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BRIEF COMMUNICATION

Allergen-specific basophil reactivity exhibits daily variations in seasonal allergic rhinitis

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Abstract

It remains poorly understood how symptoms in allergic rhinitis are most severe during overnight or early in the morning. The circadian clock consisting of a network of several 'clock genes' including *Clock* drives daily rhythms in physiology. This study showed that allergen-induced surface CD203c expression on basophils in seasonal allergic rhinitis caused by Japanese cedar pollen exhibited a time-of-day-dependent variation associated with temporal variations in canonical circadian clock gene expression. We also found that bone-marrow-derived basophils (BM basophils) generated from wild-type mice exhibited a time-of-day-dependent variation in IgE-mediated IL-4 and histamine production, which was not observed in BM basophils generated from *Clock*-mutated mice. Therefore, allergen-specific basophil reactivity shows daily variations depending on the circadian clock activity in basophils, which could partly explain temporal symptomatic variations in allergic rhinitis. Additionally, circadian variations in CD203c expression should be considered for interpretation of this biomarker in clinical research.

Symptoms in allergic rhinitis are often most severe during overnight or early in the morning ('morning attack'), which results in poor daytime quality of life (1, 2). However, it remains poorly understood how the prominent ~24-h symptomatic variations occur in allergic rhinitis.

To explore the cellular mechanism(s) behind the daily symptomatic variations in allergic rhinitis, we determined whether allergen-specific basophil reactivity exhibited a time-of-day-dependent variation in patients with seasonal allergic rhinitis (SAR). Because the circadian clock consisting of an autoregulatory transcriptional network driven by several 'clock genes' including *Clock* and *Period* controls physiological processes that vary across the day–night cycle (3), we also determined whether the circadian clock was functional in basophils and played a role in a time-of-day-dependent variation in allergen-specific basophil reactivity.

Materials and methods

Additional Supporting Information on materials and methods may be found in the online version of this article.

Patients

Eighteen volunteers diagnosed as SAR caused by Japanese cedar pollen (JCP) (a mean age of 30.5 years) and 11 normal subjects (a mean age of 33.27 years) were recruited at University of Yamanashi Hospital (Yamanashi, Japan) with written informed consent (Tables S1 and S2). The SAR patients were diagnosed by medical doctors based on elevated serum levels of specific IgE to Japanese cedar pollen (JCP) (CAP-FEIA; SRL, Tokyo, Japan) and repeated symptoms in the pollen season without performing skin prick test or nasal provocations with pollen. None of the subjects exhibited symptoms of SAR and were treated with any medication 1 month before and during this study. This study was approved by the ethics committee of University of Yamanashi Faculty of Medicine.

Results and discussion

CD203c is an activation marker upregulated by cross-linking of FcεRI in human basophils (4, 5). To determine whether allergen-specific basophil reactivity exhibited a time-of-day-

dependent variation in SAR, we examined allergen-induced CD203c expression on basophils obtained at AM 7:00 and PM 7:00 (19:00) from patients with JCP pollinosis.

Incubation of whole blood samples with the concentration of 0.3 μg/ml, but not 0.03 or 0.003 μg/ml, of JCP extract significantly induced CD203c expression on basophils from all JCP pollinosis patients tested (Figs 1A,B and S1). Thus, we considered this concentration of JCP extract (0.3 μg/ml) as an optimal dose for basophil stimulation in this study so that we used this dose for the following analysis. Stimulation of whole blood samples with JCP extract (0.3 μg/ml) or anti-IgE antibody significantly increased the frequency of $CD203c^+$ basophils at 7:00 compared with that at 19:00 (Fig. 1A,B). In contrast, the frequency of $CD203c^+$ basophils stimulated with calcium ionophore A23187 was comparable between at 7:00 and at 19:00 (Fig. 1A,B). The time-of-day-dependent variation in JCP-induced basophil CD203 expression was reproduced on different days in individual SAR subjects (Fig. S2 and data not shown). Interestingly, basophils from normal subjects also showed a time-of-day-dependent variation in CD203c expression upon stimulation with anti-IgE antibody, but showed comparable induction of CD203c by A23187 between at 7:00 and at 19:00 (Fig. 1C). The mRNA expres-

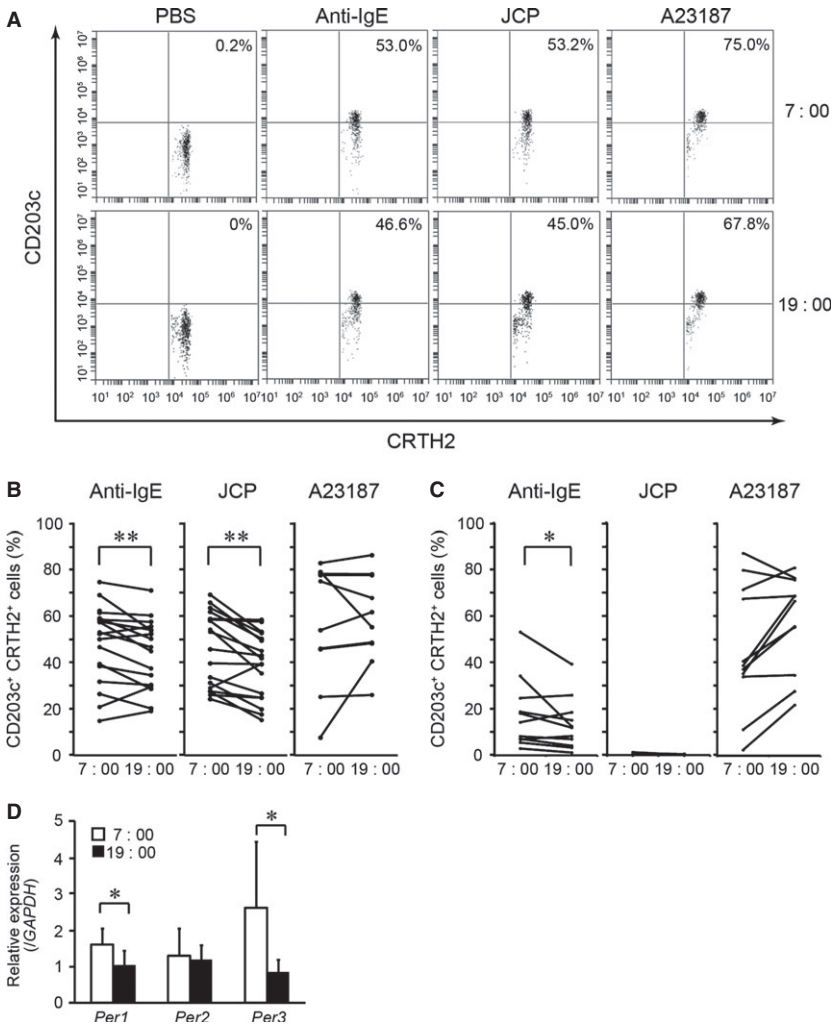

Figure 1 Allergen-induced upregulation of CD203c expression on SAR basophils shows a time-of-day-dependent variation. (A, B) Whole blood samples collected at 7:00 and 19:00 from patients with SAR were analyzed quantitatively by FACS analysis for CD203c expression on basophils following stimulations with anti-IgE antibody ($n = 18$), JCP ($n = 18$), or A23187 ($n = 10$). Representative FACS data from one subject were shown in (A), and quantitative data were shown in (B). (C) Whole blood samples collected at 7:00 and 19:00 from normal subjects were quantitatively analyzed by FACS analysis for CD203c expression on basophils following stimulations with anti-IgE antibody, JCP, or A23187 ($n = 11$). Quantitative data are shown. (D) Basophils were isolated at 7:00 and 19:00 from patients with SAR, and qPCR analysis for Per1, Per2, and Per3 was performed. The values represent the means ± SD ($n = 3$ per group). $^*P < 0.05$, $^{**}P < 0.01$.

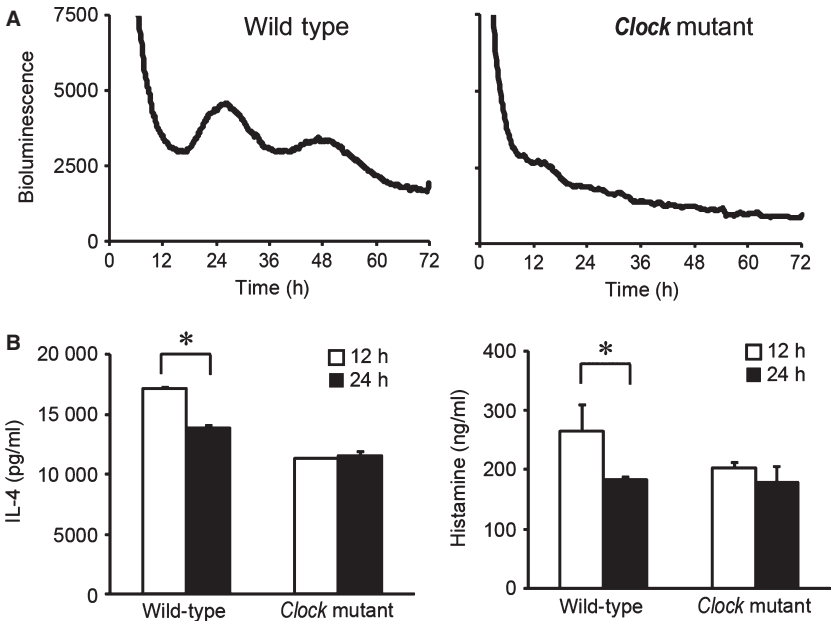

Figure 2 The circadian molecular clock in basophils is functional and temporally gates IgE-dependent IL-4 and histamine production in mouse basophils. (A) Monitoring of BM basophils from $Per2^{LUC}$ knock-in mice ($PER2^{LUC}$ BM basophils) with or without a loss-of-function mutation of *Clock* for 72 h by the detection of bioluminescence. (B) IgE-mediated IL-4 and histamine production from the 12 or 24-h cultured (after medium change for synchronization) $PER2^{LUC}$ BM basophils with or without a loss-of-function mutation of *Clock* ($n = 3$). Values represent the mean ± SD. $^*P < 0.05$. Similar results (A and B) are obtained in two independent experiments.

sion levels of *Per1* and *Per3*, but not *Per2*, exhibited a time-of-day-dependent variation at 7:00 and at 19:00 in SAR basophils (Fig. 1D). Thus, allergen-specific basophil reactivity showed a time-of-day-dependent variation in patients with JCP pollinosis associated with temporal variations in canonical clock gene expression. Because stimulation of basophils from normal subjects with anti-IgE antibody also showed a time-of-day-dependent variation in CD203c expression, allergen/IgE-mediated basophil reactivity may generally exhibits circadian variations.

To determine whether the circadian clock was functional in basophils and played a role in a time-of-day-dependent variation in allergen-specific basophil reactivity, we generated bone-marrow-derived basophils (6) from $Per2^{LUC}$ knock-in mice which express PERIOD2 (PER2) as a luciferase fusion protein (7) ($PER2^{LUC}$ BM basophils) and examined the kinetics of PER2 protein and IgE-mediated IL-4 and histamine production in $PER2^{LUC}$ BM basophils with or without a loss-of-function mutation of *Clock* (8).

$PER2^{LUC}$ BM basophils showed daily oscillations in $PER2^{LUC}$ protein levels following synchronization by a media change (9, 10), suggesting that basophils had functional clockwork (Fig. 2A). The extent of IgE-mediated IL-4 and histamine production was significantly higher in the 12-h cultured (after a media change for synchronization) $PER2^{LUC}$ BM basophils than in the 24-h cultured $PER2^{LUC}$ BM basophils, which was absent in $PER2^{LUC}$ BM basophils with *Clock* mutation (Fig. 2B).

The current results suggest that allergen-specific basophil reactivity exhibits a time-of-day-dependent variation in SAR. Given that BM basophils have functional clockwork and IgE-mediated activation of BM basophils shows temporal variations relying on *Clock*, we propose that allergen-specific basophil reactivity shows daily variations depending on the circadian clock activity in basophils, which may partly explain temporal symptomatic variations in allergic rhinitis. As direct stimulation of Ca^{2+} signaling with A23187, but not JCP or anti-IgE antibody, failed to show temporal variations in CD203c upregulation (Fig. 1), the circadian clock in basophils may temporally regulate FcεRI signaling at the levels upstream of Ca^{2+} signaling. The precise mechanisms how the circadian clock temporally gates allergen-/IgE-mediated signaling in basophils remain to be investigated.

This study has several limitations, a major one being that it remains unclear whether basophils play a role in the pathophysiology of allergic rhinitis. Diurnal functional variations in vessels, glands, nerves, hormones such as cortisol, mast cells, and eosinophils might be of more importance for the temporal variations in allergic rhinitis symptoms (1, 2), and basophils might be more like a surrogate marker for the symptomatic variations. Previous studies suggest circadian functions in mast cells and eosinophils (9–14). Thus, it may be also important to investigate whether these cells show temporal variations in allergen-specific reactivity in SAR patients.

By testing outside the pollen season, we were able to avoid the influence of allergen exposure on basophil reactivity; however, allergen exposure might be of much greater significance for diurnal symptomatic variations. This issue should be also investigated, together with a study on correlation of basophil CD203c expression with clinical parameters in allergic rhinitis.

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Author contributions

N.A. and A.N. designed the study. N.A., Y.N., and K.I. performed the *in vitro* and *in vivo* experiments and analyzed the data. H.O., K.O., and S.S. supervised and contributed reagents/materials/analysis tools for the *in vitro* and *in vivo* experiments. N.A., Y.N., and A.N. wrote the paper. All authors reviewed the manuscript.

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Conflicts of interest

The authors declare no financial conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Allergen-induced CD203c expression on SAR basophils stimulated with low concentrations of JCP.

Figure S2. Representative FACS data regarding reproducibility of daily variations in basophil CD203 expression assessed on different days in an individual subject.

Table S1. SAR patient profile (n = 18).

Table S2. Normal subject profile (n = 11).

Methods S1. Materials and methods.

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