Circadian regulation of allergic reactions by the mast cell clock in mice

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Background: It remains elusive how allergic symptoms exhibit prominent 24-hour variations. In mammals the circadian clocks present in nearly all cells, including mast cells, drive the daily rhythms of physiology. Recently, we have shown that the circadian clocks drive the daily rhythms in IgE/mast cell–mediated allergic reactions. However, the precise mechanisms, particularly the specific roles of the mast cell–intrinsic clockwork in temporal regulation, remain unclear.
Objective: We determined whether the mast cell clockwork contributes to the temporal regulation of IgE/mast cell–mediated allergic reaction.
Methods: The kinetics of a time of day–dependent variation in passive cutaneous anaphylactic reactions were compared between mast cell–deficient mice reconstituted with bone marrow–derived cultured mast cells generated from mice with a wild-type allele and a dominant negative type mutation of the key clock gene *Clock*. We also examined the temporal responses of wild-type and *Clock*-mutated bone marrow–derived cultured mast cells to IgE stimulation *in vitro*. Furthermore, factors influencing the mast cell clockwork were determined by using *in vivo* imaging.
Results: The *Clock* mutation in mast cells resulted in the absence of temporal variations in IgE-mediated degranulation in mast cells both *in vivo* and *in vitro* associated with the loss of temporal regulation of FcεRI expression and signaling. Additionally, adrenalectomy abolished the mast cell clockwork *in vivo*.
Conclusion: The mast cell–intrinsic clockwork, entrained by humoral factors from the adrenal gland, primarily contributes to the temporal regulation of IgE/mast cell–mediated allergic reactions. Our results reveal a novel regulatory mechanism for IgE-mediated mast cell responses that might underlie the circadian pathophysiology in patients with allergic diseases.
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Key words: *The circadian clock, mast cells, IgE, allergy, mouse*

Abbreviations used
BMCMC: Bone marrow–derived cultured mast cell
ChIP: Chromatin immunoprecipitation
mMCP: Mouse mast cell protease
PCA: Passive cutaneous anaphylaxis
PER2::LUC: Period2::Luciferase
siRNA: Small interfering RNA
ZT: Zeitgeber time

Allergic diseases are characterized by symptoms that exhibit prominent 24-hour variations.[1,2] For instance, in patients with allergic rhinitis, the symptoms are worse overnight or early in the morning ("morning attack") and often compromise nighttime sleep, resulting in a poor daytime quality of life.[3] Although these phenomena have been recognized for decades,[4,5] the precise mechanisms remain unclear.

The circadian clocks drive daily rhythms in physiology that enable organisms to keep track of the time of day. In mammals the light-entrained central oscillator located in the suprachiasmatic nucleus of the hypothalamus synchronizes the peripheral oscillators present in nearly all cell types, including mast cells, through neural and endocrine pathways.[6-11] The mechanisms of rhythm generation are based on transcriptional-translational feedback loops, wherein 2 transcription factors, CLOCK and BMAL1, activate the transcription of the Period *(Per)* and Cryptochrome *(Cry)* genes. The PER and CRY proteins in turn inhibit their own expression by repressing CLOCK/BMAL1 activity.[6-9]

In a classical mouse model of IgE/mast cell–mediated allergic reaction (passive cutaneous anaphylactic [PCA] reaction), we have recently shown that there are time of day–dependent variations that rely on the normal activity of a key clock gene, *Period2* (*Per2*),[10] suggesting that the circadian clocks drive the daily rhythms in IgE/mast cell–mediated allergic reactions. However, the precise mechanisms, particularly the specific roles of the mast cell–intrinsic clockwork in the temporal regulation, have been unclear. This study aimed to determine whether the mast cell clockwork contributes to the temporal regulation of IgE/mast cell–mediated allergic reactions.

METHODS

For more information, see the Methods section in this article's Online Repository at www.jacionline.org.

Mice

Male 5- to 6-week-old C57BL/6 mice, mast cell–deficient WBB6F1-W/Wv mice (Japan SLC, Tokyo, Japan), PER2::Luciferase (PER2::LUC) knock-in mice (C57BL/6 background),[12] and C57BL/6 $Clock^{\Delta 19/\Delta 19}$ mice[13] were bred under specific pathogen-free conditions. $Clock^{\Delta 19/\Delta 19}$ mice have an

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A to T point mutation in the 5′ splice site of intron 19 and, as a consequence, an in-frame deletion of the entire exon 19 ($Clock^{\Delta 19/\Delta 19}$), which results in loss of normal transcriptional activity.[13] This autosomal dominant mutation eventually provokes arrhythmicity in mice. All mice were housed under 12-hour light/12-hour dark conditions (the light was turned on at 6 AM, which is Zeitgeber time [ZT] 0, and the light was turned off at 6 PM, which is ZT12) with *ad libitum* access to food and water for at least 2 weeks.

Preparation of bone marrow–derived cultured mast cells

Bone marrow–derived cultured mast cells (BMCMCs) were generated from the femoral bone marrow cells of male mice.[14]

PCA reaction

Mast cell–deficient WBB6F1-W/Wv mice were reconstituted with subcutaneous injections (dorsal skin) of BMCMCs (1.5×10^6 per mouse) derived from wild-type C57BL/6 mice or C57BL/6 $Clock^{\Delta 19/\Delta 19}$ mice. Six weeks after reconstitution, the mice were sensitized subcutaneously in the BMCMC-injected dorsal skin region with mouse anti-TNP IgE (0.5 μg/ 20 μL; BD Biosciences, San Jose, Calif) to induce a PCA reaction. Saline alone was used as a negative control. The mice were then challenged intravenously 24 hours later with 50 μg of DNP-BSA (Cosmo Bio, Tokyo, Japan) with 0.5% Evans blue dye. Quantitative analysis of the PCA reaction was performed, as previously described.[10]

Statistical analysis

The statistical analyses were performed by using the unpaired Student *t* test for 2-group comparisons and ANOVA for comparison of more than 2 groups. Statcel3 software (OMS Publishing, Saitama, Japan) was used for the analysis. A *P* value of less than .05 was considered significant, unless otherwise indicated.

RESULTS

The mast cell clock times PCA reactions

To determine whether the mast cell–intrinsic clockwork contributes to the temporal regulation of IgE/mast cell–mediated allergic reactions, we compared the kinetics of a time of day–dependent variation in the PCA reaction between mast cell–deficient W/Wv mice subcutaneously reconstituted with BMCMCs generated from wild-type mice and mice with a dominant negative–type mutation of *Clock* ($Clock^{\Delta 19/\Delta 19}$ mice).[13] There were comparable levels of cell-surface FcεRIα and c-kit expression (without synchronization, please see below), mouse mast cell protease (mMCP) 5 and mMCP-6 mRNA expression, and Syk and Lyn protein expression and similar morphology between wild-type and *Clock*-mutated BMCMCs (see Fig E1 in this article's Online Repository at www.jacionline.org), suggesting that the *Clock* mutation did not affect the differentiation of mast cells. We also confirmed that the numbers of mast cells in the skin were comparable between mice reconstituted with wild-type BMCMCs and those reconstituted with *Clock*-mutated BMCMCs (see Fig E2 in this article's Online Repository at www.jacionline.org).

The extent of PCA reactions showed a time of day–dependent variation in control mice, with a clear nadir around the onset of night (10 PM, which was ZT16; Fig 1, *A* and *B*), as described previously in conventional wild-type mice.[10] This variation was absent in mast cell–specific *Clock*-mutated mice (Fig 1, *A* and *B*). In contrast, the daily profiles and levels of serum corticosterone and IgE were comparable between the mice (Fig 1, *C* and *D*). These results suggest that the mast cell–intrinsic clockwork is critical to the daily rhythm generation in the PCA reaction.

IgE-mediated mast cell responses show temporal variations *in vitro*

To support the *in vivo* findings, we examined the temporal responses of mast cells to IgE stimulation by using BMCMCs generated from knock-in mice expressing a PER2::LUC fusion protein (PER2::LUC BMCMCs),[12] wild-type mice, and $Clock^{\Delta 19/\Delta 19}$ mice[13] *in vitro*. We noted that the time window when the mast cell clockwork was functional appeared to be very limited (approximately 0-36 hours after medium change) in *in vitro* culture conditions based on the monitoring of bioluminescent emission of PER2::LUC BMCMCs (see Fig E3 in this article's Online Repository at www.jacionline.org).[10] This might be due to a lack of oscillator coupling in the dissociated cell cultures, leading to damping of the ensemble rhythm at the population level.[15] Therefore we compared the extent of IgE-mediated degranulation in mast cells between 12-hour cultured PER2::LUC BMCMCs (after a medium change) with the nadir of the PER2::LUC protein level and 24-hour cultured PER2::LUC BMCMCs with the peak PER2::LUC protein level. A simple medium change is a trigger to synchronize the circadian clocks in peripheral cells *in vitro*.[6-9] We avoided using potent reagents to synchronize peripheral clocks (eg, cyclic AMP activators and dexamethasone) for the *in vitro* experiments because such reagents affect IgE-mediated signaling in mast cells independent of "clock" function.[16,17]

The extent of IgE-mediated β-hexosaminidase release was significantly higher in the 12-hour cultured PER2::LUC BMCMCs than in the 24-hour cultured PER2::LUC BMCMCs (Fig 2, *A*). More detailed kinetic studies using the 6-, 12-, 18-, and 24-hour cultured BMCMCs after the medium change showed similar findings in wild-type BMCMCs but not in *Clock*-mutated BMCMCs (Fig 2, *B*). There were little differences in spontaneous β-hexosaminidase release (ie, without IgE stimulation) between wild-type and *Clock*-mutated BMCMCs at the representative time points (see Fig E4 in this article's Online Repository at www.jacionline.org). In contrast, the extent of IgE-mediated β-hexosaminidase release was comparable between the 48- and 60-hour cultured wild-type BMCMCs (Fig 2, *C*), both of which appeared to no longer have functional clockwork (see Fig E3). The extent of IgE-mediated IL-13 production also showed similar temporal variations in 12- and 24-hour cultured wild-type BMCMCs (Fig 2, *D*). Consistently, the extent of IgE-dependent intracellular Ca^{2+} mobilization and the total tyrosine phosphorylation levels of cell lysates were higher in the 12-hour cultured wild-type BMCMCs than those in the 24-hour cultured wild-type BMCMCs, which were absent in *Clock*-mutated BMCMCs (Fig 2, *E* and *F*). Interestingly, FcεRI-independent stimulation of the 12- and 24-hour cultured wild-type BMCMCs (and also *Clock*-mutated BMCMCs) with calcium ionophore A23187 showed comparable β-hexosaminidase release (Fig 2, *G*). Collectively, the IgE-mediated degranulation and signaling in mast cells showed temporal variations *in vitro*, and disruption of the mast cell clockwork by either a *Clock* mutation or long-term culture without a medium change (synchronization) abolished the variations.

FcεRIβ transcription is under circadian control by the mast cell clock

The high-affinity IgE receptor (FcεRI) on mast cells consists of 3 subunits (FcεRI α, β, and γ chains), and IgE binds to FcεRIα with a high affinity, whereas the β and γ chains transduce the extracellular signals into the intracellular signaling pathways,