

Circadian clock gene *Period2* regulates a time-of-day-dependent variation in cutaneous anaphylactic reaction

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Background: IgE-mediated immediate-type skin reaction shows a diurnal rhythm, although the precise mechanisms remain uncertain. *Period2* (Per2) is a key circadian gene that is essential for endogenous clockworks in mammals.

Objective: This study investigated whether Per2 regulates a time-of-day-dependent variation in IgE-mediated immediate-type skin reaction.

Methods: The kinetics of a passive cutaneous anaphylactic reaction were compared between wild-type mice and mice with a loss-of-function mutation of Per2 (mPer2^{m/m} mice). The effects of adrenalectomy, aging, and dexamethasone on the kinetics of a passive cutaneous anaphylactic reaction were also examined. In addition, the extent of IgE-mediated degranulation in bone marrow-derived mast cells (BMMCs) was compared between wild-type and mPer2^{m/m} mice.

Results: A time-of-day-dependent variation in a passive cutaneous anaphylactic reaction observed in wild-type mice was absent in mPer2^{m/m} mice and in adrenalectomized and aged mice associated with the loss of rhythmic secretion of corticosterone. In addition, mPer2^{m/m} mice showed decreased sensitivity to the inhibitory effects of dexamethasone on the passive cutaneous anaphylactic reactions. IgE-mediated degranulation in BMMCs was comparable between wild-type and mPer2^{m/m} mice, but Per2 mutation decreased sensitivity to the inhibitory effects of dexamethasone on IgE-mediated degranulation in BMMCs.

Conclusion: A circadian oscillator, Per2, regulates a time-of-day-dependent variation in a passive cutaneous anaphylactic reaction in mice. Per2 may do so by controlling the rhythmic secretion of glucocorticoid from adrenal glands and/or by gating the glucocorticoid responses of mast cells to certain times of the day (possibly when Per2 levels are high in mast cells). (J Allergy Clin Immunol 2011;127:1038-45.)

Key words: Circadian rhythm, clock, Period2, IgE, mast cell, anaphylactic reaction, mouse

Abbreviations used

BMMC: Bone marrow-derived mast cell
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
Per2: Period2
Per2::LUC: Period2::Luciferase
SCN: Suprachiasmatic nucleus
ZT: Zeitgeber time

Circadian rhythms, which are recognized in many organisms from yeast to mammals, are daily oscillations of multiple biological processes including the sleep-wake cycle, blood pressure, and behavioral patterns driven by an endogenous clock.¹⁻⁴ The mammalian master circadian clock is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus. The biological clock also resides in peripheral organs such as the liver, lung, skin, and adrenal glands, and it thereby regulates local physiology (termed “peripheral clocks”). The core cellular oscillators within the SCN ultimately generate circadian rhythms in physiology and behavior by coordinating oscillations of peripheral clocks through neural and endocrine pathways. The fundamental mechanisms of rhythm generation are cell-autonomous, highly conserved in the SCN and peripheral cells, and created and maintained by transcriptional-translational negative feedback loops, whereby the protein products of the *Period* and *Cryptochrome* genes periodically suppress their own expression.

IgE-mediated immediate-type reaction is an acute-onset pathology triggered by the exposure of mast cells to specific environmental allergens.⁵ Individuals sensitized with a given allergen produce IgE antibodies against the allergens and bind to the high-affinity IgE receptor FcεRI expressed on mast cells. The binding of the allergen induces the IgE-FcεRI complexes on mast cells to be cross-linked, thereby leading to mast cell activation.⁶⁻⁸ Activated mast cells undergo degranulation and release chemical mediators, such as histamine, that are responsible for the development of IgE-mediated immediate-type reaction or other types of anaphylactic reactions.

Previous studies suggest that IgE-mediated immediate-type reactions in the skin show diurnal variations. In 1976, Miller and Church⁹ found anaphylactic sensitivity in the pinna of a mouse to be subject to diurnal variation. Seery et al¹⁰ reported that the magnitude of cutaneous hypersensitivity reactions to allergens in patients with nocturnal asthma exhibits a circadian rhythm. Although rhythmic humoral signals from the adrenal glands, such as cortisol, are implicated in the diurnal variations, the precise molecular mechanisms still remain uncertain.

This study investigated whether *Period2* (Per2), a key clock gene, regulates a time-of-day-dependent variation in IgE-mediated

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immediate-type skin reactions. This was investigated by comparing the kinetics of a passive cutaneous anaphylactic reaction between normal mice and mice with a loss-of-function mutation of Per2 (mPer2^{m/m} mice), displaying a loss of persistence in circadian rhythmicity.¹¹ Adrenalectomized mice were also used to determine whether humoral signals derived from the adrenal glands are involved in the time-of-day-dependent variation observed in a passive cutaneous anaphylactic reaction in mice.

METHODS

Mice

Adult Per2 mutant mice (mPer2^{m/m}),¹¹ 1 to 3 months old, backcrossed over 10 generations onto ICR mice, the wild-type ICR mice purchased from Japan SLC, Tokyo, Japan, and Per2::Luciferase (Per2::LUC) transgenic mice (The Jackson Laboratory, Bar Harbor, Me),¹² were bred under specific pathogen-free conditions and 12-hours light/12-hours dark conditions (the light was turned on at 6:00 AM, zeitgeber time [ZT] 0, and the light was turned off at 6:00 PM, ZT12) with *ad libitum* access to food and water, for at least 2 weeks. The mPer2^{m/m} mice carry an in-frame deletion in the PER-ARNT-SIM-B domain and are deficient in mPer2-mediated transcriptional regulation.¹¹ All animal experiments were performed by using male mice and were approved by the Institutional Review Board of the University of Yamanashi.

Induction of passive cutaneous and systemic anaphylactic reactions

The mice were sensitized subcutaneously in the dorsal skin with mouse anti-TNP IgE (0.5 μ g/20 μ L; BD Bioscience, San Diego, Calif) to induce a passive cutaneous anaphylactic reaction. Saline alone was used as a negative control. The mice were then challenged intravenously 24 hours later with 50 μ g DNP-BSA (Cosmo Bio, Tokyo, Japan) with 0.5% Evans blue dye. Vascular permeability was visualized 30 minutes later by the blue staining of the injection sites on the reverse side of the skin. These staining sites were digitalized by using a high-resolution color camera (digital camera LUMIX DE-991; Panasonic, Tokyo, Japan), and the images were saved in a Windows (Microsoft, Seattle, Wash) photo viewer as 8-bit color-scale Joint Photographic Experts Group (.jpg) files. The open source ImageJ 1.43 software package (NIH, Bethesda, Md) was used for image analysis. Briefly, color-scale images exported from Windows photo viewer were converted for hue/saturation/brightness stack type images by using the Image tool. Thereafter, the hue/saturation/brightness stack image was split into hue, saturation, and brightness images, respectively. Only blue-stained were selected from the hue image by using the threshold tool. These images were then combined with the saturation image, and the density values for the blue-stained areas were measured by using the analyze tool. Water-soluble dexamethasone at the indicated doses (Sigma-Aldrich, St Louis, Mo) was intraperitoneally injected 2 hours before the DNP-BSA challenge in this article's Fig E1 in the Online Repository at www.jacionline.org.

A passive systemic anaphylactic reaction was induced in mice as previously described with some modifications.¹³ Briefly, mice were sensitized with an intravenous injection of 20 μ g with mouse anti-TNP IgE in 0.2 mL PBS. The mice were intravenously challenged with 1000 μ g DNP-BSA in 0.2 mL PBS 24 hours later. Their rectal temperature was measured with a digital thermometer (Shibaura Electronics, Tokyo, Japan) every 5 minutes after the challenge.

Serum IgE and corticosterone levels

Serum levels of total IgE and corticosterone were determined by using the mouse IgE ELISA kit (R&D, Minneapolis, Minn, or Morinaga Institute of Biological Science, Kanagawa, Japan) or AssayMax Corticosterone

ELISA kit (AssayPro, Charles, Mo), respectively, according to the manufacturers' instructions.

Adrenalectomy

Bilateral adrenalectomy was carried out via a dorsal approach under ketamine/xylazine anesthesia as previously described.¹⁴ Briefly, the skin on the back was shaved and disinfected, and an incision of approximately 1 cm was made above and parallel to the spinal cord. The adrenals were removed from the surrounding fat tissue through a small opening in the muscle layer left and right of the spinal cord. The mice were subsequently provided with a 0.9% saline solution instead of water to maintain appropriate mineral balance.

Preparation of bone marrow-derived mast cells

Bone marrow-derived mast cells (BMMCs) were generated from the femoral bone marrow cells of female mice as previously described.¹⁵ Diff-Quik stain (a modified May-Giemsa staining; Sysmex Ltd, Hyogo, Japan) was used to identify mast cells morphologically.

Fluorescence-activated cell sorting staining of BMMCs

BMMCs were incubated for 15 minutes with rat-antimouse antibodies to CD16/32 (2.4G; BD Biosciences) to block nonspecific binding and then stained with fluorescein isothiocyanate-conjugated antimouse Fc ϵ RI α (MAR-1; eBioscience, San Diego, Calif) and phycoerythrin-conjugated antimouse c-kit (2B8; BD PharMingen, San Diego, Calif) in PBS for 30 minutes on ice.

After being washed with PBS, the stained cells (live-gated on the basis of forward and side scatter profiles) were analyzed on a FACSCalibur (BD Biosciences), and data were processed using the CellQuest program (BD Biosciences).

Measurement of bioluminescence in BMMCs generated from Per2::LUC mice

BMMCs generated from Per2::LUC mice were placed in a 35-mm Petri dish and incubated at 37°C, and bioluminescence was monitored at 10-minute intervals for 1 minute by using a dish-type luminometer (Kronos DioAB-2550; ATTO Inc, Tokyo, Japan) as previously described.¹⁶

Quantitative real-time PCR

Quantitative real-time RT-PCR with specific primers and probes for mouse Per2, Fc ϵ RI α , Fc ϵ RI β , and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Applied Biosystems, Foster City, Calif) was performed by using the AB7300 real-time PCR system (Applied Biosystems) as previously described.¹⁷

β -Hexosaminidase release assay

The β -hexosaminidase release assay was performed as previously described.¹⁵ Briefly, BMMCs (5×10^6 cells/mL) incubated for 1 hour at 4°C with 1 μ g/mL anti-DNP mouse IgE mAb (BD Biosciences) were stimulated for 40 minutes at 37°C with 1 μ g/mL antimouse IgE (BD Biosciences). After centrifugation, supernatants were collected, and total cell lysates were obtained by 1% Triton X-100. The percentage of β -hexosaminidase release was calculated as previously described.¹⁵ BMMCs were pretreated with water-soluble dexamethasone at the indicated doses 2 hours before the stimulation in Fig E1.

Data analysis

The statistical analysis was performed by using unpaired Student *t* test. A value of *P* < .05 was considered to be significant.

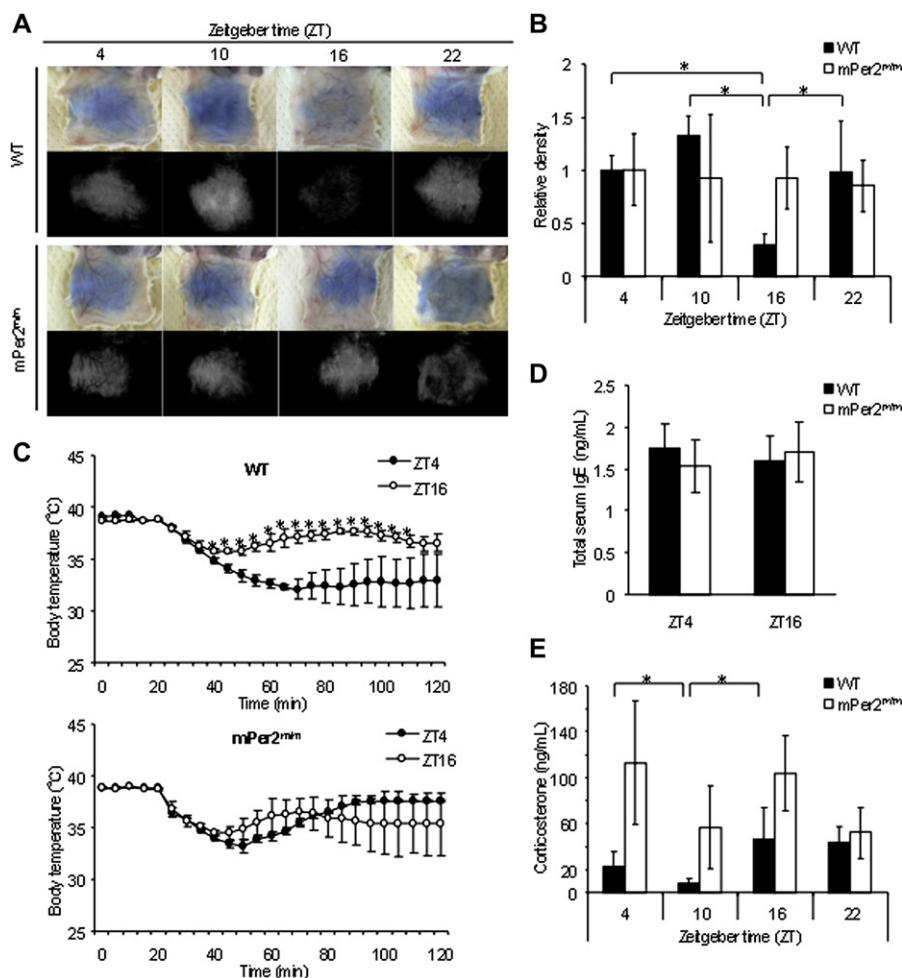


FIG 1. A passive cutaneous anaphylactic reaction in mice displays a time-of-day-dependent variation, which is dependent on Per2. **A**, Representative pictures of the skin color reactions after the allergen challenge (upper panels) and the digitalized images for the density value evaluation of the blue-stained areas (lower panels). **B**, A quantitative analysis of the skin color reactions ($n = 4$). **C**, Changes in rectal temperature over time after the allergen challenge ($n = 4$). **D**, The total serum IgE levels obtained at 10:00 AM (ZT4) and 10:00 PM (ZT16; $n = 4$). **E**, The serum corticosterone levels at the indicated time points in wild-type (WT) and mPer2^{m/m} mice ($n = 4$). Values represent the mean $\pm$ SD. * $P < .05$.

RESULTS

A passive cutaneous anaphylactic reaction in mice displays a time-of-day-dependent variation relying on Per2

The kinetics of a passive cutaneous anaphylactic reaction was observed in wild-type mice and in mice with a loss-of-function mutation of Per2 (mPer2^{m/m} mice). Wild-type and mPer2^{m/m} mice subcutaneously sensitized with anti-TNP IgE were intravenously challenged with DNP-BSA (and Evans blue solution) at various time points with 6-hour intervals. The extent of a passive cutaneous anaphylactic reaction was assessed by a quantitative analysis of the extent of the skin color reactions by using an imaging software program. The extent of the passive cutaneous anaphylactic reaction showed a time-of-day-dependent variation in wild-type mice, with a clear nadir around the onset of night (10:00 PM [ZT16]; Fig 1, A and B). In contrast, the time-of-day-dependent variation of the skin reactions was absent in the mPer2^{m/m} mice.

The kinetics of the passive systemic anaphylactic reaction were also compared between wild-type and mPer2^{m/m} mice to validate these findings further (Fig 1, C). Wild-type mice challenged with

the antigen at 10:00 PM (ZT16) showed a smaller drop in the extent of rectal temperatures than the mice challenged at 10:00 AM (ZT4). Again, such a time-of-day-dependent variation in the passive systemic anaphylactic reaction was absent in the mPer2^{m/m} mice.

The serum IgE levels were examined in wild-type and mPer2^{m/m} mice at 10:00 AM (ZT4) and 10:00 PM (ZT16; Fig 1, D), because IgE upregulates FcεRI expression on mast cells and enhances the ability of mast cells to release chemical mediators in response to challenge with IgE and specific antigens.¹⁸ The serum IgE levels were comparable between the wild-type and mPer2^{m/m} mice both at 10:00 AM (ZT4) and 10:00 PM (ZT16). These results suggest that an IgE-mediated passive cutaneous anaphylactic reaction displays a time-of-day-dependent variation in mice, relying on Per2.

A time-of-day-dependent variation in a passive cutaneous anaphylactic reaction is absent in adrenalectomized mice

The lack of Per2 function results in a loss of persistence in a daily rhythmic secretion of humoral endocrine factors from

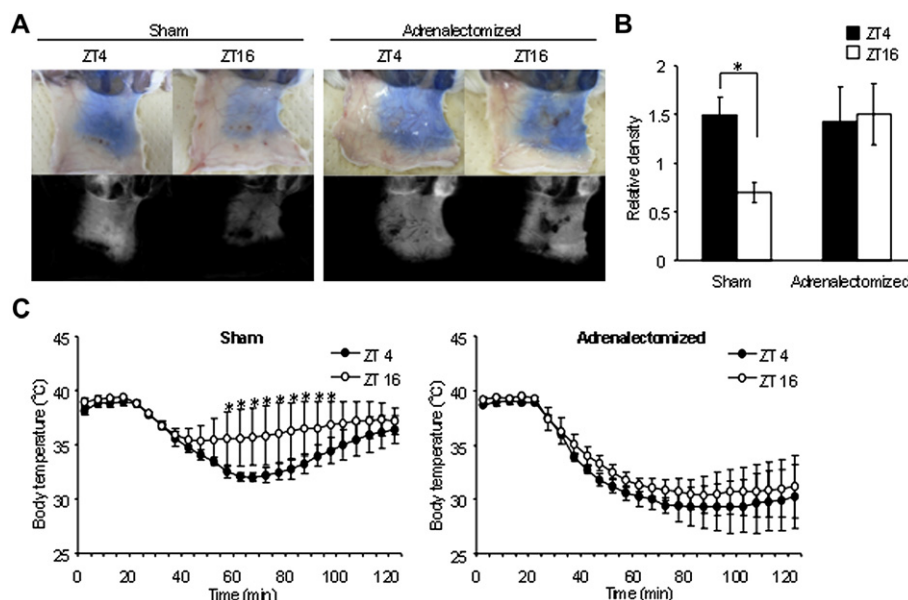


FIG 2. A time-of-day-dependent variation in a passive cutaneous anaphylactic reaction is absent in adrenalectomized mice. **A**, Representative pictures of the skin color reactions after the allergen challenge (upper panels) and the digitalized images for the density value evaluation of the blue-stained areas (lower panels). **B**, A quantitative analysis of the skin color reactions ($n = 4$). **C**, Changes in the rectal temperature over time after the allergen challenge ($n = 4$). Values represent the mean $\pm$ SD. * $P < .05$.

adrenal glands such as cortisol (corticosterone in rodents).^{19,20} Humoral endocrine factors are implicated in the diurnal variations of the IgE-mediated immediate skin reactions in mice and human beings.^{9,10} Consistent with a previous study,^{19,20} the serum corticosterone levels showed a robust daily variation in wild-type mice, with a peak around the onset of night (around 10:00 PM [ZT16]), whereas this daily variation was absent in the *mPer2^{m/m}* mice (Fig 1, E).

Therefore, to determine whether rhythmic humoral signals from the adrenal glands primarily control the time-of-day-dependent variations observed in the passive cutaneous anaphylactic reaction in mice, the effects of adrenalectomy on the skin reaction were examined. There was no time-of-day-dependent variation in a passive cutaneous anaphylactic reaction at 10:00 AM (ZT4) and 10:00 PM (ZT16) in adrenalectomized mice, in contrast with sham-operated mice (Fig 2, A and B). The time-of-day-dependent variation in a passive systemic anaphylactic reaction was also absent in adrenalectomized mice (Fig 2, C). These results indicate that adrenalectomy attenuates the time-of-day-dependent variation in a passive cutaneous (and also systemic) anaphylactic reaction in mice. Therefore, the time-of-day-dependent variation in a passive cutaneous anaphylactic reaction requires the presence of adrenal glands.

Time-of-day-dependent variation in a passive cutaneous anaphylactic reaction is absent in aged mice

Circadian rhythmicity including the daily rhythmic secretion of humoral endocrine factors from adrenal glands may degenerate with increasing age in mammals.^{21,22} Therefore, aged mice may not show an apparent time-of-day-dependent variation in passive cutaneous anaphylactic reactions in association with reduced rhythmic changes in the serum corticosterone levels. This

possibility was investigated by comparing the extent of a passive cutaneous anaphylactic reaction in young and aged mice. Little time-of-day-dependent variation in a passive cutaneous anaphylactic reaction at 10:00 AM (ZT4) and 10:00 PM (ZT16) was observed in 30-week-old mice compared with 8-week-old mice (Fig 3, A and B). The daily variation in serum corticosterone levels was absent in 30-week-old mice compared with 8-week-old mice (Fig 3, C). The serum IgE levels were comparable between 8-week-old and 30-week-old mice at both 10:00 AM (ZT4) and 10:00 PM (ZT16; Fig 3, D). These results suggest that the time-of-day-dependent variation in a passive cutaneous anaphylactic reaction is attenuated in aged mice in association with aberrant rhythmic secretion of corticosterone.

BMMCs from *mPer2^{m/m}* mice show levels of IgE-mediated β -hexosaminidase release comparable to those of wild-type mice

The *in vivo* results described can be also attributed to possible differences in the intrinsic activity of mast cells between wild-type and *mPer2^{m/m}* mice. It was therefore important to determine whether the *Per2* mutation affects IgE-mediated degranulation in mast cells. As a result, the levels of IgE-mediated β -hexosaminidase release were compared between BMMCs generated from wild-type and *mPer2^{m/m}* mice.

The 5-week-cultured BMMCs exhibited comparable levels of the cell surface Fc ϵ RI α and c-kit expression between wild-type and *mPer2^{m/m}* mice (Fig 4, A). In addition, the 5-week-cultured BMMCs did not show any apparent difference in morphology between wild-type and *mPer2^{m/m}* mice (Fig 4, B). These results suggest that the *Per2* mutation does not affect the differentiation of mast cells.

To investigate whether BMMCs have a circadian oscillation of *Per2*, the molecular oscillations in BMMCs generated from

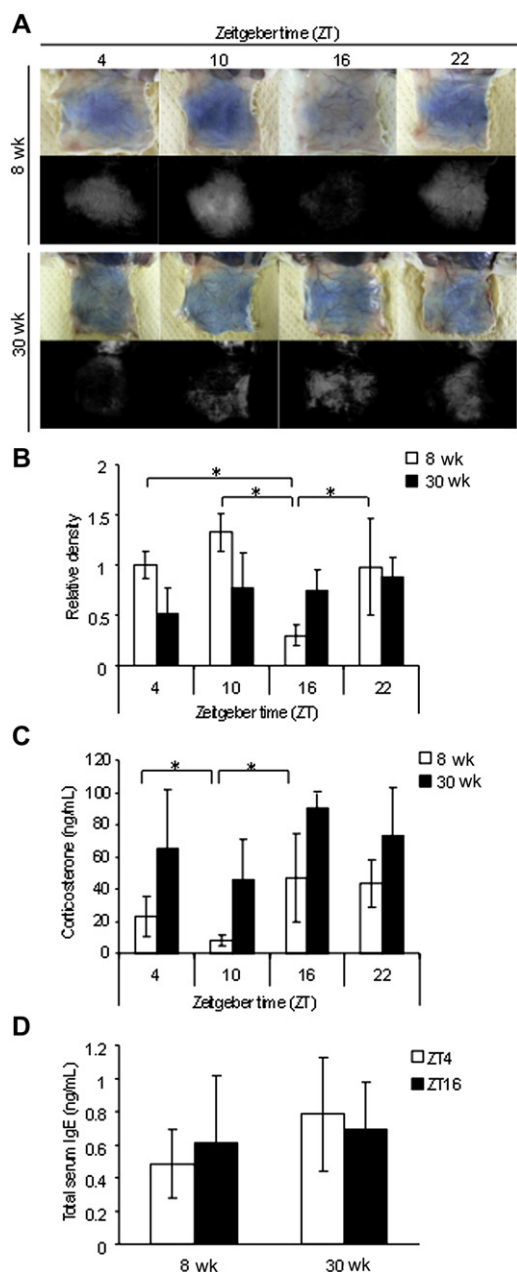


FIG 3. A time-of-day-dependent variation in a passive cutaneous anaphylactic reaction is absent in aged mice. **A**, Representative pictures of the skin color reactions after the allergen challenge (upper panels) and the digitalized images for the density value evaluation of the blue-stained areas. **B**, A quantitative analysis of the skin color reactions ($n = 4$). **C**, Serum corticosterone levels in 8-week-old and 30-week-old wild-type mice ($n = 4$). **D**, The total serum IgE levels obtained at 10:00 AM (ZT4) and 10:00 PM (ZT16; $n = 4$). Values represent the mean $\pm$ SD. * $P < .05$.

knock-in mice expressing a Per2::LUC fusion protein¹² were observed. Monitoring bioluminescence emission in consistent culture conditions revealed that BMMCs generated from the Per2::LUC knock-in mice displayed a circadian rhythmicity of Per2::LUC expression (Fig 4, C). In addition, a real-time PCR analysis showed that the Per2 transcript had a circadian oscillation in wild-type BMMCs, whereas FcεRI α-chain and β-chain transcripts showed stable expression levels (Fig 4, D). These

results suggest that Per2 is rhythmically expressed in wild-type BMMCs. However, the levels of IgE-mediated β-hexosaminidase release in BMMCs were comparable between wild-type and mPer2^{m/m} mice with IgE stimulation at any time point (Fig 4, E; data not shown). These results suggest that the BMMCs had an autonomous clock as indicated by a circadian oscillation of both Per2 protein and transcript, but the clock did not affect IgE-mediated degranulation of BMMCs.

Per2 may also regulate mast cell responses to glucocorticoid

The *in vivo* results obtained from mPer2^{m/m} mice, adrenalectomized mice, and aged mice suggest that a time-of-day-dependent variation in the passive cutaneous anaphylactic reaction may be associated with the rhythmic secretion of corticosterone under the control of Per2.^{19,20} However, a recent study also suggested that Per2 plays a role not only in generating circadian patterns of glucocorticoid release but also in mediating specific actions of glucocorticoid.²³ It is therefore also possible that Per2 may affect the time-of-day-dependent variation in the passive cutaneous anaphylactic reaction in mice by regulating the activity of glucocorticoid. We therefore examined the effects of various doses of dexamethasone on the passive cutaneous anaphylactic reaction in wild-type and mPer2^{m/m} mice.

The acute administration of 100 μg/kg, but not 10 μg/kg, of dexamethasone decreased the extent of the passive cutaneous anaphylactic reaction at 10:00 AM (ZT4) in wild-type mice, but the inhibitory effect was not observed in mPer2^{m/m} mice (Fig 5, A and B). A high dose of dexamethasone (1 mg/kg) decreased the extent of the passive cutaneous anaphylactic reaction at 10:00 AM (ZT4) in both wild-type mice and mPer2^{m/m} mice (Fig E1, A and B).

Consistently, the levels of IgE-mediated β-hexosaminidase release were inhibited by the addition of 1 μmol/L, but not 10 nmol/L, of dexamethasone in BMMCs from wild-type mice, whereas these inhibitory effects were not observed in BMMCs from mPer2^{m/m} mice (Fig 5, C). The 10-μmol/L concentration of dexamethasone inhibited IgE-mediated β-hexosaminidase release in BMMCs from both wild-type and mPer2^{m/m} mice (Fig E1, C). These results suggest that Per2 may regulate mast cell responses to glucocorticoid, and this mechanism may thus play an additional role in the time-of-day-dependent variation in the passive cutaneous anaphylactic reaction in mice.

DISCUSSION

This study demonstrated the time-of-day-dependent variation in the passive cutaneous anaphylactic reaction observed in wild-type mice to be absent in mPer2^{m/m} mice, in adrenalectomized mice, and in aged mice in association with an aberrant daily variation of serum corticosterone levels. In addition, a loss-of-function mutation of Per2 decreased the sensitivity of mast cells to the inhibitory effects of glucocorticoid both *in vitro* and *in vivo*. On the basis of these findings, we suggest that the circadian oscillator, Per2, regulates the time-of-day-dependent variation in the passive cutaneous anaphylactic reaction in mice, and it may do so by controlling the rhythmic secretion of glucocorticoid from adrenal glands and/or by gating the glucocorticoid responses of mast cells to certain times of day (possibly when Per2 levels are high in mast cells).

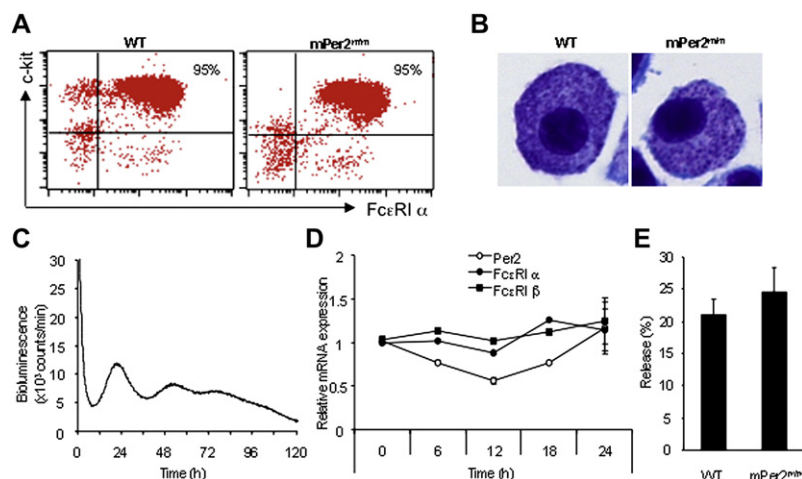


FIG 4. BMMCs from mPer2^{m/m} mice show levels of IgE-mediated β-hexosaminidase release comparable to those of wild-type (WT) mice. **A**, Flow-cytometric analysis of BMMCs with anti-FcεRI and anti-c-kit antibody. **B**, Diff-Quik staining of BMMCs. **C**, Monitoring of BMMCs from Per2::LUC knock-in mice for 120 hours by the detection of bioluminescence. **D**, Real-time PCR analysis of BMMCs from wild-type and mPer2^{m/m} mice for Per2, FcεRIα, and FcεRIβ (n = 3). **E**, Release of β-hexosaminidase from BMMCs (%; n = 3). Values represent the mean ± SD. *P < .05.

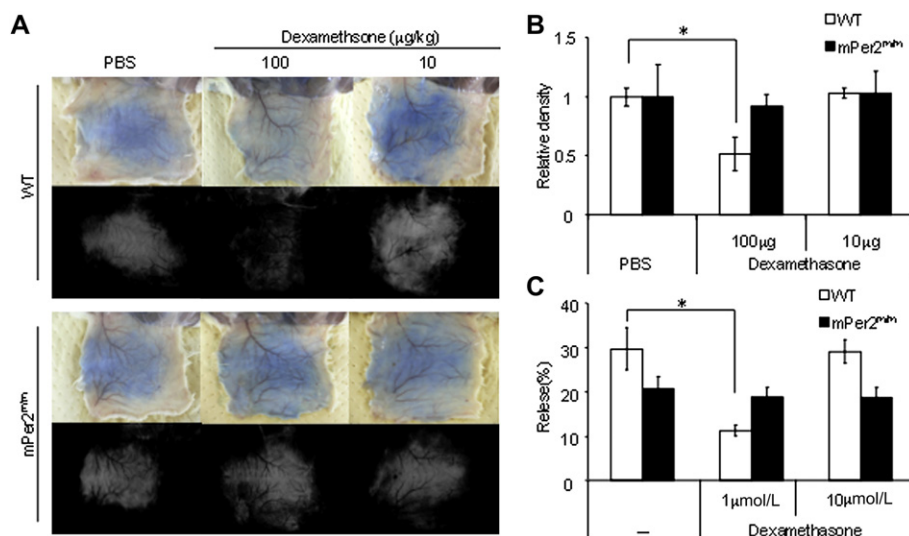


FIG 5. Per2 may regulate mast cell responses to glucocorticoid. **A**, Representative pictures of the skin color reactions after the allergen challenge with or without the indicated doses of dexamethasone as pretreatment (upper panels) and the digitalized images of the density value evaluation of the blue-stained areas (lower panels) are shown. **B**, A quantitative analysis of the skin color reactions (n = 4). **C**, The release of β-hexosaminidase from BMMCs (%) with or without pretreatment with the indicated doses of dexamethasone (n = 3). Values represent the mean ± SD. *P < .05. WT, Wild-type.

Previous studies suggest that the IgE-mediated immediate-type skin reaction shows a diurnal rhythm in mice and human beings,^{9,10} which is consistent with the current results. Given that a passive systemic anaphylactic reaction may also display a time-of-day-dependent variation, it is likely that IgE/mast cell-mediated anaphylactic reactions have diurnal rhythm regardless of the involved organs.

The results from the adrenalectomized mice strongly suggest that humoral factors released from the adrenal glands are plausible candidates to regulate the time-of-day-dependent variation

in the passive cutaneous anaphylactic reaction, because adrenal glands do not have any direct connections to mast cells distributed throughout the body. This hypothesis was supported by the finding that adrenalectomy affects both passive cutaneous and systemic anaphylactic reactions.

The plasma concentrations of cortisol exhibit a distinct circadian variation regulated by the clock system.^{1,4,24} The levels in human beings are minimal at night and rise to a maximum in the beginning of the day, whereas the patterns are reversed in mice because they are nocturnal animals.^{19,20} Therefore, the

time-of-day-dependent variation in a passive cutaneous (and also systemic) anaphylactic reaction in wild-type mice showed an inverse association with the plasma corticosterone levels. Thus, corticosterone may be a key humoral factor regulating the daily variation in mice. However, the precise involvement of corticosterone or other humoral factors, such as catecholamine, rhythmically secreted from adrenal glands²⁵ remains to be determined.

The biological clock resides not only in the SCN but also in peripheral organs such as the liver, skin, and adrenal glands.²⁶ The current study also suggests that BMMCs have their own clock, as indicated by the circadian oscillation of the mRNA and protein levels of Per2. During the submission of this article, Wang et al²⁷ also reported that several clock genes, including Per2, oscillated in BMMCs, and that IgE-dependent expression of IL-6 and IL-13 mRNAs, but not degranulation, exhibited circadian rhythms. This is largely consistent with the current results.

The precise mechanisms underlying how Per2 affects the time-of-day-dependent variation in the passive cutaneous anaphylactic reaction in mice remain to be determined. One possibility is that Per2 may do so by regulating the rhythmic secretion of humoral factors such as corticosterone from adrenal glands, as indicated in the previous studies,^{9,10} and as discussed. Alternatively, because Per2 appears to be required for appropriate mast cell responses to glucocorticoid (Fig 5), the local mast cell-intrinsic clock (the "peripheral clock") may gate the glucocorticoid responses to certain times of day, thereby regulating the time-of-day-dependent variation in passive cutaneous anaphylactic reactions. We speculate that the 2 mechanisms are not mutually exclusive and may work together, because serum corticosterone levels and Per2 expression levels in mast cells had similar circadian patterns in mice, both showing a peak at around the onset of night (Fig 1, E; see this article's Fig E2 in the Online Repository at www.jacionline.org).

Therefore, when the serum corticosterone levels are high during the day, mast cells might receive the systemic timing signals because the Per2 expression levels in mast cells are also high at this time. Per2 dysfunction in mast cells might result in the loss of appropriate responses to high serum levels of corticosterone ("disrupted coupling of corticosterone secretion and target cell sensitivity to corticosterone"), thereby blunting the time-of-day-dependent variation in the passive cutaneous anaphylactic reaction. The redundancy of such dual controls of glucocorticoid levels and sensitivity by Per2 might enhance the robustness of the circadian control of IgE-mediated mast cell responses.

It has been reported that glucocorticoids, including dexamethasone, can reset the rhythm of clock gene expression in the mouse lung or other tissues.²⁸⁻³⁰ Therefore, regarding the inhibitory effects of dexamethasone on the degranulation response and passive cutaneous anaphylactic reaction, it remains unclear whether dexamethasone directly inhibited those responses or indirectly suppressed them by resetting mast cell clock gene expression.

In summary, this study suggests that Per2, a key clock gene, regulates the time-of-day-dependent variation in the passive cutaneous anaphylactic reaction in mice. This is the first study to provide direct evidence that the circadian clock system underlies diurnal rhythmic changes observed in IgE-mediated immediate-type skin reactions. The current results also suggest that the clock system may regulate the rhythms and activities of systemic timing

signals, such as glucocorticoid, and play an important role in "synchronizing" the allergic immune responses.

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Clinical implications: The circadian clock system may underlie diurnal symptoms and laboratory parameters observed in patients with allergic diseases such as asthma, allergic rhinitis, and chronic urticaria.

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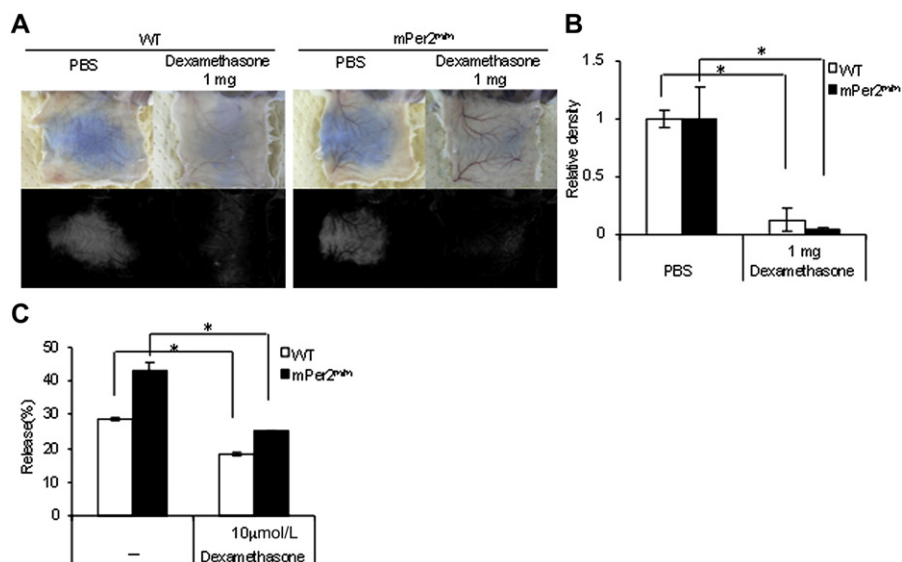


FIG E1. A large dose of dexamethasone decreases the extent of the passive cutaneous anaphylactic reaction at 10:00 AM (ZT4) in mice and inhibits the β -hexosaminidase release from BMMCs regardless of a loss-of-function mutation of Per2. **A**, Representative pictures of the skin color reactions after the allergen challenge with or without dexamethasone pretreatment (1 mg/kg/mouse; *upper panels*) and the digitalized images for the density value evaluation of the blue-stained areas (*lower panels*). **B**, A quantitative analysis of the skin color reactions ($n = 4$). **C**, The release of β -hexosaminidase from wild-type (WT) mice-derived or mPer2^{m/m} mice-derived BMMCs (%) with or without pretreatment with 10 μ mol/L dexamethasone ($n = 3$). Values represent the mean $\pm$ SD. * $P < .05$.

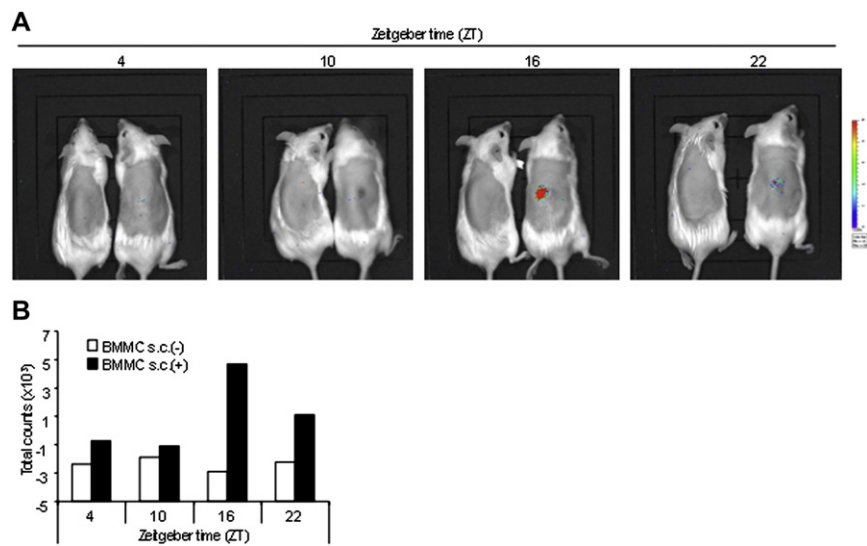


FIG E2. Expression of *Per2* in mast cells shows a circadian rhythm *in vivo*. **A**, Mast cell-deficient WBB6F1-*W/W^v* mice (Japan SLC, Tokyo, Japan) were reconstituted with subcutaneous (*s.c.*) injections of BMMCs (1.5×10^6 /mouse) derived from *Per2::Luc* transgenic mice (*right*) or with only PBS (*left*). After 6 weeks of reconstitution, 20 μ L luciferin (50 mg/mL) was subcutaneously administered at the indicated time points, and then bioluminescence emission from the mice was recorded as described previously.^{E1} Note the focal induction of luciferase activity (*red*) in the back area of the mice (the area where BMMCs were injected), with a peak at around ZT16. **B**, Quantitative analysis of the results described in **A**.