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IgE-dependent activation of human mast cells and fMLP-mediated activation of human eosinophils is controlled by the circadian clock

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ABSTRACT

Symptoms of allergic attacks frequently exhibit diurnal variations. Accordingly, we could recently demonstrate that mast cells and eosinophils – known as major effector cells of allergic diseases – showed an intact circadian clock. Here, we analyzed the role of the circadian clock in the functionality of mast cells and eosinophils. Human intestinal mast cells (hiMC) were isolated from intestinal mucosa; human eosinophils were isolated from peripheral blood. HiMC and eosinophils were synchronized by dexamethasone before stimulation every 4 h around the circadian cycle by FcεRI crosslinking or fMLP, respectively. Signaling molecule activation was examined using Western blot, mRNA expression by real-time RT-PCR, and mediator release by multiplex analysis. CXCL8 and CCL2 were expressed and released in a circadian manner by both hiMC and eosinophils in response to activation. Moreover, phosphorylation of ERK1/2, known to be involved in activation of hiMC and eosinophils, showed circadian rhythms in both cell types. Interestingly, all clock genes *hPer1*, *hPer2*, *hCry1*, *hBmal1*, and *hClock* were expressed in a similar circadian pattern in activated and unstimulated cells indicating that the local clock controls hiMC and eosinophils and subsequently allergic reactions but not *vice versa*.

1. Introduction

The circadian clock plays a key role in many physiological processes and behaviors such as in the maintenance and regulation of sleep–wake cycle, metabolism, or immunological processes (Reppert and Weaver, 2002). Interruption of circadian rhythms caused by sleep disturbances, jet lag or shiftwork resulted in biological and metabolic malfunction (De Bacquer et al., 2009), increased inflammation, emergence of malignant growth and cancer (Logan et al., 2012; Davis and Mirick, 2006; Filipski et al., 2003) as well as reduced life span.

The mammalian circadian clockwork system consists of a core oscillator located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus and peripheral tissues (Froy and Chapnik, 2007; Reppert and Weaver, 2002). At the molecular level, generation of circadian rhythms is maintained by the expression of specific clock genes, and thus, the functional interaction of transcriptional and translational feedback loops. Briefly, the transcription factor *circadian locomotor output cycles Kaput* (CLOCK) forms a heterodimer with *brain and muscle ARNT-like protein 1* (BMAL1) to activate the transcription of several genes, including *Periods 1–3* (*Per1*, *Per2*, *Per3*) and *Cryptochromes 1–2* (*Cry1*, *Cry2*) as well as clock controlled genes, upon binding to E-box containing (5′-CACGTG-3′) sequences. PER and CRY proteins oligomerize and migrate to the nucleus to act as transcription repressors resulting in the inhibition of CLOCK:BMAL1-mediated transcription (Reppert and Weaver, 2001).

The prevalence of allergic diseases, such as atopic dermatitis, asthma or allergic rhinitis has rapidly increased in the past decades especially in industrialized countries. The severity of major symptoms of allergic diseases as well as pulmonary function are frequently exacerbated between midnight and morning and exhibit a prominent 24 h variation (Smolensky et al., 2007; Durrington et al., 2014). These findings assume a role for the biological clock in allergic reactions and a novel approach of new therapeutic strategies and appropriate treatment within chronopharmacology.

Abbreviations: BMAL, *brain and muscle ARNT-like protein*; CLOCK, *circadian locomotor output cycles Kaput*; CRY, *Cryptochromes*; fMLP, *N-formyl-methionyl-leucyl-phenylalanine*; hiMC, human intestinal mast cells; MC, mast cells; PER, *Periods*; SCN, suprachiasmatic nucleus.

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Mast cells (MC) and eosinophils are known to be involved as major effector cells in type I allergic reactions, and in many other acute and chronic diseases (Minai-Fleminger and Levi-Schaffer, 2009; Bischoff, 2007). Within the allergic response, MC carry out their inflammatory effects by producing and releasing a variety of pre-stored (histamine, β-hexosaminidase or proteases) as well as *de novo* synthesized mediators, such as lipid mediators, proinflammatory cytokines and chemokines in response to activation *via* the high-affinity FcεRI (Bischoff, 2007). Eosinophils are capable of producing the granule-stored proteins major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin/eosinophil protein X (EDN/EPX) as well as lipid mediators, growth factors, proinflammatory chemokines and cytokines (Kariyawasam and Robinson, 2006; Hogan et al., 2008).

A circadian variation of serum mast cell tryptase (Dugas-Breit et al., 2005) and plasma histamine (Friedman et al., 1989) concentrations with lower levels in the afternoon and nocturnal peaks has been reported. Several studies suggested that some eosinophil-specific mediators, including ECP, and EPX/EDN displayed a diurnal variation in sputum, urine and serum in patients with allergic rhinitis (Wolthers and Heuck, 2003). Recently, we demonstrated a functional circadian clock in human MC and eosinophils. Clock genes as well as mast cell-specific molecules, such as tryptase, FcεRI α-chain and cKit were expressed in a circadian manner (Baumann et al., 2013). Similarly, an oscillatory expression pattern of the eosinophil-specific molecules ECP and EPX/EDN was observed in human eosinophils.

The aim of this study was to analyze the role of the molecular clock in the functionality of MC and eosinophils in response to activation and consequently, their relevance in allergic reactions.

2. Material and methods

2.1. Isolation and culture of human intestinal mast cells

Human intestinal mast cells (hiMC) were isolated from intestinal mucosa of macroscopically normal surgery specimens of patients who underwent bowel resection because of cancer. Intestinal tissue is a good source to obtain primary human MC. The procedure of combined mechanic and enzymatic tissue digestion is described in detail elsewhere (Sellge and Bischoff, 2006). The study has been approved by the local ethics committee. The obtained cell suspension was cultured overnight in medium RPMI 1640 + GlutaMax™ I (Gibco® Life-Technologies, Darmstadt, Germany) supplemented with 10% fetal calf serum (Biochrom AG, Merck Millipore, Berlin, Germany), 100 μg/ml gentamycin (Biochrom AG), 100 μg/ml streptomycin and 100 U/ml penicillin (PAN Biotech, Aidenbach, Germany), and 2.5 μg/ml amphotericin B (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). Afterwards, hiMC were purified by positive selection using CD117 microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany). HiMC were cultured in the presence of 25 ng/ml recombinant human stem cell factor and 2 ng/ml IL-4 (PeproTech, London, UK). HiMC purity was controlled by FcεRI expression using flow cytometry and FITC-labelled anti-human FcεRIα antibody (BioLegend, San Diego, CA, USA). FcεRI expression and purity of the cells was >99% (Fig. S1).

Supplementary Fig. S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molimm.2014.10.026.

2.2. Isolation and culture of human peripheral blood eosinophils

Human peripheral blood eosinophils were isolated from healthy volunteers by density gradient centrifugation of Pancoll (PAN-Biotech) and purified by negative selection using CD16 microbeads (Miltenyi Biotech). Permission to take a blood sample was obtained from the local ethics committee. After isolation and purification of eosinophils, cells were resuspended in RPMI 1640 (Gibco® Life-Technologies), 10% fetal calf serum (Biochrom AG), and 100 μg/ml streptomycin and 100 U/ml penicillin (PAN-Biotech).

2.3. In vitro treatment of hiMC and human peripheral blood eosinophils

Purified hiMC and eosinophils were synchronized with 40 μM dexamethasone for 2 h to reset the biological clock (Sherman and Froy, 2008). To activate hiMC by FcεRI crosslinking, cells were loaded with 0.1 μg/ml myeloma IgE (Calbiochem®, Merck Millipore, Darmstadt, Germany) for 90 min, and were stimulated with 1 μg/ml anti-human IgE every 4 h around the circadian cycle. To analyze the phosphorylation level of phospho ERK1/2, cells were activated for 10 min, as well as for 90 min or 6 h to determine the relative mRNA expression of clock genes and chemokines as well as the release of mediators in collected supernatants, respectively. Eosinophils were incubated with 5 μg/ml cytochalasin B (Sigma–Aldrich Chemie GmbH) for 10 min prior to the addition of 30 ng/ml IL-5 (PeproTech) for 1 h before stimulation with 100 nM *N-formyl-methionyl-leucyl-phenylalanine* (fMLP) (Sigma–Aldrich Chemie GmbH) every 4 h over a period of 24 h (Takafuji et al., 1991). Eosinophils were stimulated for 2 min to quantify phosphorylation levels of ERK1/2 (Zhu and Bertics, 2011), as well as for 90 min to measure the expression of clock genes and expression and release of chemokines.

2.4. RNA isolation, cDNA synthesis, and real-time RT-PCR

Total RNA of hiMC and eosinophils was isolated using RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. To quantify the expression levels of clock genes as well as chemokines, real-time RT-PCR was assayed as described in detail (Feuser et al., 2012). The following sense and anti-sense primers were applied. *hPer1*: 5′-GGA CCG ACC CCT CAT GCT-3′, 5′-CCC GCC AAC TGC AGA ATC T-3′; *hPer2*: 5′-TAA CTG TAG CTT CAG CGC GT-3′, 5′-ACC TGT GTA AGC ACA CAC AC-3′; *hBmal1*: 5′-ATT GAA ACA CCT CAT TCT CAG GG-3′, 5′-CTT ACG ACA AAC AAA AAT CCA TCT G-3′; *hClock*: 5′-ACA GCT GCT GAC AAA AGC CAA-3′, 5′-TGT GTT TAT ACG ATT ATC TGA CCC AGA AA-3′; *hCry1*: 5′-CAA TGG TGA ACC ATG CTG AG-3′, 5′-GCC TCC ATT CCC ATT AGG AT-3′; *hCxcl8*: 5′-CTG AGA GTG ATT GAG AGT GG-3′, 5′-ACA ACC CTC TGC ACC CAG TT-3′; *hCcl2*: 5′-CTT CTG TGC CTG CTG CTC AT-3′, 5′-CGG AGT TTG GGT CTT GTC C-3′. The expression levels of target genes were normalized using the constitutively expressed house-keeping gene *hGapdh* (5′-TGG TCT CCT CTG ACT TCA AAC-3′, 5′-CCT GTT GCT GTA GCC AAA TT-3′). Primers were validated by a melting curve of the amplificates, and reaction mixture without cDNA was used as a negative control. All reactions were performed using iQ™5 Cycler (Bio-Rad Laboratories, Munich, Germany) and the relative quantification was calculated by the $2^{-\Delta\Delta Ct}$ method.

2.5. Release of β-hexosaminidase and de novo synthesized chemokines

The released β-hexosaminidase was quantified by a colorimetric enzyme assay as accurately described (Schwartz et al., 1979). To determine the amount of chemokines including CXCL8, and CCL2, the Bioplex multibead cytokine assay (Bio-Rad Laboratories) was used in accordance with the manufacturer's instructions and analyzed with the MAGPIX® system (Bio-Rad Laboratories).