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Constant light exposure impairs immune tolerance development in mice

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ABSTRACT

Background: An intrinsic daily physiological rhythm called circadian rhythm has been indicated to affect the immune system and its related diseases. Immune tolerance development is closely associated with the onset of immunological disorders. However, the effect of circadian rhythm in the mechanisms of immune tolerance development has not yet been fully understood.
Objective: The purpose of this study was to investigate the effects of circadian rhythm disruption on the development of immune tolerance by the perturbation of light environment, using a mouse model of neonatally induced cutaneous tolerance.
Methods: Mice were kept under constant light (LL) or light-dark (LD) conditions, and hapten was applied at 2 days after birth. Six weeks later, hapten was reapplied to abdominal skin, followed by hapten application to ear skin 5 days later.
Results: The ear-swelling responses and cell infiltration into inflamed skin significantly increased in LL mice compared with those in LD mice. Interestingly, the percentage and the number of $Foxp3^{+}$-regulatory T cells notably decreased in inflamed skin and draining lymph nodes of LL mice compared with that in LD mice. Loss-of-function mutation of a key circadian gene, *Bmal1*, also exacerbated the ear-swelling responses and cell infiltration into inflamed skin in mice.
Conclusion: These results suggest that circadian rhythm may be implicated in immune tolerance development in allergic inflammation.

1. Introduction

Circadian rhythms are biological processes that recur on an approximately twenty-four-hour cycle in living systems, and are closely related to many behavioral and physiological changes, such as sleep-wake cycle, body temperature, metabolism, and hormone secretion [1,2]. Accumulating evidence suggests that circadian rhythm disruption is a possible risk factor for various types of health problems such as sleep disorders, metabolic diseases and cancer; therefore, it is important to understand the physiology and pathophysiology of the mammalian circadian clock. In the mammalian clock system, the central pacemaker resides in the suprachiasmatic nucleus (SCN) of the hypothalamus, coordinating cell-autonomous molecular oscillators throughout the body to perform tissue-specific functions. Mammalian circadian clock is composed of positive and negative transcriptional-translational feedback loops including the genes circadian locomotor output cycles kaput (*Clock*), *Bmal1*, *Period (Per1, 2, 3)*, and *Cryptochrome (Cry1, 2)* [1–4]. To keep pace with the solar day-night cycle, the master clock in the SCN can be entrained by light received from photoreceptors in the retina [1,2]. Therefore, environmental light condition strongly affects the circadian physiological rhythms via perturbation of the master circadian clock in the SCN.

Abbreviations: CFSE, carboxyfluorescein succinimidyl ester; CHS, contact hypersensitivity; CLOCK, circadian locomotor output cycles kaput gene; CRY, cryptochrome; DC, dendritic cell; FITC, fluorescein isothiocyanate; IL, interleukin; KO, knockout; LD, light-dark; LL, constant light; LNs, lymph nodes; PE, phycoerythrin; PE-Cy7, phycoerythrin-cyanine7; PER, period; SCN, suprachiasmatic nucleus; TNCB, 2,4,6-trinitro-1-chlorobenzene; Treg, regulatory T cell; WT, wild-type.

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Clinical symptoms in patients with immunological disorders such as atopic dermatitis, urticaria, and asthma, often depend on diurnal variations [5]. Cutaneous hypersensitivity reactions peak at noon in patients with nocturnal asthma [6]. Some experimental studies have shown that mutation in the circadian clock genes greatly affects immune responses [7–9]. In a mouse model of arthritis, lack of both *Cry1* and *Cry2* induces the exacerbation of symptoms with increased serum levels of tumor necrosis factor [7]. In addition, mutation in the circadian clock gene *Per2* affects the immediate-type hypersensitivity reactions such as passive cutaneous anaphylactic reaction via disturbance of the rhythmic secretion of glucocorticoids from adrenal glands and increasing responses of mast cells to glucocorticoids [8]. Furthermore, mutation in the circadian clock gene *Clock* results in increase of the contact hypersensitivity (CHS) response with elevated numbers of mast cells in inflamed skin [9]. These findings suggest that the circadian clock is likely to be involved in the mechanisms of immune responses.

Regulatory mechanisms are closely related to the development of immunological disorders [10]. Regulatory T (Treg) cells, which are deeply involved in the induction and maintenance of immune tolerance, are present at high frequency in fetal or neonatal skin and lymphoid tissues [11,12]. In addition, the stimulation of naïve T cells during the neonatal period strongly induces antigen-specific Treg cell generation, and the ability of Treg cell generation gradually decreases within 2 weeks of birth in mice [13]. Furthermore, the application of a contact sensitizer to neonatal skin leads to the generation of antigen-specific tolerance [14,15]. These findings suggest that regulatory processes may tend to be induced in early life. The levels of interleukin (IL)-10, which is important for tolerance induction, are high during late gestation and at birth [16]. Interestingly, polymorphisms in IL-10 affect Th1/Th2 cytokine secretion and Treg cell generation in neonates and increase the risk of wheeze or atopic symptoms in childhood [17]. In addition, autoimmune gastritis develops in 20–60% of mice following removal of the thymus, where natural Treg cells are generated [10], at 3 days after birth [15]. Taking these findings together, Treg cells, especially those generated during early life, are deeply involved in the onset of immunological disorders.

Molecular mechanisms responsible for generating circadian rhythms develop gradually from the prenatal to the postnatal period in mammals [18,19]. Developing biological clocks during early life may be vulnerable to disruption by constant light [20]. Under constant light conditions, clock genes expression became almost no rhythms in both the SCN and peripheral tissues [21–23]. Constant exposure of preterm infants to light in neonatal intensive care units affects the development of various functions, leading to disturbances of physiology and behavior, such as sleep-wake patterns and postnatal weight gain [24,25]. As mentioned above, immune tolerance in early life has important roles in the later onset of immunological disorders. However, the roles of circadian systems in the development of immune tolerance in neonates have not yet been clarified. Although existing pharmacological treatments can control the symptoms of immunological diseases, there is no way to improve the natural course of these diseases. Therefore, elucidation of the circadian clock function in tolerance induction during early life may lead to the development of a new therapeutic strategy against these diseases.

Exposure to constant light, which is a form of lighting stress, may mimic a certain human lifestyle, such as shift workers or constant lighting conditions at home in our night-active modern society. We evaluated the influence on the immune tolerance development as a phenomenon that is observed under constant light conditions. By using a mouse model of neonatally induced cutaneous tolerance, we first examined whether impairment of the circadian clock by constant light conditions from the gestational or neonatal period affects cutaneous hypersensitivity response. We then examined the population and function of Treg cells in inflamed skin and draining lymph nodes (LNs) of mice exposed to constant light. We also investigated the effects of loss-of-function mutation of a key circadian gene, *Bmal1* on cutaneous hypersensitivity response.

2. Materials and methods

2.1. Mice

BALB/c mice were obtained from Shimizu Laboratory Supplies (Kyoto, Japan). *Bmal1* knockout (KO) mice and C57BL/6J wild-type (WT) mice were obtained from Jackson laboratories. All mice were housed in a condition of foods and water was freely accessible. The experimental procedure here was approved by the Committee for Animal Research of Kyoto Prefectural University of Medicine.

2.2. Constant light exposure

The day of delivery was designated as postnatal day 0 (P0). Neonatal mice (P1) or pregnant mice (embryonal day (E)14) started to be kept under constant light (LL) or 12hr-light/12hr-dark (LD) conditions (Fig. 1), and were weaned on P28. Mice were kept under LL or LD conditions until 7 weeks of age. For behavior analysis, LL or LD grown mice were placed individually in the cage at an age of 4 weeks, and their behaviors were analyzed using wheel running counting systems (Muromachi Kikai Co. Ltd., Tokyo, Japan). Calculation was performed using Clock Lab software (Actimetrics, Wilmette, IL).

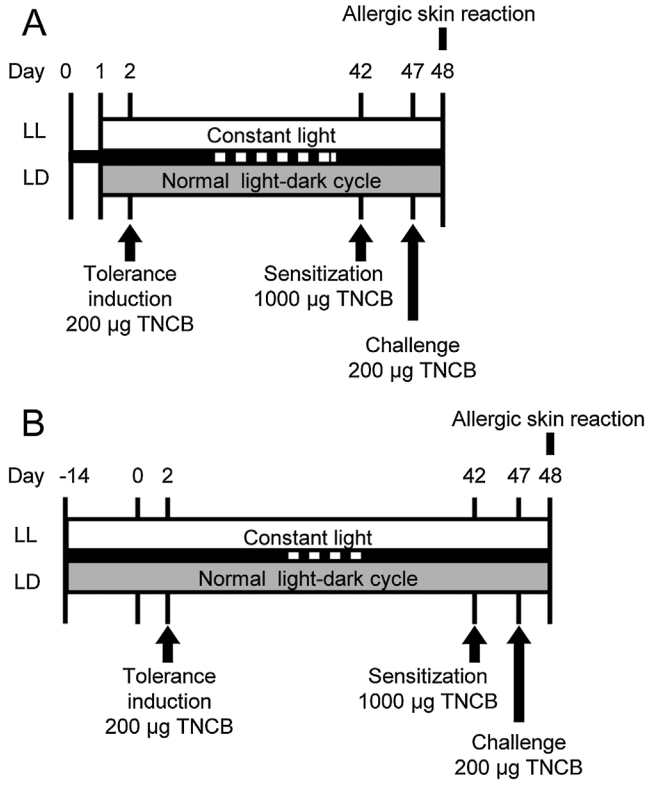

Fig. 1. Protocols of constant light exposure from the neonatal and gestational period. Neonatal (P1) mice (A) or pregnant (E14) mice (B) were kept under LD or LL conditions. Pups were treated with TNCB at postnatal day 2 for tolerance induction. Six weeks later, mice were sensitized, and then challenged with TNCB.