumbell, R., O. Matveeva, and H. Oster, *Circadian Clocks, Stress, and Immunity.* Front Endocrinol (Lausanne), 2016. **7**: p. 37.
15. Bonilla, F.A. and H.C. Oettgen, *Adaptive immunity.* J Allergy Clin Immunol, 2010. **125**(2 Suppl 2): p. S33-40.
16. Cooper, M.D. and M.N. Alder, *The evolution of adaptive immune systems.* Cell, 2006. **124**(4): p. 815-22.
17. Sallusto, F. and A. Lanzavecchia, *The instructive role of dendritic cells on T-cell responses.* Arthritis Res, 2002. **4 Suppl 3**: p. S127-32.
18. Jameson, S.C. and D. Masopust, *Understanding Subset Diversity in T Cell Memory.* Immunity, 2018. **48**(2): p. 214-226.
19. Silver, A.C., et al., *Circadian expression of clock genes in mouse macrophages, dendritic cells, and B cells.* Brain Behav Immun, 2012. **26**(3): p. 407-13.
20. Bollinger, T., et al., *Circadian clocks in mouse and human CD4+ T cells.* PLoS One, 2011. **6**(12): p. e29801.
21. Fortier, E.E., et al., *Circadian variation of the response of T cells to antigen.* J Immunol, 2011. **187**(12): p. 6291-300.

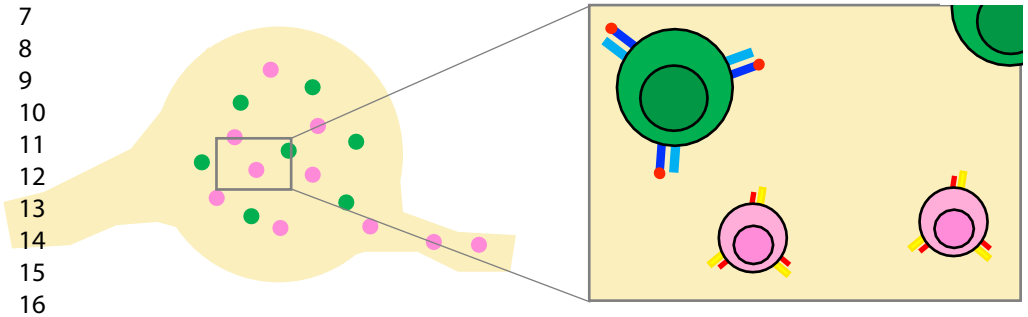
22. Keller, M., et al., *A circadian clock in macrophages controls inflammatory immune responses.* Proceedings of the National Academy of Sciences, 2009. **106**(50): p. 21407-21412.
23. Yang, X.O., et al., *T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma.* Immunity, 2008. **28**(1): p. 29-39.
24. Ivanov, II, et al., *The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells.* Cell, 2006. **126**(6): p. 1121-33.
25. Abo, T., et al., *Studies on the bioperiodicity of the immune response. I. Circadian rhythms of human T, B, and K cell traffic in the peripheral blood.* J Immunol, 1981. **126**(4): p. 1360-3.
26. Besedovsky, L., J. Born, and T. Lange, *Endogenous glucocorticoid receptor signaling drives rhythmic changes in human T-cell subset numbers and the expression of the chemokine receptor CXCR4.* Faseb J, 2014. **28**(1): p. 67-75.
27. Born, J., et al., *Effects of sleep and circadian rhythm on human circulating immune cells.* J Immunol, 1997. **158**(9): p. 4454-64.
28. Dimitrov, S., et al., *Cortisol and epinephrine control opposing circadian rhythms in T cell subsets.* Blood, 2009. **113**(21): p. 5134-43.
29. Zhao, Y., et al., *Uncovering the mystery of opposite circadian rhythms between mouse and human leukocytes in humanized mice.* Blood, 2017.
30. Druzd, D., et al., *Lymphocyte Circadian Clocks Control Lymph Node Trafficking and Adaptive Immune Responses.* Immunity, 2017. **46**(1): p. 120-132.
31. Rosenblum, M.D., K.A. Remedios, and A.K. Abbas, *Mechanisms of human autoimmunity.* J Clin Invest, 2015. **125**(6): p. 2228-33.
32. He, W., et al., *Circadian Expression of Migratory Factors Establishes Lineage-Specific Signatures that Guide the Homing of Leukocyte Subsets to Tissues.* Immunity, 2018. **49**(6): p. 1175-1190 e7.
33. Moon, J.J., et al., *Naive CD4(+) T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude.* Immunity, 2007. **27**(2): p. 203-13.
34. Suzuki, K., et al., *Adrenergic control of the adaptive immune response by diurnal lymphocyte recirculation through lymph nodes.* J Exp Med, 2016. **213**(12): p. 2567-2574.
35. Shimba, A., et al., *Glucocorticoids Drive Diurnal Oscillations in T Cell Distribution and Responses by Inducing Interleukin-7 Receptor and CXCR4.* Immunity, 2018. **48**(2): p. 286-+.
36. Hoebe, K., E. Janssen, and B. Beutler, *The interface between innate and adaptive immunity.* Nature Immunology, 2004. **5**(10): p. 971-974.
37. Pashine, A., N.M. Valiante, and J.B. Ulmer, *Targeting the innate immune response with improved vaccine adjuvants.* Nature Medicine, 2005. **11**(4): p. S63-S68.
38. Silver, A.C., et al., *The circadian clock controls toll-like receptor 9-mediated innate and adaptive immunity.* Immunity, 2012. **36**(2): p. 251-61.
39. Phillips, A.C., et al., *Preliminary evidence that morning vaccination is associated with an enhanced antibody response in men.* Psychophysiology, 2008. **45**(4): p. 663-666.
40. Long, J.E., et al., *Morning vaccination enhances antibody response over afternoon vaccination: A cluster-randomised trial.* Vaccine, 2016. **34**(40): p. 4842-4842.
41. Taylor, D.J., et al., *Is Insomnia a Risk Factor for Decreased Influenza Vaccine Response?* Behavioral Sleep Medicine, 2017. **15**(4): p. 270-287.

42. Lange, T., et al., *Sleep enhances the human antibody response to hepatitis A vaccination.* Psychosomatic Medicine, 2003. **65**(5): p. 831-835.
43. Lange, T., S. Dimitrov, and J. Born, *Effects of sleep and circadian rhythm on the human immune system.* Neuroendocrine Immunology in Rheumatic Diseases, 2010. **1193**: p. 48-59.
44. Hopwood, T.W., et al., *The circadian regulator BMAL1 programmes responses to parasitic worm infection via a dendritic cell clock.* Sci Rep, 2018. **8**(1): p. 3782.
45. Rijo-Ferreira, F., et al., *Sleeping sickness is a circadian disorder.* Nat Commun, 2018. **9**(1): p. 62.
46. Prior, K.F., et al., *Host circadian rhythms are disrupted during malaria infection in parasite genotype-specific manners.* Sci Rep, 2019. **9**(1): p. 10905.
47. Prior, K.F., et al., *Timing of host feeding drives rhythms in parasite replication.* PLoS Pathog, 2018. **14**(2): p. e1006900.
48. Hirako, I.C., et al., *Daily Rhythms of TNFalpha Expression and Food Intake Regulate Synchrony of Plasmodium Stages with the Host Circadian Cycle.* Cell Host Microbe, 2018. **23**(6): p. 796-808 e6.
49. Early, J.O., et al., *Circadian clock protein BMAL1 regulates IL-1beta in macrophages via NRF2.* Proc Natl Acad Sci U S A, 2018. **115**(36): p. E8460-E8468.
50. Dobson, R. and G. Giovannoni, *Multiple sclerosis - a review.* Eur J Neurol, 2019. **26**(1): p. 27-40.
51. Fletcher, J.M., et al., *T cells in multiple sclerosis and experimental autoimmune encephalomyelitis.* Clin Exp Immunol, 2010. **162**(1): p. 1-11.
52. Harding, K., et al., *Seasonal variation in multiple sclerosis relapse.* J Neurol, 2017. **264**(6): p. 1059-1067.
53. Lavtar, P., et al., *Association of circadian rhythm genes ARNTL/BMAL1 and CLOCK with multiple sclerosis.* PLoS ONE, 2018. **13**(1): p. e0190601.
54. Gustavsen, S., et al., *Shift work at young age is associated with increased risk of multiple sclerosis in a Danish population.* Mult Scler Relat Disord, 2016. **9**: p. 104-9.
55. Hemmers, S. and A.Y. Rudensky, *The cell-intrinsic circadian clock is dispensable for lymphocyte differentiation and function.* Cell Rep, 2015. **11**(9): p. 1339-1349.
56. Sutton, C.E., et al., *Loss of the molecular clock in myeloid cells exacerbates T cell-mediated CNS autoimmune disease.* Nat Commun, 2017. **8**(1): p. 1923.
57. Amir, M., et al., *REV-ERBalpha Regulates TH17 Cell Development and Autoimmunity.* Cell Rep, 2018. **25**(13): p. 3733-3749 e8.
58. Holgate, S.T., *Innate and adaptive immune responses in asthma.* Nat Med, 2012. **18**(5): p. 673-83.
59. Litinski, M., F.A. Scheer, and S.A. Shea, *Influence of the Circadian System on Disease Severity.* Sleep Med Clin, 2009. **4**(2): p. 143-163.
60. Martin, R.J., et al., *Airways inflammation in nocturnal asthma.* Am Rev Respir Dis, 1991. **143**(2): p. 351-7.
61. Durrington, H.J., et al., *Time of Day Affects Eosinophil Biomarkers in Asthma: Implications for Diagnosis and treatment.* Am J Respir Crit Care Med, 2018.
62. Zaslona, Z., et al., *The circadian protein BMAL1 in myeloid cells is a negative regulator of allergic asthma.* Am J Physiol Lung Cell Mol Physiol, 2017. **312**(6): p. L855-L860.
63. Harkness, J.A., et al., *Circadian variation in disease activity in rheumatoid arthritis.* Br Med J (Clin Res Ed), 1982. **284**(6315): p. 551-4.

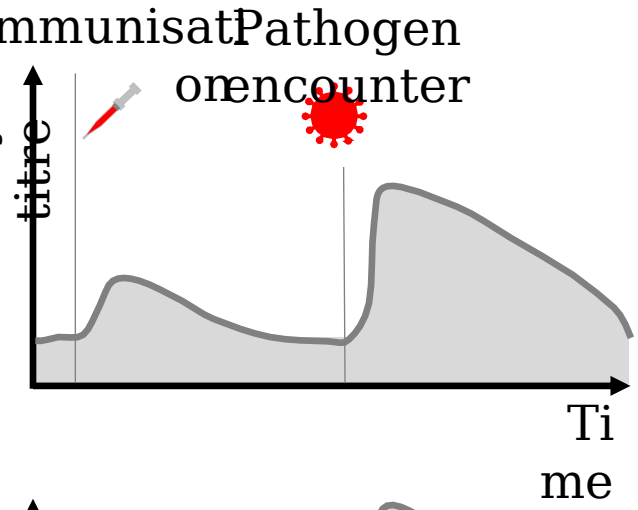
64. Perry, M.G., et al., *Overnight variations in cortisol, interleukin 6, tumour necrosis factor alpha and other cytokines in people with rheumatoid arthritis.* Ann.Rheum.Dis., 2009. **68**(1): p. 63-68.
65. Poolman, T.M., et al., *Rheumatoid arthritis reprograms circadian output pathways.* Arthritis Res Ther, 2019. **21**(1): p. 47.
66. Hand, L.E., et al., *The circadian clock regulates inflammatory arthritis.* FASEB J, 2016. **30**(11): p. 3759-3770.
67. Hashiramoto, A., et al., *Mammalian clock gene Cryptochrome regulates arthritis via proinflammatory cytokine TNF-alpha.* J.Immunol., 2010. **184**(3): p. 1560-1565.


Bone marrow
Thymus
Haematopoietic stem cell
T cells
B cells
antibody production
CD8+
(cytotoxic)
CD4+
(Helper)
Th1
Th2
Th17
Treg
Memory
CD8+
Memory
CD4+

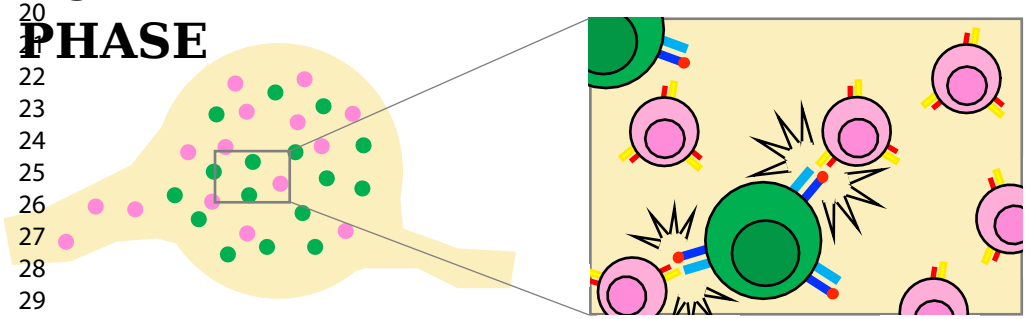
REST PHASE



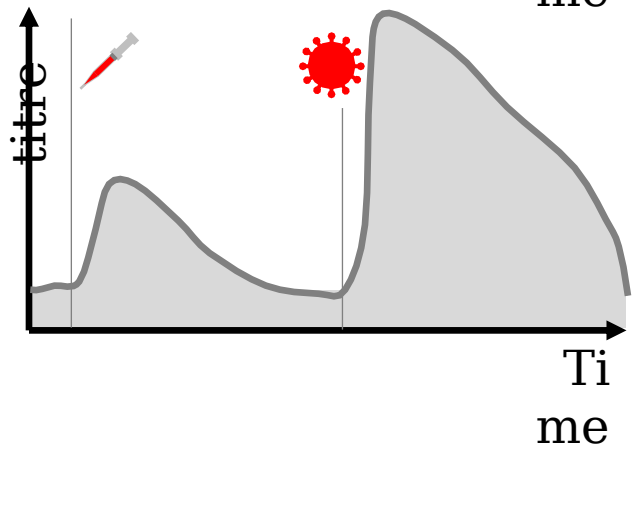
Lower chance of interaction with T cells



ACTIVE PHASE



Higher chance of interaction with T cells



Lymph node

T cells

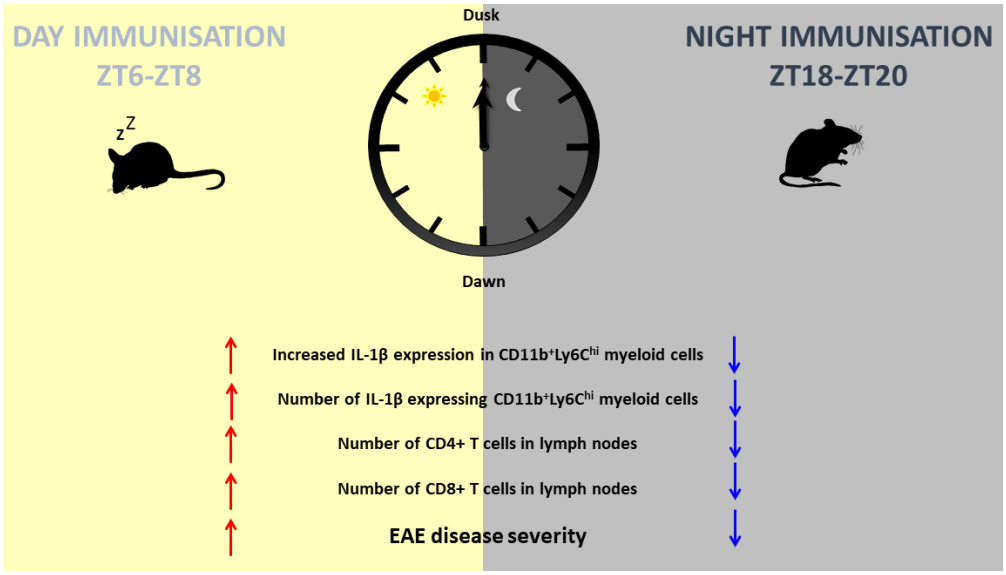
DCs

MHCII + antigen

CD80 /86

TCR

CD28



303x172mm (96 x 96 DPI)

Gene	Deletion	Adaptive Immune Phenotype
Bmal1	Myeloid	Exacerbation of EAE symptoms and loss of time-of-day protection [50] Increased migration of CD11b+ myeloid cells into the CNS during EAE [50] Increased Th1 and Th17 response to EAE [50]
	CD4+ T cells	Loss of rhythmic trafficking of lymphocytes to lymph nodes [27] Loss of diurnal variation in disease progression following EAE induction [27]
	CD11c+ Dendritic cells	Loss of circadian control over Trichurs muris expulsion [41]
Nr1d1	Global	Exacerbation of EAE symptoms [51] Increased infiltration of CD4+ T cells into CNS during EAE [51] Increased severity of Colitis [51]
Rorα sg/sg	Global	Impaired Th17 cell development [22]
Rorγ	Global	Impaired Th17 cell development [23]
Rorα/γ	Global	No Th17 cell population [22] Mice are resistant to EAE [22]
Cry1/Cry2	Global	Increased Antibody induced arthritis severity [61]

Table 1. Adaptive immune phenotype of circadian mutant mice.