



BASIC SCIENCE FOR THE CHEST PHYSICIAN

The circadian clock and asthma

Hannah J Durrington,¹ Stuart N Farrow,¹ Andrew S Loudon,² David W Ray¹

¹Faculty of Medical and Human Sciences, Institute of Human Development, Manchester, UK

²Faculty of Life Sciences, University of Manchester, Manchester, UK

Correspondence to

Dr Hannah J Durrington, Faculty of Medical and Human Sciences, Institute of Human Development, Manchester M13 9PT, UK; hannahd@doctors.org.uk

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ABSTRACT

It is characteristic of asthma that symptoms worsen overnight, particularly in the early hours of the morning. Nocturnal symptoms in asthma are common and are an important indicator for escalation of treatment. An extensive body of research has demonstrated that nocturnal symptoms of cough and dyspnea are accompanied by circadian variations in airway inflammation and physiologic variables, including airflow limitation and airways hyper-responsiveness. The molecular apparatus that underpins circadian variations, controlled by so called 'clock' genes, has recently been characterised. Clock genes control circadian rhythms both centrally, in the suprachiasmatic nucleus of the brain and peripherally, within every organ of the body. Here, we will discuss how clock genes regulate circadian rhythms. We will focus particularly on the peripheral lung clock and the peripheral immune clock and discuss how these might relate to both the pathogenesis and treatment of asthma.

CIRCADIAN RHYTHM

Circadian rhythms are autonomous, self-sustained, oscillations of approximately 24 h in biological processes, entrained to environmental cues. Our ability to generate circadian rhythms has allowed us to anticipate environmental changes in order to optimise our survival.

ASTHMA AND CIRCADIAN RHYTHM**Circadian variation in asthma symptoms**

Symptoms of asthma frequently show exacerbation in the early hours of the morning, at around 04:00. Sudden death in asthma also tends to occur at this time. In a survey of 7729 patients with asthma, 74% awoke at least once per week with asthma symptoms, 64% reported nocturnal asthma symptoms at least three times per week, and approximately 40% of patients experienced symptoms nightly.¹

Circadian variation in lung physiology

Although lung function has been shown to fluctuate over the 24-h period in healthy individuals, these fluctuations are far more pronounced in patients with nocturnal asthma, with the difference in forced expiratory volume in 1 s (FEV₁) (and also peak expiratory flow rate (PEFR)) between wakefulness and sleep exceeding 15%.¹

Circadian variation in airway inflammation

Kraft *et al* performed bronchoscopy and transbronchial biopsy in subjects with nocturnal or non-nocturnal asthma at 16:00 (the peak of lung function) and 04:00 (when airflow limitation was at its worst). In tissue

from subjects with nocturnal asthma, there was a circadian variation in the number per unit volume of alveolar eosinophils, with a significantly greater number present at 04:00 vs 16:00. Increases in alveolar eosinophils in subjects with nocturnal asthma correlated with nocturnal decrements in FEV₁. A circadian increase in tissue macrophages was also observed in alveolar tissue from subjects with nocturnal asthma.¹ Although patients with nocturnal asthma typically meet criteria for moderate or severe persistent asthma, circadian changes in lung function can be seen in patients with more mild asthma. In 2004, Kelly *et al* investigated circadian changes in airway inflammation in patients with mild atopic asthma (mean FEV₁ of 93%±4% of predicted value). In this patient population bronchoalveolar lavage fluid contained increased numbers of macrophages, neutrophils and CD4 T lymphocytes at 04:00 versus 16:00. Additionally, the percentage of CD4 T lymphocytes in the 4:00 lavage fluid was inversely correlated with 04:00 FEV₁.²

Chronotherapeutics

The timing of drug treatments in asthma (chronotherapeutics) is governed by the circadian nature of asthma. Interestingly, in asthmatic patients given prednisolone at 15:00 (rather than at 08:00 or 20:00), there is a significant increase in FEV₁ and a decrease in airway inflammatory cells, compared with controls. Furthermore, an inhaled steroid given once a day between 15:00 and 17:30 was superior to once daily, 08:00 dosing and comparable to dosing four times a day.¹

The circadian rhythm is inextricably linked to the pathogenesis of asthma. An understanding of how circadian rhythms are generated, will allow us an intriguing insight into the biology of asthma.

THE CIRCADIAN CLOCK

Over the last decade the field of chronobiology has rapidly advanced. In the 1970s, researchers used mutagenesis screens to identify single-locus mutations which affected circadian period in *Drosophila*, leading to the discovery of the first 'clock gene' (PERIOD). Similar approaches in mammals identified other members of what we now recognise is a largely conserved genetic cluster driving circadian rhythms in insects and mammals.³

Initially the 'central' clock in the suprachiasmatic nucleus (SCN) of the mammalian brain was defined. Light enters through the retina of the eye, causing electrical signals to pass through the retinal hypothalamic tract, which are converted to chemical signals in the SCN. Light signals and other physiological factors, such as feeding cues, entrain the central circadian clock. It was initially assumed that all physiological processes were 'driven' by

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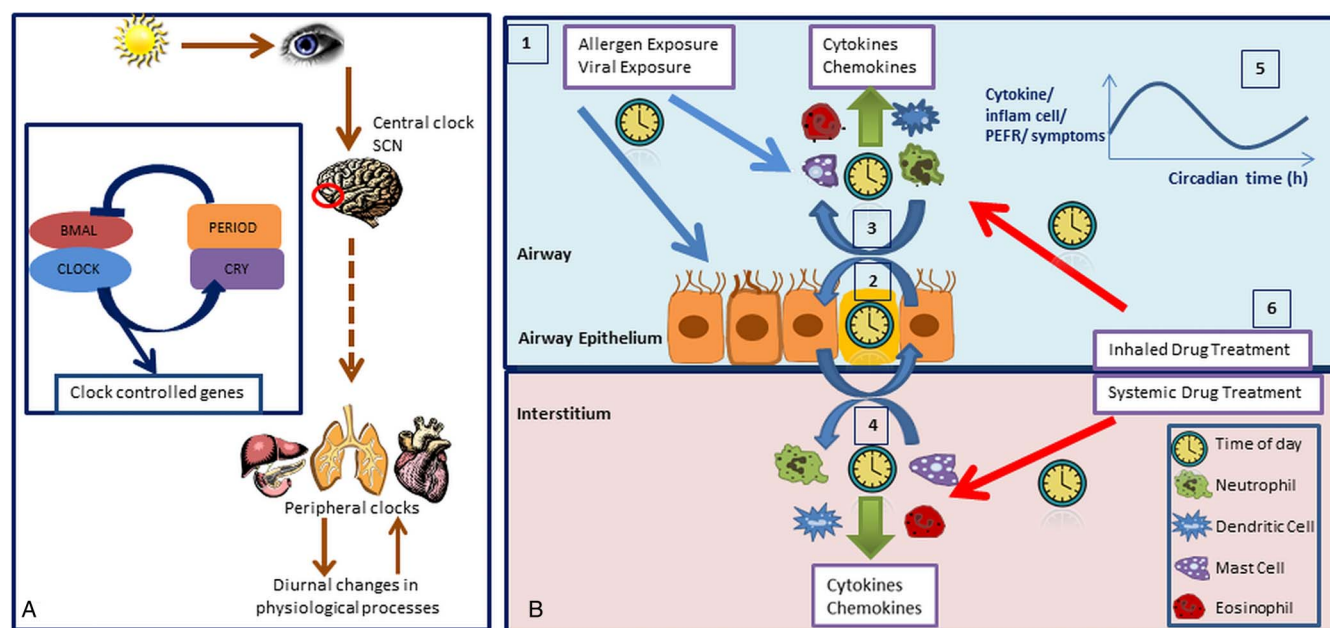


Figure 1 (A) The output from the central circadian clock in the suprachiasmatic nucleus (SCN) of the brain serves as a source of timing information to permit peripheral clocks to 'track' day and night, as they lack light input. The molecular circadian clock consists of interlocking transcriptional and translational feedback loops, culminating in the rhythmic expression of core clock genes. (B) The time of day that an asthma exacerbation is triggered by exposure to an allergen or virus may have a significant effect on the magnitude of the host inflammatory response (1). The peripheral lung clock resides in the Clara cell in the murine lung (2). Crosstalk between the lung clock and the clocks of immune cells present in the airway (3) and in the lung interstitium (4) cause circadian variation in local cytokines, chemokines, inflammatory cell recruitment and in asthmatic symptoms and lung physiology (5). These day-to-day variations may lead to more chronic changes in the gating of the immune response in the asthmatic lung. The timing of administration of both inhaled and systemic medications is critical in the effective treatment of asthma (6).

neural and hormonal output signals from the 'central' clock. However, the discovery of 'peripheral' clocks within every tissue and organ of the body has opened a new understanding of how the clock works. The SCN essentially serves as a source of timing information to permit peripheral clocks to 'track' day and night, as they lack light input. However, the signals used to transmit this time signal can also be activated by other processes, for example, glucocorticoid is both the timing signal and an important immune, and metabolic signal. Each peripheral clock can adapt to its own external and internal stimuli (figure 1A).

CLOCK MECHANISM

Both central and peripheral clocks use the same molecular machinery to 'time' the day. Interlocking repressing and activating transcriptional and translational feedback loops with a built-in 'delay' mechanism culminate in the approximately 24 h rhythmic expression and activity of a set of core clock genes in each organ. CLOCK and BMAL1 promote the transcription of Period (Per1, 2) and Cryptochrome (Cry1, 2) genes. As protein levels increase, PER and CRY associate and translocate into the nucleus directly repressing CLOCK/BMAL1, thereby inhibiting their own transcription. Enzymic degradation of PERIOD and CRYPTOCHROME proteins provides a delay mechanism prior to the onset of the next transcriptional cycle. The expression of positive factors, CLOCK and BMAL1, and negative factors, PER and CRY, are held in antiphase of one another providing circadian timing at the molecular level (figure 1A). Outputs from the molecular clock are generated through transcription or repression of target genes.

BMAL1 is regulated by rhythmic interaction with REV-ERB α . REV-ERB α , a nuclear hormone receptor and core clock gene is a critical regulator of inflammation and metabolism. Drug

targeting of REV-ERB α represents an exciting option for manipulation of the clock in disease states.

The importance of understanding the role of the peripheral clocks in each tissue and their relationship to one another is paramount in order to better comprehend the role of peripheral clocks in circadian physiology. This has been aided by the generation of clock gene knockout mice.

THE LUNG CLOCK

Work from our laboratory has demonstrated the presence of a peripheral clock within the lung and specifically localised this clock to the Clara cell of the mouse bronchial epithelium.⁴

Disruption of circadian rhythms in mice, to mimic chronic jet lag or shift work, caused an alteration in lung mechanics and clock gene expression in the lung in a sexually dimorphic manner.⁵

Sukumaran *et al* conducted a genome-wide analysis of diurnal/nocturnal patterns in mRNA expression from lungs of rats kept in a tightly controlled 12:12 h light-dark cycle. They showed cyclic oscillations in the expression of genes associated with extracellular matrix, cytoskeleton, cell cycle and apoptosis, suggesting that the repair and turnover of these components in lung are directly or indirectly under the regulation of the molecular clock. Many of the growth factor ligands and receptors involved in the direct regulation of these processes and the maintenance of the homeostasis in lung also showed circadian-like oscillations in expression. Genes coding for inflammatory molecules, including chemokine ligands (Cxc12) showed circadian-like oscillations in expression in lung, suggesting that the molecular clock could directly or indirectly regulate the immune response in the organ. Furthermore, genes that are involved in the metabolism of xenobiotics showed circadian-like oscillations in expression in lung.⁶

The implications of circadian variation within the lung are far-reaching, and now imply that the time of day that samples are taken from patients, in the clinic and for research purposes, needs to be considered.

THE IMMUNE CLOCK

Circadian variations in immune parameters such as lymphocyte proliferation, antigen presentation and cytokine gene expression have been described. Recently, an association between the molecular circadian clock and the immune system and inflammation has been recognised. Our group has shown that the macrophage clockwork provides temporal gating of systemic responses to endotoxin, and, we have identified REV-ERB α as the key link between the clock and immune function.⁷

Furthermore, toll-like receptor 9 (TLR9) expression and function have been recently shown to be modulated by core circadian molecular clock components. In a vaccination model using TLR9 ligand as adjuvant, mice immunised at the time of enhanced TLR9 responsiveness presented weeks later with an improved adaptive immune response. In a TLR9-dependent mouse model of sepsis, disease severity was dependent on the timing of sepsis induction, coinciding with the daily changes in TLR9 expression and function.⁸

Therefore, the timing of an attack on the lung immune system by an allergen or virus might significantly affect the ability of the lung to mount an adequate immune response. In this scenario one might imagine crosstalk between the peripheral clocks of the lung and the immune system in coordinating such a response (figure 1B).

COULD THE MOLECULAR CIRCADIAN CLOCK PLAY A ROLE IN THE PATHOGENESIS OF ASTHMA?

First, the molecular clock may be responsible for the marked circadian variability in symptoms, airway physiology and

inflammation that occurs in asthma on a day-to-day basis. Asthmatic airways may be more sensitive to the output of the molecular clock or the amplitude of the clock might be increased in asthma. Second, if the molecular clock is responsible for 'gating' the immune response within the lung, then chronic airway inflammation, typical of asthma, might be caused through disruption of the molecular clock. Future research using clock gene knockout mice, and eventually, in patients with asthma might provide the answers to these intriguing questions.

Competing interests None.

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